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REVIEW ARTICLE

Cacao as a Globalised Functional Food: Review on Cardiovascular Effects of Chocolate Consumption

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Abstract: Polyphenols have increasingly been studied for their influence on cardiometabolic health. Since cacao and chocolate can be a rich source of polyphenols, they too have been investigated for their supposed health benefits. In the first part of this paper the history of the food and medicinal uses of the cacao plant was briefly examined. A particular emphasis has been placed on the analysis of the process of transformation of the cacao seed into chocolate, since many of the processing steps involved have important consequences on the final product's content in polyphenols and flavanols, and hence on the purported beneficial activity of chocolate. This evaluation is also of great importance in interpreting the results of the epidemiological and clinical studies. The relevant literature was surveyed in the second part of the paper, and the results for the consumption of polyphenol-rich chocolate are that it is well correlated to a reduction of the overall cardiovascular risk, and of arterial blood pressure in hypertensive subjects. Less corroborated are the positive effects on vascular endothelial health, on blood lipids and on lipid peroxidation. There are also preliminary but promising results for a positive action on insulin sensitivity, platelet function and inflammation. It remains to be seen whether and how these results can be translated into pragmatic guidelines on the health benefits of the consumption of commercial chocolate bars or products.

Keywords: Antioxidants, Cardiovascular, Chocolate, Flavanols, Polyphenols, *Theobroma*.

INTRODUCTION

Cocoa and chocolate are commercial products of enormous economic importance. Between 2006 and 2007 the world production of cocoa was of 3.4 millions of tons per year, with Ivory Coast (38%), Ghana (18%), Indonesia (15%), Nigeria (6%), Cameroon (5%) as primary general producers; the primary consumers are the Netherlands (12,8%), the US (11,5%), Germany (10%), Ivory Coast (10%), and Malaysia (7,5%) [1].

Because of this economic importance, and of the widespread use of cocoa and chocolate, it is important to evaluate the health claims for cocoa, and how well they translate into health claims for commercial chocolate products.

Cocoa is obtained from the seeds of the cacao plant (*Theobroma cacao* L.) a small diploid perennial tree in the family Malvaceae (previously Sterculiaceae), native to the deep tropical region of the Americas, and the most economically important species of the *Theobroma* genus [2]. Thus, we use the term Cacao to refer to the fruit tree and its natural seeds, and the term "Cocoa" to indicate the same substance after the processes of roasting and grinding. Chocolate is the food prepared from roasted seeds of cacao.

Traditional Uses

The cacao plant was probably first domesticated in north-eastern Ecuador around 4,000 BCE, but the natives only consumed the sweet fruit pulp, fresh or as an ingredient of fermented beverages, and not the intensely bitter seeds [3].

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Chocolate first appeared in southern Mexico or in northern Guatemala, likely first as a bitter medicine and only secondarily as a food, between 1,900 and 1,500 Before Current *Era* (BCE) [3] or, according to more conservative estimates, around 1,000 BCE [4]. What is undoubtable is that cacao was a central player in Mayan and Aztecan mythology [5]. It has been suggested that during the Classical Mayan period (250-900 Current Era), cocoa was drunk hot and frothing, with added vanilla (*Vanilla planifolia*) and chili (*Capsicum annum*) [6]. Cocoa was also used for stomach and intestinal complaints, for infections (mixed with *Castilla elastica*), for childhood diarrhoea (5 beans), for fever and faintness (8-10 beans ground together with maize kernels and *Calliandra anomala*). When someone suffered from productive cough cacao seeds were used together with *Piper sanctum* Miq., *Chiranthodendron pentadactylon* and *Vanilla planifolia*. In terms of therapeutic uses, over the centuries four claims have been consistently attached to "health chocolate" [5]: *i)* weight and physical strength gain in feeble and emaciated patients, *ii*) Central Nervous System (CNS) stimulant for apathetic, delicate or exhausted individuals (including children and pregnant and nursing women), *iii)* Calming for overstimulated people and *iv)* improves all manner of digestive and eliminative complaints, including weak stomach, intestine and kidneys. It was also consistently used as a pharmacological binder and masking agent.

Chemical Composition

Cocoa seeds contain lipids, carbohydrates and a complex mixture of active chemical compounds, most notably the methylxantines that give it its stimulant effects: theobromine, caffeine, trigonelline, theophylline, *etc*. [7]. However, the compounds that show a significant correlation with the cardiometabolic health effects belong to the polyphenol class. Chemical data for dry seeds in the literature give a range of 6-18% of polyphenols, mainly composed of flavanols. Around 10% of total polyphenols is made of monomers like the flavanol-3-ols (ca. 3,5%) (−)- epicatechin and (+)-catechin, and the remaining 90% is made of oligomers and polymers. Oligomeric procyanidins, which together with catechins seem to be the most active molecules, include procyanidins B1, B2, B5, and C1; other relevant flavanols are anthocyanins and flavonol glycosides (including quercetin glycosides, *etc*.) [7 - 10]. Given the health relevance of this class of chemicals, it is of primary importance to examine the effects of the manufacturing steps on quantity and diversity of these molecules in the final products.

Chocolate Manufacturing and Polyphenols

The process used on the seed of *Theobroma cacao* to produce various end products has an important impact on the organoleptic, nutritional and health-modifying characteristics of the material. Since many data on chemical composition and biological activities refer to the raw seeds or to intermediate materials, while the commercially available products are usually end-stage products like cocoa powder and chocolate bars, it is of great importance to study these processes to estimate the quantitative and qualitative changes implicated, lest attribute to the final product activities which belong to the raw or intermediate materials. Fresh or *Lavado* cocoa beans (unfermented washed and dried beans) contain the highest amount of polyphenols, about 1.3% of their weight calculated as (-)-epicatechin (the dominant monomer), plus some small quantities of (+)-catechin [11]. After drying there is a health-related loss of both (-)-epicatechin and (+)-catechin and the appearance of measurable amounts of (-)-catechin. The spontaneous three- to six-day fermentation of the cocoa mass (necessary in order to develop the precursors of chocolate flavour) [12] cause an overall loss of the 80% of all polyphenols [7], in particular a further reduction of the level of (-)-epicatechin and (+)-catechin [11], converted to a largely insoluble red-brown material [13] and the formation of more (-)-catechin. Roasting (again necessary in order to develop the precursors of chocolate flavour) causes a small loss in (-)-epicatechin but a large increase in (-)-catechin, presumably due to epimerization due to the temperature of roasting [11]. Dutching (a series of alkalinizing steps) causes loss of around 60% of (-)-epicatechin and the (relative) increase in (-)-catechin, which becomes the dominant flavan-3-ol monomer [14]. Of all the processing stages certainly the two most important in terms of chemical content modification are the fermentation and the Dutching steps. They have been reported to result in the loss of as much as 90% of the cocoa flavanols, and much of the increase in the level of (-)-catechin observed may be the result of heat-related epimerization from (-)-epicatechin [11]. One interesting consequence of these polyphenols-degrading steps is that the content in (-)-epicatechin tends to be normalised, no matter what the initial content was, to a very similar, and low, content [15]. Hence, it doesn't seem particularly useful to choose a starting material with elevated content in epicatechin (for instance by selecting specific *Theobroma cacao* varieties), because this content would still be normalised by the processing steps. A better way to control, and enhance, the content in polyphenols, would instead involve working on the fermentation process, by selecting specific microbial populations with lower impact on polyphenols, to limit heat production during fermentation and drying to reduce the epimerization, and perhaps to avoid Dutching (which is an avoidable step in chocolate production). In a Spanish paper, the authors have explored a different

strategy: they have chosen to block the degradation of polyphenols by treating the seeds with hot water, inactivating the enzymes that degrade (by means of oxidation) the polyphenols in the seeds, and then avoiding the fermentation and roasting steps altogether, thereby maintaining the original polyphenol concentration. They however have obtained a product which is completely different from either cocoa powder or chocolate, and probably too bitter to be used as a food [16].

Bioavailability

Since cocoa polyphenols, and in particular the flavan-3-ols, have been linked to the antioxidant and health effects of cocoa, it is clearly necessary to review the data concerning their bioavailability in humans after oral consumption, their metabolism at gastrointestinal level and their possible distribution after absorption. The bioavailability of cocoa polyphenols (monomeric flavonoids like (-)-epicatechin, dimeric and trimeric procyanidins) as measured in human studies has been reviewed by Rimbach [10], according to which plasma concentrations after ingestion were often very low, in the nanomolar or low micromolar range, manifold lower than those concentrations used in cultured cells *in vitro*, and transient in nature, given that the mean apparent elimination half-life for total flavanols was around 3.6 hours, and that plasma concentration peaked at 2–4 hours [8, 17]. Given this relatively short half-life, it is not surprising that twice daily dosing with the cocoa drink for 2 weeks did not result in any significant increase in steady state plasma flavanol concentrations [17]. In a randomised, double-blind, placebo-controlled trial an increase in plasma epicatechin concentrations was observed, from around 25 nmol/L baseline to around 200 nmol/L after 2 weeks of daily ingestion of polyphenol-rich cocoa (PRC) (46 grams chocolate; 46 mg epicatechin). In a separate experiment, a single dose of 120 grams of PRC (around 120 mg catechins) the concentrations peaked at 200 and 227 nmol/L two hours after consumption [18]. The bioavailability of the monomers differed amongst different monomers: (-)-epicatechin plasma concentration was higher than that of (+)-catechin, which was higher than that of (-)-catechin, and in general the plasma concentration of catechins was less than 10% of that of epicatechins [8], while the dimeric oligomers seem to generally have a very limited availability (and limited to the epicatechin-derived B2- and B5-dimers) [8, 11] and it is unclear whether or not higher oligomeric procyanidins are absorbed, or to what extent intestinal breakdown products are [10, 11]. While untreated cocoa powder flavanol composition is dominated by (-)-epicatechin (the most bioavailable of all the flavan-3-ols), even slightly processed (Dutched) cocoa powder shows dominance by (-)-catechin, which is the least bioavailable of all the catechins. This change will therefore have an effect on the cocoa powder flavanol-derived health effects [11].

Milk is a common ingredient used to dissolve cacao powder in chocolate drinks, or combined to cocoa in commercial chocolate bars, hence it is of great clinical interest to determine whether it and its components have an effect on the bioavailability of polyphenols and catchins. Although milk as a base for dissolving cacao powder has caused a decrease in urinary excretion of flavan-3-ol metabolites, and in some studies seems to have reduced cocoa plasma antioxidant activity [19] the evidence about this effect is still contradictory. In 24 subjects, milk proteins, mixed with chocolate polyphenols (2.45 g and 2 g, respectively), sugar and cocoa butter (as a drink in 200 mL of water), caused no increase or decrese in average blood catechin and epicatechins levels, compared to the the control (a drink without milk proteins) when measured for 8 hours. While they seemed to accelerate absorption, this acceleration was so slight to be clinically non significant [20].

A randomised crossover trial on 21 healthy subjects compared the absorption of (-)-epicatechin from cocoa powder from two active drinks, one combining 40 g of cocoa powder and 250 ml of whole milk and the other 40 g of cocoa powder and 250 ml of water, plus one control (250 ml of whole milk). The plasma levels of the metabolite (-)-epicatechin-glucuronide were lower for the milk drink (273.7 nmol/l) than for the water drink (330.44 nmol/l), although the difference did not reach statistical significance ($p = 0.076$) [21]. The same authors measured the levels of (2)-epicatechin metabolites in urines after consumption of the same drinks, and found very similar concentrations of one (2)-epicatechin glucuronide and three (2)-epicatechin sulfates, but noted a difference in excretion profiles, thereby suggesting that milk can affect catechins metabolism and excretion [22].

A study on nine healthy subjects consuming 250 mL of a milk- or water-based cocoa drink containing 45 micromol (-)-epicatechin and (-)-catechin, showed only minor differences in plasma concentration of catechins metabolites, but showed a reduction in concentration of urinary metabolites (from 18.3% to 10.5% of the ingested dose) in the milk-based drink group [21].

The preliminary consensus seems to be that milk does not significantly affect human catechins bioavailability and pharmacokinetics [23, 24] (although in a study on animal midels milk seemed to reduce slightly the bioavailability [25])

and the same seems true for lipid and protein rich diets, while concurrent consumption of carbohydrates and sucrose could increase flavonols bioavailability [10, 25, 26].

After ingestion, cocoa flavanols and procyanidins seem to pass unchanged the gastric acidic conditions, and they tend to exist in the mesenteric circulation in their conjugated form. In fact ingested (−)-epicatechin is metabolised, into a wide range of structurally related (−)-epicatechin metabolites (SREM). The majority of procyanidins pass unabsorbed to the large intestine where they may influence and be influenced by the active microbiota, which in fact can disrupt the flavonoid structure leading to the formation of breakdown products like simple phenolics and ring-fission metabolites, which might be more easily absorbed and contribute to the health effects [8, 27]. It has been shown that rat intestinal microbiota can increase the hydrolysis and hence the absorption of the polyphenols, while at the same time the products of hydrolysis can affect the growth of intestinal bacterial species and increase the expression of the intestinal Toll-like receptors (TLRs) thereby modifying the intestinal immune response [28]. A recent paper by Ottaviani has shown that the predominant SREM found postprandially in the systemic circulation of humans are (−)-epicatechin-3′-β-D-glucuronide, (−)-epicatechin-3′-sulfate, and 3′-O-methyl-(−)-epicatechin-5/7-sulfate, and hence that any attempt to link flavanol ingestion and health effects has to take on board the notion that these are the molecules likely to be active [29]. It is noteworthy that since conjugation with glucoronic acid and sulphate blocks radical scavenging hydroxyl groups, it is unlikely that conjugated catechins can exhibit radical scavenging activities [10]. This bioavailability issue needs concern only effects supposedly mediated by systemic activities of cocoa polyphenols, while it is not relevant for effects mediated by action at intestinal sites [30, 31].

Cocoa and Cardiometabolic Health: Summary of Research

A substantial body of epidemiological literature suggests that the regular consumption of foods rich in flavonoids is associated with a reduced risk of chronic diseases, coronary heart disease, stroke, myocardial infarction and certain cancers, in the general population, in men and women, and in postmenopausal women [32 - 46]. A number of recent randomised, controlled clinical trials have suggested that cocoa has various cardioprotective activities, although the biochemical mechanisms behind this effect have not been conclusively identified [14]. Specifically, mechanisms that explain polyphenol-rich cocoa (PRC) effects may be:

− Antioxidation, inhibiting LDL-cholesterol (LDL-C) oxidation and LOX activity
− Lowering of LDL-C and triglyceride, and raising of HDL-cholesterol (HDL-C)
− Lowering of both systolic and diastolic blood pressure (BP) (possibly *via* improvement of arterial elasticity and stimulation of endothelial nitric oxide synthase (eNOS))
− Prevention of blood coagulation by inhibiting platelet aggregation (possibly *via* modulation of cell signalling (NFκB and AP1) and gene expression)
− Inhibition of inflammation, cell adhesion proteins, chemotactic factors and metalloproteinases *via* NFκB and AP1
− Vasodilation
− Improved insulin sensitivity and decrease insulin resistance
− Improved cognitive function and mood

Specifically, the improvement in endothelial function, the insulin resistance and the reduction in inflammation have been proposed as particularly relevant [47]. A randomized controlled trial measured the effects on healthy volunteers of receiving either dark chocolate or flavanol-free white chocolate for 3 days after an oral glucose tolerance test. The group receiving dark chocolate showed a reduction in impairment of endothelial function (increase in flow-mediated dilation, reduction in wave reflections and endothelin-1) and lipooxidative stress (reduction in 8-iso-PGF(2α)) caused by the oral glucose tolerance test [48]. The effcets on insulin resistance might be secondary to a modification of the expression of signaling markers, transcription factors and metabolic mediators, such as ERK, Akt, PPARγ and CEBPα [49] and as tAMPKα, GLUT4 and UCP in skeletal muscle and adipose tissue [50].

The reduction in inflammation seems to be due to a modification of the eicosanoid cascade *via* phospholipase A2, arachidonic acid and COX-2 [51] and in the reduction in expression of adhesion molecules such as VLA-4, CD40, CD36, endothelium-derived P-selectin and intercellular adhesion molecule-1 [52].

In Fig. (**1**) the cardiovascular effect of polyphenols are summarized.

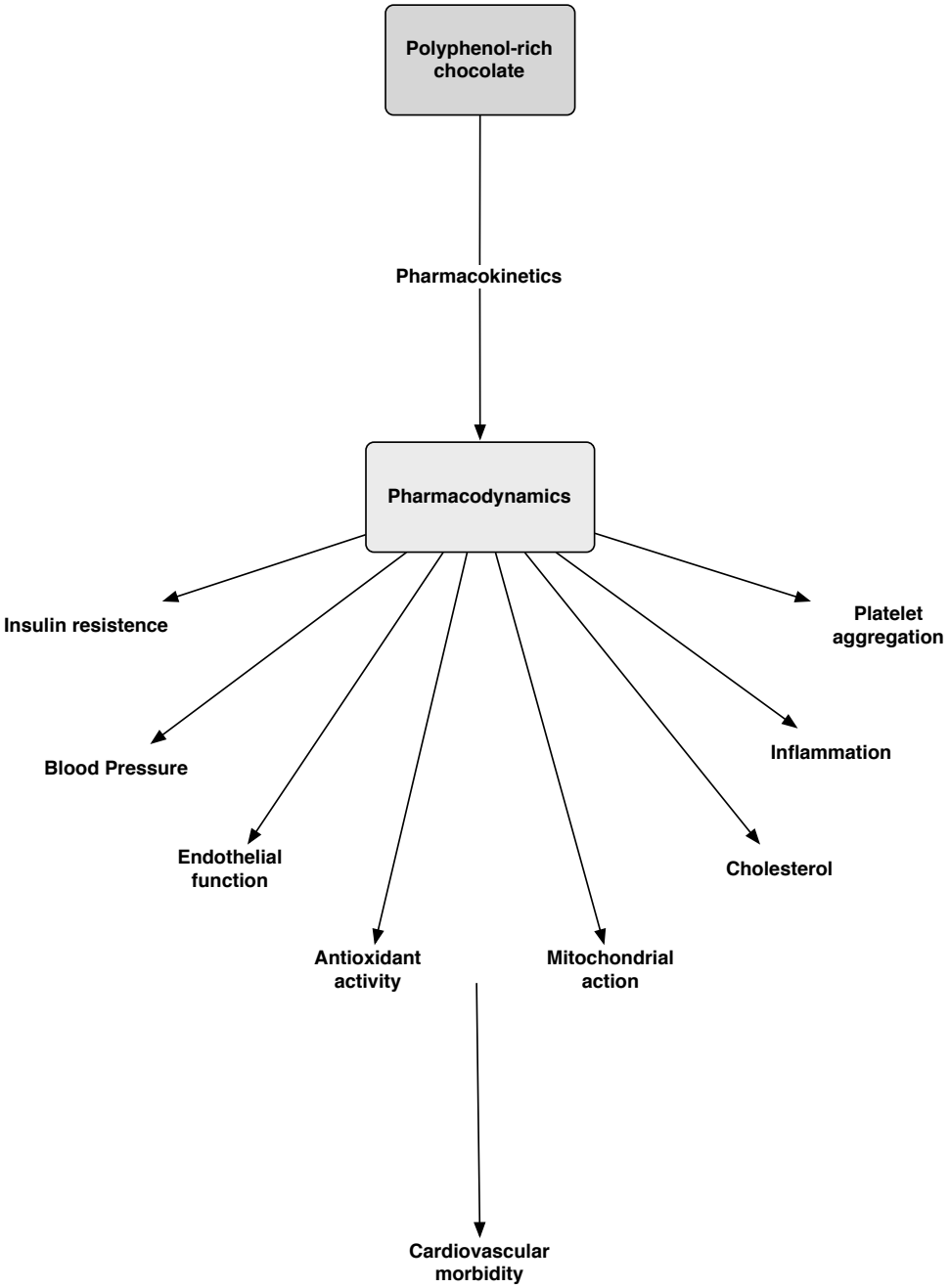

Fig. (1). Summary of polyphenols' cardiovascular effects.

Epidemiology

Epidemiological evidence specific for cocoa intake is increasing, and it statistically links the cardiovascular protective effects of cocoa to their high flavanol content. As cocoa is the main flavanol-delivering ingredient in chocolate, the findings from cocoa intervention studies may be used, with certain limitations kept in mind, to construct a feasible physiological link between chocolate consumption and cardiovascular risk reduction. Evidence from epidemiological studies show association between decreased risk of stroke and cardiovascular disease (including reductions in morbidity and mortality) and the long-term consumption of chocolate/cacao. Studies of the Kuna populations in Panama, who consume large amounts of a natural cocoa beverage (not chocolate as we know it), have found lower BP, better renal function and decreased cardiovascular mortality relative to control populations [53].

Similar investigations in American and European populations have demonstrated significant associations between chocolate intake and improved BP as well as cardiovascular and all-cause mortality; Buijsse and colleagues have presented an observational study on 19,357 middle-aged German participants of both sexes without cardiovascular disease, that shows an inverse relationship between chocolate consumption and cardiovascular disease risk [54]. In a previous cross-sectional long-term study on healthy elderly Dutch men, cocoa intake was inversely associated with BP and 15-year cardiovascular and all-cause mortality [55]. Two other cross-sectional trials have shown positive associations. In one on 4,970 men and women it was found an inverse association (independent of traditional risk factors) between frequent (1-5+ times/week) chocolate consumption and prevalent CHD, compared to subjects who did not report any chocolate intake [56]. A second one on 2,217 participants in the NHLBI Family Heart Study showed that chocolate consumption was inversely associated with prevalent calcified atherosclerotic plaque in the coronary arteries in a dose-response manner [56]. A third one, on 351 subjects, didn't find significant associations. It analysed the association between cocoa intake and various arterial stiffness measures and found that non-cocoa consumers had statistically significant higher pulse wave velocity and greater cardiovascular risk, but showed no differences in the other measures, and even these differences disappeared after adjustment in multivariate analysis for age, gender, the presence of diabetes, systolic BP and antihypertensive and lipid-lowering drug use [57]. Other European cohort studies have suggested an inverse relation between the consumption of cocoa or chocolate and cardiovascular diseases [58]. A prospective cohort study conducted on 31,823 women for 9 years showed an association between moderate habitual chocolate intake and lower rate of heart failure hospitalisation or death, specifically a 26% reduction for 1-3 servings of chocolate per month and a 32% reduction for 1-2 servings per week. However, it showed an increased ratio of heart failure (23%) for intake of one or more servings per day. The authors evaluated that most of the chocolate consumed in the studies analysed had low concentrations of polyphenols (approximately 30% cocoa solids) and that might explain the results for high chocolate consumption, where the sugar and fat content might counteract the beneficial effects of cocoa. Dark chocolate (polyphenol-rich chocolate or PRC) might show better protective effects [59]. In a prospective cohort study attempting to assess the effects of chocolate intake on risk of preeclampsia, 2567 pregnant women (2351 normotensive, 58 preeclamptic and 158 with gestational hypertension) were studied. Chocolate intake in the 1st trimester and 3rd trimester was associated with reduced odds of preeclampsia, while it was associated with reduced odds of gestational hypertension only in the 1st trimester [60]. Not all the prospective studies were unambiguously positive: in one on post-menopausal American women, there was a statistically weaker relation between flavonoid/chocolate consumption and cardiovascular diseases (CVD) [61] and in a second one there was no statistically significant association [62]. Even considering the problems of population studies in pinpointing a specific causal factor, the epidemiological literature unambiguosly identifies a correlation between chocolate consumption and reduced CVD risk, reduced CV mortality, reduced coronary heart diseases, and reduced arterial blood pressure.

Antioxidant Activity

In vitro and cultured cells studies showed that cocoa polyphenols, and in particular epicatechin, may act as free radical scavengers, prevent and/or inhibit NADPH-dependent lipoperoxidation, LDL oxidation, and inhibit LOX activity (one order of magnitude less potently than epigallocatechin gallate) [8, 10, 63]. In chocolate there is a high correlation between the cocoa content and the antioxidant activity [64]. Four weeks of cocoa supplementation in obese-diabetic rats resulted in a significant reduction in a marker of oxidative stress (plasma 8-isoprostane), and in an increase in activity of superoxide dismutase [65]. Similar results were showed in human studies, were cocoa polyphenols decreased LDL oxidation, improved plasma antioxidative status and reduced various biomarkers of lipid peroxidation. It is however unlikely that cocoa polyphenols can act as free radical scavengers *in vivo*, because they are mainly present in the conjugated form and because of the relatively low bioavailability of these compounds, which means they would be in a kinetically unfavourable condition compared to other plasma antioxidants like vitamin C, vitamin E, bilirubin and urate, present at much higher concentration [10]. It has been proposed that flavanols might not act directly as antioxidants, but interfere with the endogenous redox systems or with nuclear transcription factors like NF-kB, involved with oxidant and inflammatory cascade [66]. In a recent study it was in fact shown that epicatechin, administered for 10 days at a dose (1 mg/kg) unlikely to elicit a direct antioxidant activity, had a protective (antiapoptotic) effect, suggesting an action *via* receptor-mediated pathways; 3′-O-methyl epicatechin, a non antioxidant catechin, showed a similar antiapoptotic effect [67]. It has also been suggested that the effect of cocoa supplementation on LDL-C, making it more resistant to *in vitro* oxidation, could be the result of changes in the LDL-C particles surface rendering them less susceptible to oxidation [8].

Anti-inflammatory Activity

In vitro studies have shown that some cocoa polyphenols mildly inhibit LOX pathways, down regulate the production of pro-inflammatory cytokines (IL1β, IL2, IL4, IL6 and TNF-α), and inhibit iNOS gene expression *via* NFκB and AP1. Polyphenols might also inhibit activation of T and B cells [68]. In a feeding trial a cocoa-enriched diet (procyanidin content of 147 mg pro dose) increased the expression of vasodilatory and anti-platelet prostacyclins and decrease that of pro-inflammatory, vasoconstrictive, pro-aggregant leukotrienes, compared the consumption of a low procyanidin (3.3 mg) chocolate [66]. A study on animal models evaluated the effects of cocoa supplementation on adipose tissue inflammation. The mices were fed either a high-fat diet, a high fat diet with with 8% unsweetened cocoa powder, or a low-fat diet for 18 weeks. Compared to the group fed a high-fat diet only, the group fed also cocoa powder showed reduced adipose tissue inflammation, as showed by reduced levels of mRNA coding for TNF-α, IL-6, iNO-synthase, and EMR1, and by decreased levels of NF-κB. The study suggests that the reduction in inflammation is at least partially due to a modification of the eicosanoid cascade, showed by reduced levels of phospholipase A2, arachidonic acid and of the proinflammatory enzyme COX-2 by 53% and 55% [51].

In a randomized trial on high-risk volunteers, comparing the ingestion of cocoa powder plus milk to milk alone, the effects of cocoa on inflammatory biomarkers was investigated. In the cocoa plus milk group there was a statistically significant lowering effect on the expression of adhesion molecules involved with the inflammatory process, such as VLA-4, CD40, and CD36 in monocytes, endothelium-derived P-selectin and intercellular adhesion molecule-1, compared to the milk only group [52].

Effects on Cardiovascular Disease Risk

Ding and colleagues conducted a systematic review of experimental, observational, and clinical studies (from 1966 to 2005) of relations between cocoa, cacao, chocolate, stearic acid, flavonoids and CVD risk (coronary heart disease (CHD), stroke), concluding that cocoa and chocolate may exert beneficial effects on CVD risk and that flavonoid content of chocolate may reduce risk of cardiovascular mortality, *via* effects on BP, inflammation, platelet function, HDL, and LDL oxidation [66]. A more recent meta-analysis of only clinical studies demonstrated a chocolate-associated reduction in CVD risk of 37% and a reduction in stroke risk of 29% [69]. Shrime and colleagues conducted a systematic review and meta-analysis of 24 randomised, controlled trials, to evaluate the short-term effect of flavonoid-rich cocoa (FRC) on cardiovascular health, and concluded that there is a promising association between FRC consumption and the reduction of many CVD risk factors [70]. An even more recent systematic review and meta-analysis of randomised trials by Hooper and colleagues on the effects of chocolate and flavan-3-ols consumption for the prevention of CVD examined 42 acute or short-term chronic RCTs. Acute and chronic chocolate consumption was associated with beneficial effects on flow mediated dilation (FMD), insulin and insulin resistance, regardless of the dose consumed [71]. A best case scenario analysis using a Markov model on published meta-analyses was used to evaluate the treatment effects of dark chocolate consumption, compared to no treatment, on the absolute number of cardiovascular events in people with hypertension and metabolic syndrome, with no history of CVD and not receiving antihypertensive therapy. The analysis showed that daily consumption of 100 grams of dark chocolate can reduce cardiovascular events by 85 per 10,000 subjects treated over 10 years [72]. In summary, the meta-analysis show a good correlation between chocolate consumption and reduction in CVD risk and in CV events, and a relevant correlation between polyphenol content and chocolate cardiovascular effects. Three clinical studies not surveyed in the above mentioned reviews are summarised in Table **1**. They all point towards a beneficial endothelial influence of cocoa consumption, in terms of vasodilation in response to shear stress, a relevant atherogenic risk indicator, while one of them points towards a reduction in BP [73]. However, only one of these studies is of sufficient quality [74].

Effects on Arterial Pressure

Although there are several epidemiological studies that demonstrate a lower risk of CVD with increasing amounts of cocoa intake possibly through lowering peripheral BP, the majority of adequately controlled cocoa intervention trials have not been able to confirm this. Early studies examining the effect of cocoa ingestion on BP reported mixed results. A preliminary clinical trial (cross-over design) suggests that consuming 100 grams/day of PRC might modestly reduce systolic and diastolic BP in 13 elderly patients with isolated systolic hypertension after 14 days of intervention [75]. Grassi and colleagues found that 46-105 grams of PRC providing 213-500 mg of polyphenols/day modestly reduced both systolic and diastolic BP in normotensive and hypertensive subjects after 7 consecutive days of cocoa consumption [76]. Even lower amounts of cocoa (6.3 grams providing 30 mg polyphenols) decreased systolic and diastolic BP in a

sample of healthy persons with above-optimal BP [77, 78]. Another study also demonstrated beneficial effects of PRC on BP in healthy subjects after a 2-week intervention [79]. In fact, two early meta-analyses found a BP-reducing effect of chocolate [66, 80]. Later reviews were however less clear-cut: a meta-analysis by Egan, on PRC/cocoa and its effects on BP identified 13 studies, 6 with a double-blind design and of higher quality, and 7 with an open-label design and judged of lower quality. While a BP-lowering effect was reported in 6 out of 7 of the low-quality studies, a lowering effect on diastolic BP was found only in one out of the 6 higher-quality studies [81]. In the same year the review by Ried and colleagues analysed 13 RCTs for the effects of flavanol-rich chocolate on BP in hypertensive and normotensive individuals. The meta-analysis revealed a BP-reducing effect, superior to placebo, for the hypertensive or prehypertensive subgroups but not for the normotensive one. The effective dosages spanned from 30 mg to 1000 mg/die of flavanols, and the treatment duration from 2 or 18 weeks [82]. The systematic review and meta-analysis by Shrime and colleagues concluded that there is a statistically significant association between FRC (flavonoid-rich cocoa) consumption and reduction in systolic BP [70], and the review by Hooper and colleagues found that chocolate consumption was associated with moderate reductions in diastolic BP and mean arterial pressure, and doses >50 mg epicatechin/day resulted in greater effects on systolic and diastolic BP, but the strength of the evidence was reduced because of the heterogeneity and general design issues for many trials [71]. The meta-analyses show a correlation between flavonoid rich chocolate consumption and moderate reduction in BP, while this reduction seems limited to hypertensive or prehypertensive subjects. The results are somewhat unclear due to the heterogeneity of the trials examined. Of the studies not surveyed in the metanalyses one, a randomised, double blind, placebo controlled study on 23 healthy older adults, compared the effects of four different quantities of cocoa (2, 5, 13, or 26 grams) *versus* a placebo on BP. Small changes in BP were present at various time points during the experiments, but responses were similar on placebo and experimental days [74]. Two other studies were instead mainly positive, but their designs had many methodological problems [73, 83].

Table 1. Clinical studies on the beneficial effect of cocoa on endothelial function.

Number and status of subjects	Control	Design	Jadad score	Duration	Material	Endpoint	P value	Effect	References
23 Healthy	Placebo	Randomised, double blind, placebo controlled	3	2 hours	Cocoa: 5, 13, 26 grams	Endothelial function	< 0.05	Dose dependent increase of brachial artery flow-mediated dilation: maximum Δ2.5 ± 0.4%	Monahan, Feehan, Kunselman *et al*. 2011
32 prehypertensive	25 grams/day of white chocolate	Parallel randomised clinical trial	2	15 days	30 grams/day of 70% cocoa chocolate	Systolic and diastolic BP; NO serum levels	NO serum levels:=0.001 SBP: =0.001 DBP: =0.308	Increase of NO serum levels Reduction in SBP and DBP significantly strong negative correlation between NO and SBP; significantly moderate negative correlation between NO and DBP	Sudarma, Sukmaniah, Siregar 2011
22 stage 1 hypertensive	N	Pre-post intervention clinical trial	1	6 weeks (2 run-in, 4 treatment)	50 grams/day of chocolate (70% cocoa).	Endothelial function	=0.01	Increase of reactive hyperemia index: 1.94 ± 0.18 to 2.22 ± 0.08	Nogueira Lde, Knibel, Torres *et al*. 2012

Effects on Blood Lipids and Body Weight

Cocoa polyphenols fed at 1% to rats on a high-cholesterol diet reduced plasma total cholesterol levels and cholesterol and triglyceride hepatic depositions. The authors suggest that this activity might be due to the ability of catechins to inhibit intestinal absorption of dietary cholesterol and triglyceride. The inhibition is correlated with the degree of polymerisation, and is probably caused by catechins co-precipitating with cholesterol, thereby making it insoluble [84]. In the systematic review by Ding and colleagues on experimental, observational, and clinical studies, the authors concluded that the of body of short-term randomised feeding trials showed an association between chocolate consumption and anti-inflammatory and anti-platelet effects, higher HDL levels and decreased LDL oxidation, while the trials on stearic acid showed it is cholesterol-neutral. These last studies were however marred with many

methodological limitations [66]. The systematic review and meta-analysis by Shrime and colleagues found that chocolate consumption was associated to a decrease of LDL-C, and an increase of HDL-C, whereas total cholesterol, TG, and C-reactive protein serum levels remained the same [70]. Hooper and colleagues, however, found only marginally significant effects on LDL-C and HDL-C, and noticed that the strength of evidence was lowered due to unclear reporting for allocation concealment, dropouts, missing data on outcomes, and heterogeneity in biomarker results in some studies [71]. Two studies were not included in the meta-analyses. A parallel-design, randomised placebo-controlled trial on 118 type 2 diabetic patients tested the effect of PRC against placebo for a year. The active group showed significant improvements in lipoprotein status and markers of insulin sensitivity and an attenuation of the estimated 10-year risk of CHD in postmenopausal women receiving standard therapy for type 2 diabetes [85]. A low quality and preliminary study showed no significant changes in lipid profile, biomarkers of inflammation, and oxidised LDL after PRC consumption [86]. Saturated fats are thought to contribute to atherosclerosis (increasing the total cholesterol and LDL-C levels), and cocoa is rich in fats (40-60% of dry weight) and in particular in saturated fats like stearic acid (33-34%) and palmitic acid (25-26%) [87]. It has, however, been confirmed in a series of meta-analyses that stearic acid is a non-atherogenic type of saturated fat, since it doesn't increase LDL-C or TC, while there is contrasting evidence on the effects on HDL-C and lipoprotein A, and on coagulation and thrombosis [66]. It is not known why cocoa butter should be non-atherogenic, but it has been proposed this is due to its complex chemical structure: almost all of the stearic acid in cocoa butter is present as symmetrical triacylglycerol, a structural feature which slows down its intestinal absorption, thereby reducing the dyslipidemic effects. It has also been proposed that there is a relatively high *in vivo* transformation of stearic acid to mono-saturated oleic acid, which is considered hypocholesterolaemic and cardioprotective [88]. Given that the vast majority of studies show stearic acid has beneficial or neutral effects on BP, and does not adversely affect established traditional lipid risk factors, it appears unlikely that it would adversely affect CVD risk [66]. It must be emphasised that in an early study stearic acid lowered cholesterol in healthy men when used in substitution of myristic acid from dairy butter, but did not seems to reduce cholesterol in absolute terms [89]. Because of these results it can be preliminarily concluded that while stearic acid is better than other saturated fats, there is no solid evidence for a reduction in CVD risk correlated to consumption of cocoa butter alone [90, 91].

A 2014 review suggests that cocoa polyphenols regulate lipid metabolism by regulating the expression of genes involved in energy metabolism, such as the AdipoQ gene, possibly by activating transcription factors (NF-kB, AP-1) and nuclear receptors (PPARγ, LXR) [92].

In a study on obese mice fed a high-fat diet, cocoa polyphenols reduced the body weight gain and fat accumulation of the models. The same cocoa polyphenols showed, *in vitro* on preadipocytes, to inhibit insuline receptor kinase activity, cellular differentiation and lipid accumulation, by blocking signaling markers and transcription factors, such as ERK, Akt, PPARγ and CEBPα [49]. In another study on animal models fed a high-fat diet, evaluating and comparing long-term exposure to various cocoa flavanols, found that an oligomeric procyanidins-rich fraction was more effective than monomeric or the polymeric procyanidins-rich fractions in preventing weight gain, fat mass, impaired glucose tolerance, and insulin resistance [93].

In another animal study a liquid cocoa extract (containing 4.3% catechin, 6.1% epicatechin, 39.4% procyanidins) was successful in suppressing the pathogenic consequences of a high-fat diet (hyperglycemia, glucose intolerance, fat accumulation in white adipose tissue) probably *via* modification of the expression of different metabolic mediators such as tAMPKα, GLUT4 and UCP in skeletal muscle and adipose tissue [50].

Effects on Insulin

Improvements in insulin sensitivity have been observed following clinical dietary interventions with flavanol-containing foods and beverages [94]. A systematic review and meta-analysis of 24 randomised, controlled trials evaluated the effect of flavonoid-rich cocoa (FRC) on CVD risk factors, and found a decrease in insulin resistance [70]. Reductions in fasting serum insulin levels and insulin resistance were also reported in a meta-analysis of 42 studies, 4 of which included people with impaired glucose tolerance or diabetes. These changes were seen with 19-54 grams of cocoa per day, or 46-100 grams of PRC per day [71].

Effects of Platelet Aggregation

According to Ding and colleagues cocoa polyphenols, and in particular catechin and epicatechin, have shown *in vitro* anti-platelet effects quantitatively similar to that of aspirin [66]. PRC reduced markers for platelet aggregation (fibrinogen-binding glycoprotein IIb-IIIa) [95], and 40 grams of PRC caused coronary vasodilatation, improved

coronary vasomotion and decreased platelet adhesion, all effects which were correlated with serum content in epicatechin [96].

Negative Effects of Cocoa Consumption

Literature on potential adverse effects of chocolate consumption are still scarce. Few studies remark negative action on strength of lower esophageal sphincter (LES): ingestion of cocoas seems to decrease LES pressure, thus worsening gastroesophageal reflux [97]. Moreover, chocolate has been related to allergic reactions as dermatitis and food-induced vasculitis in children [98, 99].

Methodological Remarks

According to Heiss and Kelm [94] both epidemiological studies and clinical ones on cocoa and chocolate consumption suffer from various shortcomings:

- In many of the epidemiological studies chocolate consumption was only estimated as one item on the food-frequency questionnaire, making it impossible to arrive at definitive conclusions on the associated intake of potential bioactives, given the high variability of cocoa and flavanol content.
- Flavanols exist in distinct stereochemical configurations, which can influence bioactivity and which can in turn be modified by food processing, but these modifications have not been evaluated properly.
- Very few of the clinical studies had a rigorous study design: many were not placebo-controlled; many were done on healthy subjects; most of them were underpowered, a fact that reduced their statistical power; they often tested the association for a short time, and used *ex vivo* biomarkers and surrogate endpoints; and finally, compliance of the volunteers as well as plasma polyphenol concentrations have been rarely reported.
- The products used in the trials often contain much higher polyphenol contents than most of the commercially available products, making it very difficult to extrapolate the results to normal chocolate consumption. Given that higher polyphenols content translates into a more bitter flavour, it also remains to be seen whether a supplementation with PRC is feasible in reality.

These methodological limitations mean that claims for prevention of CVD are based on projection of changes in known risk factors for CVD rather than establishing actual changes in disease, and while epidemiological studies suggest that long-term consumption of flavonoids in general lowers the risk of coronary heart disease mortality, the next step is large scale, long term randomised placebo-controlled studies (ideally with a cross-over design) and prospective studies which specifically confirm cocoa's on-going influence on cardiovascular health [10].

CONCLUSION

There is an increasing evidence that cocoa-based products can exert a positive influence on health, in particular on the “natural history” of cardiometabolic diseases, mainly thanks to their flavanol content. These results are consistent with the general epidemiological and clinical literature on polyphenol-rich foods. Given that chocolate is a widespread food commodity, there has been an interest in analysing the possible health consequences of its consumption, and the ways in which it could be used. These positive results notwithstanding, there are still many difficulties in translating the clinical results into pragmatic, operative guidelines. The main obstacle is the difference in polyphenols content between the commercial chocolate bars or drinks and the products used in clinical trials. Rarely can in fact the commercial brands provide therapeutic amounts of polyphenols, given that a normal milk chocolate bar can provide 15-16 mg of polyphenols for 100 grams of product. Moreover, high levels of commercial chocolate consumption for long periods of time might have unknown implications for tooth health, weight gain and diabetic complications, because of the high sugar and saturated fatty acids content. Cacao powder or dark chocolate bars (with at least 70% cocoa content) could be a richer and healthier source of polyphenols, but they are not as widespread and common as milk chocolate products. Innovative cocoa products, engineered to contain more polyphenols, either *via* genetic selection, enzymes inactivation, manufacturing changes, and combinations thereof are another option, but they are even less likely than dark chocolate bars to become a very common food commodity, because, as has been already stressed, polyphenol content and palatability do not go easily hand in hand. As it stands now, there are either products with a vast appeal, cheap and easily available but which do not provide sufficient amounts of polyphenols, or products that can provide the right amount of polyphenols but have an appeal only for a restricted sector of the population, are expensive and are not easily available. It could be proposed that PRC is a useful functional food in those parts of the world where cocoa has been

used for a long time, is part of the culture and is appreciated even in its more bitter versions. In these areas of the world the introduction of newly engineered products could be made acceptable. It would be instead much more difficult to offer such products in those areas of the world where chocolate is a more recent acquisition and/or where it has almost always been consumed as a very sweet, low cocoa product.

CONFLICT OF INTEREST

The author reports no conflict of interest. The author alone is responsible for the content and writing of the paper.

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REFERENCES

[1] International Trade Centre. Cocoa: A guide to Trade Practice. Geneva: ITC 2001.

[2] Tropicos. Available from: http://www.tropicos.org.

[3] Powis TG, Cyphers A, Gaikwad NW, Grivetti L, Cheong K. Cacao use and the San Lorenzo Olmec. Proc Natl Acad Sci USA 2011; 108(21): 8595-600.
[http://dx.doi.org/10.1073/pnas.1100620108] [PMID: 21555564]

[4] Macri MJ. Tempest in a chocolate pot: Origins of the word Cacao. In: Grivetti LE, Shapiro H-Y, Eds. Chocolate: History, culture, and heritage. New Jersey: Wiley and Sons 2009.
[http://dx.doi.org/10.1002/9780470411315.ch2]

[5] Grivetti LE. Medicinal chocolate in New Spain, Western Europe, and North America. In: Grivetti LE, Shapiro H-Y, Eds. Chocolate: History, culture, and heritage. New Jersey: Wiley and Sons 2009.
[http://dx.doi.org/10.1002/9780470411315.ch6]

[6] Grivetti LE, Shapiro H-Y, Eds. G V. Cacao use in Yucatan among the pre-hispanic Maya. Chocolate: History, culture, and heritage. New Jersey: Wiley and Sons 2009.

[7] Trognitz B, Cros E, Assemat S, *et al.* Diversity of cacao trees in Waslala, Nicaragua: associations between genotype spectra, product quality and yield potential. PLoS One 2013; 8(1): e54079.
[http://dx.doi.org/10.1371/journal.pone.0054079] [PMID: 23349790]

[8] Galleano M, Oteiza PI, Fraga CG. Cocoa, chocolate, and cardiovascular disease. J Cardiovasc Pharmacol 2009; 54(6): 483-90.
[http://dx.doi.org/10.1097/FJC.0b013e3181b76787] [PMID: 19701098]

[9] Lamuela-Raventos RM. Review: Health Effects of Cocoa Flavonoids. Food Sci Technol Int 2005; 11(3): 159-76.
[http://dx.doi.org/10.1177/1082013205054498]

[10] Rimbach G, Melchin M, Moehring J, Wagner AE. Polyphenols from cocoa and vascular health-a critical review. Int J Mol Sci 2009; 10(10): 4290-309.
[http://dx.doi.org/10.3390/ijms10104290] [PMID: 20057946]

[11] Hurst WJ, Krake SH, Bergmeier SC, Payne MJ, Miller KB, Stuart DA. Impact of fermentation, drying, roasting and Dutch processing on flavan-3-ol stereochemistry in cacao beans and cocoa ingredients. Chem Cent J 2011; 5(1): 53.
[http://dx.doi.org/10.1186/1752-153X-5-53] [PMID: 21917164]

[12] Illeghems K, De Vuyst L, Papalexandratou Z, Weckx S. Phylogenetic analysis of a spontaneous cocoa bean fermentation metagenome reveals new insights into its bacterial and fungal community diversity. PLoS One 2012; 7(5): e38040.
[http://dx.doi.org/10.1371/journal.pone.0038040] [PMID: 22666442]

[13] Ruzaidi A, Amin I, Nawalyah AG, Muhajir H, Pauliena M, Muskinah M. Hypoglycaemic properties of Malaysian Cocoa (Theobroma Cacao) polyphenols-rich extract. Int Food Res J 2008; 15(3): 305-12.

[14] Crozier SJ, Preston AG, Hurst JW, *et al.* Cacao seeds are a "Super Fruit": A comparative analysis of various fruit powders and products. Chem Cent J 2011; 5: 5.
[http://dx.doi.org/10.1186/1752-153X-5-5] [PMID: 21299842]

[15] Andres-Lacueva C, Monagas M, Khan N, *et al.* Flavanol and flavonol contents of cocoa powder products: influence of the manufacturing process. J Agric Food Chem 2008; 56(9): 3111-7.
[http://dx.doi.org/10.1021/jf0728754] [PMID: 18412367]

[16] Tomas-Barberan FA, Cienfuegos-Jovellanos E, Marín A, *et al.* A new process to develop a cocoa powder with higher flavonoid monomer content and enhanced bioavailability in healthy humans. J Agric Food Chem 2007; 55(10): 3926-35.
[http://dx.doi.org/10.1021/jf070121j] [PMID: 17439235]

[17] Muniyappa R, Hall G, Kolodziej TL, Karne RJ, Crandon SK, Quon MJ. Cocoa consumption for 2 wk enhances insulin-mediated vasodilatation without improving blood pressure or insulin resistance in essential hypertension. Am J Clin Nutr 2008; 88(6): 1685-96.

[http://dx.doi.org/10.3945/ajcn.2008.26457] [PMID: 19064532]

[18] Engler MB, Engler MM, Chen CY, *et al.* Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr 2004; 23(3): 197-204.
[http://dx.doi.org/10.1080/07315724.2004.10719361] [PMID: 15190043]

[19] Monagas M, Khan N, Andres-Lacueva C, *et al.* Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. Am J Clin Nutr 2009; 90(5): 1144-50.
[http://dx.doi.org/10.3945/ajcn.2009.27716] [PMID: 19776136]

[20] Keogh JB, McInerney J, Clifton PM. The effect of milk protein on the bioavailability of cocoa polyphenols. J Food Sci 2007; 72(3): S230-3.
[http://dx.doi.org/10.1111/j.1750-3841.2007.00314.x] [PMID: 17995820]

[21] Roura E, Andrés-Lacueva C, Estruch R, *et al.* Milk does not affect the bioavailability of cocoa powder flavonoid in healthy human. Ann Nutr Metab 2007; 51(6): 493-8.
[http://dx.doi.org/10.1159/000111473] [PMID: 18032884]

[22] Roura E, Andrés-Lacueva C, Estruch R, Lourdes Mata Bilbao M, Izquierdo-Pulido M, Lamuela-Raventós RM. The effects of milk as a food matrix for polyphenols on the excretion profile of cocoa (-)-epicatechin metabolites in healthy human subjects. Br J Nutr 2008; 100(4): 846-51.
[http://dx.doi.org/10.1017/S0007114508922534] [PMID: 18257943]

[23] Mullen W, Borges G, Donovan JL, *et al.* Milk decreases urinary excretion but not plasma pharmacokinetics of cocoa flavan-3-ol metabolites in humans. Am J Clin Nutr 2009; 89(6): 1784-91.
[http://dx.doi.org/10.3945/ajcn.2008.27339] [PMID: 19403635]

[24] van den Bogaard B, Draijer R, Westerhof BE, van den Meiracker AH, van Montfrans GA, van den Born BJ. Effects on peripheral and central blood pressure of cocoa with natural or high-dose theobromine: a randomized, double-blind crossover trial. Hypertension 2010; 56(5): 839-46.
[http://dx.doi.org/10.1161/HYPERTENSIONAHA.110.158139] [PMID: 20823377]

[25] Neilson AP, Sapper TN, Janle EM, Rudolph R, Matusheski NV, Ferruzzi MG. Chocolate matrix factors modulate the pharmacokinetic behavior of cocoa flavan-3-ol phase II metabolites following oral consumption by Sprague-Dawley rats. J Agric Food Chem 2010; 58(11): 6685-91.
[http://dx.doi.org/10.1021/jf1005353] [PMID: 20446738]

[26] Neilson AP, George JC, Janle EM, *et al.* Influence of chocolate matrix composition on cocoa flavan-3-ol bioaccessibility *in vitro* and bioavailability in humans. J Agric Food Chem 2009; 57(20): 9418-26.
[http://dx.doi.org/10.1021/jf902919k] [PMID: 19780539]

[27] Tzounis X, Rodriguez-Mateos A, Vulevic J, Gibson GR, Kwik-Uribe C, Spencer JP. Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. Am J Clin Nutr 2011; 93(1): 62-72.
[http://dx.doi.org/10.3945/ajcn.110.000075] [PMID: 21068351]

[28] Massot-Cladera M, Pérez-Berezo T, Franch A, Castell M, Pérez-Cano FJ. Cocoa modulatory effect on rat faecal microbiota and colonic crosstalk. Arch Biochem Biophys 2012; 527(2): 105-12.
[http://dx.doi.org/10.1016/j.abb.2012.05.015] [PMID: 22663919]

[29] Ottaviani JI, Momma TY, Kuhnle GK, Keen CL, Schroeter H. Structurally related (-)-epicatechin metabolites in humans: assessment using de novo chemically synthesized authentic standards. Free Radic Biol Med 2012; 52(8): 1403-12.
[http://dx.doi.org/10.1016/j.freeradbiomed.2011.12.010] [PMID: 22240152]

[30] Gu Y, Hurst WJ, Stuart DA, Lambert JD. Inhibition of key digestive enzymes by cocoa extracts and procyanidins. J Agric Food Chem 2011; 59(10): 5305-11.
[http://dx.doi.org/10.1021/jf200180n] [PMID: 21495725]

[31] Rodríguez-Ramiro I, Ramos S, López-Oliva E, *et al.* Cocoa-rich diet prevents azoxymethane-induced colonic preneoplastic lesions in rats by restraining oxidative stress and cell proliferation and inducing apoptosis. Mol Nutr Food Res 2011; 55(12): 1895-9.
[http://dx.doi.org/10.1002/mnfr.201100363] [PMID: 21953728]

[32] Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 2005; 81(1)(Suppl.): 317S-25S.
[PMID: 15640497]

[33] Arts IC, Hollman PC, Feskens EJ, Bueno de Mesquita HB, 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(2): 227-32.
[PMID: 11470725]

[34] Arts IC, Jacobs DR Jr, Harnack LJ, Gross M, Folsom AR. Dietary catechins in relation to coronary heart disease death among postmenopausal women. Epidemiology 2001; 12(6): 668-75.
[http://dx.doi.org/10.1097/00001648-200111000-00015] [PMID: 11679795]

[35] Geleijnse JM, Launer LJ, Van der Kuip DA, Hofman A, Witteman JC. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. Am J Clin Nutr 2002; 75(5): 880-6.
[PMID: 11976162]

[36] 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(8878): 1007-11.
[http://dx.doi.org/10.1016/0140-6736(93)92876-U] [PMID: 8105262]

[37] Hertog MG, Kromhout D, Aravanis C, *et al.* Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 1995; 155(4): 381-6.
[http://dx.doi.org/10.1001/archinte.1995.00430040053006] [PMID: 7848021]

[38] Joshipura KJ, Hu FB, Manson JE, *et al.* The effect of fruit and vegetable intake on risk for coronary heart disease. Ann Intern Med 2001; 134(12): 1106-14.
[http://dx.doi.org/10.7326/0003-4819-134-12-200106190-00010] [PMID: 11412050]

[39] Keli SO, Hertog MG, Feskens EJ, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Arch Intern Med 1996; 156(6): 637-42.
[http://dx.doi.org/10.1001/archinte.1996.00440060059007] [PMID: 8629875]

[40] Knekt P, Kumpulainen J, Järvinen R, *et al.* Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 2002; 76(3): 560-8.
[PMID: 12198000]

[41] Liu S, Lee IM, Ajani U, Cole SR, Buring JE, Manson JE. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men: The Physicians' Health Study. Int J Epidemiol 2001; 30(1): 130-5.
[http://dx.doi.org/10.1093/ije/30.1.130] [PMID: 11171873]

[42] Liu S, Manson JE, Lee IM, *et al.* Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. Am J Clin Nutr 2000; 72(4): 922-8.
[PMID: 11010932]

[43] Mursu J, Voutilainen S, Nurmi T, Tuomainen TP, Kurl S, Salonen JT. Flavonoid intake and the risk of ischaemic stroke and CVD mortality in middle-aged Finnish men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Br J Nutr 2008; 100(4): 890-5.
[http://dx.doi.org/10.1017/S0007114508945694] [PMID: 18377681]

[44] Steinberg FM, Bearden MM, Keen CL. Cocoa and chocolate flavonoids: implications for cardiovascular health. J Am Diet Assoc 2003; 103(2): 215-23.
[http://dx.doi.org/10.1053/jada.2003.50028] [PMID: 12589329]

[45] Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991; 2(5): 325-57.
[http://dx.doi.org/10.1007/BF00051672] [PMID: 1834240]

[46] Yochum L, Kushi LH, Meyer K, Folsom AR. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. Am J Epidemiol 1999; 149(10): 943-9.
[http://dx.doi.org/10.1093/oxfordjournals.aje.a009738] [PMID: 10342803]

[47] Grassi D, Desideri G, Mai F, *et al.* Cocoa, glucose tolerance, and insulin signaling: cardiometabolic protection. J Agric Food Chem 2015; 63(45): 9919-26.
[http://dx.doi.org/10.1021/acs.jafc.5b00913] [PMID: 26126077]

[48] Grassi D, Desideri G, Necozione S, *et al.* Protective effects of flavanol-rich dark chocolate on endothelial function and wave reflection during acute hyperglycemia. Hypertension 2012; 60(3): 827-32.
[http://dx.doi.org/10.1161/HYPERTENSIONAHA.112.193995] [PMID: 22851734]

[49] Min SY, Yang H, Seo SG, Shin SH, Chung MY, Kim J, *et al.* Cocoa polyphenols suppress adipogenesis *in vitro* and obesity *in vivo* by targeting insulin receptor. Int J Obesity 2013; 37(4): 584-92.
[http://dx.doi.org/10.1038/ijo.2012.85]

[50] Yamashita Y, Okabe M, Natsume M, Ashida H. Prevention mechanisms of glucose intolerance and obesity by cacao liquor procyanidin extract in high-fat diet-fed C57BL/6 mice. Arch Biochem Biophys 2012; 527(2): 95-104.
[http://dx.doi.org/10.1016/j.abb.2012.03.018] [PMID: 22465028]

[51] Gu Y, Yu S, Park JY, Harvatine K, Lambert JD. Dietary cocoa reduces metabolic endotoxemia and adipose tissue inflammation in high-fat fed mice. J Nutr Biochem 2014; 25(4): 439-45.
[http://dx.doi.org/10.1016/j.jnutbio.2013.12.004] [PMID: 24561154]

[52] Monagas M, Khan N, Andres-Lacueva C, *et al.* Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. Am J Clin Nutr 2009; 90(5): 1144-50.
[http://dx.doi.org/10.3945/ajcn.2009.27716] [PMID: 19776136]

[53] Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. Antioxid Redox Signal 2011; 15(10): 2779-811.
[http://dx.doi.org/10.1089/ars.2010.3697]

[54] Buijsse B, Weikert C, Drogan D, Bergmann M, Boeing H. Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. Eur Heart J 2010; 31(13): 1616-23.
[http://dx.doi.org/10.1093/eurheartj/ehq068] [PMID: 20354055]

[55] Buijsse B, Feskens EJ, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. Arch Intern Med 2006; 166(4): 411-7.
[PMID: 16505260]

[56] Djoussé L, Hopkins PN, Arnett DK, *et al.* Chocolate consumption is inversely associated with calcified atherosclerotic plaque in the coronary arteries: the NHLBI Family Heart Study. Clin Nutr 2011; 30(1): 38-43.
[http://dx.doi.org/10.1016/j.clnu.2010.06.011] [PMID: 20655129]

[57] Recio-Rodríguez JI, Gómez-Marcos MA, Patino-Alonso MC, Agudo-Conde C, Rodríguez-Sánchez E, García-Ortiz L. Cocoa intake and arterial stiffness in subjects with cardiovascular risk factors. Nutr J 2012; 11: 8.
[http://dx.doi.org/10.1186/1475-2891-11-8] [PMID: 22325068]

[58] Janszky I, Mukamal KJ, Ljung R, Ahnve S, Ahlbom A, Hallqvist J. Chocolate consumption and mortality following a first acute myocardial infarction: the Stockholm Heart Epidemiology Program. J Intern Med 2009; 266(3): 248-57.
[http://dx.doi.org/10.1111/j.1365-2796.2009.02088.x] [PMID: 19711504]

[59] Mostofsky E, Levitan EB, Wolk A, Mittleman MA. Chocolate intake and incidence of heart failure: a population-based prospective study of middle-aged and elderly women. Circ Heart Fail 2010; 3(5): 612-6.
[http://dx.doi.org/10.1161/CIRCHEARTFAILURE.110.944025] [PMID: 20713904]

[60] Saftlas AF, Triche EW, Beydoun H, Bracken MB. Does chocolate intake during pregnancy reduce the risks of preeclampsia and gestational hypertension? Ann Epidemiol 2010; 20(8): 584-91.
[http://dx.doi.org/10.1016/j.annepidem.2010.05.010] [PMID: 20609337]

[61] Mink PJ, Scrafford CG, Barraj LM, *et al.* Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr 2007; 85(3): 895-909.
[PMID: 17344514]

[62] Hu FB, Stampfer MJ, Willett WC. No evidence for a link between consumption of chocolate and coronary heart disease. Am J Clin Nutr 2000; 72: 1059-60.
[PMID: 11010953]

[63] Gómez-Juaristi M, González-Torres L, Bravo L, Vaquero MP, Bastida S, Sánchez-Muniz FJ. Beneficial effects of chocolate on cardiovascular health. Nutr Hosp 2011; 26(2): 289-92.
[PMID: 21666964]

[64] Carlsen MH, Halvorsen BL, Holte K, *et al.* The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutr J 2010; 9: 3.
[http://dx.doi.org/10.1186/1475-2891-9-3] [PMID: 20096093]

[65] Jalil AM, Ismail A. Polyphenols in cocoa and cocoa products: is there a link between antioxidant properties and health? Molecules 2008; 13(9): 2190-219.
[http://dx.doi.org/10.3390/molecules13092190] [PMID: 18830150]

[66] Ding EL, Hutfless SM, Ding X, Girotra S. Chocolate and prevention of cardiovascular disease: a systematic review. Nutr Metab (Lond) 2006; 3: 2.
[http://dx.doi.org/10.1186/1743-7075-3-2] [PMID: 16390538]

[67] Panneerselvam M, Tsutsumi YM, Bonds JA, *et al.* Dark chocolate receptors: epicatechin-induced cardiac protection is dependent on delta-opioid receptor stimulation. Am J Physiol Heart Circ Physiol 2010; 299(5): H1604-9.
[http://dx.doi.org/10.1152/ajpheart.00073.2010] [PMID: 20833967]

[68] Barnett CF, De Marco T. A chocolate a day keeps the doctor away? J Physiol 2011; 589(Pt 24): 5921-2.
[http://dx.doi.org/10.1113/jphysiol.2011.222109] [PMID: 22174142]

[69] Buitrago-Lopez A, Sanderson J, Johnson L, *et al.* Chocolate consumption and cardiometabolic disorders: systematic review and meta-analysis. BMJ 2011; 343: d4488.
[http://dx.doi.org/10.1136/bmj.d4488] [PMID: 21875885]

[70] Shrime MG, Bauer SR, McDonald AC, Chowdhury NH, Coltart CE, Ding EL. Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies. J Nutr 2011; 141(11): 1982-8.
[http://dx.doi.org/10.3945/jn.111.145482] [PMID: 21956956]

[71] Hooper L, Kay C, Abdelhamid A, *et al.* Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. Am J Clin Nutr 2012; 95(3): 740-51.
[http://dx.doi.org/10.3945/ajcn.111.023457] [PMID: 22301923]

[72] Zomer E, Owen A, Magliano DJ, Liew D, Reid CM. The effectiveness and cost effectiveness of dark chocolate consumption as prevention therapy in people at high risk of cardiovascular disease: best case scenario analysis using a Markov model. BMJ 2012; 344: e3657.
[http://dx.doi.org/10.1136/bmj.e3657] [PMID: 22653982]

[73] Sudarma V, Sukmaniah S, Siregar P. Effect of dark chocolate on nitric oxide serum levels and blood pressure in prehypertension subjects. Acta Med Indones 2011; 43(4): 224-8.
[PMID: 22156352]

[74] Monahan KD, Feehan RP, Kunselman AR, Preston AG, Miller DL, Lott ME. Dose-dependent increases in flow-mediated dilation following acute cocoa ingestion in healthy older adults. J Appl Physiol 2011; 111(6): 1568-74.
[http://dx.doi.org/10.1152/japplphysiol.00865.2011] [PMID: 21903881]

[75] Taubert D, Berkels R, Roesen R, Klaus W. Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. JAMA 2003; 290(8): 1029-30.
[http://dx.doi.org/10.1001/jama.290.8.1029] [PMID: 12941673]

[76] Grassi D, Necozione S, Lippi C, *et al.* Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation

in hypertensives. Hypertension 2005; 46(2): 398-405.
[http://dx.doi.org/10.1161/01.HYP.0000174990.46027.70] [PMID: 16027246]

[77] Taubert D, Roesen R, Lehmann C, Jung N, Schömig E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. JAMA 2007; 298(1): 49-60.
[http://dx.doi.org/10.1001/jama.298.1.49] [PMID: 17609490]

[78] Vlachopoulos CV, Alexopoulos NA, Aznaouridis KA, *et al.* Relation of habitual cocoa consumption to aortic stiffness and wave reflections, and to central hemodynamics in healthy individuals. Am J Cardiol 2007; 99(10): 1473-5.
[http://dx.doi.org/10.1016/j.amjcard.2006.12.081] [PMID: 17493484]

[79] Grassi D, Desideri G, Croce G, Tiberti S, Aggio A, Ferri C. Flavonoids, vascular function and cardiovascular protection. Curr Pharm Des 2009; 15(10): 1072-84.
[http://dx.doi.org/10.2174/138161209787846982] [PMID: 19355949]

[80] Taubert D, Roesen R, Schömig E. Effect of cocoa and tea intake on blood pressure: a meta-analysis. Arch Intern Med 2007; 167(7): 626-34.
[http://dx.doi.org/10.1001/archinte.167.7.626] [PMID: 17420419]

[81] Egan BM, Laken MA, Donovan JL, Woolson RF. Does dark chocolate have a role in the prevention and management of hypertension?: commentary on the evidence. Hypertension 2010; 55(6): 1289-95.
[http://dx.doi.org/10.1161/HYPERTENSIONAHA.110.151522] [PMID: 20404213]

[82] Ried K, Sullivan T, Fakler P, Frank OR, Stocks NP. Does chocolate reduce blood pressure? A meta-analysis. BMC Med 2010; 8: 39.
[http://dx.doi.org/10.1186/1741-7015-8-39] [PMID: 20584271]

[83] Solà R, Valls RM, Godàs G, *et al.* Cocoa, hazelnuts, sterols and soluble fiber cream reduces lipids and inflammation biomarkers in hypertensive patients: a randomized controlled trial. PLoS One 2012; 7(2): e31103.
[http://dx.doi.org/10.1371/journal.pone.0031103] [PMID: 22383996]

[84] Osakabe N, Yamagishi M. Procyanidins in theobroma cacao reduce plasma cholesterol levels in high cholesterol-fed rats. J Clin Biochem Nutr 2009; 45(2): 131-6.
[http://dx.doi.org/10.3164/jcbn.07-34] [PMID: 19794919]

[85] Curtis PJ, Sampson M, Potter J, Dhatariya K, Kroon PA, Cassidy A. Chronic ingestion of flavan-3-ols and isoflavones improves insulin sensitivity and lipoprotein status and attenuates estimated 10-year CVD risk in medicated postmenopausal women with type 2 diabetes: a 1-year, double-blind, randomized, controlled trial. Diabetes Care 2012; 35(2): 226-32.
[http://dx.doi.org/10.2337/dc11-1443] [PMID: 22250063]

[86] Nogueira Lde P, Knibel MP, Torres MR, Nogueira Neto JF, Sanjuliani AF. Consumption of high-polyphenol dark chocolate improves endothelial function in individuals with stage 1 hypertension and excess body weight. Int J Hypertens 2012; 2012: 147321.
[http://dx.doi.org/10.1155/2012/147321]

[87] Lima LJ, van der Velpen V, Wolkers-Rooijackers J, Kamphuis HJ, Zwietering MH, Nout MJ. Microbiota dynamics and diversity at different stages of industrial processing of cocoa beans into cocoa powder. Appl Environ Microbiol 2012; 78(8): 2904-13.
[http://dx.doi.org/10.1128/AEM.07691-11] [PMID: 22327588]

[88] Rimbach G, Egert S, de Pascual-Teresa S. Chocolate: (un)healthy source of polyphenols? Genes Nutr 2011; 6(1): 1-3.
[http://dx.doi.org/10.1007/s12263-010-0185-7] [PMID: 21437025]

[89] Kris-Etherton PM, Derr J, Mitchell DC, *et al.* The role of fatty acid saturation on plasma lipids, lipoproteins, and apolipoproteins: I. 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(1): 121-9.
[http://dx.doi.org/10.1016/0026-0495(93)90182-N] [PMID: 8446039]

[90] Connor WE. Harbingers of coronary heart disease: dietary saturated fatty acids and cholesterol. Is chocolate benign because of its stearic acid content? Am J Clin Nutr 1999; 70(6): 951-2.
[PMID: 10584037]

[91] Hu FB, Stampfer MJ, Manson JE, *et al.* Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr 1999; 70(6): 1001-8.
[PMID: 10584044]

[92] Ali F, Ismail A, Kersten S. Molecular mechanisms underlying the potential antiobesity-related diseases effect of cocoa polyphenols. Mol Nutr Food Res 2014; 58(1): 33-48.
[http://dx.doi.org/10.1002/mnfr.201300277] [PMID: 24259381]

[93] Dorenkott MR, Griffin LE, Goodrich KM, *et al.* Oligomeric cocoa procyanidins possess enhanced bioactivity compared to monomeric and polymeric cocoa procyanidins for preventing the development of obesity, insulin resistance, and impaired glucose tolerance during high-fat feeding. J Agric Food Chem 2014; 62(10): 2216-27.
[http://dx.doi.org/10.1021/jf500333y] [PMID: 24559282]

[94] Heiss C, Kelm M. Chocolate consumption, blood pressure, and cardiovascular risk. Eur Heart J 2010; 31(13): 1554-6.
[http://dx.doi.org/10.1093/eurheartj/ehq114] [PMID: 20462977]

[95] Rein D, Paglieroni TG, Wun T, *et al.* Cocoa inhibits platelet activation and function. Am J Clin Nutr 2000; 72(1): 30-5.
[PMID: 10871557]

[96] Flammer AJ, Hermann F, Sudano I, *et al.* Dark chocolate improves coronary vasomotion and reduces platelet reactivity. Circulation 2007; 116(21): 2376-82.
[http://dx.doi.org/10.1161/CIRCULATIONAHA.107.713867] [PMID: 17984375]

[97] Wright LE, Castell DO. The adverse effect of chocolate on lower esophageal sphincter pressure. Am J Dig Dis 1975; 20(8): 703-7.
[http://dx.doi.org/10.1007/BF01070826] [PMID: 239592]

[98] Businco L, Falconieri P, Bellioni-Businco B, Bahna SL. Severe food-induced vasculitis in two children. Pediatr Allergy Immunol 2002; 13(1): 68-71.
[http://dx.doi.org/10.1034/j.1399-3038.2002.00097.x]

[99] Steinman HA, Potter PC. The precipitation of symptoms by common foods in children with atopic dermatitis. Allergy Proc 1994; 15(4): 203-10.
[PMID: 7806078]