



www.chocolateusa.org



American Cocoa Research Institute



American Cocoa Research Institute

*A Resource Guide to
Chocolate and Health*



THE CHOCOLATE MANUFACTURERS ASSOCIATION (CMA)

The Chocolate Manufacturers Association (CMA) has served as the premier trade group for manufacturers and distributors of cocoa and chocolate products in the United States since 1923. The association was founded to fund and administer research, promote chocolate to the general public and serve as an advocate of the industry before Congress and government agencies. CMA supports science-based approaches to a healthy lifestyle that promote energy balance through diet and activity choices. Please visit our website at www.chocolateusa.org to learn more about us.

THE AMERICAN COCOA RESEARCH INSTITUTE (ACRI)

The American Cocoa Research Institute (ACRI) was founded in 1947. It is the research arm of the Chocolate Manufacturers Association of America (CMA) and is devoted to research in scientific areas related to cocoa and chocolate.

OUR COMMITMENT TO COCOA FARMING FAMILIES

The U.S. cocoa and chocolate industry is working actively to help cocoa farming families around the world. Industry members have formed partnerships with producing countries, non-government organizations (NGOs), government agencies and development organizations to foster economic and social development as well as environmental conservation in the cocoa-growing regions of West Africa, Asia and the Americas. Industry-supported programs are improving farm family incomes, working to ensure responsible labor practices, expanding access to education, and protecting the tropical ecosystem. The Chocolate Manufacturers Association proudly supports the World Cocoa Foundation, a unique partnership of industry, government, international agencies and NGOs. The Foundation plays a leading role in helping cocoa farming families by developing and managing on-the-ground programs and acting as a forum for broad discussion of the cocoa farming sector's needs. For more information, go to www.worldcocoafoundation.org.

A Resource Guide to Chocolate and Health

For centuries, cocoa, the main ingredient in chocolate, has been used to promote health and well-being. Ancient civilizations referred to cocoa as “a gift from the gods” and used it as a highly coveted food and as a type of currency. *Theobroma cacao*, the scientific name for the cacao tree, means “food of the gods.” The word “cacao” came from the Spanish adaptation of the Olmec word “kakaw-tl.” Today, this widely enjoyed and special food has come full circle as the health-giving properties recognized by ancient civilizations are now revealed to us through science.

Over the past ten years, our knowledge of foods and their relationship to health has grown tremendously. The recognized benefits of food and nutrients now go beyond avoiding deficiencies to maintaining health, preventing disease and even treating health problems.

An array of protective, bioactive substances in plant foods collectively called phytochemicals has been identified. The power of these naturally occurring plant components and our knowledge of their impact on human health is just starting to be realized. Ultimately, this knowledge may improve public health by encouraging consumption of a nutrient-rich diet that is full of variety and balance. In addition, food manufacturers are taking steps to retain these natural compounds throughout the manufacturing process.

Studies have begun to show the potential health benefits of cocoa, chocolate and their naturally occurring phytochemicals and to uncover the various mechanisms involved. This review provides a look at the research on cocoa and chocolate and how this long revered food may offer much more than just delicious taste.

Contents

- I. Composition of Cocoa and Chocolate**
What makes it so special?..... 1
 - A. MACRONUTRIENTS**
 - i. Stearic Acid
 - B. MINERALS**
 - C. FLAVANOLS**
 - i. Bioavailability of Flavanols
 - D. METHYLXANTHINES—CAFFEINE AND THEOBROMINE**
- II. Vascular Health and Cocoa/Chocolate Overview**..... 4
 - A. CARDIOVASCULAR HEALTH**
 - i. Anti-platelet
 - ii. Anti-inflammatory
 - iii. Endothelial Function
 - iv. Antioxidant Activity
 - B. BLOOD PRESSURE**
 - C. RENAL HEALTH**
 - D. COGNITION**
 - E. IMMUNE HEALTH**
 - F. DIABETES**
 - G. EXERCISE PERFORMANCE**
- III. Weight Management**..... 8
- IV. Anti-Cancer Effects**..... 8
- V. Chocolate Myths Revealed**..... 9
 - A. ALLERGIES**
 - B. CRAVINGS AND MOOD**
 - C. MIGRAINES**
 - D. ACNE**
 - E. BEHAVIOR AND HYPERACTIVITY**
 - F. DENTAL HEALTH**
- VI. References**..... 11



I. Composition of Cocoa and Chocolate

What makes it so special?

MACRONUTRIENTS

The nutrients in cocoa and chocolate-containing products will depend on both the amount and type of ingredients derived from the cocoa bean and the amount and type of other “non-chocolate” ingredients (e.g., sugar, milk, etc.) (**Table 1**). Of the macronutrients in the cocoa bean, the fat content and fatty acid composition are of most interest. Cocoa butter, the fat naturally contained in cocoa beans, is predominantly made up of three fatty acids: oleic acid (36%), a monounsaturated fat, stearic acid (35%) and palmitic acid (26%), both saturated fats (**Figure 1**). Despite its saturated fat content, chocolate has been shown to have a neutral effect on blood cholesterol levels. In a study by Kris-Etherton and colleagues participants were fed 10 ounces of chocolate per day for approximately one month as part of a calorie-controlled diet. The participants’ cholesterol

remained the same on the chocolate-containing diet as on the non-chocolate-containing diet. This similarity persisted despite the chocolate-containing diet having a higher saturated fat content (20% of calories) than the non-chocolate diet (14% of calories).¹ A subsequent study included a daily substitution of a 1.6-ounce milk chocolate bar in place of a high carbohydrate snack as part of the National Cholesterol Education Program/American Heart Association Step One Diet.² The substitution of the chocolate bar did not adversely affect LDL-cholesterol levels. The overall neutral effect on blood cholesterol levels may be explained by the combined effect of oleic acid, which has been shown to have a beneficial effect on serum lipids, and stearic acid’s atypical actions as a saturated fat.^{3,4}

Stearic Acid

Stearic acid is an 18-carbon saturated fatty acid similar to oleic and linoleic fatty acids in length. Stearic acid is desaturated by the liver into oleic acid. These factors may partially explain stearic acid’s atypical saturated fatty acid effects on various cardiovascular disease risk markers.⁵ Studies comparing serum lipid and lipoprotein effects of stearic acid to oleic acid and linoleic acid have shown no to little difference.^{6,7,8} Additionally, recent studies have shown that stearic acid has effects similar to oleic and linoleic acids on markers of thrombosis, and may even provide a benefit.^{9,10,11}

Figure 1 • Lipid Composition of Cocoa Butter

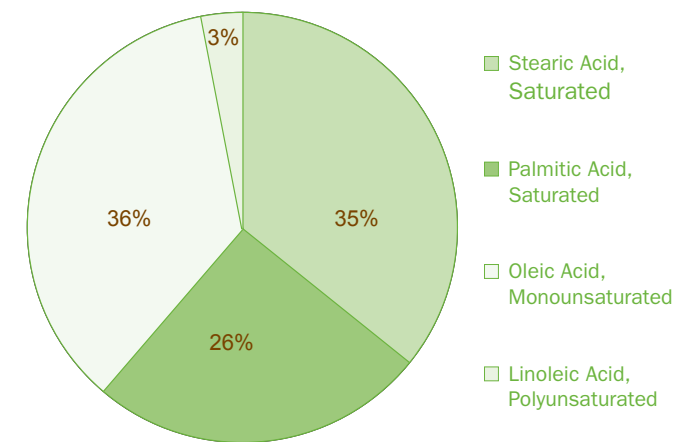


Table 1 • Macronutrient content of cocoa and chocolate products

	Cocoa Mix Powder	Cocoa Powder, Unsweetened	Milk Chocolate Bar	Semisweet (Dark) Chocolate Bar	Semisweet Chocolate Chips	Baking Chocolate, Unsweetened
Serving	1 Envelope (28 g / 1 oz)	1 Tbsp (5 g / 0.18 oz)	1 Bar (40 g / 1.4 oz)	1 Bar (40 g / 1.4 oz)	1 Tbsp (15 g / 32 pcs)	1/2 Square (14 g / 0.5 oz)
Fat (g)	1.1	0.7	11.9	12.0	4.5	7.3
Saturated Fat (g)	0.7	0.4	5.7	7.1	2.7	4.5
Cholesterol (mg)	0	0	9	0	0	0
Carbohydrate (g)	23.4	2.7	23.8	5.2	9.5	4.2
Fiber (g)	1.0	1.7	1.4	2.4	0.9	2.3
Sugars (g)	20.2	0.1	20.6	21.8	8.2	0.1
Protein (g)	1.9	1.0	3.1	1.7	0.6	1.8

Source: U.S. Department of Agriculture, Agricultural Research Service, USDA Nutrient Data Laboratory. 2005. USDA National Nutrient Database for Standard Reference, Release 18.

MINERALS

As depicted in **Table 2**, cocoa and chocolate naturally contain several minerals, including copper, magnesium, potassium and calcium, that may positively impact blood pressure and markers of cardiovascular disease (CVD) risk. Mineral bioavailability is reasonably good despite the high phytate content of cocoa and chocolate.^{12,13} Fermentation of the cocoa beans and further processing causes hydrolysis of the minerals from the phytates, facilitating bioavailability of the minerals.

Copper

Cocoa and chocolate are important and significant sources of copper and were reported to have contributed 9.4% of the daily copper intake in 1990.¹⁴ Copper has numerous functions in the body including increasing iron absorption, is a key component of enzymes that form collagen, converts dopamine to epinephrine, and is a component of superoxide dismutase—one of the most powerful endogenous antioxidants. Generally, copper deficiencies are associated with infants and those undergoing intestinal surgery or dialysis. During the early stages of development, copper deficiency can result in cardiovascular abnormalities. Additionally, low dietary intake may contribute to the development of vascular disease later in life.^{15,16,17}

Magnesium

One serving of cocoa or chocolate can contain from 4%-11.5% of the daily value for magnesium. Studies show magnesium intake to have beneficial effects on a multitude of chronic metabolic disorders including insulin resistance, type 2 diabetes, CVD and hypertension.¹⁸

Table 2 • Mineral content (in milligrams) and % of Daily Value (%DV) for cocoa and chocolate products

	Cocoa Mix Powder		Cocoa Powder, Unsweetened		Milk Chocolate Bar		Semisweet (Dark) Chocolate Bar		Semisweet Chocolate Chips		Baking Chocolate, Unsweetened	
Serving	1 envelope (28 g / 1 oz)		1 Tbsp (5 g / 0.18 oz)		1 bar (40 g / 1.4 oz)		1 bar (40 g / 1.4 oz)		1 Tbsp (15 g / 32 pcs)		1/2 square (14 g / 0.5 oz)	
Calcium	3.7	(0.4%)	6	(0.6%)	76	(7.6%)	13	(1.3%)	5	(0.5%)	14	(1.4%)
Copper	0.1	(5.0%)	0.2	(9.4%)	0.2	(9.8%)	0.3	(14.0%)	0.1	(5.2%)	0.4	(22.5%)
Iron	0.3	(1.8%)	0.7	(3.9%)	0.9	(5.2%)	1.2	(6.9%)	0.5	(2.6%)	2.4	(13.3%)
Magnesium	23	(5.8%)	25	(6.2%)	25	(6.3%)	46	(11.5%)	17	(4.3%)	46	(11.5%)
Phosphorus	88	(8.8%)	37	(3.7%)	83	(8.3%)	53	(5.3%)	20	(2.0%)	56	(5.6%)
Potassium	199	(5.7%)	76	(2.2%)	149	(4.2%)	146	(4.2%)	55	(1.6%)	116	(3.3%)
Zinc	0.4	(2.7%)	0.3	(2.3%)	0.8	(5.4%)	0.6	(4.3%)	0.2	(1.6%)	1.4	(9.0%)

Source: U.S. Department of Agriculture, Agricultural Research Service, USDA Nutrient Data Laboratory. 2005. USDA National Nutrient Database for Standard Reference, Release 18.

Potassium

Cocoa and chocolate are natural sources of potassium and contain about 2%-4% of the daily value in one typical serving. An inverse relationship between potassium intake and blood pressure and stroke-related mortality has been observed in several studies.^{19,20,21} The strong body of research was validated by an authoritative statement by the National Academies of Science and resulted in a United States Food and Drug Administration (FDA) health claim stating, “Diets containing foods that are good sources of potassium and low in sodium may reduce the risk of high blood pressure and stroke.”

Calcium

One serving of milk chocolate provides approximately 8% of the daily value for calcium. Epidemiologic and intervention studies have also demonstrated an inverse relationship between calcium intake and blood pressure.^{22,23,24}

Together these minerals contribute to the overall natural mineral content of cocoa and chocolate. Additionally, evidence shows a beneficial cardiovascular impact when minerals co-exist with other nutrients in a balanced and healthy diet.^{20,25,26}

FLAVANOLS

Cocoa beans are actually seeds from the fruit of the *Theobroma cacao* tree. Often mistaken for a legume, cocoa beans belong to the Sterculia plant family and contain large amounts of polyphenolic compounds. Flavonoids are a family of polyphenolic compounds commonly found in a variety of plant-based foods and beverages. Cocoa beans contain several flavonoids but are particularly rich in a specific class of flavonoids called flavan-3-ols or flavanols (**Figure 2**). Foods known to be concentrated sources of flavanols include apples, blueberries, cranberries, beans, spices, nuts, purple grapes, red wine, teas and cocoa and chocolate. These compounds originally were studied for their aroma and flavor contributions in foods. Today, increasing attention is given to these compounds for their possible health benefits.

Cocoa is particularly rich in both monomeric flavanols, specifically epicatechin and catechin, and oligomeric flavanols, the procyanidins (**Figure 3**).^{27,28} Unlike many other foods and beverages, cocoa and chocolate can contain an array of the various sized flavanol compounds.^{28,29} Interestingly, it has been postulated that the degree of polymerization of the flavanols may affect bioavailability and biological activity.^{30,31,32} Cocoa bean handling and processing can affect the amount and type of flavonoids in finished cocoa and chocolate products. Several factors can have an impact, including cultivar type, geographical origin, agricultural and post-harvesting practices and processing.³³ Today, cocoa and chocolate manufacturers and organizations are working to better understand and preserve the naturally occurring nutrients found in the cocoa bean.

Bioavailability of Flavanols

Epicatechin and catechin plasma concentrations have been shown to increase in a dose-dependent manner after consumption of various amounts of flavanol-rich chocolate (27 g to 80 g).^{34,35} The absorption of procyanidins, the oligomeric flavanols, is less understood than the monomeric flavanols but has been observed.³⁶

Figure 2 • Major classes of flavonoids

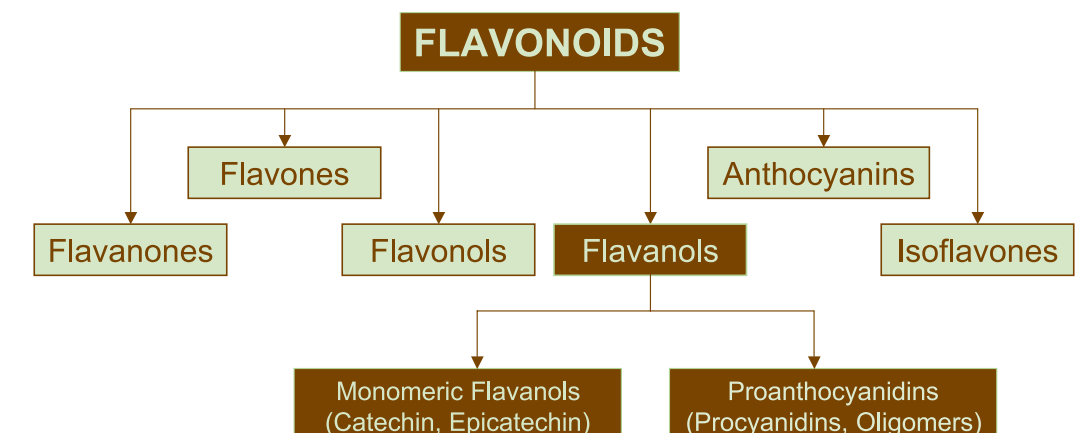
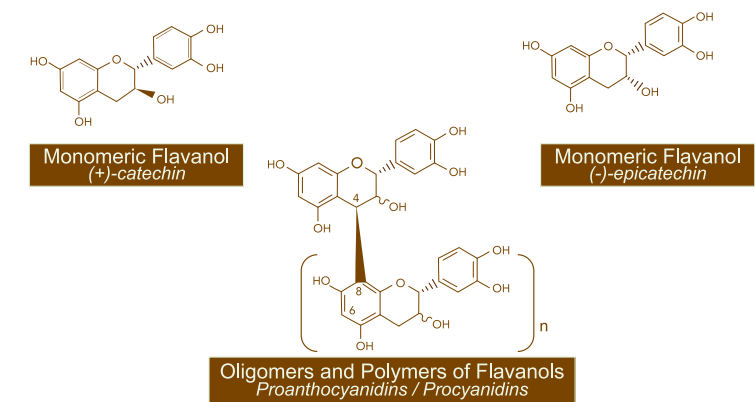


Figure 3 • Examples of cocoa flavanol structures



Epicatechin is the predominant flavanol found in plasma. The half-life is relatively short with plasma epicatechin levels peaking at two hours and returning to baseline values within six to eight hours after consuming a flavanol-rich cocoa or chocolate. Holt and colleagues detected the presence of procyanidin dimers in human plasma 30 minutes after participants consumed 26.4 g of cocoa as a beverage. The larger oligomers are believed to be degraded in the gut into epicatechin monomers and then absorbed.³⁶ These studies demonstrate that the consumption of flavanol-rich cocoa and chocolate does increase plasma flavanol levels.

Studies have also shown that the smaller oligomers, dimers and trimers, can be transported across the cell layer intact. Schroeder and colleagues demonstrated the presence of (-)-epicatechin in murine aortic endothelial cells, providing further evidence that flavanols may provide protective benefits both intracellularly and extracellularly.³⁷ Further identification and characterization studies are needed to truly understand flavanol metabolism as flavanol metabolites and bioactive forms are still not well understood or recognized.

Also important to understanding the bioavailability and bioactivity of flavanols is the potential interaction with

other nutrients. Carbohydrate content has been shown to improve flavanol absorption.³⁸ Protein interaction has also come into question. One study reported that the proteins in milk may negatively affect flavanol absorption.³⁹ A second study, more rigorously controlled for matrix and macronutrient composition, found no difference in epicatechin plasma concentrations when participants consumed a milk versus a water-based cocoa beverage.⁴⁰ Researchers concluded that the presence of milk in cocoa and chocolate products does not counteract the absorption or biological activity of flavanols.

METHYLXANTHINES— CAFFEINE AND THEOBROMINE

Chocolate contains relatively small amounts of caffeine ranging from 3-10 mg in a typical 1.4-ounce milk chocolate bar, 2-7 mg in an 8-ounce glass of chocolate milk, 7-50 mg in 1.4-ounce dark chocolate bar, and 17 mg in a half-ounce of unsweetened baking chocolate. These

levels are similar to a cup of decaffeinated coffee. In comparison, an 8-ounce cup of brewed coffee contains about 135 mg of caffeine. In total, children consume on average 1 mg of caffeine per kg of body weight from all sources, with chocolate contributing about 1.5% of children's total caffeine intake.^{41,42}

The major methylxanthine in chocolate and cocoa, however, is theobromine, ranging from 81 mg in a 1.4-ounce milk chocolate bar to 193 mg in 1.4-ounces of semi-sweet chocolate.⁴³ Theobromine, unlike caffeine, has little stimulating effect on the human central nervous system.⁴⁴ Interestingly, theobromine has recently been shown to suppress sensory nerve activation and cough. In a double-blind, placebo-controlled study, theobromine inhibited capsaicin-induced cough with no adverse effects.⁴⁵ Additionally, theobromine has been used to treat arteriosclerosis and hypertension.⁴⁶ It has been postulated that theobromine may have contributed to the positive impact of cocoa and chocolate on blood pressure observed in recent studies.⁴⁷

II. Vascular Health and Cocoa/Chocolate Overview

The vascular benefits of cocoa and chocolate have been under investigation for over 15 years. Studies have shown the ability of flavanols to reduce platelet activation, improve endothelial function and increase plasma antioxidant status.⁴⁸ The results suggest that cocoa and chocolate may positively influence various mechanisms that impact vascular health, thereby benefiting the cardiovascular system, blood pressure, renal health, cognition, immune function, diabetes and ultimately overall health and well-being.

CARDIOVASCULAR HEALTH

The thrust of the chocolate research has focused on the impact of the flavanols on cardiovascular health. Epidemiological studies show that flavonoid-rich diets are associated with a decreased risk for heart disease and stroke.^{49,50}

In cultures where cocoa is a significant part of the diet, such as the Kuna Indians living off the coast of Panama, significant cardiovascular benefits are observed. This

population's low incidence of age-related hypertension is believed to be the result of a very high intake of flavanol-rich cocoa beverages.⁵¹ A recent study in an elderly population found that habitual cocoa intake was inversely associated with hypertension, and both cardiovascular and all causes of mortality.⁵² Clinical studies have shown a promising picture by discovering potential mechanisms behind the flavanols' impact on cardiovascular health. The multi-factorial effects of flavanols against various risk factors associated with cardiovascular disease include anti-platelet, anti-inflammatory, and antioxidant actions as well as improved endothelial function.

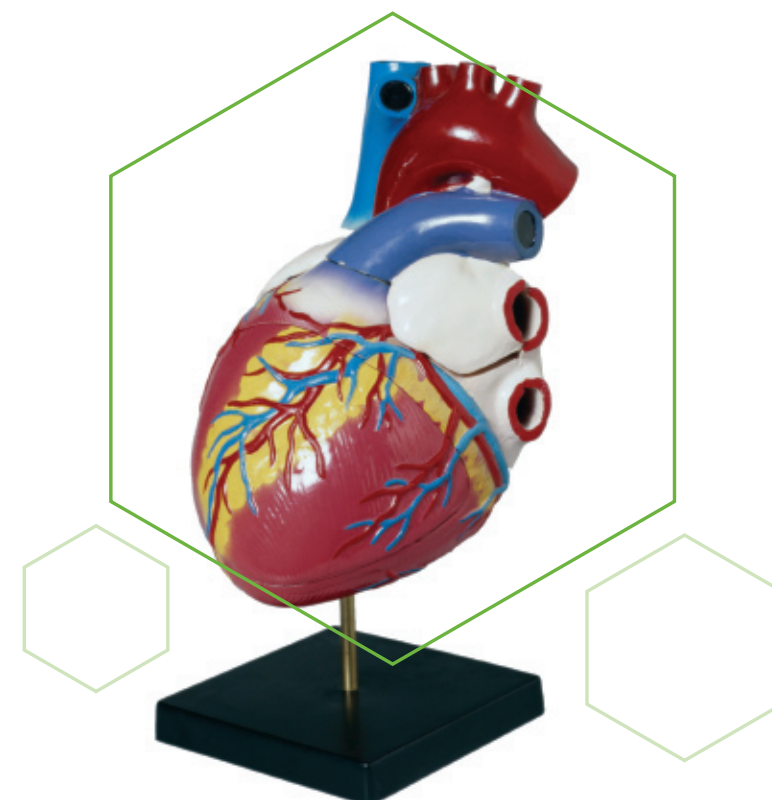
Anti-platelet

Flavanols have been shown to reduce the tendency for platelets to aggregate.^{48,53,54,55} Platelet reactivity and aggregation can lead to the development of thrombosis, contributing to various cardiovascular diseases, including heart attack, stroke and peripheral vascular disease. The ability for cocoa flavanols to modulate platelet reactivity is similar to the benefit seen with

taking a daily baby aspirin. In fact, a study comparing platelet function at two hours and six hours after taking a baby aspirin, a flavanol-rich cocoa beverage or a combination of the two showed that cocoa had similar effects on platelets as taking a baby aspirin. The combination resulted in the greatest reduction in platelet reactivity, suggesting an additive effect.⁵⁶ Several studies suggest that dietary flavanols affect platelet function through a multitude of pathways.^{53,54,56,57,58,59} One potential mechanism for reducing platelet activation is chocolate's ability to favorably alter eicosanoid synthesis. After ingestion of 37 g of a commercially available, high-flavanol chocolate bar, measurement of the eicosanoid ratio of leukotriene to prostacyclin was favorably altered.⁶⁰ Another study showed a similar effect on eicosanoid ratio with a reduction in stimulated platelet reactivity when participants were fed 25 g of semisweet chocolate chips.⁵⁵ These studies as well as others demonstrate that the consumption of a single serving of flavanol-rich chocolate can have positive short-term effects on platelet function.

Anti-inflammatory

Dietary flavonoids suppress pro-inflammatory mediators and enhance an anti-inflammatory mediator, nitric oxide.⁶¹ Initially, it was believed that cocoa flavanols decreased inflammatory compounds primarily by their ability to scavenge free radicals. However, several non-antioxidant effects are now being elucidated. As mentioned above, cocoa flavanols beneficially alter the eicosanoid ratio by reducing proinflammatory leukotrienes.^{53,55,56,60,61} Additionally, cocoa flavanols have been shown to increase anti-inflammatory nitric oxide species in plasma.^{61,62}



Endothelial Function

The vascular endothelium is a dynamic tissue involved in regulating processes to maintain the actions and tone of the blood vessels and cardiovascular system. Endothelial dysfunction is a key factor in cardiovascular disease and is characterized by a decrease in nitric oxide activity and impaired flow-mediated vasodilation.⁴⁰ Randomized trials have shown that consuming a high-flavanol dark chocolate or cocoa beverage may significantly impact the markers associated with endothelial dysfunction, thereby improving endothelial health. Nitric oxide is the principal compound responsible for vasodilation in arteries and its release is crucial to maintaining vascular tone. Several studies have shown that when participants are given a flavanol-rich cocoa beverage or chocolate bar, there is an acute increase in the production of nitric oxide, positively impacting vasodilation and thus improving blood flow.^{61,63,64,65,66} This increase has been seen in both healthy volunteers as well as those with risk factors for atherosclerosis. Additionally, studies looking directly at endothelial function have shown that flavanol-rich dark chocolate increases flow-mediated vasodilation, impacting blood flow.⁶⁶ Several of the studies eliciting a short-term positive benefit included single servings of commercially available cocoa and chocolate products.

Antioxidant Activity

In addition to the direct actions on the endothelium, flavanols potentially reduce the development of atherosclerosis through their indirect actions as antioxidants. When LDL particles are oxidized they can cause injury to arterial walls, which then attract foam cells and other immune responses, and ultimately lead to plaque formation and atherosclerosis.⁶⁷ Cocoa flavanols have been shown to protect LDL cholesterol from oxidation.^{68,69} Healthy subjects were fed a 36.9 g dark chocolate bar and 31 g of cocoa powder as a drink for six weeks, with a six-week washout period. The subjects experienced a protective effect against LDL oxidation as evidenced by an increase in LDL oxidation time in the presence of a free radical initiator.⁷⁰ An earlier study also showed a dose-dependent decrease in LDL oxidation susceptibility as well as an improved plasma antioxidant capacity after the ingestion of 27 g, 53 g and 80 g of procyanidin-rich chocolate.⁷¹ Recently, a study using the Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbit showed cocoa powder to not only reduce LDL susceptibility to oxidation but to also suppress the development of atherosclerotic lesions.⁷² This preliminary research in an animal model is very promising, indicating a potential preventive role for cocoa. Further clinical trials are needed to better understand cocoa's impact on atherosclerosis.

A recent survey of commercially available chocolate products showed that antioxidant capacity and procyanidins were found in varying amounts, depending on product type.⁷³ Oxygen radical absorbance capacity (ORAC) data show that cocoa and chocolate have a potent antioxidant capacity, especially in comparison to other flavanol-containing foods (**Figure 4**).⁷⁴ These data are consistent with studies that show the ingestion of cocoa elicits a rise in plasma antioxidant capacity and thus a reduction in the susceptibility of cell membranes to oxidation.^{75,76} The biological significance of consuming foods with increased antioxidant capacity is still under investigation.

BLOOD PRESSURE

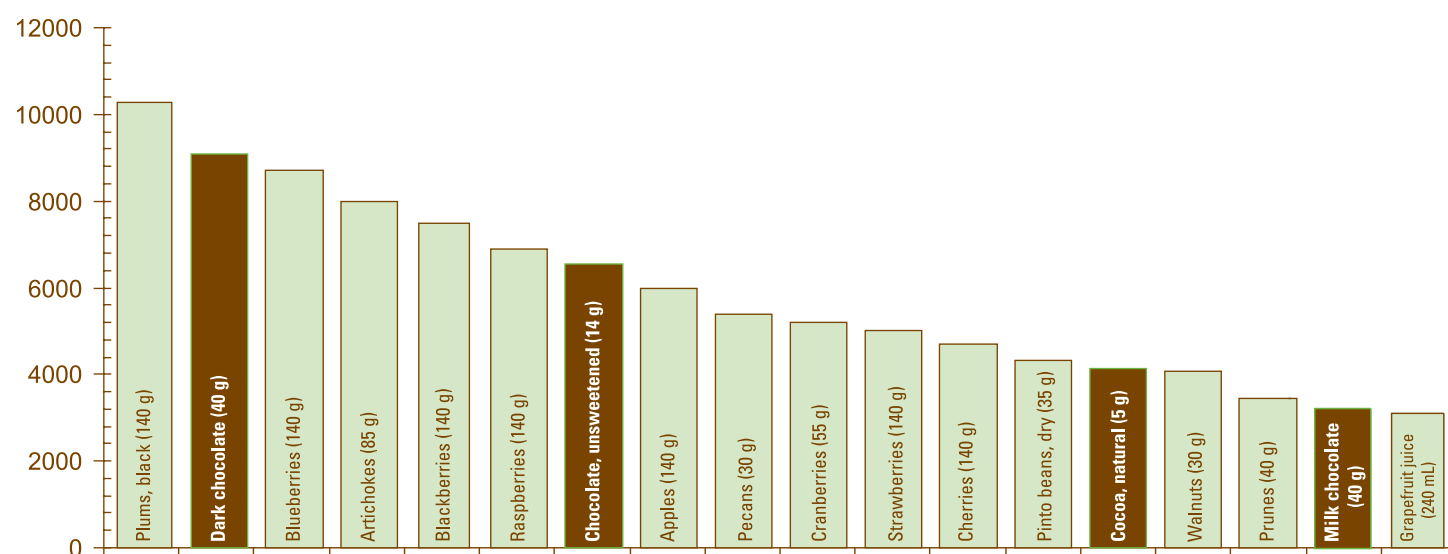
As mentioned previously, epidemiological evidence from the Kuna Indians of Panama suggest the potential anti-hypertensive benefits of cocoa flavanols. More recently, studies using flow-mediated dilation have shown improved endothelial function and increased blood flow in the brachial artery. Direct measures of blood pressure show that a flavanol-rich dark chocolate bar may reduce systolic and diastolic blood pressure in both healthy and hypertensive subjects.^{77,78,79,80} Two studies have tested cocoa or dark chocolate consumption in people with high blood pressure. One study resulted in both a systolic and diastolic blood pressure reduction following ingestion of flavanol-rich cocoa for 10 days.⁷⁷ The other found similar results after 15 days of feeding 100 g of dark chocolate.⁷⁸ Two additional studies have reported blood pressure values for individuals with normal blood pressure fed cocoa or chocolate. In one study, while both groups maintained blood pressure levels within the normal range, those that consumed dark chocolate, but not white chocolate, had reduced systolic blood pressure.⁷⁹ Another study on healthy, male soccer play-

ers found reduced diastolic and mean blood pressure in those that consumed 105 g of flavanol-containing milk chocolate for 14 days.⁸⁰ The reductions in blood pressure were attributed to the ability of flavanols to solicit a nitric oxide dependent vasorelaxation.

RENAL HEALTH

Blood pressure plays a crucial role in maintaining healthy renal function. In fact, high blood pressure can be a sign of impaired renal function. To work properly the kidney must be able to sufficiently regulate fluid volume, excrete waste and balance electrolytes. However, when the many tiny blood vessels of the kidney suffer endothelial damage, similar to the damage that can occur in the blood vessels surrounding the heart, the function of the kidney and electrolyte balance can be disrupted and exacerbate hypertension. Substances such as angiotensin converting enzyme (ACE) inhibitors are often prescribed to treat hypertension and will protect the kidneys by lowering blood pressure. In a similar way, eating a diet rich in foods that help promote healthy blood pressure can also serve to maintain renal function. As discussed in the previous section on blood pressure, cocoa flavanols have been shown to reduce blood pressure in both hypertensive and healthy subjects with nitric oxide dependent vasorelaxation as the mechanism of action.^{78,79} A recent in vitro study evaluated the activity of ACE in the presence of flavanol-rich food components, such as a chocolate extract.⁸¹ The researchers found that ACE activity was inhibited by foods rich in flavanols, including a chocolate extract rich in procyanidins. The occurrence of this inhibition needs further study; however, this supports the blood pressure lowering ability of flavanol-rich chocolate and thus its potential role in maintaining renal function.

Figure 4 • Comparison of antioxidant capacity of foods (Oxygen Radical Absorbance Capacity assay, micromoles TE per serving)¹



¹ Chocolate and cocoa products analyzed by Gu et al. (2005).⁷³ Other foods are from Wu et al. (2004).⁷⁴ Serving sizes based on the FDA's Reference Amounts Customarily Consumed.

COGNITION

Research is shedding light on the role of oxidative stress and inflammation as major contributors to the behavioral and cognitive decline seen with aging.⁸² The central nervous system is vulnerable to the effects of oxidative stress, especially as people age. In addition, inflammatory reactions involving cytokine factors appear to play a key role in the impairment of working memory. It is believed that flavanols, as antioxidants and with their effects on anti-inflammatory agents, may have a role to play in maintaining cognitive function. Although this area of study for flavonoids is in its infancy, there is some research to date in animal models that indicates this possibility. In proceedings from the First International Conference on Polyphenols and Health, researchers presented data suggesting the anti-inflammatory polyphenolic compounds found in fruits and vegetables were efficacious in reducing the deleterious effects of brain aging. When rats were given flavonoid extract supplements, researchers observed improved performance on water maze tests, indicating an improvement in memory. They also found significantly lower concentrations of reactive oxygen species in the brains of the treatment group than in the control group.⁸² A similar study in mice using a green tea catechin supplement showed improvement in working memory in treated mice with prior cerebral atrophy.⁸³ Although much still needs to be investigated, including the underlying issues that contribute to cognitive decline, there is certainly a potential role for cocoa flavanols in addressing reduced cognition-related conditions.

IMMUNE HEALTH

Recent research has focused on understanding the interaction between diet and immune function. Diseases, such as Celiac disease, have been discovered that are either caused or aggravated by certain foods eliciting an immune response. Additionally, conditions characterized by a hyperactive immune system such as autoimmune or chronic inflammatory diseases may benefit from dietary therapy.^{84,85,86} Also of interest is the impact of diet on healthy people to maintain health and avoid disease. Cocoa procyanidins may influence immune function indirectly through their ability to act as antioxidants, thereby inhibiting reactive oxygen species, and directly by affecting the secretion of immune reaction mediators, cytokines.^{86,87,88,89} Cytokines are central to the initiation of inflammation in humans.⁹⁰ In vitro studies have shown that cocoa extracts and the individual flavanol monomers and procyanidins modulate cellular cytokine profiles. Further research is needed to understand the ability of flavanols to moderate an immune response. However, this preliminary research indicates that flavanols could have a therapeutic benefit for some states of immune hyperactivity.

DIABETES

The effect of flavanols on endothelial function, circulation and atherosclerosis can potentially greatly benefit those with diabetes as vascular issues and impaired circulation are prominent health conditions for this population. An emerging area of study for cocoa flavanols is their role in insulin sensitivity and diabetes. Because insulin sensitivity is partly dependent on insulin-mediated nitric oxide release, flavanols may decrease insulin resistance by impacting nitric oxide availability. Pilot studies have measured insulin sensitivity via oral glucose tolerance tests (OGTT) after ingestion of a high-flavanol chocolate bar for 15 days.^{78,79} Compared to baseline, fasting glucose levels and insulin levels were significantly lower after the treatment period. With the growing population of individuals with diabetes, it is an interesting area of study; however, larger studies in diabetic populations are needed to confirm these results.

EXERCISE PERFORMANCE

In strenuous physical exercise the body experiences an increase in oxidative stress as evidenced by an increase in F(2)-isoprostanes. Research shows that when participants consume a high-flavanol cocoa drink after strenuous physical exercise, plasma F(2)-isoprostanes are significantly lower. These results suggest that flavanols may decrease exercise-induced oxidative stress.⁹¹ A subsequent study conducted on male soccer players showed that daily consumption of 3.7-ounces (105 g) of milk chocolate decreased blood pressure and markers associated with oxidant stress.⁸⁰



III. Weight Management

The rising incidence of overweight and obesity, with its associated complications, is a clear public health issue facing the nation and the world. With nearly two-thirds of U.S. adults at a Body Mass Index (BMI) that puts them at increased risk for health complications, achieving and maintaining a healthy weight is an important goal when making nutrition recommendations. Recent Dietary Guidelines emphasize the importance of balancing food intake with physical activity to maintain a healthy weight. The revised Food Guide Pyramid (**Figure 5**) indicates the importance of adequacy and variety among the basic food groups, while leaving room for discretionary calories to be used for foods that provide enjoyment.

Various surveys show that calories available for consumption and actual calorie intakes have increased over the past 20 to 25 years, but no one food group accounts for all of the increase. Although candy availability and intake also have increased over this same period, candy intake remains a small part of the total energy intake. Dietary survey data show that candy's contribution to total energy intake is less than 2% of total calories.⁹²

Chocolate and other highly palatable foods are often eliminated from weight control diets. However, when the pleasurable aspects of eating are eliminated, many people are unable to comply with a weight loss diet. A crucial element in adhering to a healthy diet is to include favorite foods and eliminate feelings of deprivation. It is important for those trying to manage their weight to exercise portion control and moderation with all foods. Chocolate can be very satisfying and easily fit into a nutritious diet that balances calories in and calories out.

A newer area of interest in nutrition is the glycemic response of foods and how they impact feelings of fullness and the role in weight management. Chocolate is a low to moderate glycemic index food, meaning that it elicits a relatively low affect on blood sugar levels.⁹³ It is theorized that a smaller rise in blood sugar, as with low to moderate glycemic index foods, can help with satiety.

Preliminary studies in animals point to the possibility that cocoa may directly impact fat mass. Early studies on rats have shown that adding cocoa to the diet may decrease visceral adipose tissue.^{94,95} In one study researchers fed rats a high-fat diet supplemented with either cocoa powder or a control powder.⁹⁵ They found that the ingestion of the cocoa powder suppressed the high-fat diet-induced weight gain observed with the control rats. After looking at DNA assays, they determined that cocoa decreased the expression of genes responsible for the production of compounds involved in fatty acid transport and fatty acid synthesis. More research is needed to determine if there is a potential antiobesity role for cocoa.

Figure 5 • The revised Food Guide Pyramid



www.mypyramid.gov

IV. Anti-Cancer Effects

Preliminary studies have investigated the potential for the larger molecular procyanidins to inhibit the proliferation of human breast cancer cells. In one study, synthesized procyanidins, identical to compounds isolated from cocoa, were found to arrest the cell cycle in human breast cancer cells through an unknown mechanism.⁹⁶ A follow-up study revealed that a cocoa-derived,

pentameric procyanidin selectively inhibited cellular proliferation in breast cancer cells via site-specific dephosphorylation or down-regulation of several cell cycle regulatory proteins.⁹⁷ Further studies, including in vivo data, are needed to draw conclusions on the potential anticancer effects of flavanols.

V. Chocolate Myths Revealed

ALLERGIES

A food allergy is a reaction by the body's immune system to a substance in food – usually a protein. To accurately diagnose a food allergy, a double-blind placebo-controlled food challenge test should be administered. Of the approximately 1-2% of American adults and 5-8% of children that have a true food allergy, 90% are allergic to either milk, eggs, peanuts, tree nuts, soy, wheat, fish or shellfish.^{98,99}

Chocolate is an uncommon food allergen. However, chocolate candies may contain other ingredients that can elicit allergic reactions including milk, soy, gluten, peanuts and tree nuts. Processed food products are now required to indicate if they contain one of the top eight allergens on the food label.

CRAVINGS AND MOOD

The word “craving” connotes a physiological need that is somehow manifested through specific appetites. Such connections between specific appetites for foods and true physiological need are rarely supported scientifically. Chocolate is among the most preferred flavors and people commonly report the desire to eat chocolate and chocolate-flavored foods. This preference for chocolate has contributed to numerous myths about a “craving for chocolate.” The theories include that there is some particular substance in chocolate that can elevate mood. It was once thought that foods high in carbohydrate could improve mood by releasing tryptophan, a precursor of the neurotransmitter serotonin, which is involved in mood; however, research in this area does not support this theory. In general, foods high in both carbohydrate and fat that are “craved” are not those that would best stimulate tryptophan availability.

It was also suggested that food cravings may be the result from the body's need for certain nutrients, such as magnesium in chocolate, or that there is some bioactive compound in chocolate that affects neurotransmitters in the brain. None of these hypotheses have held up to scientific scrutiny.¹⁰⁰ A more recent study looked at the role of the methylxanthines, caffeine and theobromine, on the enjoyment of chocolate.¹⁰¹ Participants consumed a capsule containing similar amounts of methylxanthines as in a 50 gram chocolate bar along with a drink. They then rated various sensory

descriptors of the drink. The researchers found that those consuming the drink with the methylxanthine capsules liked the drink more and more over time as compared to those that had a placebo capsule. They concluded that methylxanthines in chocolate may contribute to an enjoyment of chocolate, especially the more acquired taste for dark chocolate. However, earlier research, which included participants who claimed to “crave” chocolate at least once a week, does not support these results. By feeding cocoa powder (which contains all of cocoa's bioactive ingredients including methylxanthines), milk chocolate, cocoa butter, white chocolate (which does not contain any cocoa bioactives) or a placebo, researchers found that participants' reported cravings were only satisfied by the milk and white chocolate, not the cocoa powder.¹⁰² This study concluded and agrees with other researchers that the desire for chocolate is more likely a result of the enjoyable flavor and sensory experience and not due to a specific compound contained in chocolate.

MIGRAINES

Migraines and headaches have long been rumored to be triggered by chocolate consumption. Studies have not been able to demonstrate this relationship. In several studies, there was no difference in occurrence of subsequent headaches in headache or migraine sufferers given chocolate or a placebo.¹⁰³ Even in those migraine sufferers who believed themselves as sensitive to chocolate as a headache trigger a relationship was not found. The reason that chocolate is frequently cited as a trigger by migraine sufferers may lie in other



aspects of chocolate. Women are more likely than men to suffer from migraines and women more often report strong desires for chocolate, especially during menses. Fluctuating estrogen levels associated with the menstrual cycle are clearly associated with incidence of migraines.

ACNE

Chocolate's implication in the etiology of acne was widespread in the medical community until recently. However, studies going as far back as the 1960s have failed to show any relationship between chocolate consumption and the development of acne. An extensive research review in the *Journal of the American Medical Association* on chocolate and acne stated "diet plays no role in acne treatment in most patients...even large amounts of chocolate have not clinically exacerbated acne."¹⁰⁴

BEHAVIOR AND HYPERACTIVITY

The notion that candy and foods containing sugar, such as chocolate, are associated with hyperactivity has been explored in depth. An extensive review of research studies indicates that there is no relationship between consumption of sugar-containing foods and adverse effects on behavior. In addition, studies which specifically tested chocolate and candy failed to show any relationship between ingestion of these foods and hyperactive behavior in children with and without Attention-Deficit Hyperactivity Disorder.¹⁰⁵

DENTAL HEALTH

Dental health and the prevention of tooth decay involve a number of factors including oral hygiene, fluoride intake, genetics and diet. It has been well established that foods containing fermentable carbohydrate can promote dental caries formation. However, a factor in the cariogenicity of the food depends on the time spent in the mouth for oral bacteria to metabolize the acids and promote decay.¹⁰⁶ Although sweetened chocolate does contain fermentable carbohydrate, it clears the mouth relatively quickly and has not been found to contribute to the development of dental caries.¹⁰⁷ A recent study, which investigated the response of plaque to chocolates of varying cocoa contents, found that all chocolate types had a lower acidic affect on dental plaque than sucrose.¹⁰⁸ In another study, researchers looked at the intake of children and specifically found that regular brushing (twice a day) with a fluoride toothpaste has a greater impact on the development of dental caries in children than restricting sugary foods.¹⁰⁹ In fact, they found no association between consumption of chocolate confectionery and the development of caries. Interestingly, the flavanols in cocoa may contribute to dental health. Cocoa flavanols have been shown to decrease plaque deposition through anti-bacterial and anti-glucosyltransferase activity.^{110,111} Research also suggests that the flavanols can mediate immune factors (cytokines). This includes the stimulation of immunoglobulin A, which could result in reduction in risk of dental caries and periodontal disease in the oral cavity.¹¹²



Chocolate...
There's nothing quite like it!

VI. References



- Kris-Etherton PM, Derr JA, Mitchell DC. The role of fatty acid saturation on plasma lipids, lipoproteins and apolipoproteins. Effects of whole food diets high in cocoa butter, olive oil, soybean oil, dairy butter and milk chocolate on the plasma lipids of young men. *Metabolism* 1993;42:130-4.
- Kris-Etherton PM, Derr JA, Mustad VA, Seligson FH, Pearson TA. A milk chocolate bar/day substituted for a high carbohydrate snack increases high density lipoprotein cholesterol in young men on a NCEP/AHA Step One diet. *Am J Clin Nutr* 1994;60(6):1037S-42S.
- Judd JT, Baer DJ, Clevidence BA, Kris-Etherton P, Muesing RA, Iwane M. Dietary cis and trans monounsaturated and saturated FA and plasma lipids and lipoproteins in men. *Lipids* 2002 Feb;37(2):123-31.
- Kris-Etherton PM, Mustad VA. Chocolate feeding studies: a novel approach for evaluating the plasma lipid effects of stearic acid. *Am J Clin Nutr* 1994;60(suppl.):1029S-36S.
- Steinberg FM, Bearden MM, Keen CL. Cocoa and chocolate flavonoids: implications for cardiovascular health. *J Am Diet Assoc* 2003 Feb;103(2):215-23.
- Rivellese AA, Maffettone A, Vessby B, Uusituppe M, Hermansen K, Berglund L, Loueranta A, Meyer BJ, Riccardi G. Effects of dietary saturated, monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy subjects. *Atherosclerosis* 2003;167:149-58.
- Thijssen MA, Hornstra G, Mensink RP. Stearic, oleic and linoleic acids have comparable effects on markers of thrombotic tendency in healthy human subjects. *J Nutr* 2005;135:1805-11.
- Bonanome A, Grundy SM. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N Engl J Med* 1988;318(19):1244-8.
- Kelly FD, Sinclair AJ, Mann NJ, Turner AH, Abedin L, Li D. A stearic acid-rich diet improves thrombogenic and atherogenic risk factor profiles in healthy males. *Eur J Clin Nutr* 2001;55:88-96.
- Thijssen MA, Mensink RP. Small differences in the effects of stearic acid, oleic acid, and linoleic acid on the serum lipoprotein profile of humans. *Am J Clin Nutr* 2005 Sep;82(3):510-6.
- Tholstrup T, Marckmann P, Jespersen J, Sandstrom B. Fat high in stearic acid favourably affects blood lipids and factor VII coagulant activity in comparison with fats high in palmitic acid or high in myristic and lauric acids. *Am J Clin Nutr*. 1994;59:371-7.
- Harland BF, Oberleas D. Phytate and zinc contents of coffees, cocoas, and teas. *J Food Sci* 1985;50:832-3, 42.
- Aremu CY, Abara AE. Hydrocyanate, oxalate, phytate, calcium and zinc in selected brands of Nigerian cocoa beverage. *Plant Foods Human Nutr* 1992;42:231-7.
- Joo S, Kies C, Schnepf M. Chocolate and chocolate-like products: impact on copper status of humans. *J Appl Nutr* 1995;47:67-77.
- Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. Washington, DC: *National Academy Press* 1999.
- Klevay LM, Milne DB. Low dietary magnesium increases supraventricular ectopy. *Am J Clin Nutr* 2002;75:550-4.
- Geleijnse JM, Grobbee DE, Kok FJ. Impact of dietary and lifestyle factors on the prevalence of hypertension in Western populations. *J Hum Hypertens* 2005 Dec;19 Suppl 3:S1-4.
- Song Y, Ridker PM, Manson JE, Cook NR, Buring JE, Liu S. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005 Jun;28(6):1438-44.
- Khaw K-T, Barrett-Connor E. Dietary potassium and stroke-associated mortality. *N Engl J Med* 1987;316:235-40.
- Ascherio A, Hennekens C, Willett WC, Sacks M, Rosner B, Manson J, Witteman J, Stampfer MJ. Prospective study of nutritional factors, blood pressure and hypertension among US women. *Hypertension* 1996;27:1065-72.
- Whelton PK, He J, Cutler JA, Bancati FL, Appel LJ, Follman D, Klag MJ. Effects of oral potassium on blood pressure: meta-analysis of randomized controlled clinical trials. *JAMA* 1997;277:1624-32.
- McCarron DA, Reusser ME. Are low intakes of calcium and potassium important causes of cardiovascular disease? *Am J Hypertens* 2001;14:206S-12S.
- Birkett NJ. Comments on a meta-analysis of the relation between dietary calcium intake and blood pressure. *Am J Epi* 1998;148:223-8.
- Griffith L, Guyatt GH, Cook RJ, Bucher HC, Cook DJ. The influence of dietary and nondietary calcium supplementation on blood pressure: an updated metaanalysis of randomized clinical trials. *Am J Hypertens* 1999;12:84-92.
- McCarron DA, Morris CD, Henry HJ, Stanton J. Blood pressure and nutrient intake in the United States. *Science* 1984;224:1392-98.
- Appel L, Moore F, Obarzanek E, Vollmer WN, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH, Karanja N. A clinical trial of the effects of dietary patterns on blood pressure. *N Engl J Med* 1997;1117-24.
- USDA Database for the Flavonoid Content of Selected Foods. Beltsville: U.S. Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Nutrient Data Laboratory, 2003:1-79.



28. Hammerstone JF, Lazarus S, Mitchell A, Rucker R, Schmitz HH. Identification of procyanidins in cocoa (Theobroma cacao) and chocolate using high-performance liquid chromatography/mass spectrometry. *J Agric Food Chem* 1999;47:490-6.
29. Prior R, Liwei G, Johnson C, Kelm M, Hammerstone J, Schmitz H, Bhagwat, Holden J. Flavanol and procyanidin composition of cocoa, chocolate and other plant foods. Presented at XXII International Conference on Polyphenols, 25-28 Aug 2004; Helsinki, Finland.
30. Mao TK, Powell JJ, Van de Water J, Keen CL, Schmitz HH, Gershwin ME. The influence of cocoa procyanidins on the transcription of interleukin-2 in peripheral blood mononuclear cells. *Int J Immunol* 1999;15(1):23-9.
31. Schewe T, Sadik c, Klotz L, Yoshimoto t, Kuhn H, Sies H. Polyphenols of cocoa: Inhibition of mammalian 15-lipoxygenase. *Biol Chem* 2001 Dec;382:1687-96.
32. Weisburger JH. Chemopreventive effects of cocoa polyphenols on chronic diseases. *Exp Biol Med* (Maywood) 2001 Nov;226(10):891-7.
33. Haslam E. Quinone tanning and oxidative polymerization. In: Haslam E. *Practical Polyphenolics: From structure to molecular recognition and physiological action*. Cambridge, MA: Cambridge University Press, 1998:335-73.
34. Richell M, Tavazzi I, Enslin M, Offord EA. Plasma kinetics in man of epicatechin from black chocolate. *Eur J Clin Nutr* 1999;53(1):22-6.
35. Wang JF, Schramm DD, Holt RR, Ensunsa JL, Fraga CG, Schmitz HH, Keen CL. A dose response effect from chocolate consumption on plasma epicatechin and oxidative damage. *J Nutr* 2000;130:2115S-9S.
36. Holt RR, Lazarus SA, Sullards MC, Zhu QY, Schramm DD, Hammerstone JF, Fraga CG, Schmitz HH, Keen CL. Procyanidin dimmer B2 (epicatechin-[4beta-8]epicatechin) in human plasma after the consumption of a flavanol-rich cocoa. *Am J Clin Nutr* 2002;76:798-804.
37. Schroeder P, Klotz LO, Sies H. Amphiphilic properties of (-)-epicatechin and their significance for protection of cells against peroxynitrite. *Biochem Biophys Res Commun* 2003;307:69-73.
38. Schramm DD, Karim M, Schrader HR, Holt RR, Kirkpatrick NJ, Polagruto JA, Ensunsa JL, Schmitz HH, Keen CL. Food effects on the absorption and pharmacokinetics of cocoa flavanols. *Life Sciences* 2003;73:857-69.
39. Serafini M, Bugianesi R, Maiani G, Valtuena S, De Santis S, Crozier A. Plasma antioxidants from chocolate. *Nature* 2003 Aug 28;424(6952):1013.
40. Schroeter H, Holt RR, Orozco TJ, Schmitz HH, Keen CL. Nutrition: milk and absorption of dietary flavanols. *Nature* 2003;426:787-8.
41. Barone JJ, Roberts HR. Caffeine consumption. *Food Chem Toxicol* 1996 Jan;34(1):119-29.
42. A Scientific Status Summary by the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition. Chicago, IL: *Institute of Food Technologists*; 1987.
43. U.S. Department of Agriculture, Agricultural Research Service, USDA Nutrient Data Laboratory. 2004. USDA National Nutrient Database for Standard Reference, Release 18.
44. Weisburger JH, Chung FL. Mechanisms of chronic disease causation by nutritional factors and tobacco products and their prevention by tea polyphenols. *Food Chem Toxicol* 2002 Aug;40(8):1145-54.
45. Usmani OS, Belvisi MG, Patel HJ, Crispino N, Birrell MA, Korbonits M, Korbonits D, Barnes PJ. Theobromine inhibits sensory nerve activation and cough. *FASEB J* 2005 Feb;19(2):231-3.
46. Smit HJ, Faffan EA, Rogers PJ. Methylxanthines are the psychopharmacologically active constituents of chocolate. *Psychopharmacology (Berl)* 2004;176:412-9.
47. Kelly CJ. Effects of theobromine should be considered in future studies. *Am J Clin Nutr* 2005 Aug;82(2):486-7; author reply 487-8.
48. Pearson DA, Holt RR, Rein D, Paglieroni T, Schmitz HH, Keen CL. Flavanols and platelet reactivity. *Clin Dev Immunol* 2005 Mar;12(1):1-9.
49. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 1993;342:1007-11.
50. Arts I, Hollman P, Feskens E, Bueno de Mesquita H, Kromhout D. Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study. *Am J Clin Nutr* 2001;74:227-32.
51. Hollenberg N, Martinez G, McCullough M, Meinking T, Passan D, Preston M, Rivera A, Taplin D, Vicaria-Clement M. Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. *Hypertension* 1997;29:171-6.
52. Buijsse B, Feskens EJM, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality. *Arch Intern Med* 2006;166:411-7.
53. Rein D, Paglieroni TG, Wun T, Pearson DA, Schmitz HH, Gosselin R, Keen CL. Cocoa inhibits platelet activation and function. *Am J Clin Nutr* 2000;72:30-5.
54. Murphy KJ, Chronopoulos AK, Singh I, Francis MA, Moriarty H, Pike MJ, Turner AH, Mann NJ, Sinclair AJ. Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. *Am J Clin Nutr* 2003;77:1466-73.
55. Holt RR, Schramm DD, Keen CL, Lazarus SA, Schmitz H. Flavonoid-rich chocolate and platelet function. *JAMA* 2002;287:2212-3.
56. Pearson D, Paglieroni T, Rein D, Wun T, Schramm DD, Wang JF, Holt RR, Gosselin R, Schmitz HH, Keen CL. The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. *Thromb Res* 2002;106:191-7.
57. Keevil JG, Osman HE, Reed JD, Folts JD. Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. *J Nutr* 2000;130:53-6.
58. Rein R, Lotito S, Holt RR, Keen CL, Schmitz HH, Fraga CG. Epicatechin in human plasma: In vivo determination and effect of chocolate consumption on plasma antioxidant capacity. *J Nutr* 2000;130(8S):2109S-14S.
59. Freedman JE, Parker C 3rd, Li L, Perlman JA, Frei B, Ivanov V, Deak LR, lafrati MD, Folts JD. Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. *Circulation* 2001 Jun 12;103(23):2792-8.
60. Schramm DD, Wang JF, Holt RR, Ensunsa JL, Gonsalves JL, Lazarus SA, Schmitz HH, German JB, Keen CL. Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. *Am J Clin Nutr* 2001;73:36-40.
61. Sies H, Schewe T, Heiss C, Kelm M. Cocoa polyphenols and inflammatory mediators. *Am J Clin Nutr* 2005 Jan;81(1 Suppl):304S-12S.
62. Karim M, McCormick K, Kappagoda TC. Effects of cocoa extracts on endothelium dependent relaxation. *Am J Clin Nutr*. 2000;130(8S):2105S-8S.
63. Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, Kwak HK, Milbury P, Paul SM, Blumber J, Mietus-Snyder ML. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J Am Coll Cardio* 2004;23:197-204.
64. Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M. Vascular effects of cocoa rich in flavan-3-ols. *JAMA* 2003;290:1030-1.
65. Fisher NDL, Hughes M, Gerhard-Herman M, Hollenberg NK. Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens* 2003;21:2281-6.
66. Vlachopoulos C, Aznaouridis K, Alexopoulos N, Economou E, Andreadou I, Stefanadis C. Effect of dark chocolate on arterial function in healthy individuals. *Am J Hypertens* 2005 Jun;18(6):785-91.
67. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989;320:915-24.
68. Wan Y, Vinson JA, Etherton TD, Proch J, Lazarus S, Kris-Etherton P. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *Am J Clin Nutr* 2001;74:596-602.
69. Osakabe N, Baba S, Yasuda A, Iwamoto T, Kamiyama M, Takizawa T, Itakura H, Kondo K. Daily cocoa intake reduces the susceptibility of low-density lipoprotein to oxidation as demonstrated in healthy human volunteers. *Free Radic Res* 2001;34:93-99.
70. Mathur S, Devaraj S, Grundy SM, Jialal I. Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. *J Nutr* 2002 Dec;132(12):3663-7.
71. Wang JF, Schramm DD, Holt RR, Ensunsa JL, Fraga CG, Schmitz HH, Keen CL. A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. *J Nutr* 2000;130(8S):2115S-119S.
72. Kurosawa T, Itoh F, Nozaki A, Nakano Y, Katsuda S, Osakabe N, Tsubone H, Kondo K, Itakura H. Suppressive effect of cocoa powder on atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic rabbits. *J Atheroscler Thromb* 2005;12(1):20-8.
73. Gu L, Wu X, Ou B, Harnly J, Prior RL. Procyanidin content and total antioxidant capacity of chocolate and cocoa products. 2005 Experimental Biology and XXXV International Congress of Physiological Sciences meeting abstracts [on CD-ROM]. The *FASEB Journal* 2005; 19:Abstract #598.20.
74. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agric Food Chem* 2004 Jun 16; 52(12):4026-37.
75. Zhu QY, Schramm DD, Gross HB, Holt RR, Kim SH, Yamaguchi T, Kwik-Urbe CL, Keen CL. Influence of cocoa flavanols and procyanidins on free radical-induced human erythrocyte hemolysis. *Clin Dev Immunol* 2005 Mar;12(1):27-34.
76. Verstraeten SV, Hammerstone JF, Keen CL, Fraga CG, Oteiza PI. Antioxidant and membrane effects of procyanidin dimers and trimers isolated from peanut and cocoa. *J Agric Food Chem* 2005 Jun 15;53(12):5041-8.
77. Taubert D, Berkels R, Roesen R, Klaus W. Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. *JAMA* 2003;290:1029-30.
78. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, Desideri G, Blumberg JB, Ferri C. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension* 2005 Aug;46(2):398-405.
79. Grassi D, Lippi C, Necozione S, Desideri G, Ferri C. 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* 2004;81:611-4.
80. Fraga CG, Actis-Goretta L, Ottaviani JI, Carrasquedo F, Lotito SB, Lazarus S, Schmitz HH, Keen CL. Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. *Clin Dev Immunol* 2005 Mar;12(1):11-7.
81. Actis-Goretta L, Ottaviani JI, Fraga CG. Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. *J Agric Food Chem* 2006 Jan 11;54(1):229-34.
82. Joseph JA, Shukitt-Hale B, Casadesus G. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* 2005 Jan;81(1 Suppl):313S-6S.
83. Unno K, Takabayashi F, Kishido T, Oku N. Suppressive effect of green tea catechins on morphologic and functional regression of the brain in aged mice with accelerated senescence (SAMP10). *Exp Gerontol* 2004 Jul;39(7):1027-34.



84. Kubena KS and McMurray DN. Nutrition and the immune system: A review of nutrient-nutrient interactions. *J Am Diet Assoc* 1996;96:1156-64.
85. Field CJ. Use of T cell function to determine the effect of physiologically active food components. *Am J Clin Nutr* 2000;71:170S-5S.
86. Ramiro E, Franch A, Catellote C, Andres-Lacueva C, Izquierdo-Pulido M, Castell M. Effect of Theobroma cacao flavonoids on immune activation of a lymphoid cell line. *Br J Nutr* 2005;93:859-866.
87. Sabongi C, Suzuki N, Sakane T. Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans in vitro. *Cell Immunol* 1997;177:129-36.
88. Mao TK, Powell JJ, Van De Water J, Keen CL, Schmitz HH, Gershwin ME. The influence of cocoa procyanidins on the transcription of interleukin-2 in peripheral blood mononuclear cells. *Int J Immunother* 1999;XV:23-29.
89. Mao T, Van de Water J, Keen CL, Schmitz HH, Gershwin ME. Cocoa procyanidins and human cytokine transcription and secretion. *J Nutr* 2000;130:2093S-99S.
90. Dinarello CA. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Intern Rev Immunol* 1998;16:57-499.
91. Wiswedel I, Hirsch D, Kropf S, Gruening M, Pfister E, Schewe T, Sies H. Flavanol-rich cocoa drink lowers plasma F(2)-isoprostane concentrations in humans. *Free Radic Biol Med* 2004 Aug 1;37(3):411-21.
92. Seligson FH. U.S. candy consumption and contribution to calorie intake. *Manufacturing Confectioner* 2005;85 (S, Supplement):1-11.
93. Foster-Powell K, Holt SHA, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. *Am J Clin Nutr* 2002;76(1):5-56.
94. Matsui N, Ito R, Nishimura E, Yoshikawa M, Kato M, Kamei M, Shibata H, Matsumoto I, Abe K, Hashizume S. Ingested cocoa can prevent high-fat diet-induced obesity by regulating the expression of genes for fatty acid metabolism. *Nutrition* 2005 May;21(5):594-601.
95. Kamei M, Yoshikawa M, Hashizime S. New application of cocoa to mitigation of peripheral intolerance to cold. *Food Ind Food Sci J* 2003;300:4-13.
96. Kozikowski AP, Tuckmantel W, Bottcher G, Romanczyk LJ Jr. Studies in polyphenol chemistry and bioactivity. 4.(1) Synthesis of trimeric, tetrameric, pentameric, and higher oligomeric epicatechin-derived procyanidins having all-4beta,8-interflavan connectivity and their inhibition of cancer cell growth through cell cycle arrest. *J Org Chem* 2003 Mar 7;68(5):1641-58.
97. Ramljak D, Romanczyk LJ, Metheny-Barlow LJ, Thompson N, Knezevic V, Galperin M, Ramesh A, Dickson RB. Pentameric procyanidin from Theobroma cacao selectively inhibits growth of human breast cancer cells. *Mol Cancer Ther* 2005 Apr;4(4):537-46.
98. Sampson HA. Peanut allergy. *New Eng J Med* 2002;346:1294-9.
99. Sicherer SH, Munoz-Furlong A, and Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: A 5-year follow-up study." *J Allergy Clin Immunol* 2003;112(6):1203-7.
100. Yanovski S. Sugar and fat: cravings and aversions. *J Nutr* 2003 Mar;133(3):835S-7S.
101. Smit HJ, Blackburn RJ. Reinforcing effects of caffeine and theobromine as found in chocolate. *Psychopharmacology* 2005 Aug;181(1):101-6.
102. Michner W, Rozin P. Pharmacological versus sensory factors in the satiation of chocolate craving. *PhysiolBehav* 1994;56(3):419-22.
103. Marcus DA, Scharff L, Turk D, Gourley L. A double-blind provocative study of chocolate as a trigger of headache. *Cephalalgia* 1997;17:855-8.
104. Minkin W, Cohen HJ. Effect of chocolate on acne vulgaris. *JAMA* 1970 Mar 16;211(11):1856.
105. Krummel DA, Seligson FH, Guthrie HA. Hyperactivity: is candy causal? *Crit Rev Food Sci Nutr* 1996 Jan;36(1-2):31-47.
106. Kashket S, Van Houte J, Lopez LR, Stocks S. Lack of correlation between food retention on the human dentition and consumer perception of food stickiness. *J Dent Res* 1991;70(10):1314-9.
107. Kashket S, Zhang J, Van Houte J. Accumulation of Fermentable Sugars and Metabolic Acids in Food Particles that Become Entrapped on the Dentition. *J Dent Res* 1996;75(11):1885-91.
108. Verakaki E, Duggal MS. A comparison of different kinds of European chocolates on human plaque pH. *Eur J Paediatr Dent* 2003 Dec;4(4):203-10.
109. Gibson S, Williams S. Dental caries in pre-school children: associations with social class, toothbrushing habit and consumption of sugars and sugar-containing foods. Further analysis of data from the National Diet and Nutrition Survey of children aged 1.5-4.5 years. *Caries Res* 1999;33(2):101-13.
110. Matsumoto M, Tsuji M, Okuda J, Sasaki H, Nakano K, Osawa K, Shimura S, Ooshima T. Inhibitory effects of cacao bean husk extract on plaque formation in vitro and in vivo. *Eur J Oral Sci* 2004 Jun;112(3):249-52.
111. Kashket S, Paolino VJ, Lewis DA, van Houte J. In-vitro inhibition of glucosyltransferase from the dental plaque bacterium *Streptococcus mutans* by common beverages and food extracts. *Arch Oral Biol* 1985;30(11-12):821-6.
112. Mao TK, Van de Water J, Keen CL, Schmitz HH, Gershwin ME. Effect of cocoa flavanols and their related oligomers on the secretion of interleukin-5 in peripheral blood mononuclear cells. *J Med Food* 2002;5(1):17-22.