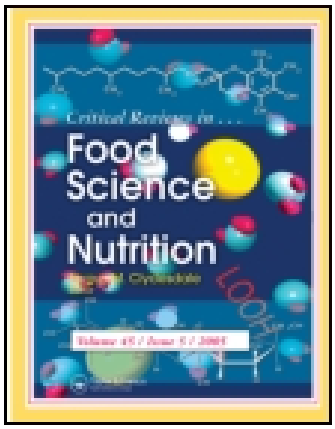


Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Cocoa Phytochemicals: Recent Advances in Molecular Mechanisms on Health

Jiyoung Kim [a b c], Jaekyoon Kim [a], Jaesung Shim [a], Chang Yong Lee [d], Ki Won Lee [a b c e] & Hyong Joo Lee [a b c]

[a] WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, Seoul, 151-742, Republic of Korea

[b] Center for Food and Bioconvergence Technology, Seoul National University, Seoul, 151-921, Republic of Korea

[c] Advanced Institutes of Convergence Technology, Seoul National University, Suwon, 443-270, Republic of Korea

[d] Department of Food Science, Cornell University, Geneva, NY, 14456, USA

[e] Research Institute of Bio Food Industry, Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang, 232-916, Republic of Korea

[f] Advanced Institutes of Convergence Technology, Seoul National University, Suwon, Gyeonggi-do, 443-270, Republic of Korea

Accepted author version posted online: 24 May 2013.Published online: 28 Feb 2014.

To cite this article: Jiyoung Kim, Jaekyoon Kim, Jaesung Shim, Chang Yong Lee, Ki Won Lee & Hyong Joo Lee (2014) Cocoa Phytochemicals: Recent Advances in Molecular Mechanisms on Health, Critical Reviews in Food Science and Nutrition, 54:11, 1458-1472, DOI: 10.1080/10408398.2011.641041

To link to this article: http://dx.doi.org/10.1080/10408398.2011.641041

Critical Reviews in Food Science and Nutrition, 54:1458–1472 (2014)

ISSN: 1040-8398 / 1549-7852 online
DOI: 10.1080/10408398.2011.641041

Taylor & Francis
Taylor & Francis Group

Cocoa Phytochemicals: Recent Advances in Molecular Mechanisms on Health

JIYOUNG KIM,[1,2,3] JAEKYOON KIM,[1] JAESUNG SHIM,[1] CHANG YONG LEE,[4] KI WON LEE,[1,2,3,5] and HYONG JOO LEE[1,2,3]

[1]WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Republic of Korea
[2]Center for Food and Bioconvergence Technology, Seoul National University, Seoul 151-921, Republic of Korea
[3]Advanced Institutes of Convergence Technology, Seoul National University, Suwon 443-270, Republic of Korea
[4]Department of Food Science, Cornell University, Geneva, NY 14456, USA
[5]Research Institute of Bio Food Industry, Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang 232-916, Republic of Korea
[6]Advanced Institutes of Convergence Technology, Seoul National University, Suwon, Gyeonggi-do 443-270, Republic of Korea

Recent reports on cocoa are appealing in that a food commonly consumed for pure pleasure might also bring tangible benefits for human health. Cocoa consumption is correlated with reduced health risks of cardiovascular diseases, hypertension, atherosclerosis, and cancer, and the health-promoting effects of cocoa are mediated by cocoa-driven phytochemicals. Cocoa is rich in procyanidins, theobromine, (−)-epicatechin, catechins, and caffeine. Among the phytochemicals present in consumed cocoa, theobromine is most available in human plasma, followed by caffeine, (−)-epicatechin, catechin, and procyanidins. It has been reported that cocoa phytochemicals specifically modulate or interact with specific molecular targets linked to the pathogenesis of chronic human diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, obesity, diabetes, and skin aging. This review summarizes comprehensive recent findings on the beneficial actions of cocoa-driven phytochemicals in molecular mechanisms of human health.

Keywords Cocoa, cardiovascular diseases, cancer, neurodegeneration, obesity and diabetes, skin aging

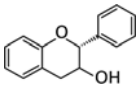
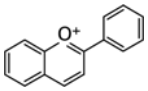
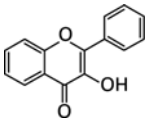
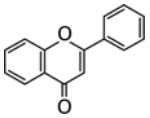
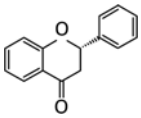
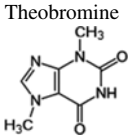
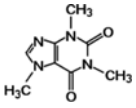
INTRODUCTION: COCOA PHYTOCHEMICALS

Cocoa contains numerous phytochemicals that are mostly flavonoid and nonflavonoid phenols, and methylxanthines as listed in Table 1 (Smit et al., 2004; Ramiro-Puig and Castell, 2009; Ptolemy et al., 2010). Flavonoids in cocoa can be subdivided into several classes based on the degree of hydroxylation and oxidation of the rings (Table 1). The primary flavonoids in cocoa are flavan-3-ols, the monomeric (–)-epicatechin and catechins, and the polymeric procyanidins (Figs. 1 and 2, Steinberg et al., 2003). The basic chemical structure of a cocoa flavonoid flavanol consists of two aromatic rings linked via an oxygenated heterocycle (Fig. 2A, Steinberg et al., 2003). Cocoa flavanol monomer (−)-epicatechin and catechins can form links between C4 and C8, allowing them to assemble as dimers, oligomers, and polymers (Fig. 2B, Manach et al., 2004). The polymers of (−)-epicatechin and catechins are procyanidins, also known as condensed tannins, which, through the formation of complexes with salivary proteins, are responsible for the astringency of cocoa (Manach et al., 2004).

Procyanidins are the most abundant phytochemicals in cocoa and chocolate products, with reported levels ranging from 1.08 to 85.36 mg/g (Table 2, Counet et al., 2006). They can be dimeric or polymeric combinations of (−)-epicatechin and catechins, with chains of up to and over 10 units in cocoa (Cooper et al., 2007). Among flavonoids, (−)-epicatechin and catechins are the next most abundant in cocoa and cocoa products (Table 2, Miller et al., 2006). The content of catechins is lower than (−)-epicatechin in most cocoa products (Gu et al., 2006). A recent study demonstrated that the catechin in chocolate is predominantly the (−)-catechin isomer, rather than the (+)-catechin isomer that is present in most other foods (Gotti et al.,

Address correspondence to Hyong Joo Lee, WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea. E-mail: leehyjo@snu.ac.kr or Ki Won Lee, WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-921, Republic of Korea. E-mail: kiwon@snu.ac.kr

Table 1 Phytochemicals contained in cocoa

	(A) Flavonoid phenols
Flavanols 	(−)-Epicatechin (−)-Catechin (+)-Catechin (−)-Epicatechin-3-*O*-gallate (+)-Gallocatechin (−)-Epigallocatechin Procyanidin B_1 (epicatechin-(4$\beta\rightarrow$8)-catechin) Procyanidin B_2 (epicatechin-(4$\beta\rightarrow$8)-epicatechin) Procyanidin B_2 -*O*-gallate (epicatechin-3-*O*-gallate-(4$\beta\rightarrow$8)-epicatechin) Procyanidin B_2 -3,3-di- *O*-gallate (epicatechin-3-*O*-gallate-(4$\beta\rightarrow$8)-epicatechin-3-*O*-gallate Procyanidin B_3 (catechin-(4$\alpha\rightarrow$8)-catechin) Procyanidin B_4 (catechin-(4$\alpha\rightarrow$8)-epicatechin) Procyanidin B_4-3-*O*-gallate (catechin-(4$\beta\rightarrow$8)-epicatechin-3-*O*-gallate) Procyanidin C_1 (epicatechin-(4$\beta\rightarrow$8)-epicatechin-(4$\beta\rightarrow$8)-epicatechin) Procyanidin D (epicatechin-(4$\beta\rightarrow$8)-epicatechin-(4$\beta\rightarrow$8)-epicatechin-(4$\beta\rightarrow$8)-epicatechin)
Anthocyanins 	3-α-L-Arabinosidyl cyaniding 3-β-D-Galactosidyl cyaniding
Flavonols 	Quercetin Quercetin-3-*O*-arabinoside Quercetin-3-*O*-galactoside Isoquercetin (quercetin-3-*O*-glucoside)
Flavones 	Luteolin Luteolin-7-*O*-hyperoside Orientin Iso-orientin Vitexin Isovitexin
Flavanones 	Naringenin Naringenin-7-*O*-glucoside
	(B) Nonflavonoid phenols
Phenolic acids	Chlorogenic acid Vanillic acid Coumaric acid Phloretic acid Caffeic acid Ferulic acid Phenylacetic acid Syringic acid
Other	Resveratrol Piceid Clovamide Deoxyclovamide Dideoxyclovamide
	(C) Methylxanthines
Theobromine 	
Caffeine 	

Modified from Ramiro-Puig and Castell, 2009.

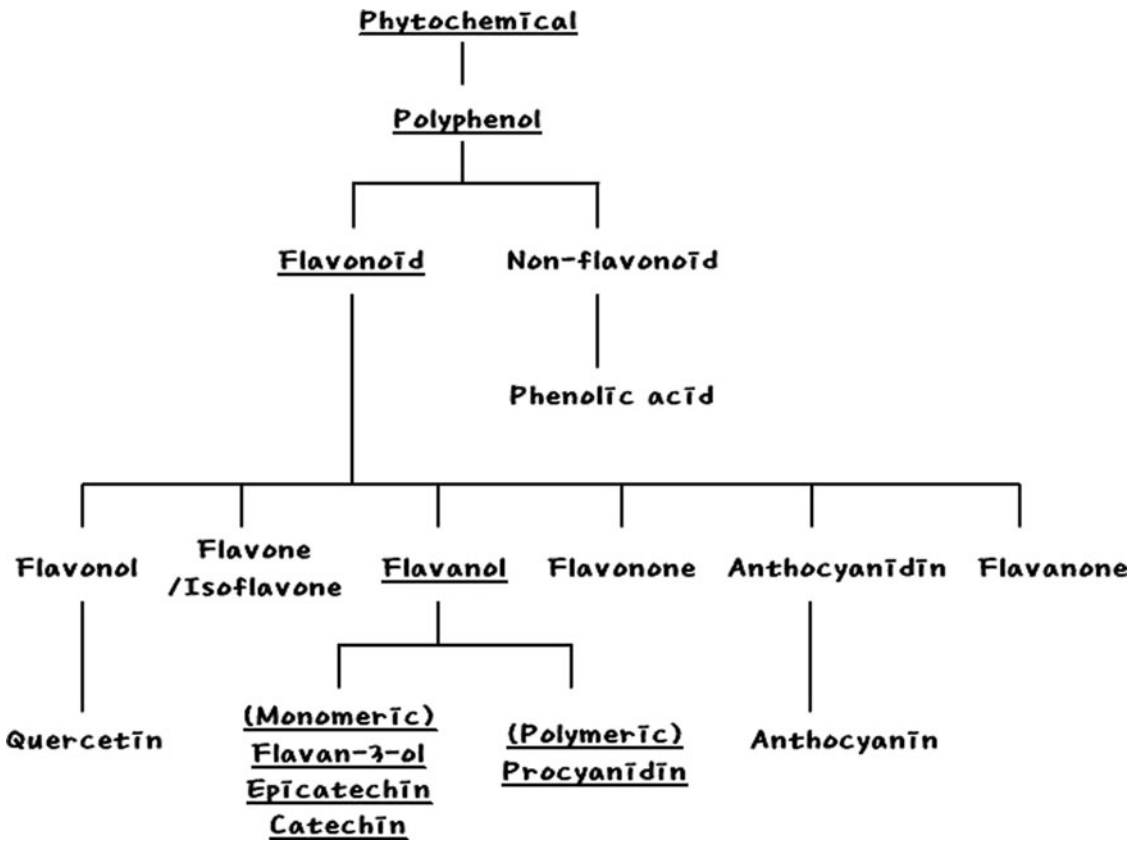


Figure 1 Major classes of flavonoids. Flavonoids include flavonols (such as quercetin), flavones, isoflavones (genistein and diadzein), flavonones, anthocyanidins, flavanones, and flavanols, such as epicatechin, catechin, and procyanidin (also termed proanthocyanidin). The predominant classes of flavonoids in cocoa are (−)-epicatechin, catechins, and procyanidins (indicated with underline).

2006). The (−)-catechin is likely formed from epimerization at the 2 position of (−)-epicatechin during cocoa processing (Gotti et al., 2006; Cooper et al., 2007). It was reported that the concentrations of (−)-epicatechin can be used to predict the content of total polyphenols in cocoa as well as procyanidin B2 and C1 (Cooper et al., 2007). In smaller amounts, traces of (+)-gallocatechin and (−)-epigallocatechin have been found in cocoa (Table 1, Nazaruddin et al., 2006). Anthocyanins are also present in somewhat lower amounts in cocoa, which give rise to the purple color of unfermented cocoa beans (Pettipher, 1986). The anthocyanin fraction consists mainly of 3-α-L-arabinosidyl cyanidin and 3-β-D-galactosidyl cyanidin (Forsyth, 1952). Cocoa also contains flavonols such as quercetin and its derivatives (an arabinoside, a galactoside, and a glucoside) (Ramiro-Puig and Castell, 2009).

Although disregarded, cocoa products contain high amounts of methylxanthine theobromine and caffeine (Tables 1 and 2). Theobromine (3,7-dimethylxanthine) is a metabolite of caffeine (1,3,7-trimethylxanthine), amounts in cocoa products similar to those of procyanidins (Smit et al., 2004). Dark chocolate contains 240–520 mg and milk chocolate 65–160-mg theobromine per 50-g portion (Smit and Blackburn, 2005). A 50-g bar of dark chocolate contains 17- to 36-mg caffeine, compared with coffee and tea, of which typical servings contain between 40- and 130-mg caffeine (Smit and Blackburn, 2005). The nonflavonoid phenols *trans*-resveratrol and *trans*-piceid were found at concentrations of at least 0.5 and 1.2 μg/ml in cocoa liquor and at least 0.4 and 1 μg/ml in dark chocolate, respectively (Table 1, Counet et al., 2006).

The concentrations of all phytochemicals can vary tremendously between cocoa beans and cocoa-containing foods, depending on the source of the beans and the processing conditions (Engler and Engler, 2006; Andres-Lacueva et al., 2008). The concentration of flavanols markedly decreases during the conventional manufacturing process from fresh cocoa seeds to the final product (Engler and Engler, 2006; Andres-Lacueva et al., 2008). The ratio and type of polyphenols found in the beans are unlikely to be exactly the same as those found in the final product (Cooper et al., 2007). For example, cocoa flavanol monomer (−)-epicatechin content ranges from 1.24 to 16.52 mg/g in cocoa beans, but it is only found from 0.116 to 6.778 mg/g in cocoa powder and from 0.023 to 2.270 mg/g in chocolate (Table 2).

BIOAVAILABILITY OF COCOA PHYTOCHEMICALS

Bioavailability differs greatly from one phytochemical to another, so that the most abundant phytochemicals in our diet

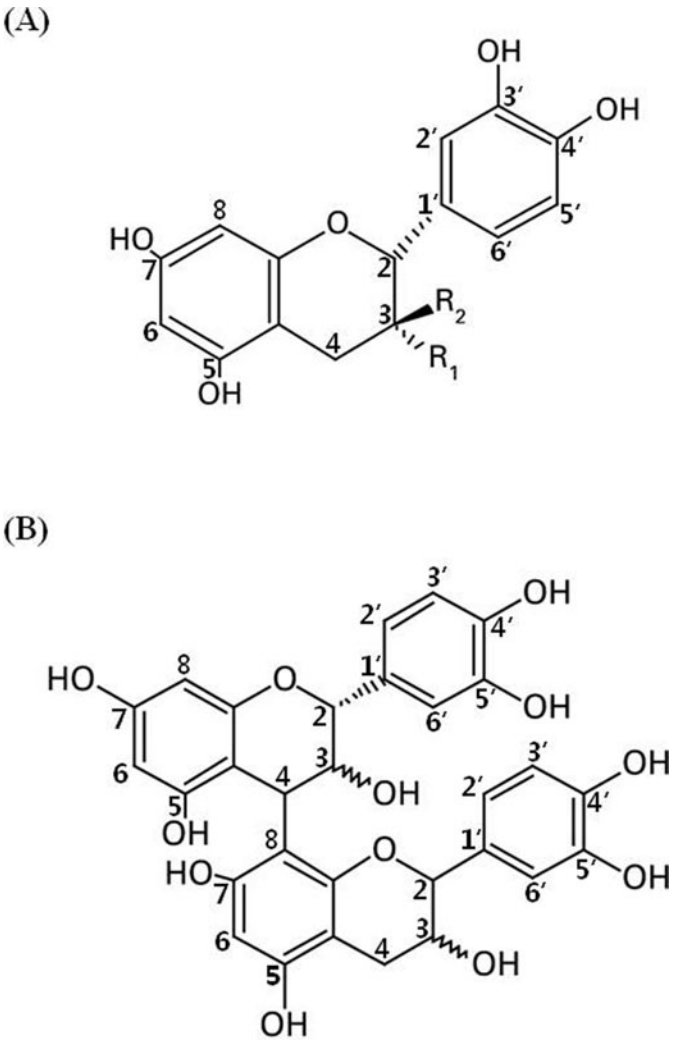


Figure 2 Chemical structure of the main cocoa flavonoids. (A) R_1 = OH corresponds to (−)-epicatechin and R_2 = OH to (+)-catechin. (B) Dimeric procyanidin ($4\beta \rightarrow 8$). Modified from (Ramiro-Puig and Castell, 2009).

are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Manach et al., 2005). Beside molecular size, other important factors modulating the in vivo efficacy of phytochemicals must be considered, including their metabolic conversion and accumulation in tissues and urinary elimination (Schewe et al., 2008).

Human bioavailability studies of phytochemicals derived from cocoa products found that theobromine, caffeine, and (−)-epicatechin were predominantly present in human plasma (Table 3). Although neglected in previous studies, theobromine and caffeine are much more bioavailable than (−)-epicatechin. After consumption of 80 g of dark chocolate, methylxanthine theobromine was found at concentrations of up to 63.35 μM at 2.6 hours in human plasma (Richelle et al., 1999). Caffeine, another methylxanthine found in cocoa, was found at levels up to 25.3 μM in human plasma 1.5 hours after consumption of 41-g chocolate (Ptolemy et al., 2010). In contrast, 0.7 μM (−)-epicatechin was found after consumption of 80 g of black chocolate (Richelle et al., 1999). The major circulating metabolites of (−)-epicatechin are (−)-epicatechin-3′-*O*-glucuronide, 4′-*O*-methyl-(−)-epicatechin-3′-*O*-glucuronide, and 4′-*O*-methyl-(−)-epicatechin-5- or 7-*O*-glucuronide (Natsume et al., 2003). Catechins appear to be far less bioavailable than (−)-epicatechin when both are consumed together in cocoa products (Holt et al., 2002).

In vitro and in vivo animal studies confirmed that polymerization of flavanols greatly impairs intestinal absorption (Deprez et al., 2001; Donovan et al., 2002). Polymeric procyanidins in cocoa are not absorbed as such, and larger units than the dimer are unlikely to be able to cross the gut barrier (Manach et al., 2005). Even though the absorption of cocoa procyanidin dimers was approximately 100-fold lower than that of the flavanol monomers (Holt et al., 2002), a study demonstrated the presence of procyanidin dimers in plasma within 30 minutes postconsumption of flavanol-rich cocoa (Holt et al., 2002). It was suggested that the biological effects of cocoa may be attributable to actions of procyanidin metabolites that can be more readily absorbed. The incubation of purified and ^{14}C-labeled procyanidin oligomers with human colonic microflora led to the formation of various polyphenol-derived phenolic acids, *m*-hydroxyphenylpropionic acid, *m*-hydroxyphenylacetic acid, and their *p*-hydroxy isomers, *m*-hydroxyphenylvaleric acid, phenylpropionic acid, phenylacetic acid, and benzoic acid (Deprez et al., 2000). Some of these compounds, *m*-hydroxyphenylpropionic acid, *m*-hydroxyphenylacetic acid, as well as *m*-hydroxybenzoic acid, were shown to be increased in human urine after consumption of procyanidin-rich chocolate (Rios et al., 2003).

The overall effects of cocoa phytochemicals may potentially accumulate. Recently, it was shown that the effects of cocoa polyphenols on flow-mediated dilation of blood vessels can be cumulative if taken in high doses on a daily basis for one week, and with a return to baseline after a week washout (Heiss et al., 2007). A 12-week cocoa study showed an approximately 8-fold increase in catechin excretion and an approximately 10-fold increase in (−)-epicatechin excretion in the cocoa group (Baba et al., 2007b).

MOLECULAR MECHANISMS ON HEALTH

In the 16th century, Aztec Emperor Montezuma called cocoa 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) (Corti et al., 2009). As noted, cocoa has been used to treat anemia, mental fatigue, tuberculosis, fever, gout, kidney stones, and even poor sexual appetite (Dillinger et al., 2000; Corti et al., 2009). Several health effects of cocoa have been considered, including improved heart, kidney, and bowel function, facilitated digestion, and stimulation of the nervous system (Dillinger et al., 2000; Corti et al., 2009).

Table 2 Phytochemical content in cocoa beans and cocoa products

	Cocoa beans (mg/g)	Cocoa powder (mg/g)	Chocolate (mg/g)	Cocoa beverage (mg/100 ml)
Procyanidin	n/a	18.35–27.75	1.081–85.36	106–111
(−)-Epicatechin	1.24–16.52	0.116–6.778	0.023–2.270	9.2–59
Catechins	0.05–0.46	0.081–0.896	0.006–0.992	10.7–15
Epicatechin gallate	n/a	n/a	0.005–0.006	n/a
Catechin gallate	n/a	n/a	0.077–0.094	n/a
Epigallocatechin	n/a	n/a	0.032–0.119	n/a
Gallocatechin	n/a	n/a	0.164–0.231	n/a
Epigallocatechin gallate	n/a	n/a	0.437–0.462	n/a
Theobromine	11.1–24.0	15.2–25.0	1.20–6.67	128.9–222
Caffeine	2.0–2.9	0.907–2.5	0.170–0.778	8.6–14
Ref.	(Wollgast and Anklam, 2000; Pura Naik, 2001; Caligiani et al., 2007)	(Osakabe et al., 2001; Pura Naik, 2001; Pearson et al., 2002; Miller et al., 2006; Andres-Lacueva et al., 2008; Miller et al., 2009)	(Pura Naik, 2001; Mursu et al., 2004; Fraga et al., 2005; Counet et al., 2006; Miller et al., 2009)	(Heiss et al., 2005; Fisher and Hollenberg, 2006; Heiss et al., 2007)

Note: n/a, not applicable.

Table 3 Bioavailability studies of phytochemicals derived from cocoa products

Source	Dose	Plasma concentration	*T*max (hours)	Ref.
Chocolate	Chocolate: 41 g (theobromine: 188 mg, caffeine: 26 mg)	Theobromine: 43.2–67.5 μmol/L caffeine: 4.6–25.3 μmol/L	1.5	(Ptolemy et al., 2010)
Chocolate	Theobromine: 370 mg caffeine: 72 mg	Theobromine: 8.05 μmol/L caffeine: 1.57 μmol/L	1.5–2	(Mumford et al., 1996)
Chocolate	Chocolate: 40, 80 g (theobromine: 405, 810 mg (−)-epicatechin: 82, 164 mg)	Theobromine: 35.33 ± 4.96, 63.35 ± 6.60 μmol/L (−)-epicatechin: 355 ± 100, 675 ± 196 nmol/L	2–2.6	(Richelle et al., 1999)
Chocolate	Chocolate: 80 g	(−)-Epicatechin: 257 nmol/L	2	(Rein et al., 2000)
Chocolate	(−)-Epicatechin: 220 mg	(−)-Epicatechin: 4.77 μmol/L	2	(Baba et al., 2000)
Chocolate	Chocolate: 27, 53, 80 g ((−)-epicatechin: 46, 92, 138 mg)	(−)-Epicatechin: 133 ± 27, 258 ± 29, 355 ± 49 nmol/L	2	(Wang et al., 2000)
Cocoa beverage	Cocoa: 26.4 g (monomer: 323 mg, dimer: 256 mg)	(−)-Epicatechin: 5.92 ± 0.6 μmol/L catechin: 0.16 ± 0.03 μmol/L procyanidin B2 dimer: 41 ± 4 nmol/L	2	(Holt et al., 2002)
Cocoa beverage	(−)-Epicatechin: 220 mg	(−)-Epicatechin: 4.92 μmol/L	2	(Baba et al., 2000)
Cocoa beverage	Cocoa: 31g (flavanol: 450 mg)	Flavanol (sum of (−)-epicatechin, catechin, 4′-*O*-methyl-catechin, and 3′-*O*-methyl-catechin): 765 ± 73 nmol/L	∼1.5	(Muniyappa et al., 2008)
Cocoa beverage	Flavanol: 352∼370 mg	(−)-Epicatechin: 19 ± 6 nmol/L (−)-epicatechin-7-β-D-glucuronide: 39 ± 13 nmol/L 4′-*O*-methyl-(−)-epicatechin: 41 ± 10 nmol/L 4′-*O*-methyl-(−)-epicatechin-β-D-glucuronide: 287 ± 58 nmol/L catechin: 18 ± 3 nmol/L		(Heiss et al., 2005)
Cocoa beverage	Flavanols: 528 mg ((−)-epicatechin + catechin: 40%, procyanidin: 60%)	Total flavanol: over 300 nmol/L ((−)-epicatechin: 10.4% (−)-epicatechin-7-β-D-glucuronide: 14.8% 4′-*O*-methyl-(−)-epicatechin: 10.1% 4′-*O*-methyl-(−)-epicatechin-β-D-glucuronide: 52.2% Catechin: 10.2%)	2	(Heiss et al., 2006)
Cocoa beverage	Cocoa beverage: 0.125 g/kg bw (catechin + (−)-epicatechin: 1.53 mg/kg bw)	Flavanol: 296.4∼343.7 ng/ml	1.7	(Schramm et al., 2003)

Note: *T*max = time to max concentration, bw = body weight, Sub = subject, Ref. = reference.

Cocoa products have an antioxidant capacity higher than that of other flavanol-rich foods and beverages, such as apples, red wine, and brewed black tea (Lee et al., 2003). After oral intake of cocoa, both the flavanol content and the total antioxidant capacity in plasma increase (Corti et al., 2009). Theobromine, caffeine, (−)-epicatechin, catechins, and oligomeric procyanidins in cocoa have strong antioxidative effects (Osakabe et al., 1998; Lee, 2000; Weisburger, 2001; Lv et al., 2010). Cocoa polyphenols fractionated from commercial cocoa powder exhibit a dose-dependent free radical-scavenging activity (Lee et al., 2006). Polyphenols extracted from commercial cocoa inhibited ultraviolet-induced DNA oxidation (Ottaviani et al., 2002). An extract of cocoa seeds rich in polyphenols counteracted the increased level of lipid peroxide in rats fed a vitamin E-deficient diet (Yamagishi et al., 2001). Cocoa procyanidin oligomers have been shown to block the effects of peroxynitrite (Arteel et al., 2000). Due to the natural antioxidant flavonoids contained in cocoa, chocolate was found to be resistant to spoilage (Engler and Engler, 2006).

Data from numerous studies suggest that caffeine and flavanols can effectively modify the inflammatory process (Cooper et al., 2008; Brothers et al., 2010; Lv et al., 2010). Caffeine is a nonspecific antagonist at adenosine receptors and adenosine has a known role in the propagation of inflammation (Chavez-Valdez et al., 2009; Brothers et al., 2010). Caffeine has been shown to inhibit tumor necrosis factor-α (TNF-α) production by decreasing TNF-α expression via adenosine receptor blockade (Dray et al., 2007; Chavez-Valdez et al., 2009). On the other hand, cocoa flavonoids have been shown to inhibit lipoxygenases, the key enzymes of leukotriene synthesis (Sies et al., 2005). Comprehensive experimental evidence has demonstrated that cocoa monomer catechins and oligomeric procyanidins have a significant anti-inflammatory effect, inhibit neutrophil oxidative burst, and reduce the expression of adhesion molecules (Mao et al., 2000; Selmi et al., 2008). Cocoa-derived (−)-epicatechin and (+)-catechin, and dimeric flavanols reduce nuclear factor-κB (NF-κB) activation, resulting in reduced production of TNF-α and interleukin-2 (IL-2) (Mao et al., 2002; Mackenzie et al., 2004; Ramiro et al., 2005; Mackenzie and Oteiza, 2006). Although further studies are awaited, the impact of cocoa blocking NF-κB activation might constitute a common trait to all cocoa anti-inflammatory effects (Selmi et al., 2008). Monomeric through pentameric flavanols in cocoa can enhance the secretion of the anti-inflammatory cytokine IL-5, which might protect against chronic infection (Selmi et al., 2008). Specific cocoa flavanols may preferentially stimulate immunoglobulin A, which could in turn reduce the risk for dental caries and infections (Selmi et al., 2008).

Besides antioxidative and anti-inflammatory activity, there are several other beneficial effects of phytochemicals derived from cocoa in the pathogenesis of chronic human diseases. In the following sections, we summarize the beneficial actions of cocoa-driven phytochemicals in cardiovascular diseases, cancer, neurodegenerative diseases, obesity, and diabetes, as well as skin aging.

Cardiovascular Diseases

Recent research demonstrates that consumption of cocoa, particularly rich in flavanols, is beneficial to cardiovascular health and disease (Steinberg et al., 2003; Buijsse et al., 2006; Schroeter et al., 2006). Platelets function to maintain vascular integrity. However, increased platelet reactivity and aggregation in the presence of endothelial dysfunction can lead to the development of arterial thrombosis and the progression of atherosclerosis (Ross, 1999; Steinberg et al., 2003). It has been proposed that cocoa inhibits platelet activation and aggregation (Heptinstall et al., 2006). Cocoa flavanol supplementation for 28 days significantly increased plasma (−)-epicatechin and catechin and decreased platelet function (Murphy et al., 2003). In healthy volunteers, consuming 100-g dark chocolate reduced platelet aggregation, an effect not seen after ingestion of white chocolate or milk chocolate (Innes et al., 2003). Dark chocolate decreased not only platelet aggregation but also adhesion in young healthy smokers (Hermann et al., 2006). On the other hand, cocoa catechin and (−)-epicatechin reduced the expression of glycoprotein IIb/IIIa, which aids in platelet activation (Pearson et al., 2002).

Hypercholesterolemia especially increases concentrations of low-density lipoprotein (LDL) cholesterol and leads to the development of atherosclerosis (Stamler et al., 1986; Shepherd et al., 1995). On the other hand, a negative correlation between plasma high-density lipoprotein (HDL) cholesterol and cardiovascular disease has been demonstrated (Gordon et al., 1989; Mursu et al., 2004). Consumption of a flavanol-rich chocolate or phenolic substances derived from cocoa powder contributes to a reduction in LDL cholesterol and an increase in HDL cholesterol in human plasma (Mursu et al., 2004; Fraga et al., 2005; Baba et al., 2007a, 2007b). It was reported that cocoa powder was a potent antioxidant for LDL oxidation (Osakabe et al., 2001; Wan et al., 2001; Baba et al., 2007a, 2007b). At 5-μmol/L gallic acid equivalents (GAE), cocoa phenols inhibited LDL oxidation by 75%, whereas pure catechin (5 μmol/L) inhibited oxidation by 87% (Waterhouse et al., 1996). Cocoa flavonoids also interact with myeloperoxidase, a prooxidant enzyme that is thought to be involved in peroxidation of LDL (Berliner and Heinecke, 1996; Podrez et al., 2000). The myeloperoxidase-mediated peroxidation of LDL was effectively blocked by the cocoa polyphenol (−)-epicatechin, the corresponding procyanidins, and other flavonoids (Kostyuk et al., 2003; Kraemer et al., 2004). Caffeine was also reported to inhibit LDL peroxidation (Lee, 2000).

Expression and activation of promatrix metalloproteinase-2 (pro-MMP-2) play pivotal roles in the migration and invasion of human aortic vascular smooth muscle cells, which is strongly linked to atherosclerosis (Lee et al., 2008). It has been reported that cocoa procyanidin fraction and procyanidin B2 directly bind with mitogen-activated protein kinase kinase (MEK1), inhibit thrombin-induced MEK1 activity, and subsequently decrease the expression of pro-MMP-2 (Fig. 3, Lee et al., 2008). In addition, cocoa procyanidin fraction and procyanidin B2 directly inhibited membrane type-1 (MT1)-MMP activity,

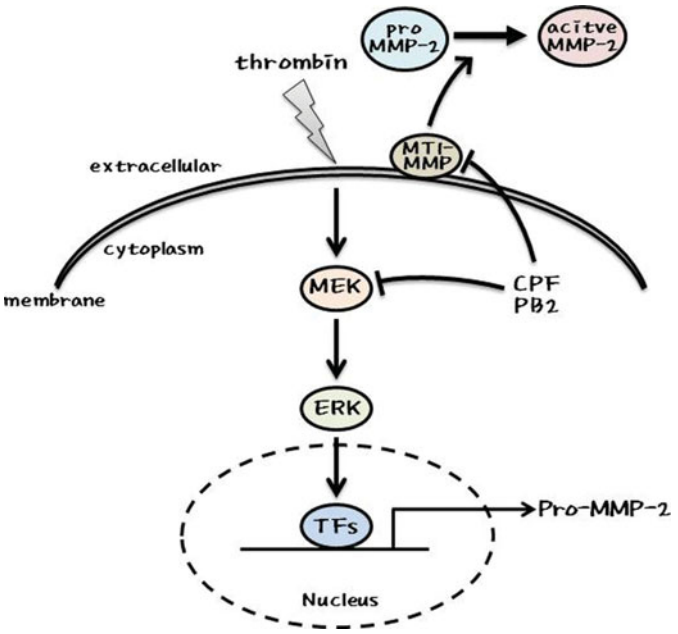


Figure 3 Cocoa procyanidin fraction (CPF) and procyanidin B2 (PB2) inhibited the production of matrix metalloproteinase-2 (MMP-2) in vascular smooth muscle cells (Lee et al., 2008). CPF and PB2 directly bound to mitogen-activated protein kinase kinase (MEK) and inhibited its activity, which finally signals to activate extracellular signal-regulated kinase (ERK) and transcription factors (TFs) to express pro-MMP-2. CPF and PB2 also suppressed the activity of membrane type-1 (MT1)-MMP, which promotes the conversion of pro-MMP-2 to active MMP-2. These observations suggest that cocoa procyanidins may favorably affect atherosclerosis by inhibiting the expression and the activation of pro-MMP-2. (Color figure available online.)

which promotes the conversion of pro-MMP-2 to active MMP-2 (Lee et al., 2008). These observations suggest that cocoa procyanidins may favorably affect atherosclerosis by inhibiting the expression and activation of pro-MMP-2.

Inflammation promotes endothelial dysfunction and atherogenesis, and it is now widely accepted that atherosclerosis is a chronic inflammatory disease (Ross, 1999). Homeostatic levels of transforming growth factor (TGF)-β1, a pleiotropic cytokine that plays an important role in preserving endothelial function, are important in maintaining cardiac function. Excess production of TGF-β1 can enhance atherogenesis by promoting excessive extracellular matrix accumulation and lead to cardiac fibrosis (Kenny et al., 1994; Lijnen et al., 2000). It was reported that purified cocoa flavan-3-ols and procyanidins modulated TGF-β1 secretion from resting peripheral blood mononuclear cells, and these effects might potentially benefit cardiovascular health (Mao et al., 2003). Cocoa-derived products rich in flavanols potentially modulate, in a positive way, the immune system response seen in several chronic and acute cardiovascular diseases (Selmi et al., 2006).

Intriguingly, the Kuna Indians, native to islands off the coast of Panama, drink several servings of unprocessed cocoa a day and appear to be the only known society, which is free from hypertension (Hollenberg et al., 1997). It was reported that diets rich in cocoa reduce both systolic and diastolic blood pressure (Taubert et al., 2007). Consumption of dark chocolate led to a significant increase in resting and hyperemic brachial artery diameter and exerted a beneficial effect on endothelial function in adults (Vlachopoulos et al., 2005). Chronic consumption of a flavanol-rich cocoa reduced plasma soluble vascular cell adhesion molecule-1 (VCAM-1), which might improve flow-mediated dilation and endothelial function (Wang-Polagruto et al., 2006). In a small clinical study, participants who received 46 mg of (−)-epicatechin daily for over a period of two weeks showed endothelium-dependent vasodilation (Engler et al., 2004). Compared with tea polyphenols, cocoa phenols were shown to have a greater efficacy on blood pressure-related disorders (Taubert et al., 2007).

Cocoa mediates an increase in the bioavailability of nitric oxide (NO) in the endothelium and reverses the endothelial dysfunction apparent in cardiovascular diseases (Heiss et al., 2005; Corti et al., 2009). NO from endothelium prevents leukocyte adhesion and migration, smooth muscle cell proliferation, and platelet adhesion and aggregation (Corti et al., 2009). Cocoa lowers vascular arginase activity in endothelial cells, which augments the local levels of L-arginine used to synthesize NO by endothelial NO synthase (eNOS) (Corti et al., 2009). Consequently, eNO produced by cocoa induces a relaxation of vascular smooth muscle cells, leading to vasodilation (Corti et al., 2009). It was reported that a cocoa drink high in flavanol content rapidly enhanced the circulating pool of bioactive NO by more than one third and, in turn, augmented vasodilation in human, suggesting that flavanol in cocoa contributes the effect of cocoa in increasing the bioavailability of NO and inducing vasodilation (Fisher et al., 2003; Heiss et al., 2003). Pure (−)-epicatechin ingestion augmented NO bioavailability and acutely reduced plasma levels of endothelin-1, a potent endothelium-derived vasoconstrictor (Loke et al., 2008). Acute administration of caffeine augments endothelium-dependent vasodilation through an increase in NO production (Umemura et al., 2006). It has been reported that caffeine stimulates the production of NO by triggering calcium-mediated expression of eNOS in endothelial cells (Echeverri et al., 2010).

Cancer

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 (Weisburger, 2001). It can therefore be postulated that consumption of cocoa or chocolate, which have high antioxidant activity, could be beneficial in inhibiting the complex molecular processes leading to cancer (Weisburger, 2001). It has been reported that cocoa liquor polyphenols have antimutagenic and anticlastogenic activity in vitro (Yamagishi et al., 2002). Cocoa liquor procyanidins significantly reduced the incidence and the multiplicity of lung carcinomas and thyroid adenomas developed in male rats (Yamagishi et al., 2003).

Cocoa procyanidins also inhibited mammary and pancreatic tumorigenesis in female rats (Yamagishi et al., 2002).

Water extract of white cocoa tea leaves (100–150 μg/ml), which is equivalent to 44–66 μM of gallocatechin gallate, inhibited cell proliferation in human prostate cancer cells, which correlated with cell cycle G2/M phase arrest, and resulted in induction of WAF1/p21 and KIP1/p27, and a decrease in cyclin-D1, -D2, and -E, and Cdk-2, -4, and -6 (Peng et al., 2010). When the human prostate cancer cells were implanted to athymic nude mice, oral administration of water extract of white cocoa tea (0.1 and 0.2%, wt/vol) resulted in a greater than 50% inhibition of implanted prostate tumor growth, together with an increase in WAF1/p21 and a decrease in cyclin-D1 expression in tumor tissues of these mice (Peng et al., 2010). Procyanidin-enriched cocoa extracts also caused G2/M cell cycle arrest and a 70% growth inhibition in human colon cancer cells (Carnesecchi et al., 2002). It has been reported that caffeine also induces cell cycle arrest through the protein kinase A/glycogen synthase kinase 3β pathway in human glioma cells (Ku et al., 2010). Flavonoids and caffeine in cocoa may have an antiproliferative effect on human cancer cell growth (Jourdain et al., 2006; Okano et al., 2008).

Angiogenesis plays an important role in cancer growth and metastasis formation. Adenosine is one of the most potent stimulators of neovascularization. Theobromine, as an adenosine receptor antagonist, causes significant inhibition of angiogenic activity and diminishes vascular endothelial growth factor (VEGF) production in vitro and in vivo (Barcz et al., 1998). When theobromine was subcutaneously administered to BALB/c mice in doses of 1–125 mg/kg body weight after intradermal inoculation of lung carcinoma cells, it inhibited tumor-related angiogenesis (Gil et al., 1993). Cocoa-derived theobromine also inhibited angiogenesis induced by ovarian and urothelial cancer cells through the inhibition of VEGF production (Barcz et al., 1998; Skopinska-Rozewska et al., 1998). Another methylxanthine found in cocoa, caffeine, has also been shown to suppress tumor cell invasiveness and experimental metastasis (Yang et al., 2004). Caffeine inhibited adenosine-induced accumulation of hypoxia-inducible factor-1α (HIF-1α), VEGF, and IL-8 expression in hypoxic human colon cancer cells (Merighi et al., 2007). It was found that caffeine mediates the inhibition of calcium release channel inositol 1,4,5-trisphosphate receptor (IP_3R) subtype and blocks glioblastoma invasion (Kang et al., 2010). Cocoa polyphenol extract was also found to reduce the upregulation of VEGF (Fig. 4, Kim et al., 2010). Cocoa polyphenol extract inhibited the activities of phosphoinositide 3-kinase (PI3K), MEK1, and mitogen-activated protein kinase kinase (MKK4), which induce the activation of the nuclear transcription factors NF-κB and activator protein-1 (AP-1), the key regulators of VEGF expression (Kim et al., 2010). Cocoa flavonoids procyanidins also reduced VEGF activity and angiogenic activity associated with tumor pathology in human aortic endothelial cells (Kenny et al., 2004).

It was also shown that cocoa inhibits neoplastic cell transformation by suppressing the kinase activity of MEK1 and p38 mitogen-activated protein kinase (MAPK) (Fig. 5, Lee et al., 2006; Kang et al., 2008). Cocoa procyanidin fraction or procyanidin B2 directly bound with MEK1 and inhibited the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced kinase activity of MEK1, extracellular signal-regulated kinase (ERK), p90 kDa ribosomal protein S6 kinase (p90RSK), and subsequently suppressed the activation of NF-κB and AP-1, the expression of cyclooxygenase-2 (COX-2), and neoplastic transformation of epidermal cells (Kang et al., 2008). Cocoa procyanidin fraction or procyanidin B2 also inhibited cell transformation induced by epidermal growth factor or H-Ras, both of which are known to induce MEK1 activation (Kang et al., 2008). In contrast, theobromine, the most bioavailable phytochemical after consumption of cocoa, had no effect on TPA-induced MEK1 activation, COX-2 expression, or neoplastic transformation (Kang et al., 2008).

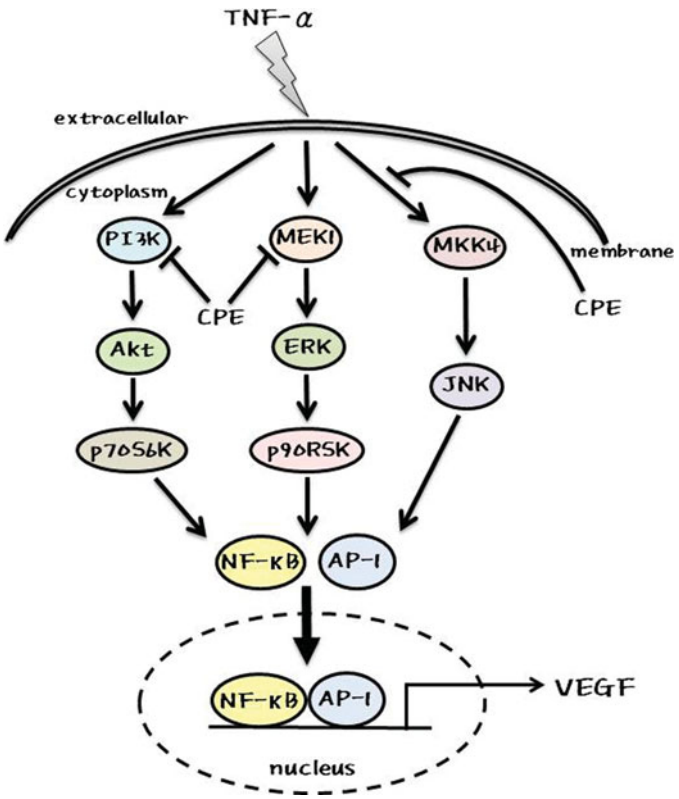


Figure 4 Cocoa polyphenol extract (CPE) suppressed tumor necrosis factor-α (TNF-α)-induced expression of vascular endothelial growth factor (VEGF) by inhibiting phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase kinase 1 (MEK1), and mitogen-activated protein kinase kinase 4 (MKK4) activities in mouse epidermal cells (Kim et al., 2010). CPE bound to PI3K and MEK1, and PI3K-mediated protein kinase B (Akt)/p70 kDa ribosomal protein S6 kinase (p70S6K) and MEK1-induced extracellular signal-regulated kinase (ERK)/p90 kDa ribosomal protein S6 kinase (p90RSK) pathway leading to activation of nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) and further expression of VEGF were attenuated by CPE. CPE also suppressed the activity of MKK4 and blocked the JNK/NF-κB/AP-1/VEGF signaling pathway. Because the expression of VEGF regulates angiogenesis in cancer, cocoa may have anticancer activity by inhibiting the expression of VEGF. (Color figure available online.)

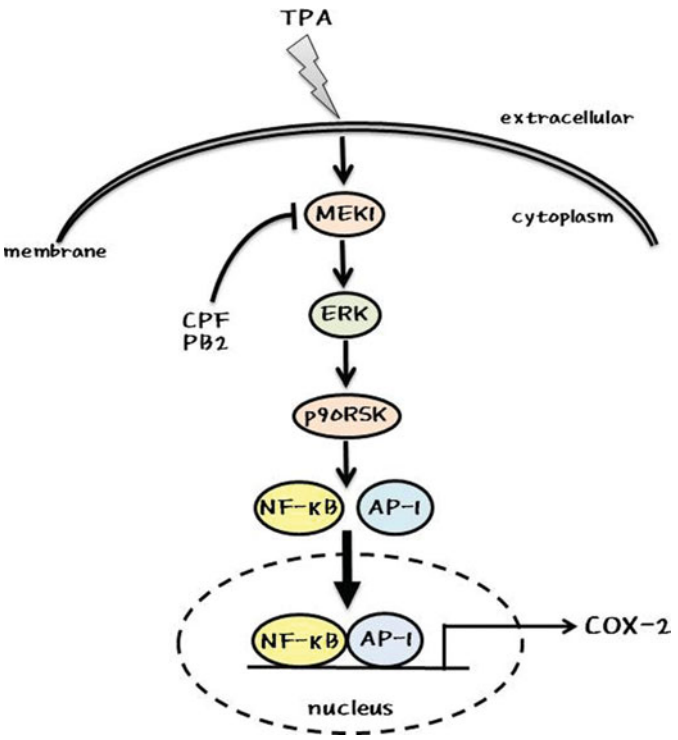


Figure 5 Cocoa procyanidin fraction (CPF) and procyanidin B2 (PB2) suppressed neoplastic cell transformation by inhibiting mitogen-activated protein kinase kinase 1 (MEK1) (Kang et al., 2008). The tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced MEK1/extracellular signal-regulated kinase (ERK)/p90 kDa ribosomal protein S6 kinase (p90RSK) signaling pathway leading to activation of nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) and expression of cyclooxygenase-2 (COX-2), which is involved in tumor promotion and inflammation, were dose dependently inhibited by CPF or PB2. (Color figure available online.)

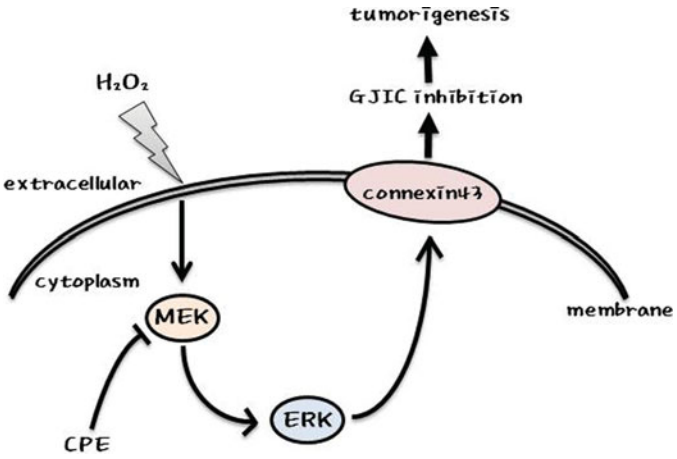


Figure 6 Cocoa polyphenol extracts (CPE) attenuated hydrogen peroxide (H_2O_2)-induced inhibition of gap-junction intercellular communication (GJIC), which is involved in tumorigenesis (Lee et al., 2010). CPE inhibited the kinase activity of mitogen-activated protein kinase kinase (MEK) and suppressed the signaling pathway of extracellular signal-regulated kinase (ERK)-connexin 43, which leads to inhibition of GJIC and tumorigenesis. (Color figure available online.)

Inhibition of gap-junction intercellular communication (GJIC) is strongly related to tumorigenesis and cocoa polyphenol extracts dose dependently attenuated hydrogen peroxide (H_2O_2)-induced inhibition of GJIC in rat liver epithelial cells (Fig. 6, Lee et al., 2010) cocoa polyphenol extracts inhibited the H_2O_2-induced activation of MEK and ERK and further attenuated the phosphorylation and internalization of connexin 43, a regulating protein of GJIC (Lee et al., 2010). Cocoa polyphenol extracts may suppress H_2O_2-induced tumorigenesis through GJIC protection.

Neurodegeneration

Consumption of cocoa flavanols has been reported to result in improvements in memory and learning (Bisson et al., 2008; Spencer, 2009; Scholey et al., 2010). Both long- and short-term memory processes were improved by the consumption of cocoa polyphenolic extract, as evaluated by month-to-month or trial-to-trial performances (Bisson et al., 2008). Available evidence indicates that (−)-epicatechin does cross the blood–brain barrier, as epicatechin glucuronide and 3′-*O*-methyl epicatechin glucuronide have been observed in rat brain for up to 10 days after oral administration of (−)-epicatechin (Abd El Mohsen et al., 2002; van Praag et al., 2007). A single acute dose (450 mg) of flavanol-rich cocoa increased local cerebral blood flow to grey matter by up to 60% at two to three hours postconsumption (Francis et al., 2006). A flavanol-rich cocoa drink may induce peripheral and cerebral vascular blood flow in a manner that may lead to the induction of angiogenesis and new nerve cell growth in the hippocampus (Spencer, 2009). It has been reported that (−)-epicatechin consumption enhances cognition and spatial memory by increasing angiogenesis and neuronal spine density in the dentate gyrus of the hippocampus (van Praag et al., 2007). On the other hand, short-term administration of cacao mass showed anxiolytic effects, with administration decreasing conditioned fear-related behavior (Yamada et al., 2009). Methylxanthines, the combination of caffeine and theobromine, are known as the psychopharmacologically active constituents of chocolate (Smit et al., 2004).

The association between caffeine intake and cognitive decline in a community-based sample of subjects aged 65 years and over was examined, and the psychostimulant properties of caffeine appeared to reduce cognitive decline in women without dementia, especially at older ages (Ritchie et al., 2007). Caffeine reliably affects cognitive and psychomotor performance, even at doses as low as 12.5 mg (Smit and Rogers, 2000). Caffeine has also been shown to be protective against Alzheimer's disease and Parkinson's disease (Chen et al., 2010; de Mendonca and Cunha, 2010; Eskelinen and Kivipelto, 2010; Prediger, 2010). Recent experimental findings have indicated that caffeine can manage the nonmotor symptoms of Parkinson's disease, which do not improve with the current dopaminergic drugs (Prediger, 2010). Stimulatory effects of caffeine are thought to be caused primarily by adenosine receptor antagonism (Nehlig et al., 1992).

There is a possible role for (−)-epicatechin in reducing neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Schroeter et al., 2000; Pan et al., 2003). In neuronal viability assays, (−)-epicatechin reduced caspase-3-mediated neuronal cell death triggered by *tert*-butyl hydroperoxide (*t*-BuOOH) or H_2O_2 (Shah et al., 2010). It has been shown that both (−)-epicatechin and catechin inhibit β-amyloid ($A\beta_{25–35}$)-induced apoptosis in neuron-like PC12 cells (Heo and Lee, 2005), and a 30-mg dose of (−)-epicatechin limits the hippocampal toxicity caused by $A\beta_{25–35}$ in rats (Cuevas et al., 2009). Furthermore, (−)-epicatechin has been found to prevent stroke damage through the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme oxygenase-1 (HO-1) pathway (Shah et al., 2010). (−)-Epicatechin-associated neuroprotection was mostly abolished in mice lacking the transcriptional factor Nrf2 or the enzyme HO-1, and in neurons derived from these knockout mice, suggesting that (−)-epicatechin exerts part of its beneficial effect through the activation of Nrf2 and an increase in the neuroprotective HO-1 enzyme (Shah et al., 2010).

Cocoa procyanidin fraction or procyanidin B2 inhibited neuronal cell death caused by H_2O_2 or 4-hydroxynonenal (HNE), as shown in Fig. 7 (Cho et al., 2008, 2009). Pretreatment with cocoa procyanidin fraction or procyanidin B2 before H_2O_2 or HNE treatment diminished proapoptotic propoly ADP ribose polymerase (pro-PARP) cleavage and increased the level of the antiapoptotic proteins Bcl-X_L and Bcl-2 compared with those treated only with H_2O_2 or HNE (Cho et al., 2008, 2009). Activation of caspase-3 by H_2O_2 or HNE was inhibited by pretreatment with cocoa procyanidin fraction or procyanidin B2 (Cho et al., 2008, 2009). It was found that cocoa procyanidin fraction or procyanidin B2 protect neuron-like cells against H_2O_2- or HNE-induced apoptosis by blocking the activity of MKK4, c-Jun N-terminal protein kinase (JNK), and p38 MAPK.

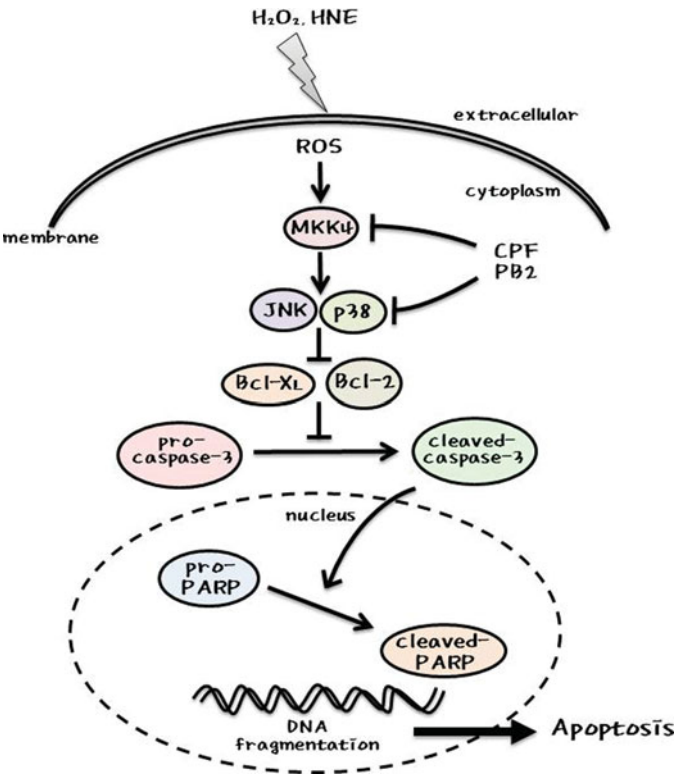


Figure 7 Cocoa procyanidin fraction (CPF) or procyanidin B2 (PB2) attenuate 4-hydroxynonenal (HNE)-induced neuron-like cell death by directly inhibiting mitogen-activated protein kinase kinase 4 (MKK4), c-Jun N-terminal protein kinase (JNK), and p38 mitogen-activated protein kinase (MAPK) (Cho et al., 2008, 2009). (Color figure available online.)

Obesity and Diabetes

It was reported that cocoa supplementation could reduce lipid profiles of normo- and hypercholesterolemic human subjects (Baba et al., 2007a). Cocoa powder, rich in polyphenols such as catechins and oligomeric procyanidins, has a hypocholesterolemic effect in humans (Yasuda et al., 2008). A high-cholesterol diet containing 1% polyphenol extract from cocoa powder significantly lowered plasma cholesterol concentrations and increased fecal cholesterol and total bile acids excretion than did a high-cholesterol diet without the extract (Yasuda et al., 2008). Procyanidin B2 (dimer), B5 (dimer), C1 (trimer), and A2 (tetramer), as well as catechin and (−)-epicatechin decrease micellar solubility of cholesterol in vitro (Yasuda et al., 2008). It has been noted that oligomeric procyanidins from cocoa powder might be the principal active components responsible for the hypocholesterolemic effect and may inhibit the intestinal absorption of cholesterol and bile acids through the decrease in micellar cholesterol (Yasuda et al., 2008).

A long-term study examining the effects of caffeine on weight reported that increases in caffeine intake may lead to reductions in long-term weight gain (Lopez-Garcia et al., 2006). The intake of caffeine progressively reduced body fat mass and body fat percentage in rats fed a high-fat diet (Kobayashi-Hattori et al., 2005). It was suggested that the caffeine intake induced lipolysis via increased levels of catecholamines (Kobayashi-Hattori et al., 2005). On the other hand, caffeine did not inhibit the differentiation of 3T3-L1 preadipocytes to mature adipocytes, but it did suppress the intracellular lipid accumulation after complete differentiation in a dose-dependent manner (Nakabayashi et al., 2008).

One study has indicated that cocoa has the ability to prevent the development of diabetes in genetically inherited diabetic rats (Tomaru et al., 2007). Experimental evidence in obese-diabetic mice suggested that cocoa prevents hyperglycemia (Corti et al., 2009). In diabetic rats, a cocoa extract diet (containing 285.6 mg total polyphenol per gram extract) for four weeks significantly lowered the serum glucose levels compared with the control (Ruzaidi et al., 2005). Consumption of dark chocolate and cocoa improves glucose metabolism, including insulin resistance and sensitivity (Grassi et al., 2005). Epidemiological studies have indicated that caffeine consumption is associated with a decreased risk of type 2 diabetes (Biessels, 2010).

Skin Aging

Consumption of high-flavanol cocoa improves skin texture, mainly its density, thickness, roughness, scaling, and hydration (Heinrich et al., 2006). A single dose of flavanol-rich cocoa leads to increase blood flow to cutaneous and subcutaneous tissues within two hours after ingestion (Gasser et al., 2008). Cocoa beverages rich in flavanols also decrease the sensitivity of human skin to ultraviolet light (Heinrich et al., 2006). Oral or topical procyanidin inhibits the ultraviolet radiation-induced erythema response (Heinrich et al., 2006). Regular consumption of cocoa rich in flavanols may help maintain skin health and confer substantial photoprotection.

Precautions and Limitations

Because of cocoa's long-term use with no reported adverse effects in human, there has not been great concern over its safety. The toxicity of the methylxanthines, specifically theobromine, is under consideration (Tarka, 1982). In humans, long-term ingestion of extremely large amounts of cocoa products (daily equivalent of 100-g cocoa powder or 1.5-g methylxanthines) results in sweating, trembling, and severe headaches (Tarka, 1982). Although cocoa has desirable effects in humans, it cannot be efficiently metabolized in many animals, including dogs and cats, and can lead to cardiac and nervous system problems, and if consumed in high quantities, even death (Tarka, 1982).

Overall, what is known so far about the actions of cocoa and cocoa products suggests that they can be considered part of a wholesome, health-promoting nutritional food (Weisburger, 2001). It should be noted that the health-promoting effects of cocoa refers to raw cocoa and, to a lesser extent, dark chocolate, as flavonoids degrade during cooking and alkalizing processes. Because high sugar intake is associated with obesity and diabetes, cocoa products with no or low sugar content are preferred. The accurate assessment of the flavanol content and its bioavailability in cocoa products is required to interpret its biological effects. Finally, larger studies with a placebo-controlled, prospective design might be needed to clarify the protective effects of cocoa and its phytochemicals on human health.

FUNDING

This work was supported by the National Leap Research Program (2010-0029233), National Research Foundation, Ministry of Education, Science and Technology, Republic of Korea. This work was also supported by the R&D program-Establishment of Infra Structure for Anti-aging Industry Support (N0000697), Ministry of Trade, Industry and Energy/Korea Institute for the Advancement of Technology, Republic of Korea.

REFERENCES

Abd El Mohsen, M. M., Kuhnle, G., Rechner, A. R., Schroeter, H., Rose, S., Jenner, P. and Rice-Evans, C. A. (2002). Uptake and metabolism of epicatechin and its access to the brain after oral ingestion. *Free Radic. Biol. Med.* **33**:1693–1702.

Andres-Lacueva, C., Monagas, M., Khan, N., Izquierdo-Pulido, M., Urpi-Sarda, M., Permanyer, J. and Lamuela-Raventos, R. M. (2008). Flavanol and flavonol contents of cocoa powder products: Influence of the manufacturing process. *J. Agric. Food Chem.* **56**:3111–3117.

Arteel, G. E., Schroeder, P. and Sies, H. (2000). Reactions of peroxynitrite with cocoa procyanidin oligomers. *J. Nutr.* **130**:2100S–2104S.

Baba, S., Natsume, M., Yasuda, A., Nakamura, Y., Tamura, T., Osakabe, N., Kanegae, M. and Kondo, K. (2007a). 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.* **137**:1436–1441.

Baba, S., Osakabe, N., Kato, Y., Natsume, M., Yasuda, A., Kido, T., Fukuda, K., Muto, Y. and Kondo, K. (2007b). 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.* **85**:709–717.

Baba, S., Osakabe, N., Yasuda, A., Natsume, M., Takizawa, T., Nakamura, T. and Terao, J. (2000). Bioavailability of (−)-epicatechin upon intake of chocolate and cocoa in human volunteers. *Free Radic. Res.* **33**:635–641.

Barcz, E., Sommer, E., Sokolnicka, I., Gawrychowski, K., Roszkowska-Purska, K., Janik, P. and Skopinska-Rozewska, E. (1998). The influence of theobromine on angiogenic activity and proangiogenic cytokines production of human ovarian cancer cells. *Oncol. Rep.* **5**:517–520.

Berliner, J. A. and Heinecke, J. W. (1996). The role of oxidized lipoproteins in atherogenesis. *Free Radic. Biol. Med.* **20**:707–727.

Biessels, G. J. (2010). Caffeine, diabetes, cognition, and dementia. *J. Alzheimers Dis.* **20**(Suppl. 1):S143–S150.

Bisson, J. F., Nejdi, A., Rozan, P., Hidalgo, S., Lalonde, R. and Messaoudi, M. (2008). Effects of long-term administration of a cocoa polyphenolic extract (Acticoa powder) on cognitive performances in aged rats. *Br. J. Nutr.* **100**:94–101.

Brothers, H. M., Marchalant, Y. and Wenk, G. L. (2010). Caffeine attenuates lipopolysaccharide-induced neuroinflammation. *Neurosci. Lett.* **480**:97–100.

Buijsse, B., Feskens, E. J., Kok, F. J. and Kromhout, D. (2006). Cocoa intake, blood pressure, and cardiovascular mortality: The Zutphen Elderly Study. *Arch. Intern. Med.* **166**:411–417.

Caligiani, A., Cirlini, M., Palla, G., Ravaglia, R. and Arlorio, M. (2007). GC-MS detection of chiral markers in cocoa beans of different quality and geographic origin. *Chirality.* **19**:329–334.

Carnesecchi, S., Schneider, Y., Lazarus, S. A., Coehlo, D., Gosse, F. and Raul, F. (2002). Flavanols and procyanidins of cocoa and chocolate inhibit growth and polyamine biosynthesis of human colonic cancer cells. *Cancer Lett.* **175**:147–155.

Chavez-Valdez, R., Wills-Karp, M., Ahlawat, R., Cristofalo, E. A., Nathan, A. and Gauda, E. B. (2009). Caffeine modulates TNF-alpha production by cord blood monocytes: The role of adenosine receptors. *Pediatr. Res.* **65**:203–208.

Chen, X., Ghribi, O. and Geiger, J. D. (2010). Caffeine protects against disruptions of the blood-brain barrier in animal models of Alzheimer's and Parkinson's diseases. *J. Alzheimers Dis.* **20**(Suppl. 1):S127–S141.

Cho, E. S., Jang, Y. J., Kang, N. J., Hwang, M. K., Kim, Y. T., Lee, K. W. and Lee, H. J. (2009). Cocoa procyanidins attenuate 4-hydroxynonenal-induced apoptosis of PC12 cells by directly inhibiting mitogen-activated protein kinase kinase 4 activity. *Free Radic. Biol. Med.* **46**:1319–1327.

Cho, E. S., Lee, K. W. and Lee, H. J. (2008). Cocoa procyanidins protect PC12 cells from hydrogen-peroxide-induced apoptosis by inhibiting activation of p38 MAPK and JNK. *Mutat. Res.* **640**:123–130.

Cooper, K. A., Campos-Gimenez, E., Jimenez Alvarez, D., Nagy, K., Donovan, J. L. and Williamson, G. (2007). Rapid reversed phase ultra-performance

liquid chromatography analysis of the major cocoa polyphenols and interrelationships of their concentrations in chocolate. *J. Agric. Food Chem.* **55**:2841–2847.

Cooper, K. A., Donovan, J. L., Waterhouse, A. L. and Williamson, G. (2008). Cocoa and health: A decade of research. *Br. J. Nutr.* **99**:1–11.

Corti, R., Flammer, A. J., Hollenberg, N. K. and Luscher, T. F. (2009). Cocoa and cardiovascular health. *Circulation*. **119**:1433–1441.

Counet, C., Callemien, D. and Collin, S. (2006). Chocolate and cocoa: New sources of trans-resveratrol and trans-piceid. *Food Chem.* **98**:649–657.

Cuevas, E., Limon, D., Perez-Severiano, F., Diaz, A., Ortega, L., Zenteno, E. and Guevara, J. (2009). Antioxidant effects of epicatechin on the hippocampal toxicity caused by amyloid-beta 25-35 in rats. *Eur. J. Pharmacol.* **616**:122–127.

de Mendonca, A. and Cunha, R. A. (2010). Therapeutic opportunities for caffeine in Alzheimer's disease and other neurodegenerative disorders. *J. Alzheimers Dis.* **20**(Suppl. 1):S1–S2.

Deprez, S., Brezillon, C., Rabot, S., Philippe, C., Mila, I., Lapierre, C. and Scalbert, A. (2000). Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J. Nutr.* **130**:2733–2738.

Deprez, S., Mila, I., Huneau, J. F., Tome, D. and Scalbert, A. (2001). Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxid. Redox. Signal.* **3**:957–967.

Dillinger, T. L., Barriga, P., Escarcega, S., Jimenez, M., Salazar Lowe, D. and Grivetti, L. E. (2000). Food of the gods: Cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *J. Nutr.* **130**:2057S–2072S.

Donovan, J. L., Manach, C., Rios, L., Morand, C., Scalbert, A. and Remesy, C. (2002). Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B3. *Br. J. Nutr.* **87**:299–306.

Dray, C., Daviaud, D., Guigne, C., Valet, P. and Castan-Laurell, I. (2007). Caffeine reduces TNFalpha up-regulation in human adipose tissue primary culture. *J. Physiol. Biochem.* **63**:329–336.

Echeverri, D., Montes, F. R., Cabrera, M., Galan, A. and Prieto, A. (2010). Caffeine's vascular mechanisms of action. *Int. J. Vasc. Med.* **2010**:834060.

Engler, M. B. and Engler, M. M. (2006). The emerging role of flavonoid-rich cocoa and chocolate in cardiovascular health and disease. *Nutr. Rev.* **64**:109–118.

Engler, M. B., Engler, M. M., Chen, C. Y., Malloy, M. J., Browne, A., Chiu, E. Y., Kwak, H. K., Milbury, P., Paul, S. M., Blumberg, J. and Mietus-Snyder, M. L. (2004). Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J. Am. Coll. Nutr.* **23**:197–204.

Eskelinen, M. H. and Kivipelto, M. (2010). Caffeine as a protective factor in dementia and Alzheimer's disease. *J. Alzheimers Dis.* **20**(Suppl. 1):S167–S174.

Fisher, N. D. and Hollenberg, N. K. (2006). Aging and vascular responses to flavanol-rich cocoa. *J. Hypertens.* **24**:1575–1580.

Fisher, N. D., Hughes, M., Gerhard-Herman, M. and Hollenberg, N. K. (2003). Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J. Hypertens.* **21**:2281–2286.

Forsyth, W. G. (1952). Cacao polyphenolic substances. I. Fractionation of the fresh bean. *Biochem. J.* **51**:511–516.

Fraga, C. G., Actis-Goretta, L., Ottaviani, J. I., Carrasquedo, F., Lotito, S. B., Lazarus, S., Schmitz, H. H. and Keen, C. L. (2005). Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. *Clin. Dev. Immunol.* **12**:11–17.

Francis, S. T., Head, K., Morris, P. G. and Macdonald, I. A. (2006). The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *J. Cardiovasc. Pharmacol.* **47**(Suppl. 2):S215–S220.

Gasser, P., Lati, E., Peno-Mazzarino, L., Bouzoud, D., Allegaert, L. and Bernaert, H. (2008). Cocoa polyphenols and their influence on parameters involved in ex vivo skin restructuring. *Int. J. Cosmet Sci.* **30**:339–345.

Gil, M., Skopinska-Rozewska, E., Radomska, D., Demkow, U., Skurzak, H., Rochowska, M., Beuth, J. and Roszkowski, K. (1993). Effect of purinergic receptor antagonists suramin and theobromine on tumor-induced angiogenesis in BALB/c mice. *Folia Biol. (Praha).* **39**:63–68.

Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D., Castelli, W. P., Knoke, J. D., Jacobs, D. R., Jr., Bangdiwala, S. and Tyroler, H. A. (1989). High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation*. **79**:8–15.

Gotti, R., Furlanetto, S., Pinzauti, S. and Cavrini, V. (2006). Analysis of catechins in Theobroma cacao beans by cyclodextrin-modified micellar electrokinetic chromatography. *J. Chromatogr. A.* **1112**:345–352.

Grassi, D., Lippi, C., Necozione, S., Desideri, G. and Ferri, C. (2005). 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.* **81**:611–614.

Gu, L., House, S. E., Wu, X., Ou, B. and Prior, R. L. (2006). Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. *J. Agric. Food Chem.* **54**:4057–4061.

Heinrich, U., Neukam, K., Tronnier, H., Sies, H. and Stahl, W. (2006). Long-term ingestion of high flavanol cocoa provides photoprotection against UV-induced erythema and improves skin condition in women. *J. Nutr.* **136**:1565–1569.

Heiss, C., Dejam, A., Kleinbongard, P., Schewe, T., Sies, H. and Kelm, M. (2003). Vascular effects of cocoa rich in flavan-3-ols. *JAMA*. **290**:1030–1031.

Heiss, C., Finis, D., Kleinbongard, P., Hoffmann, A., Rassaf, T., Kelm, M. and Sies, H. (2007). Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week. *J. Cardiovasc. Pharmacol.* **49**:74–80.

Heiss, C., Kleinbongard, P., Dejam, A., Perre, S., Schroeter, H., Sies, H. and Kelm, M. (2005). Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *J. Am. Coll. Cardiol.* **46**:1276–1283.

Heiss, C., Schroeter, H., Balzer, J., Kleinbongard, P., Matern, S., Sies, H. and Kelm, M. (2006). Endothelial function, nitric oxide, and cocoa flavanols. *J. Cardiovasc. Pharmacol.* **47**(Suppl. 2):S128–S135; discussion S172– S176.

Heo, H. J. and Lee, C. Y. (2005). Epicatechin and catechin in cocoa inhibit amyloid beta protein induced apoptosis. *J. Agric. Food Chem.* **53**:1445–1448.

Heptinstall, S., May, J., Fox, S., Kwik-Uribe, C. and Zhao, L. (2006). Cocoa flavanols and platelet and leukocyte function: Recent in vitro and ex vivo studies in healthy adults. *J. Cardiovasc. Pharmacol.* **47**(Suppl. 2):S197–S205; discussion S206–S209.

Hermann, F., Spieker, L. E., Ruschitzka, F., Sudano, I., Hermann, M., Binggeli, C., Luscher, T. F., Riesen, W., Noll, G. and Corti, R. (2006). Dark chocolate improves endothelial and platelet function. *Heart*. **92**:119–120.

Hollenberg, N. K., Martinez, G., McCullough, M., Meinking, T., Passan, D., Preston, M., Rivera, A., Taplin, D. and Vicaria-Clement, M. (1997). Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. *Hypertension*. **29**:171–176.

Holt, R. R., Lazarus, S. A., Sullards, M. C., Zhu, Q. Y., Schramm, D. D., Hammerstone, J. F., Fraga, C. G., Schmitz, H. H. and Keen, C. L. (2002). Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. *Am. J. Clin. Nutr.* **76**:798–804.

Innes, A. J., Kennedy, G., McLaren, M., Bancroft, A. J. and Belch, J. J. (2003). Dark chocolate inhibits platelet aggregation in healthy volunteers. *Platelets*. **14**:325–327.

Jourdain, C., Tenca, G., Deguercy, A., Troplin, P. and Poelman, D. (2006). In-vitro effects of polyphenols from cocoa and beta-sitosterol on the growth of human prostate cancer and normal cells. *Eur. J. Cancer Prev.* **15**: 353–361.

Kang, N. J., Lee, K. W., Lee, D. E., Rogozin, E. A., Bode, A. M., Lee, H. J. and Dong, Z. (2008). Cocoa procyanidins suppress transformation by inhibiting mitogen-activated protein kinase kinase. *J. Biol. Chem.* **283**:20664–20673.

Kang, S. S., Han, K. S., Ku, B. M., Lee, Y. K., Hong, J., Shin, H. Y., Almonte, A. G., Woo, D. H., Brat, D. J., Hwang, E. M., Yoo, S. H., Chung, C. K., Park, S. H., Paek, S. H., Roh, E. J., Lee, S. J., Park, J. Y., Traynelis, S. F. and Lee, C. J. (2010). Caffeine-mediated inhibition of calcium release channel inositol 1,4,5-trisphosphate receptor subtype 3 blocks glioblastoma invasion and extends survival. *Cancer Res.* **70**:1173–1183.

Kenny, D., Coughlan, M. G., Pagel, P. S., Kampine, J. P. and Warltier, D. C. (1994). Transforming growth factor beta 1 preserves endothelial function after multiple brief coronary artery occlusions and reperfusion. *Am. Heart J.* **127**:1456–1461.

Kenny, T. P., Keen, C. L., Jones, P., Kung, H. J., Schmitz, H. H. and Gershwin, M. E. (2004). Pentameric procyanidins isolated from Theobroma cacao seeds selectively downregulate ErbB2 in human aortic endothelial cells. *Exp. Biol. Med. (Maywood)*. **229**:255–263.

Kim, J. E., Son, J. E., Jung, S. K., Kang, N. J., Lee, C. Y., Lee, K. W. and Lee, H. J. (2010). Cocoa polyphenols suppress TNF-alpha-induced vascular endothelial growth factor expression by inhibiting phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase kinase-1 (MEK1) activities in mouse epidermal cells. *Br. J. Nutr.* **104**:957–964.

Kobayashi-Hattori, K., Mogi, A., Matsumoto, Y. and Takita, T. (2005). Effect of caffeine on the body fat and lipid metabolism of rats fed on a high-fat diet. *Biosci. Biotechnol. Biochem.* **69**:2219–2223.

Kostyuk, V. A., Kraemer, T., Sies, H. and Schewe, T. (2003). Myeloperoxidase/nitrite-mediated lipid peroxidation of low-density lipoprotein as modulated by flavonoids. *FEBS Lett.* **537**:146–150.

Kraemer, T., Prakosay, I., Date, R. A., Sies, H. and Schewe, T. (2004). Oxidative modification of low-density lipoprotein: Lipid peroxidation by myeloperoxidase in the presence of nitrite. *Biol. Chem.* **385**:809–818.

Ku, B. M., Lee, Y. K., Jeong, J. Y., Ryu, J., Choi, J., Kim, J. S., Cho, Y. W., Roh, G. S., Kim, H. J., Cho, G. J., Choi, W. S. and Kang, S. S. (2010). Caffeine inhibits cell proliferation and regulates PKA/GSK3beta pathways in U87MG human glioma cells. *Mol. Cells.* **31**:275–279.

Lee, C. (2000). Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. *Clin. Chim. Acta.* **295**:141–154.

Lee, D. E., Kang, N. J., Lee, K. M., Lee, B. K., Kim, J. H., Lee, K. W. and Lee, H. J. (2010). Cocoa polyphenols attenuate hydrogen peroxide-induced inhibition of gap-junction intercellular communication by blocking phosphorylation of connexin 43 via the MEK/ERK signaling pathway. *J. Nutr. Biochem.* **21**:680–686.

Lee, K. W., Kang, N. J., Oak, M. H., Hwang, M. K., Kim, J. H., Schini-Kerth, V. B. and Lee, H. J. (2008). Cocoa procyanidins inhibit expression and activation of MMP-2 in vascular smooth muscle cells by direct inhibition of MEK and MT1-MMP activities. *Cardiovasc. Res.* **79**:34–41.

Lee, K. W., Kim, Y. J., Lee, H. J. and Lee, C. Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J. Agric. Food Chem.* **51**:7292–7295.

Lee, K. W., Kundu, J. K., Kim, S. O., Chun, K. S., Lee, H. J. and Surh, Y. J. (2006). Cocoa polyphenols inhibit phorbol ester-induced superoxide anion formation in cultured HL-60 cells and expression of cyclooxygenase-2 and activation of NF-kappaB and MAPKs in mouse skin in vivo. *J. Nutr.* **136**:1150–1155.

Lijnen, P. J., Petrov, V. V. and Fagard, R. H. (2000). Induction of cardiac fibrosis by transforming growth factor-beta(1). *Mol. Genet Metab.* **71**:418–435.

Loke, W. M., Hodgson, J. M., Proudfoot, J. M., McKinley, A. J., Puddey, I. B. and Croft, K. D. (2008). Pure dietary flavonoids quercetin and (−)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men. *Am. J. Clin. Nutr.* **88**:1018–1025.

Lopez-Garcia, E., van Dam, R. M., Rajpathak, S., Willett, W. C., Manson, J. E. and Hu, F. B. (2006). Changes in caffeine intake and long-term weight change in men and women. *Am. J. Clin. Nutr.* **83**:674–680.

Lv, X., Chen, Z., Li, J., Zhang, L., Liu, H., Huang, C. and Zhu, P. (2010). Caffeine protects against alcoholic liver injury by attenuating inflammatory response and oxidative stress. *Inflamm. Res.* **59**:635–645.

Mackenzie, G. G., Carrasquedo, F., Delfino, J. M., Keen, C. L., Fraga, C. G. and Oteiza, P. I. (2004). Epicatechin, catechin, and dimeric procyanidins inhibit PMA-induced NF-kappaB activation at multiple steps in Jurkat T cells. *Faseb. J.* **18**:167–169.

Mackenzie, G. G. and Oteiza, P. I. (2006). Modulation of transcription factor NF-kappaB in Hodgkin's lymphoma cell lines: Effect of (−)-epicatechin. *Free Radic. Res.* **40**:1086–1094.

Manach, C., Scalbert, A., Morand, C., Remesy, C. and Jimenez, L. (2004). Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **79**:727–747.

Manach, C., Williamson, G., Morand, C., Scalbert, A. and Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **81**:230S–242S.

Mao, T., Van De Water, J., Keen, C. L., Schmitz, H. H. and Gershwin, M. E. (2000). Cocoa procyanidins and human cytokine transcription and secretion. *J. Nutr.* **130**:2093S–2099S.

Mao, T. K., Van De Water, J., Keen, C. L., Schmitz, H. H. and Gershwin, M. E. (2002). Modulation of TNF-alpha secretion in peripheral blood mononuclear cells by cocoa flavanols and procyanidins. *Dev. Immunol.* **9**:135–141.

Mao, T. K., Van De Water, J., Keen, C. L., Schmitz, H. H. and Gershwin, M. E. (2003). Cocoa flavonols and procyanidins promote transforming growth factor-beta1 homeostasis in peripheral blood mononuclear cells. *Exp. Biol. Med. (Maywood)*. **228**:93–99.

Merighi, S., Benini, A., Mirandola, P., Gessi, S., Varani, K., Simioni, C., Leung, E., Maclennan, S., Baraldi, P. G. and Borea, P. A. (2007). Caffeine inhibits adenosine-induced accumulation of hypoxia-inducible factor-1alpha, vascular endothelial growth factor, and interleukin-8 expression in hypoxic human colon cancer cells. *Mol. Pharmacol.* **72**:395–406.

Miller, K. B., Hurst, W. J., Flannigan, N., Ou, B., Lee, C. Y., Smith, N. and Stuart, D. A. (2009). Survey of commercially available chocolate- and cocoa-containing products in the United States. 2. Comparison of flavan-3-ol content with nonfat cocoa solids, total polyphenols, and percent cacao. *J. Agric. Food Chem.* **57**:9169–9180.

Miller, K. B., Stuart, D. A., Smith, N. L., Lee, C. Y., McHale, N. L., Flanagan, J. A., Ou, B. and Hurst, W. J. (2006). Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States. *J. Agric. Food Chem.* **54**:4062–4068.

Mumford, G. K., Benowitz, N. L., Evans, S. M., Kaminski, B. J., Preston, K. L., Sannerud, C. A., Silverman, K. and Griffiths, R. R. (1996). Absorption rate of methylxanthines following capsules, cola and chocolate. *Eur. J. Clin. Pharmacol.* **51**:319–325.

Muniyappa, R., Hall, G., Kolodziej, T. L., Karne, R. J., Crandon, S. K. and Quon, M. J. (2008). Cocoa consumption for 2 wk enhances insulin-mediated vasodilatation without improving blood pressure or insulin resistance in essential hypertension. *Am. J. Clin. Nutr.* **88**:1685–1696.

Murphy, K. J., Chronopoulos, A. K., Singh, I., Francis, M. A., Moriarty, H., Pike, M. J., Turner, A. H., Mann, N. J. and Sinclair, A. J. (2003). Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. *Am. J. Clin. Nutr.* **77**:1466–1473.

Mursu, J., Voutilainen, S., Nurmi, T., Rissanen, T. H., Virtanen, J. K., Kaikkonen, J., Nyyssonen, K. and Salonen, J. T. (2004). Dark chocolate consumption increases HDL cholesterol concentration and chocolate fatty acids may inhibit lipid peroxidation in healthy humans. *Free Radic. Biol. Med.* **37**:1351–1359.

Nakabayashi, H., Hashimoto, T., Ashida, H., Nishiumi, S. and Kanazawa, K. (2008). Inhibitory effects of caffeine and its metabolites on intracellular lipid accumulation in murine 3T3-L1 adipocytes. *Biofactors.* **34**:293–302.

Natsume, M., Osakabe, N., Oyama, M., Sasaki, M., Baba, S., Nakamura, Y., Osawa, T. and Terao, J. (2003). Structures of (−)-epicatechin glucuronide identified from plasma and urine after oral ingestion of (−)-epicatechin: Differences between human and rat. *Free Radic. Biol. Med.* **34**:840–849.

Nazaruddin, R., Seng, L. K., Hassan, O. and Said, M. (2006). Effect of pulp preconditioning on the content of polyphenols in cocoa beans (Theobroma cacao) during fermentation. *Industr. Crops Prod.* **24**:87–94.

Nehlig, A., Daval, J. L. and Debry, G. (1992). Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res. Brain Res. Rev.* **17**:139–170.

Okano, J., Nagahara, T., Matsumoto, K. and Murawaki, Y. (2008). Caffeine inhibits the proliferation of liver cancer cells and activates the MEK/ERK/EGFR signalling pathway. *Basic Clin. Pharmacol. Toxicol.* **102**:543–551.

Osakabe, N., Baba, S., Yasuda, A., Iwamoto, T., Kamiyama, M., Takizawa, T., Itakura, H. and Kondo, K. (2001). Daily cocoa intake reduces the susceptibility of low-density lipoprotein to oxidation as demonstrated in healthy human volunteers. *Free Radic. Res.* **34**:93–99.

Osakabe, N., Yamagishi, M., Sanbongi, C., Natsume, M., Takizawa, T. and Osawa, T. (1998). The antioxidative substances in cacao liquor. *J. Nutr. Sci. Vitaminol. (Tokyo)*. **44**:313–321.

Ottaviani, J. I., Carrasquedo, F., Keen, C. L., Lazarus, S. A., Schmitz, H. H. and Fraga, C. G. (2002). 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*. **406**:203–208.

Pan, T., Jankovic, J. and Le, W. (2003). Potential therapeutic properties of green tea polyphenols in Parkinson's disease. *Drugs Aging*. **20**:711–721.

Pearson, D. A., Paglieroni, T. G., Rein, D., Wun, T., Schramm, D. D., Wang, J. F., Holt, R. R., Gosselin, R., Schmitz, H. H. and Keen, C. L. (2002). The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. *Thromb. Res*. **106**:191–197.

Peng, L., Khan, N., Afaq, F., Ye, C. and Mukhtar, H. (2010). In vitro and in vivo effects of water extract of white cocoa tea (Camellia ptilophylla) against human prostate cancer. *Pharm. Res*. **27**:1128–1137.

Pettipher, G. L. (1986). An improved method for the extraction and quantitation of anthocyanins in cocoa beans and its use as an index of the degree of fermentation. *J. Sci. Food Agric*. **37**:289–296.

Podrez, E. A., Abu-Soud, H. M. and Hazen, S. L. (2000). Myeloperoxidase-generated oxidants and atherosclerosis. *Free Radic. Biol. Med*. **28**:1717–1725.

Prediger, R. D. (2010). Effects of caffeine in Parkinson's disease: From neuroprotection to the management of motor and non-motor symptoms. *J. Alzheimers Dis*. **20**(Suppl. 1):S205–S220.

Ptolemy, A. S., Tzioumis, E., Thomke, A., Rifai, S. and Kellogg, M. (2010). Quantification of theobromine and caffeine in saliva, plasma and urine via liquid chromatography-tandem mass spectrometry: A single analytical protocol applicable to cocoa intervention studies. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. **878**:409–416.

Pura Naik, J. (2001). Improved high-performance liquid chromatography method to determine theobromine and caffeine in cocoa and cocoa products. *J. Agric. Food Chem*. **49**:3579–3583.

Ramiro, E., Franch, A., Castellote, C., Perez-Cano, F., Permanyer, J., Izquierdo-Pulido, M. and Castell, M. (2005). Flavonoids from Theobroma cacao down-regulate inflammatory mediators. *J. Agric. Food Chem*. **53**:8506–8511.

Ramiro-Puig, E. and Castell, M. (2009). Cocoa: Antioxidant and immunomodulator. *Br. J. Nutr*. **101**:931–940.

Rein, D., Lotito, S., Holt, R. R., Keen, C. L., Schmitz, H. H. and Fraga, C. G. (2000). Epicatechin in human plasma: In vivo determination and effect of chocolate consumption on plasma oxidation status. *J. Nutr*. **130**:2109S–2114S.

Richelle, M., Tavazzi, I., Enslen, M. and Offord, E. A. (1999). Plasma kinetics in man of epicatechin from black chocolate. *Eur. J. Clin. Nutr*. **53**:22–26.

Rios, L. Y., Gonthier, M. P., Remesy, C., Mila, I., Lapierre, C., Lazarus, S. A., Williamson, G. and Scalbert, A. (2003). Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am. J. Clin. Nutr*. **77**:912–918.

Ritchie, K., Carriere, I., de Mendonca, A., Portet, F., Dartigues, J. F., Rouaud, O., Barberger-Gateau, P. and Ancelin, M. L. (2007). The neuroprotective effects of caffeine: A prospective population study (the Three City Study). *Neurology*. **69**:536–545.

Ross, R. (1999). Atherosclerosis—an inflammatory disease. *N. Engl. J. Med*. **340**:115–126.

Ruzaidi, A., Amin, I., Nawalyah, A. G., Hamid, M. and Faizul, H. A. (2005). The effect of Malaysian cocoa extract on glucose levels and lipid profiles in diabetic rats. *J. Ethnopharmacol*. **98**:55–60.

Schewe, T., Steffen, Y. and Sies, H. (2008). How do dietary flavanols improve vascular function? A position paper. *Arch. Biochem. Biophys*. **476**:102–106.

Scholey, A. B., French, S. J., Morris, P. J., Kennedy, D. O., Milne, A. L. and Haskell, C. F. (2010). Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. *J. Psychopharmacol*. **24**:1505–1514.

Schramm, D. D., Karim, M., Schrader, H. R., Holt, R. R., Kirkpatrick, N. J., Polagruto, J. A., Ensunsa, J. L., Schmitz, H. H. and Keen, C. L. (2003). Food effects on the absorption and pharmacokinetics of cocoa flavanols. *Life Sci*. **73**:857–869.

Schroeter, H., Heiss, C., Balzer, J., Kleinbongard, P., Keen, C. L., Hollenberg, N. K., Sies, H., Kwik-Uribe, C., Schmitz, H. H. and Kelm, M. (2006). (−)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc. Natl. Acad. Sci. USA*. **103**:1024–1029.

Schroeter, H., Williams, R. J., Matin, R., Iversen, L. and Rice-Evans, C. A. (2000). Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. *Free Radic. Biol. Med*. **29**:1222–1233.

Selmi, C., Cocchi, C. A., Lanfredini, M., Keen, C. L. and Gershwin, M. E. (2008). Chocolate at heart: The anti-inflammatory impact of cocoa flavanols. *Mol. Nutr. Food Res*. **52**:1340–1348.

Selmi, C., Mao, T. K., Keen, C. L., Schmitz, H. H. and Eric Gershwin, M. (2006). The anti-inflammatory properties of cocoa flavanols. *J. Cardiovasc. Pharmacol*. **47**(Suppl. 2):S163–S171; discussion S172– S176.

Shah, Z. A., Li, R. C., Ahmad, A. S., Kensler, T. W., Yamamoto, M., Biswal, S. and Dore, S. (2010). The flavanol (−)-epicatechin prevents stroke damage through the Nrf2/HO1 pathway. *J. Cereb. Blood Flow Metab*. **30**:1951–1961.

Shepherd, J., Cobbe, S. M., Ford, I., Isles, C. G., Lorimer, A. R., MacFarlane, P. W., McKillop, J. H. and Packard, C. J. (1995). Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N. Engl. J. Med*. **333**:1301–1307.

Sies, H., Schewe, T., Heiss, C. and Kelm, M. (2005). Cocoa polyphenols and inflammatory mediators. *Am. J. Clin. Nutr*. **81**:304S–312S.

Skopinska-Rozewska, E., Janik, P., Przybyszewska, M., Sommer, E. and Bialas-Chromiec, B. (1998). Inhibitory effect of theobromine on induction of angiogenesis and VEGF mRNA expression in v-raf transfectants of human urothelial cells HCV-29. *Int. J. Mol. Med*. **2**:649–652.

Smit, H. J. and Blackburn, R. J. (2005). Reinforcing effects of caffeine and theobromine as found in chocolate. *Psychopharmacology (Berl)*. **181**:101–106.

Smit, H. J., Gaffan, E. A. and Rogers, P. J. (2004). Methylxanthines are the psycho-pharmacologically active constituents of chocolate. *Psychopharmacology (Berl)*. **176**:412–419.

Smit, H. J. and Rogers, P. J. (2000). Effects of low doses of caffeine on cognitive performance, mood and thirst in low and higher caffeine consumers. *Psychopharmacology (Berl)*. **152**:167–173.

Spencer, J. P. (2009). Flavonoids and brain health: Multiple effects underpinned by common mechanisms. *Genes Nutr*. **4**:243–250.

Stamler, J., Wentworth, D. and Neaton, J. D. (1986). Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA*. **256**:2823–2828.

Steinberg, F. M., Bearden, M. M. and Keen, C. L. (2003). Cocoa and chocolate flavonoids: Implications for cardiovascular health. *J. Am. Diet Assoc*. **103**:215–223.

Tarka, S. M., Jr. (1982). The toxicology of cocoa and methylxanthines: A review of the literature. *Crit. Rev. Toxicol*. **9**:275–312.

Taubert, D., Roesen, R. and Schomig, E. (2007). Effect of cocoa and tea intake on blood pressure: A meta-analysis. *Arch. Intern. Med*. **167**:626–634.

Tomaru, M., Takano, H., Osakabe, N., Yasuda, A., Inoue, K., Yanagisawa, R., Ohwatari, T. and Uematsu, H. (2007). Dietary supplementation with cacao liquor proanthocyanidins prevents elevation of blood glucose levels in diabetic obese mice. *Nutrition*. **23**:351–355.

Umemura, T., Ueda, K., Nishioka, K., Hidaka, T., Takemoto, H., Nakamura, S., Jitsuiki, D., Soga, J., Goto, C., Chayama, K., Yoshizumi, M. and Higashi, Y. (2006). Effects of acute administration of caffeine on vascular function. *Am. J. Cardiol*. **98**:1538–1541.

Van Praag, H., Lucero, M. J., Yeo, G. W., Stecker, K., Heivand, N., Zhao, C., Yip, E., Afanador, M., Schroeter, H., Hammerstone, J. and Gage, F. H. (2007). Plant-derived flavanol (−)epicatechin enhances angiogenesis and retention of spatial memory in mice. *J. Neurosci*. **27**:5869–5878.

Vlachopoulos, C., Aznaouridis, K., Alexopoulos, N., Economou, E., Andreadou, I. and Stefanadis, C. (2005). Effect of dark chocolate on arterial function in healthy individuals. *Am. J. Hypertens*. **18**:785–791.

Wan, Y., Vinson, J. A., Etherton, T. D., Proch, J., Lazarus, S. A. and Kris-Etherton, P. M. (2001). Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *Am. J. Clin. Nutr*. **74**:596–602.

Wang, J. F., Schramm, D. D., Holt, R. R., Ensunsa, J. L., Fraga, C. G., Schmitz, H. H. and Keen, C. L. (2000). A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. *J. Nutr*. **130**:2115S–2119S.

Wang-Polagruto, J. F., Villablanca, A. C., Polagruto, J. A., Lee, L., Holt, R. R., Schrader, H. R., Ensunsa, J. L., Steinberg, F. M., Schmitz, H. H. and Keen, C. L. (2006). Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholesterolemic postmenopausal women. *J. Cardiovasc. Pharmacol.* **47**(Suppl. 2):S177–S186; discussion S206– S209.

Waterhouse, A. L., Shirley, J. R. and Donovan, J. L. (1996). Antioxidants in chocolate. *Lancet.* **348**:834.

Weisburger, J. H. (2001). Chemopreventive effects of cocoa polyphenols on chronic diseases. *Exp. Biol. Med. (Maywood).* **226**:891–897.

Wollgast, J. and Anklam, E. (2000). Review on polyphenols in Theobroma cacao: Changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Res. Int.* **33**:423–447.

Yamada, T., Yamada, Y., Okano, Y., Terashima, T. and Yokogoshi, H. (2009). Anxiolytic effects of short- and long-term administration of cacao mass on rat elevated T-maze test. *J. Nutr. Biochem.* **20**:948–955.

Yamagishi, M., Natsume, M., Osakabe, N., Nakamura, H., Furukawa, F., Imazawa, T., Nishikawa, A. and Hirose, M. (2002). Effects of cacao liquor proanthocyanidins on PhIP-induced mutagenesis in vitro, and in vivo mammary and pancreatic tumorigenesis in female Sprague-Dawley rats. *Cancer Lett.* **185**:123–130.

Yamagishi, M., Natsume, M., Osakabe, N., Okazaki, K., Furukawa, F., Imazawa, T., Nishikawa, A. and Hirose, M. (2003). Chemoprevention of lung carcinogenesis by cacao liquor proanthocyanidins in a male rat multi-organ carcinogenesis model. *Cancer Lett.* **191**:49–57.

Yamagishi, M., Osakab, N., Takizawa, T. and Osawa, T. (2001). Cacao liquor polyphenols reduce oxidative stress without maintaining alpha-tocopherol levels in rats fed a vitamin E-deficient diet. *Lipids.* **36**:67–71.

Yang, H., Rouse, J., Lukes, L., Lancaster, M., Veenstra, T., Zhou, M., Shi, Y., Park, Y. G. and Hunter, K. (2004). Caffeine suppresses metastasis in a transgenic mouse model: A prototype molecule for prophylaxis of metastasis. *Clin. Exp. Metastasis.* **21**:719–735.

Yasuda, A., Natsume, M., Sasaki, K., Baba, S., Nakamura, Y., Kanegae, M. and Nagaoka, S. (2008). Cacao procyanidins reduce plasma cholesterol and increase fecal steroid excretion in rats fed a high-cholesterol diet. *Biofactors.* **33**:211–223.