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Cacao and Human Health: from Head to Foot — A Review

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Cacao and Human Health: from Head to Foot – A Review

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(Short Title: **Cacao and Health**)

Cacao and Human Health: from Head to Foot – A Review

The cacao, as part of the wonderful nature, provides the mankind a wide variety of valuable food products and health benefits. The most known and universally relished product derived from this fruit is chocolate, an amazing and unique food for the human nutrition with records of consumption of similar products dating to 1000 years B.C. In fact, the cocoa is a complex food that includes over 300 different components. This review is designed to inform scientists, technicians, academicians, farmers, and interested communities of numerous studies that have been conducted worldwide to investigate the properties of various cocoa constituents, their relations to human health, and their potential role in the prevention and treatment of many medical conditions. The general population, for example in Brazil, despite being one of the major producers of cocoa, is poorly informed of the significant and beneficial properties of cocoa. The present review covers important topics linking cocoa to human health and show the state of the art of effect of cocoa in different systems that comprise the human body. The paper is organized based on the main human organ system and includes: Cardiovascular / Circulatory, Neurological / Nervous, Oral health, Endocrine, Lymphatic and Immunological, Respiratory, Reproductive, Dermatological systems. Scientific findings tend to confirm the historic designation of cacao as "food of the Gods".

Key Words: *Theobroma cacao* L., food, antioxidant, heart, neurology, immunology.

Introduction

The nature has a huge, effective and wonderful collection of plant species that offer several beneficial properties to human nutrition and health. These plant species represent important sources of raw materials essential for maintaining human existence and improving its quality of life.

The cocoa tree, which forms the basis for one of the world's most popular food products-chocolate, has a rich history involving many cultures and carrying important economical and social implications to millions of peoples worldwide. In recognition of its multiple health benefits, the Maya gave cocoa its ancient name "kakawa" which translates into ''Food of the Gods". The Maya of Central America are credited for being the first people to consume cocoa. The health-promoting properties of cocoa have also been celebrated by the ancient Mesoamerican society with historical records revealing more than 150 applications of cocoa for medicinal purposes. Christopher Columbus was the first European to encounter cocoa in around 1502 A.D. But it was not until a quarter of a century later that the great Spanish conqueror Hernán Cortéz introduced cocoa into Europe. By the mid-1600s, cocoa was used in Europe as a medicine that promotes health, and as a cure for all manner of aliments (Pucciarelli and Grivetti, 2008). Cocoa was particularly valued for its ability to treat upper respiratory tract conditions such as colds and coughs, enhance mental well-being and to protect against nutritional deficiencies (Pucciarelli and Grivetti, 2008; Selmi et al., 2008; Tomaru et al., 2007).

In 2005, a meeting was set at Lucerne, Switzerland, where scientists and medical experts gathered to discuss the latest evidence on the potential health properties of cocoa. Since then,

numerous studies have appeared in the literature indicating that the value of cocoa goes beyond its nutritional properties to potentially disease prevention. Chocolate have for several years been studied for its possible beneficial health effects (Cooper et al., 2008; Sarmadi et al., 2011).

Cocoa is a complex plant product that contains over 300 different constituents (Fung, 2011), even in roasted beans (Keeney, 1972). Its major components include: cocoa butter (oleic, stearic, and palmitic fatty acids), minerals (magnesium, potassium, iron and zinc), methylxanithines (theobromine and caffeine), polyphenols in addition to other compounds such as tyramine, tryptophan, and serotonin. Polyphenols are a large group of compounds found in fruits and vegetables. Recently attention has been drawn to these compounds because of their ability to improve health and prevent numerous diseases owing to their antioxidant properties and their potential anti-inflammatory and cardio-protective properties (Wollgast and Anklam, 2000). For these compounds from chocolate were directed the cause of significantly improved Chalder Fatigue Scale score, a measurement of symptoms for subjects with chronic fatigue syndrome (Sathyapalan, 2010). One class of polyphenols is the flavonoids which is present in high concentration in cocoa (Stoclet et al., 2011; Tzounis et al., 2011; Jalil and Ismail, 2008; Mehrinfar and Frishman, 2008), and because this, cacao seeds was recently called as “Super Fruit” (Crozier, 2011). The fiber of cocoa can be considered as an excellent source of dietary fiber, and therefore could be used as an ingredient in functional foods rich in fiber. Besides this, fiber cocoa would provide protection against oxidative damage through its content in phenolic compounds (epicatechin) (Lecumberri et al., 2007). The pharmacokinetics of cocoa flavanols are rapidly absorbed in the intestine and metabolized (Spencer et al., 2001).

The present reviews deals with some of the literature concerning the health properties of cocoa and its related products and constituents. It is aimed at researchers, academics, farmers, and general public who would like to gain a broad knowledge of the most recent evidence. The structure of the review spans round the main systems in the human organ and include:

1. Cardiovascular / Circulatory system
2. Neurological / Nervous system
3. Endocrine system
4. Oral health
5. Lymphatic and Immunological system
6. Dermatological system
7. Respiratory system
8. Reproductive system

Cardiovascular / Circulatory

The cradioprotective effects of cocoa can be summarized to include effects on blood pressure, endothelium function, lipid profile and platelet function (Beckett, 2008)

Blood pressure

The interest in the effects of cocoa intake on the cardiovascular system was initially triggered by the observation that the indigenous Kuna Indians, a population living in an island

off the coast of Panama, have a very low incidence of ischemic heart disease, stroke and hypertension (Bayard et al., 2007; Hollenberg et al., 1997). These Kuna Indians consumed over 5 cups of flavanol-rich cocoa containing 900mg of polyphenols per day (Hollenberg et al., 2006). These observations are supported by several European epidemiological studies. In the Zutphen Elderly cohort, in particular, habitual intake of cocoa has been found to be inversely associated with approximately 50 % reduction of 15-year cardiovascular and all-cause mortality (Buijsse et al., 2006). Individuals from the highest tertile of cocoa also had lower blood pressure compared to those from lowest tertile (Buijsse et al., 2006). Similar observations have been made in the Potsdam European Prospective Investigation into Cancer and Nutrition cohort (Buijsse et al., 2010). Consumption of polyphenol-rich chocolate has been associated with lower risk of cardiac mortality after first myocardial infarction in the Stockholm Heart Epidemiology Program (Janszky et al., 2009). Nonetheless, in a more recent cohort, only moderate habitual intake (1-2 servings per week or 1-3 servings per month) was found to be protective against heart failure while no effect was observed for cohorts members who consumed more than 3 servings of chocolate per week (Mostofsky et al., 2010). In general, the value of epidemiological findings is limited by the possible correlation between individual food or nutrient intake and other dietary and non-dietary factors that may confound associations. This may explain some of the differences in findings. Likewise, data from epidemiological studies could often under-estimate the true effect of cocoa and chocolate consumption since most cocoa products available on the market are poor in polyphenols. As such evidence from cohorts studies need to be reinforced by randomised controlled trials.

To date, findings from randomised controlled trials suggest a potential role for flavanol-rich chocolate and cocoa products in non-pharmacological treatment of high blood pressure (Hernández et al., 2011; Sanchez et al., 2010; Corti et al., 2009; Selmi et al., 2008). A recent literature evaluation by Ried et al. (2010) has concluded that dark chocolate is superior to placebo in reducing systolic hypertension or diastolic prehypertension. Taubert et al. (2007) were amongst the first researchers to report the blood-pressure lowering effects of chocolate in a randomised controlled trial. In their trial, 14-day consumption of polyphenol-rich chocolate induced a 5.1 (SD 2.4) and 1.8 (2.0) mm Hg reduction in systolic and diastolic blood pressure compared to white chocolate, respectively. This study was followed by a series of evaluations confirming on the consumption of 100g dark chocolate containing 500mg polyphenols was found to reduce blood pressure in healthy adults (Grassi et al., 2005a) and patients with essential hypertension (Grassi et al., 2005b, 2008). Recently efforts have been to develop commercially available polyphenol-rich cocoa products. One such product, CocoanOx, has been shown to produce similar reduction in blood pressure in rats to Captopril (Cienfuegos-Jovellanos et al., 2009).

Much speculation exists as to the cocoa constituent responsible for the blood-pressure lowering effects of cocoa. Earlier, studies used white chocolate as placebo which apart from not permitting effective blinding of human volunteers also differs in its nutrient composition since it does not contain minerals like magnesium and methyxanithines. In a recent study by van den Bogaard et al. (2010), theobromine-enriched cocoa was found to significantly increase 24-hour ambulatory systolic blood pressure while lowering central systolic blood pressure. These findings suggest that caution need to be exercised when interpreting the findings from earlier

cross-sectional studies. Moreover, the use of high quantities of chocolate, sometimes 100g per day, might not be feasible because of the high-fat content and energy density of chocolate. The effective polyphenol dose required to induce clinically relevant reduction in blood pressure also remains unclear. Recently, Almoosawi et al. (2010) has also shown that in overweight subjects intake of 20g of polyphenol-rich chocolate containing 500mg polyphenols produces similar reductions in blood pressure as 20g of polyphenol-rich chocolate containing 1000mg polyphenols chocolate. Regardless of these findings, evidence from long-term studies suggest that use of doses as low as 6.5g per day induces clinically relevant reduction in blood pressure by increasing bioactive nitric oxide levels (Taubert et al., 2007).

In relation to the mechanism by which polyphenol-rich chocolate reduces blood pressure it appears to be related to increased nitric oxide bioavailability, or a maintenance of optimal nitric oxide levels, that could be associated with lowering superoxide anion production in the vasculature (Fraga et al., 2011); which then translated into improvement in arterial stiffness and endothelial function, as outlined below.

Endothelium function

Cocoa and chocolate produce their cardio-protective effects via modulation of nitric oxide bioavailability, hence endothelium function. In-vitro and in-vivo studies have shown that flavanol-rich cocoa reduces the activity of arginase, the main enzyme involved in the inactivation of nitric oxide (Schnorr et al., 2008). This results in a rise in the plasma concentration of nitrose compounds, which can then be expressed as improved brachial artery flow-mediated dilation (Yokoi et al., 2011; Sies et al., 2005). Endothelial dysfunction is an early marker of

atherosclerosis development. Thus, the ability of polyphenol-rich cocoa and its products to reverse endothelial dysfunction suggests that such products could be used at early stages of disease to prevent or delay progression to cardiovascular events such as stroke. Evidence of the beneficial effects of cocoa and its related products on endothelium function are consistent and range from effects on healthy volunteers (Fisher et al., 2006a; Schroeter et al., 2006) to individuals with high cardiovascular risk factors such as hypertensives (Grassi et al., 2005b), insulin-resistant (Grassi et al., 2008) and obese subjects (Davison et al., 2008). Currently there is even data to suggest that polyphenol-rich chocolate consumption improves endothelium-dependent responses in the coronary circulation, in addition to decreasing shear stress-induced platelet adhesion in heart transplant recipients (Flammer et al., 2007). Interestingly, flavanol-rich cocoa appears to improve blood pressure and endothelial function to a greater extent in the elderly compared to younger healthier subjects (Fisher et al., 2006a). This is unsurprising elderly (Fisher et al., 2006b). Currently debate still exists as to whether polyphenol-rich products can be of any benefit to healthy young adults. What remains evident is that the timing of dietary intervention is critical to ensuring maintenance of health and disease prevention, since the use of diet, on its own, may be less effective at more advanced stages of disease.

Lipid profile and peroxidation

In a meta-analysis of eight short-term trials, consumption of cocoa was found to elicit a 5.87 mg/dL (95% CI: −11.13, −0.61; $P < 0.05$) reduction in LDL cholesterol (Jia et al., 2011). This effect was only observed in individuals who consumed low doses of cocoa and in those with high cardiovascular disease risks. By contrast, only a marginal reduction in total cholesterol

(mean reduction = -5.82 mg/dL; 95% CI: −12.39, 0.76; *P* = 0.08) was detected, and no significant change in HDL cholesterol. Similar observation, have been made in a recent systematic review which included 10 short-term trials (Tokede et al., 2011). However, in the latter meta-analysis, a significant reduction in total cholesterol was seen (mean reduction = -6.23 mg/dl (−11.60, −0.85 mg/dl). Moreover, short-term trials showed greater improvements in lipid profile compared to long-term clinical trials, possibly implying a potential physiological adaptation to high polyphenol intake with long-term intake. The minor differences between the two meta-analyses could be related to differences in the studies included in analysis. In the meta-analysis conducted by Tokede et al. (2011), both short-term (2 week) and long-term trials (up to 12 weeks) as well as studies on overweight individuals (Almoosawi et al., 2010, Davison et al., 2008) and elderly were included (Crews et al., 2008). Alternatively, inter-individual variations in the absorption of cocoa polyphenols could dictate the extent of the lipid-lowering effects of cocoa products, which may then produce differences in results. In a randomised-controlled trial involving forty-two volunteers with high cardiovascular risk, consumption of 40g of polyphenol-rich cocoa dissolved in 500ml of skimmed milk reduced oxidised LDL levels and increased HDL cholesterol (Khan et al., 2011). However, the increment in HDL cholesterol and the reduction in oxidised LDL were found to strongly correlate with urinary cocoa polyphenol metabolites excretion. More recently, consumption of polyphenol-rich cocoa was shown to even protect Type 2 diabetes patients against an atherogenic lipid profile when cocoa is consumed as part of a balanced diet. Thus, consumption of 45g of polyphenol-rich chocolate for 16 weeks was found to significantly reduce HDL cholesterol (1.16 ± 0.08 *vs* 1.26 ± 0.08 mmol/l, *P* = 0.05)

and cholesterol: HDL ratio (4.4 ± 0.4 *vs* 4.1 ± 0.4 mmol/l, $P = 0.04$) in patients with type 2 diabetes, without producing any changes in weight or glycaemic control (Mellor et al., 2010).

It is important to note that it is the polyphenol composition of cocoa and not the amount of cocoa solids that determine the beneficial properties of cocoa and chocolate (Efraim et al, 2011; Coper et al., 2008; Gu et al., 2006; Miller et al., 2006). In young (18-20 years old) male soccer players, consumption of flavonol-rich milk chocolate, with a low cocoa solids content, has been shown to be associated with improvements in several parameters associated with cardiovascular health and oxidant stress in healthy young soccer players (Fraga et al., 2005). Thus consumption of milk chocolate was found to decrease diastolic blood pressure (- 5 mm Hg), mean blood pressure (- 5 mm Hg), plasma cholesterol (-11%), LDL-cholesterol (-15%), malondialdehyde (- 12%), urate (- 11%) and lactate dehydrogenase (LDH) activity (- 11%), and to increase in vitamin E/cholesterol (+ 12%) (Fraga et al., 2005). The fatty acid compsotion of cocoa butter may also explain the lipid-lowering or neutral effects of chocolate. The main saturated fatty acid found in cacao butter is stearic acid. Stearic acid has a neutral effect on blood lipids (Hannum and Erdman, 2000). Cocoa also contains high amounts of monounsaturated fatty acids which are known to have a favorable effect on lipid profile.

In addition to improving lipid profile, cocoa polyphenols can influence LDL oxidation. Oxidative modification of LDL is a critical stage of atherogenesis, and one of the mediators is the pro-inflammatory pro-atherogenic enzyme myeloperoxidase. Micromolar concentrations of (-)-epicatechin or other flavonoids can suppress myeloperoxidase-induced lipid peroxidation of LDL (Sies et al., 2005). Consumption of cocoa fiber has also been shown to reduce lipid peroxidation in animal models (Lecumberri et al., 2007). Earlier, it was observed that soluble

cocoa fiber product in an animal model of dietary-induced hypercholesterolemia, diminished the negative impact of the cholesterol-rich diet, buffering the decrease of high density lipoprotein-cholesterol, and the increase of total and low density lipoprotein-cholesterol levels, and lipid peroxidation (malondialdehyde levels) induced by the fatty diet. The soluble fiber product also decreased triglyceride levels to values lower than those in the group fed the cholesterol-free diet (Ramos et al., 2008)

Anti-platelet

Platelet activation and aggregation play a key role in inflammatory processes and form part of the initial stages of arterial thrombosis development. Factors such as ADP, thrombin or collagen activate platelets and stimulate their aggregation while prostaglandins inhibit these processes.

Cocoa, like aspirin, possesses potential anti-thrombotic properties. The ability of polyphenol-rich cocoa and chocolate to modulate platelet function has been observed both ex-vivo (Rein et al, 2000a-c) and in-vivo (Schramm et al., 2003; Holt et al., 2002; Pearson et al., 2005). Some inconsistencies remain with regard to the magnitude of cocoa's anti-platelet effects. According to Pearson et al. (2005) cocoa has a less profound inhibitory effect on epinephrine-stimulated platelet activation and function than aspirin. However, the combination of the two produces an additive effect (Pearson et al., 2005). Heptinstall et al. (2006), on the other hand, reported similar inhibitory effects of cocoa flavanols/ metabolites and aspirin on platelet activation/aggregation, platelet-monocyte conjugate formation platelet-neutrophil conjugate formation in-vitro, with no additive effect when combining cocoa flavanols and

aspirin. Differences in the results could be attributed to differences in the techniques used to assess platelet function or variations in the products used. Regardless of these inconsistencies, several studies have revealed potential mechanisms by which cocoa ad its related products can protect against thromboemolic diseases. Cocoa procyanidins can inhibit platelet activation by altering eicosanoid synthesis. Treatment of aortic endothelial cells with chocolate procyanidins doubles keto-prostaglandin $F_{1\alpha}$ production and reduces leukotriene production by 16% (Schramm et al., 2003). When high-procyanidin chocolate (37g containing 4.0 mg/g procyanidins) is acutely consumed, this produces a 37% increase and a 29% decrease in plasma prostacyclin and leukotrienes concentrations, respectively, in healthy volunteers (Schramm et al., 2003). Cocoa can also diminish platelet function by significantly reducing P selectin expression and by lowering ADP- and collagen-induced aggregation (Murphy et al., 2003, Innes et al., 2003). This effect correlates with a rise in plasma ascorbic acid, epicatechin and catechin concentrations (Murphy et al., 2003). Pre-treatment of human aortic endothelial cells with catechin and quercetin metabolites has also been shown to influence monocyte adhesion (Koga and Meydani, 2001). According to Koga and Meydani (2001), the flavonoid metabolites, as opposed to the intact flavonoids, are responsible for the ability of flavonoids to modulate platelet function. In a double-blind, randomized study involving 22 heart transplant recipients, consumption of 40 g of dark (70% cocoa) chocolate reduced platelet adhesion decreased from 4.9+/-1.1% to 3.8+/-0.8% (P=0.04) (Flammer et al., 2007).

In a large cross-sectional analysis assessing habitual chocolate consumption in 1535 subjects, chocolate consumers showed longer *platelet function analyzer* closure times (130 *vs* 123 seconds, P=.005) and decreased *11-dehydro thromboxane B2* (175 *vs* 290 ng/mol creatinine,

P=.03) (Bordeaux et al., 2007). Chocolate remained an independent predictor of both ex vivo and in vivo platelet function testing after controlling for age, sex, education level, race, cigarette smoking, and BMI and values of glucose, blood pressure, total cholesterol, fibrinogen, and Von Willbrand Factor. Bordeaux et al. (2007) concluded that consumption of small quantities of chocolate (5.9g) can improve platelet function.

Neurological/ Nervous System

Interest in the neurological and neuroprotective effects of cocoa and chocolate polyphenols stemmed from findings that cocoa and chocolate can enhance vascular function by modulating nitric oxide bioavailability. Nitric oxide is central to the regulation of peripheral vascular. However, it also plays a key role in the cerebral circulation where it promotes brain perfusion. Studies suggest that daily intake of cocoa flavanols can enhance blood flow and perfusion of the brain via its stimulatory effect on nitric oxide bioavailability (Pate et al., 2008). Indeed, in a study conducted on thirty-four healthy elderly volunteers aged 72±6 years, Sorond (2008) used transcranial Doppler ultrasound to measure blood flow velocity in the middle cerebral artery. Sorond (2008) observed a consistent increase in blood flow velocity from 8% after 1 week of cocoa consumption to 10% following 2-week consumption of flavanol-rich cocoa. These findings were validated in a later study in which changes in middle cerebral artery flow, assessed by transcranial Doppler ultrasound, strongly correlated with changes in perfusion measured by gadolinium-enhanced MRI and arterial spin labelling (Sorond et al., 2010).

Increased blood oxygenation and blood flow to brain grey matter have also been reported (Field et al., 2011; Francis et al., 2006). These findings suggest a potential role for cocoa flavanols in the treatment and prevention of cerebrovascular diseases such as dementia and stroke.

Indeed, several studies indicate that polyphenols can reduce the risk of neurodegenerative diseases particularly those stemming from oxidative stress, such as Alzheimer's and Parkinson's disease. Incubation of neuronal cells with cocoa extract or (-)-epicatechin dose-dependently reduces reactive oxygen species production and down regulates the stress-kinases: pJNK and p38, which are involved in activation mitogen-activated protein kinase pathway (Ramiro-Puig and Castell, 2009). In models of Parkinson's disease, pretreatment of male adult Sprague-Dawley rats with cocoa extract at a dose of 100 mg/kg/day has also been reported to diminish oxidative stress-induced 6-hydroxydopamine-induced dopaminergic loss (Datla et al., 2007). Pre-treatment of neurons with cocoa extract has also been reported to reduce expression and release of calcitonin gene-related peptide expression, a factor that promotes neural inflammation (Abbey et al., 2008) and migraine development (Li et al., 2008). Cocoa also exerts protective effects against amyloid β protein-induced neurotoxicity (Yasuda et al., 2011; Heo and Lee, 2005), which is relevant to Alzheimer's disease, a neurological dementia caused by accumulation of amyloid plaques and neurofibrillary tangles in the brain and which is characterised by memory loss and progressive decline in cognitive function. Indeed, cocoa can protect against cognitive decline associated with normal aging. In old Wister-Unilever rats, 1-year oral administration of a cocoa polyphenolic extract, at a dose of 24 mg/kg per day, delays the onset of age-related cognitive deficits and maintains high urinary free dopamine levels (Bisson et al., 2008b). In a large trial involving 2031 human subjects, habitual chocolate

consumption predicted improved cognitive performance (Nurk et al., 2009). In fact, the association between chocolate consumption and cognitive performance was dose-dependent, with maximum beneficial effect achieved at intakes of ~10 g of chocolate per day (Nurk et al., 2009).

It is important to state that chocolate is also rich in fat and contains amines such as tyramine, histamine and phenylethylamine that can be linked to headaches. In a double blind study of headache was performed using chocolate as the active agent and carob as the placebo. Sixty-three women with chronic headache (50% migraine, 37.5% tension-type, 12.5% combined migraine and tension-type) participated in the study. Diaries were maintained by the subjects throughout the study, monitoring diet and headache. But contrary to the commonly held belief of patients and physicians, chocolate didn't play a significant role in trigger headaches in typical migraine, tension-type, or combined headache sufferers (Marcus, 1997). However, in patients with migraine who believed that chocolate could provoke their attacks, chocolate ingestion was followed by a typical migraine episode in 5 out of 12 patients, while none of the 8 patients challenged with placebo had an attack ($p = 0.051$) (Gibb, 1991). And in other study with four hundred twenty-nine patients that had migraine, 16.5% reported that headaches could be precipitated by cheese or chocolate, and nearly always by both. There was a statistical association between sensitivity to cheese/chocolate and to red wine in patients with migraine, and related more to migraine than to more chronic tension-type headache (Peatweld, 1995). Therefore, evidence of the association between chocolate consumption and headaches remains conflicting.

In addition to age-associated neurodegenerative diseases, cocoa and chocolate consumption can play a role in the prevention of neurological disorders such as depression. Depression is a common condition that affects a large proportion of the population nowadays. A recent study found that the addition of high-flavanol cocoa extract given to rats in a forced swimming test reduces depression (Messaoudi et al., 2008). This effect has been attributed to the conversion of tryptophan from cocoa into serotonin and the presence of some compounds in cocoa that alleviate mood, thereby protecting against depression. Raikkonen et al., (2004) evaluated prenatal frequency of chocolate consumption and its relation to intensity of psychological stress in 305 mothers. The temperament of infants at 6 months postpartum was also evaluated. Chocolate was found to produce subjective feelings of psychological well being, to reduce maternal stress and improve infant temperant. Similar observations have been made in elderly men where consumption of chocolate was associated with improved overall health and better psychological well-being (Strandberg et al., 2008). Together this evidence favors a potential role for cocoa and chocolate polyphenols in maintaining cognitive function through the life course and preventing age-related development of cerebrovascular disease.

Endocrine

Growing evidence suggests that polyphenols in cocoa can modulate the endocrine system. In studies on diabetic-obese mice, administration of cocoa liquor procyanidins for 3 weeks have been shown to dose-dependently reduce fasting glucose and fructosamine levels

(Tomaru et al., 2007). Likewise, acute reductions in postprandial glucose have been reported by Jalil et al. (2008), with this effect coinciding with an improvement in antioxidant defense mechanism as demonstrated by enhanced superoxide dismutase activity and reduction in 8-isoprostanes. Cocoa supplementation can also reduce free fatty acids, which is important since fatty acids can impair glucose metabolism. Despite this evidence, no changes in fasting glucose have yet been reported with long-term administration of cocoa extract (4 weeks) in neither mice nor humans. The reason behind these differences in effect remains to be clarified, but could be related to the short half-lives of cocoa polyphenols which leads to rapid metabolism and excretion. As a result, the benefit of cocoa consumption may lie in its transient effects on postprandial glucose levels.

Despite this evidence, recent trials in humans show that consumption of polyphenol-rich dark chocolate can improve insulin sensitivity in healthy subjects (Grassi et al., 2005a), hypertensives (Grassi et al., 2005b), insulin-resistant individuals (Grassi et al., 2008) and overweight subjects (Almoosawi et al., 2010). These effects could be attributed to the ability of polyphenol-rich dark chocolate to improve endothelium function and antioxidant status. Only two studies have so far investigated the effect of cocoa consumption on glucose and insulin regulation in diabetics. None of these studies observed any significant improvement in glucose levels or insulin resistance. Several explanations could be provided to explain such inconsistencies. First, most previous trials conducted on non-diabetic patients compared the effect of polyphenol-rich dark chocolate to polyphenol-deficient white chocolate. This implies that volunteers were not blinded to treatment allocation which may have biased the results. Additionally, white chocolate differs in its macro- and micro-nutrient composition to dark

chocolate since it does not contain methylxanithines or minerals such as magnesium. This implies that differences in the effect of dark chocolate and white chocolate may be related to components other than polyphenols. Most of the above trials were also of short duration (2 weeks) and used large amounts of chocolate (100g). Thus, the benefit of cocoa or chocolate consumption may lie in its ability to reduce diabetes-related metabolic complication as opposed to improving glycaemic control. This notion could be supported by several studies. For instance, administration of proanthocyanidins derived from cacao has been shown to inhibit diabetes-induced cataract formation, possibly by improving antioxidant activity (Osakabe et al., 2004). Similarly, supplementing the diet with 1-2% cocoa extract of diabetic rats has been shown to increase HDL-cholesterol and the reduction in LDL-cholesterol (Ruzuadi et al., 2005). In humans, Balzer et al. (2008) demonstrated that thrice-daily consumption of polyphenol-rich cocoa for 30 days reversed vascular dysfunction in medicated diabetic patients. Similarly, in a study on type 2 diabetics, daily intake of cocoa for 16 weeks raised HDL-cholesterol and reduced total cholesterol-to-HDL ratio (Mellor et al., 2010). All of these findings suggest that cocoa consumption may benefit diabetics by reducing cardiovascular risk factors.

Cocoa consumption can also be of benefit to obese individuals because of its low calorie density in comparison to chocolate. Consistent with this, cocoa polyphenols have been shown to prevent diet-induced obesity by modulating lipid metabolism, especially by decreasing fatty acid synthesis and transport systems, and enhancing thermogenesis in hepatic and white adipose cells (Matsui et al., 2005). In humans, long-term ingestion of a polyphenol-rich cocoa drink has been reported to improve endothelial function (Davison et al., 2008). Certain constituents in cocoa

also have the potential to modulate glucocorticoid metabolism which could be of relevance to obesity-related complications (Almoosawi, 2011).

Oral health

Addai et al. (2002) screened a large selection of foods for their acidogenic effects on teeth. To their surprise, they found that the Golden Tree brand of milk chocolate produced in Ghana, which contained 30% cocoa solids, was not acidogenic. This finding prompted researchers to conduct further trials in an attempt to understand the mechanisms underlying their observations.

Evidence of the protective effect of cocoa against dental caries has been, in fact, documented as early as 1985, when Paolino and Kashket observed that cocoa inhibited plaque accumulation and caries formation by reducing polysaccharide production. Since then, several studies have shown the ability of cocoa to reduce the risk of dental caries and prevent periodontal disease. Rats infected with Streptococcus sobrinus showed a significant reduction in caries scores after administration of a water-soluble cacao extract compared to infected rats given a control diet (Ito et al., 2003). This protective effect was attributed to the inhibitory activity of cocoa extract on glucan synthesis by Streptococcus. Polymeric fractions from cocoa, in particular, appear to have immunomudulatory effects on the production of cytokines IL-1β (IL-1β), IL-2 and IL-4 (Hirao et al., 2010; Mao et al., 2002). Larger oligomeric fractions (hexamer through decamer) have also been reported to inhibit IL-5 release, which may promote

immunoglobulin A (Mao et al., 2002). More recently, Percival et al. (2006) demonstrated that cocoa polyphenols can delay acid production by Streptococcus mutans. The growth of streptococcus sanguins and formation of biofilm have also been shown to be inhibited by cocoa dimer, tetramer and pentamer polymers (Percival et al., 2006). Cocoa also contains tannins which possess anti-bacterial and anti-enzymatic properties (Beckett, 2008). Recently, Ferrazzano et al. (2009) reviewed the mechanisms of the anti-cariogenic effects of cocoa polyphenols and concluded that products like cocoa may have potential applications in the prevention of dental caries. Despite this evidence, it is important to highlight that findings from the above experiments need to be interpreted with caution since the majority of cocoa and chocolate products currently available on the market are low in polyphenols and high in simple sugars which can be detrimental to oral health. Thus, the use of cocoa polyphenols as means of improving periodontal health will only be possible once appropriate formulations and products are developed.

Lymphatic and Immunological

Chronic and acute inflammation underlies the molecular basis of many cardiovascular and cerebrovascular diseases. The role of cocoa in modulating inflammatory markers and immune responses has been reviewed by several researchers (Selmi et al., 2006; Selmi et al., 2008; Ramiro-Puig and Castell, 2009). This role has been established in both in-vitro and in-vivo

studies and could be summarised to include effects on the innate and the acquired immune system.

Accordingly, isolated flavanols and procyanidins fractions have been shown to attenuate the release of inflammatory cytokines: interleukin-1b and interleukin-2 from peripheral blood mononuclear cells (Mao et al., 2002), and to promote the production of anti-inflammatory cytokines such as interleukin-4 and interleukin-5 in an oligomer length-dependent manner (Mao et al., 2002). It was also observed that the effect of cocoa flavanols and procyanidins on peripheral blood mononuclear cells depends on the immunological status of an individual. Cocoa flavonols and procyanidins stimulated peripheral blood mononuclear cells individuals with low cytokine transforming growth factor -1 but attenuated peripheral mononuclear cell isolated from individuals with high baseline transforming growth factor -1.

In addition to influencing peripheral mononuclear cell, cocoa flavonoids have been shown to affect the release of inflammatory cytokines and chemokines from macrophages (Ramiro-Puig et al., 2005). This inhibitory effect is achieved at the transcriptional level as evident by the down-regulation of TNFα, interleukin (IL) 1α, and IL-6 mRNA expression.

Besides the innate immune response, cocoa can modulate the acquired immune response. In a study conducted by Ramiro-Puig et al. (2005), incubation of cocoa extract with stimulated murine EL4.BU.OU6 cells dose-dependently inhibited IL-2Rα (CD25) expression, an early marker of lymphocyte T activation. This effect was likely to be modulated through epicatechin as both cocoa extract and epicatechin produced similar levels of inhibition. Cocoa flavonoids also induced a 3-fold rise in IL-4 release. These findings have been recently replicated in-vivo. Perez-Berezo et al. (2009) demonstrated that supplementing the diet of adult Wistar rats with a

cocoa-enriched diet for 9 weeks attenuates the Th2 immune response leading to reduced antibody synthesis. In another study, weaned rats were given cocoa (4% or 10% food intake), containing 32 mg flavonoids/g, for 3 weeks (Ramiro-Puig et al., 2007b). The diet with the highest cocoa content was found to diminish TNF-α secretion by peritoneal macrophages. The high-cocoa diet also promoted lymphocyte proliferation rate but attenuated T helper 2-related cytokines and Ig secretion and decreased the number of Th cells. Interestingly, one study has shown that the highest uptake and accumulation of cocoa metabolites occurs in lymphoid tissues, particularly the thymus, which reinforces a role for cocoa polyphenols in modulating lymphocyte composition in this tissue (Urpi-Sarda et al., 2010).

The ability of cocoa polyphenols to affect both the innate immune system and the adaptive immunity has also been recently reported by Kenny et al. (2007). Kenny et al. (2007) demonstrated that the chain length of flavanols determines the extent of cytokine release from both unstimulated and LPS-stimulated peripheral blood mononuclear cells. Long-chain flavanols, in particular, were found to increase LPS-induced synthesis of IL-1β, IL-6, IL-10, and TNF-α. By contrast, both long-chain and short-chain flavanols increased expression of the B cell markers CD69 and CD83.

Cocoa polyphenols have been speculated to act as general leukotriene inhibitors. Schewe et al. (2001) demonstrated that (-)-epicatechin and its low-molecular procyanidins, i.e. dimers and to a lesser extent trimers through pentamers, inhibit both dioxygenase and LTA_4 synthase activities of human 5-lipoxygenase. In earlier studies, Schewe et al. (2001) reported inhibition of 15-lipoxygenase-1, an important catalyst of lipid peroxidation, by (–)-epicatechin and cocoa procyanidins (Schewe et al., 2001). Here, higher oligomers exhibited greater inhibitory activity

than monomers and medium-sized oligomers. Cocoa flavonoids were also found to inhibit mammalian 12-lipoxygenases (Schewe et al., 2001).

It is important to observe that the value of some of the above findings is limited by the in-vitro design of these studies and the use of pharmacological doses of cocoa polyphenols. Nevertheless, because macrophages play an important role in the innate immune response and inflammation and are implicated in the development of atherosclerotic lesions and cancer cell proliferation, a potential role for cocoa polyphenols in preventing atherosclerosis and cancer development could be speculated. Likewise, as suggested by Ramiro-Puig et al. (2007a), cocoa's capacity to modulate macrophage cytokine secretion and lymphocyte function could be relevant to hypersensitivity and autoimmunity. Consequently, further research is required to replicate these findings in humans and to evaluate the clinical implications of these findings. Finally, it could be stated that some authors have also speculated a role for cocoa in the prevention of malaria by virtue of cocoa's ability and the ability of cocoa constituents (polyphenols, magnesium and zinc) to improve nitric oxide bioavailability, increase antioxidant status and to boost the immune system (Addai, 2010).

Immunostimulation have been also observed as a health-promoting effect of the microflora. Among effective prebiotics, cacao-derived flavonols was identified affecting the growth of select gut microflora in humans to a healthy composition (Tzounis, 2011).

Dermatological

In the past, chocolate has often been viewed as a food that promotes acne production. However, this common belief has not been supported by scientific evidence. Among the first to question the link between chocolate and acne were researchers from the University of Missouri in the 1960s (Grant and Anderson, 1965). Grant and Anderson failed in their attempt to induce an acne flare-up in eight individuals with mild to moderate acne by feeding them a large amount of chocolate. The authors discredited the assertion that chocolate causes acne. In a study examining the effect of chocolate intake on acne, 65 participants consumed 112g of dairy-free cocoa-enriched bars of chocolate each day for 4 months. Researchers found no significant change in acne production (Ferdowsian, 2010; Fulton et al., 1969). These findings were consistent with other studies (Anderson, 1971; Grant and Anderson, 1965). In an extensive review of research on chocolate and acne (Fries, 1978), it was concluded that the general trend of published reports suggested that chocolate ingestion was unrelated to the cause of acne. In last years it was observed the effect of other foods in acne occurrence. A positive association with acne for intake of total milk and skim milk was identified in a study involving 47,355 women (Adebamowo, 2005), later reinforced in an evaluation with 6,094 girls, aged 9-15 (Adebamowo, 2006) and with 4273 boys, where a positive association between intake of skim milk and acne was found (Adebamowo, 2008). This finding suggested that skim milk contains hormonal constituents, or factors that influence endogenous hormones, in sufficient quantities to have biological effects in consumers (Danby, 2005; Melnik, 2009). Therefore, other compounds from chocolate, but not cocoa, can promote acne occurrence giving the mistaken conclusion of chocolate as an acne promoter.

More attention has been drawn to the dermatological properties of cocoa polyphenols recently. Dietary flavanols from cocoa have been shown to contribute to endogenous photoprotection, to improve dermal blood circulation, and to affect cosmetically relevant skin surface and skin hydration (Heinrich et al., 2006). Accordingly, consumption of high-flavanol cocoa for 12 weeks has been shown to decrease skin roughness and scaling compared to low-flavanol cocoa (Heinrich et al., 2006). This effect is potentially achieved through an increase in blood flow in cutaneous and subcutaneous tissues which leads to higher skin density and hydration (Heinrich et al., 2006). In another study the acute effects of a single dose of flavanol-rich cocoa on dermal microcirculation was investigated (Neukam et al., 2007). Flavanol-rich cocoa consumption acutely increased dermal blood flow and oxygen saturation. Pre-treatment with cocoa polyphenolic extract have also been demonstrated to confer a significant protection against oxidation of cultured human HepG2 cells submitted to oxidative stress induced by tert-butylhydroperoxide (t-BOOH) (Martin et al., 2008). There is also evidence that flavonoid-rich products contribute to the protection of skin against UV-induced damage at the molecular and cellular level, thereby improving overall skin conditions (Stahl, 2011). This finding has also been observed by Jorge et al. (2011) who showed that long term cocoa ingestion leads to an increased resistance against UV-induced erythema and a lowered transepidermal water loss **(**Jorge et al., 2011). A cosmetic formulation elaborated with 10% ozonized theobroma oil was also observed as protector against oxidant radiation in exerting beneficial effects in the restoring of the antioxidant activity on the skin of rats previously irradiated with ultraviolet light (Sanches et al., 2011).

Respiratory

This area of research has not been widely investigated. One study has reported that dietary supplementation of cacao liquor proanthocyanidins prevents lung injury induced by diesel exhaust particles in mice (Yasuda et al., 2008). This effect was achieved through down-regulation of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 and correlated with a decline in oxidative stress and lipid peroxidation (Yasuda et al., 2008). The authors concluded that these findings have potential relevance to asthma, tuberculosis and others respiratory conditions. Other evaluation, showed that flavanol-rich cocoa has been acted to reverse endothelial dysfunction, measured as flow-mediated dilation (FMD) of the brachial artery, in smokers (Heiss et al., 2005).

Reproductive

Most of the beneficial effects of cocoa on the reproductive system have been observed in animal models. The highest accumulation of epicaetechin metabolites occurs in the testes which has important implication for cancer prevention (Urpi-Sarda et al., 2010). Administration of a diet containing 0.5 to 2% cocoa-rich flavanols to male rats for 2 weeks have been shown to dose-dependently reduce oxidative DNA damage in rat testes (Urpi-Sarda et al., 2010). An in-vitro study evaluated the inhibitory effect of different cocoa polyphenols extracts, alone or combined

with beta-sitosterol (a common phytosterol which plays a protective role in cancer development) on human prostate cancer. The results showed that cocoa polyphenols extracts have antiproliferative effects on prostate cancer cell growth (Jourdain et al., 2006). The effect of a commercially-available polyphenol-rich cocoa on prostate carcinogenesis was evaluated in sixty Wistar-Unilever rats. Rats were treated orally with cocoa powder 24mg/kg or 48mg/kg or vehicle, daily for nine months. Cocoa markedly reduced prostate cancer incidence and increased the life span of the rats (Melnyk et al., 2011; Bisson et al., 2008a; Bisson et al., 2006). The authors concluded that polyphenol-rich cocoa can prevent prostate hyperplasia induced by testosterone propionate and therefore may be beneficial in the treatment of benign prostatic hyperplasia.

Final Considerations

Chocolate induces, in general, a feeling of pleasure as postulate by scientists (Fung, 2011). Because its composition cocoa mass remains solid at room temperature, but when consumed, its fat content absorbs heat from the mouth and melts at body temperature, producing the effect of 'melt in your mouth'.

Eating chocolate, as part of a healthful balanced diet, could potentially provide a beneficial enjoyable way to improving wellbeing. Chocolate can be a very nutritional component in food and the knowledge of its various medicinal properties represents a stimulus to those involved with its production, processing and consumption.

Recently science has advanced significantly in improving our understanding of the various features of chocolate that contribute to its popularity, and its after-effects of consumption on human health have also been extensively studied. Although its marketing as a health product is not a priority, eating in moderation, mainly the darker forms could potentially have many beneficial results. But one thing is certain, both from a scientific viewpoint as sensory, chocolate is enjoyed as a favorite food for most of the people.

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