The sweet side of dark chocolate for chronic kidney disease patients

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Abstract

Chocolate is a widely appreciated foodstuff with historical appreciation as a food from the gods. In addition to its highly palatable taste, it is a rich source of (poly)phenolics, which have several proposed salutogenic effects, including neuroprotective anti-inflammatory, anti-oxidant and cardioprotective capabilities . Despite the known benefits of this ancient foodstuff, there is a paucity of information on the effects of chocolate on in the context of chronic kidney disease (CKD). This review focusses on the potential salutogenic contribution of chocolate intake, to mitigate inflammatory and oxidative burden in CKD, its potential,, for cardiovascular protection and on the maintenance of diversity in gut microbiota, as well as clinical perspectives, on regular chocolate intake by CKD patients.

Keywords: chronic kidney disease, dark chocolate, oxidative stress, inflammation, gut microbiota.

Introduction

(Poly)phenolics are natural botanical compounds that, *in planta*, act against pathogens and protect the plants from ultraviolet radiation (Manach et al. 2004). In humans, dietary (poly)phenolic intake has been proposed to facilitate anti-inflammatory, antimicrobial and immunomodulatory effects (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Marzocchella et al., 2011). Tea, wine, coffee, vegetables, fruits, nuts, cereals and cocoa (often as chocolate) are common sources of (poly)phenolics in the diet (Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005).

Chocolate, a cocoa (*Theobroma cacao L*.) based product, is considered a good dietary source of (poly)phenolic bioactives,, of which proanthocyanidins are the most abundant, followed by monomeric flavan-3-ols, catechins and anthocyanins. Cocoa is also source of fatty acids, microelements and methylxanthine alkaloids (Aprotosoaie, Luca, & Miron, 2016; Seem, Yuan, & Tou, 2019). A number of studies have shown that chocolate consumption, has neuroprotective, anti-inflammatory and cardioprotective (blood pressure, endothelial function) effects, which include modulation of endothelium-dependent vasodilation and increase cerebral blood flow (Mayorga-Gross & Esquivel, 2019; Verna, 2013).

Many of these complications are common in chronic kidney disease (CKD) patients, and are associated with high cardiovascular risk and premature mortality. The role of foods rich in bioactive compounds as an alternative therapeutic to mitigate complications nicluding inflammation, oxidative stress and dysbiosis in CKD patients, has been discussed previously (Mafra, Borges, et al., 2019). A range of studies have identified optimized nutrition as one of the best strategies to prevent or reduce the complications in CKD. For example, cranberry consumption known historically as a strategy to treat urinary tract infections, can also be used to decrease inflammation and oxidative stress in CKD (De Almeida Alvarenga et al., 2019). Brazilian açai fruit has also been suggested as a food with anti-inflammatory and anti-oxidant action, and is a rich source of flavonoids (Martins et al., 2018). Moreover, studies with curcumin have shown interesting anti-inflammatory effects in CKD (Alvarenga et al., 2018; Alvarenga et al.

2020). Thus, much recent attention has focused on using (poly)phenolics in renal nutrition (Vargas et al. 2018).

Chocolate has many health benefits, but its' quality and salutogenic effects depend on the percentage of cocoa. Hence dark chocolate, which contains more cocoa bean solids of >80% of the total weight and cocoa butter, demonstrates the most health (Montagna et al., n.d.).Few studies, however, have been published regarding the effects of chocolate in patients with CKD (Rassaf et al., 2016). The aim of this review is to discuss the role of dark chocolate, as a source of cocoa rich in bioactive flavanol food, as a treatment option for CKD patients.

Chocolate

Chocolate is an ancient food, whose name is derived from the Nahuatl word *cacahuatl*. The Mayo-Chinchipe people cultivated cacao as long as 5,300 years ago in central America. For some religions, cocoa was considered to be of divine origin and gifted by the Gods (Christenson, 2007; Favazza, 1984; Maiorana, 2007). Chocolate was often consumed as a drink made from roasted and ground cocoa beans heated in water and flavored with pepper and cinnamon to mask the bitter taste of the beans. For a period, cocoa beans were also used as a bargaining chip by emperors (Verna, 2013). Initially, the cocoa-based drink was used only by male adults, especially rulers, priests and warriors, and it was considered a valuable and exclusively noble food (Maiorana, 2007). Over time the use of chocolate in cooking, as well as medicine spread to the Old World. The salutogenic effects claimed to date for chocolate have included anti-asthmatic (Physitian, 2020), calmative (All, 2020), anti-pyrogenic (De, 2020) and anti-oncogenic effects (Juárez López, 2012). Due to increased demand for the product, cocoa plantations have expanded in the Caribbean and Philippines (Cards et al., 2020; Kitto, 1920; Maiorana, 2007). In addition to planting cocoa, the dispersal of cocoa seeds in the wild occurs through animal vectors, such as monkeys and parrots (Maiorana, 2007).

Chocolate bioactives include methylxanthine and (poly)phenolics. In fact, cocoa is an important source of dietary (poly)phenolics (principally proanthocyanidins, catechins and anthocyanidins) (Wollgast & Anklam, 2000). The proanthocyanidins are classified as dimeric procyanidins type B and trimeric type C, flavonols, anthocyanins, stilbenoids,

phenolic acid derivatives, amides and amines. The procyanidins are composed of two, or more chemical bonds of catechin or epicatechin units.

The beneficial effects to human health have been attributed to the darker varieties of chocolate, with higher cocoa content compared to milk chocolate: the number of phenolic compounds and flavonoids in dark chocolate is around five times higher than milk chocolate and white chocolate. A common feature of all chocolate products is the high energy profile (30 g fat per 100g), with sugar content around 52 g per 100g in milk varieties (USDA). Both higher sugar and fat contents are therefore relevant for potential adverse health effects associated with high consumption (Petyaev & Bashmakov, 2017; Mello et al 2015)

Flavonoids present in cocoa display significant anti-oxidant capacity and the position of hydroxyl groups in the molecular structure seems to be a key factor for such activity. The configuration, substitution and total number of hydroxyl groups influence the antioxidant activity, such as radical scavenging and metal ion chelation capacity. The hydroxyl configuration of the B ring is the most important factor in the elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS), because it donates hydrogen and an electron to the hydroxyl, peroxyl and peroxynitrite radicals, stabilizing them and forming a more stable flavonoid radical (Cao, Sofic, & Prior, 1997; Kumar & Pandey, K., 2013).

Cocoa flavonoids, mainly epicatechin, are important mediators of cellular anti-oxidant and anti-inflammatory responses mainly to increase the mRNA expression Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. There are some mechanisms that can explain these benefits. Firstly, (poly)phenolics provide a substrate for the production of alkyl catechols, known as Nrf2 agonists (Senger, Li, Jaminet, & Cao, 2016; Stenvinkel, Meyer, Block, Chertow, & Shiels, 2019). Alkyl catechols and catechol are generated from some Lactobacillus species such as *L. plantarum and L. brevis, L. collinoides*, wich posses phenolic acid decarboxylase e cinnamoyl esterase. These compounds can liberate Nrf2 from Keap1in the cytoplasm, which can be translocated to the nucleous and increase the heme oxygenase-1 (HO-1), NAD(P) H:quinone oxidoreductase1 (NQO1), and glucose-6-phosphate dehydrogenase (G6PD) mRNA expression (Senger et al. 2016).

Secondly, (poly)phenolics present in cocoa can downregulate the phosphorylation of mitogen- activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3K) (Ali, Ismail, & Kersten, 2014; Cordero-Herrera, Martín, Goya, & Ramos, 2015; Rodríguez-Ramiro, Ramos, Bravo, Goya, & Martín, 2012), which can trigger the release of Nrf2 from the complex through phosphorylation of Nrf2 (Eggler, Gay, & Mesecar, 2008; Huang, Nguyen, & Pickett, 2000). Additionally, epicatechin mediates anti-inflammatory effects *in vitro* via inhibition of nitrite formation, inhibit ion of IL-1β -induced expression of iNOS, by blocking the nuclear p65 subunit of NF-κB (M. J. Kim et al., 2004) and via regulation of NF-κB, activating protein 1 (AP-1), decreasing the expression of a family of genes that encode por-inflammatory proteins (Ali et al., 2014). Furthermore, epicatechin can inhibit the IkB-kinases, leading to the suppression of the phosphorylation of IkB, inactivating nuclear factor-κB, thus attenuating the inflammatory reaction due reduction of cytokines synthesis (Li et al. 2019; Prince et al. 2019) (**Figure 1**).

Consequently, (poly)phenolics demonstrate positive cardiovascular effects, through action on endothelial vasodilation and platelet aggregation (Heiss et al., 2010; Keen, 2001; Kluknavsky et al., 2016; Taubert, 2008; Wang, Feltham, Suh, & Jones, 2019). This has been demonstrated in type-2 diabetic patients, with high-polyphenol chocolate ingested prior to induced acute transient hyperglycemia (Mellor et al 2013). Correspondingly, the effects on the endothelium were accompanied by a decrease in the expression of oxidative stress markers. In addition, the ability to regulate mitochondrial structure and function may contribute to the cardiac protection attributed to epicatechin (Chidambaram, et al. 2018). As polyphenolic compounds present in cocoa demonstrate these cardiovascular health benefits (Magrone, Russo, & Jirillo, 2017), there has been increased interest in cocoa within the pharmaceutical and nutraceutical spheres (Gammone et al., 2018).

Despite the many benefits demonstrated in these studies, the efficacity of this approach is limimited, in that dietary acquired (poly)phenolics have low bioavailability (Prakash, Basavaraj, & Chidambara Murthy, 2019; Zugravu & Otelea, 2019). Among

these (poly)phenolics, epicatechin, however, seems to be better absorbed than others (Williamson, 2009). After ingestion, epicatechin released from the food matrix becomes bio-accessible and absorbed into the gastrointestinal tract. In enterocytes, aglycones can be conjugated by phase II enzymes, resulting in sulfated/methylated and/or glucuronidated to form conjugated enzymes. These bioactive metabolites reach the systemic circulation and are subsequently excreted via urine or bile (Prakash et al., 2019).

Some unabsorbed epicatechins reach the large intestine and are further excreted in the faeces. The low absorption of epicatechins may be offset by local positive effects (Zugravu & Otelea, 2019). As colonic microbes can transform unabsorbed (poly)phenolics into low molecular weight metabolites (Gonthier et al., 2003; Monagas et al., 2010), which act as Nrf2 agonists (Senger et al. 2016) dietary composition and gut microbe diversity are critical mediators of any salutogenic effects.

Chocolate and the microbiome

The "microbiome" is generally used to collectively term the microbes and their associated genomes, that present as a community within a given location (e.g. the gut) (Marchesi & Ravel, 2015). The alternative term "microbiota" is used to describe the microbes *per se*, and "microbiome" genomes, or collective coding capacity. The gut microbiota comprises the largest population of microorganisms colonizing the human body (Qin et al., 2010). Its activities can release nutrients for the body through the fermentation of nondigestible foods within the large intestine (Cani, Everard, & Duparc, 2013). In addition, its compositional diversity and abundance prevent establishment of undesirable pathogens within the gut (Cigarran Guldris, González Parra, & Cases Amenós, 2017).

Deviations from a normal core microbiome to an altered microbiome (dysbiosis) (Hooks & O'Malley, 2017) have been associated with a range of diseases, including chronic kidney disease (CKD), neurodegenerative diseases, obesity and cardiovascular disease. Changes in thmicrobial profile occur due to altered intestinal transit (constipation), reduced dietary fiber intake, use of phosphate binders, antibiotics, decreased protein absorption, high red meat intake, low intake of fruit and vegetables and oral iron supplementation (Mafra et al. 2019; O'Toole & Shiels, 2020). Pro-inflammatory

and pro-oxidant microbial metabolites from alterations within the core gut microbiome contribute to both inflammatory burden and uremic toxicity (Mafra et al. Claesson et al. 2012) and bioactive compounds such as (poly)phenolics modulate the gut microbiota in humans (Mafra, Borges, et al., 2019; Shiels, Buchanan, Selman, & Stenvinkel, 2019; Stenvinkel et al., 2019; Tzounis et al 2011; Istas et al. 2019; Guglielmetti et al 2020). Although nutritional therapy based on bioactive compounds, including (poly)phenolics has been discussed previously (Mafra, Borges, et al., 2019), there are no studies showing efficacy for cocoa-derived (poly)phenolics in CKD. Due to the benefits provided to the host, (poly)phenolics have been included as a prebiotic, non-digestible compounds responsible for modulating the composition of the intestinal microbiota (Bindels, Delzenne, Cani, & Walter, 2015; Marchesi et al., 2016). Thus, inclusion of (poly)phenolics in the food regime provides health benefits to the host (Oteiza, Fraga, Mills, & Taft, 2018; Tomás-Barberán, Selma, & Espín, 2016).

One of the main mechanisms by which cocoa derived (poly)phenolics modulate the gut microbiota is through support of colonization by *Lactobacillus* spp. and *Bifidobacterium* spp (Tzounis et al, 2011). Subsequent metabolism of phenylacetic, phenylpropionic and valerolactones (poly)phenolics results in activation of anti-inflammatory pathways (Fraga, Croft, Kennedy, & Tomás-Barberán, 2019; Magrone et al., 2017; Tomás-Barberán & Espín, 2019). These activities are modulated through chemical reactions such as hydrolysis (ester hydrolysis and *O*-deglycosylations), cleavage (C-ring cleavage, delactonization and demethylation) and reduction, principally through dihydroxylation and double-bond reduction (Espín, González-Sarrías, & Tomás-Barberán, 2017).

As many (poly)phenolics derived from the diet cannot be absorbed due to their glycosyl moieties they rely on the intestinal microbiota to yield the aglycone, and smaller phenolic acids as downstream catabolites (Tomás-Barberán & Espín, 2019). Consumption of (poly)phenolics also promotes the growth of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Roseburia* spp., which enhance host metabolic capabilities (Anhê et al., 2015; Neyrinck et al., 2017; Roopchand et al., 2015). *Akkermansia muciniphila* has been studied for its ability to restore mucus production as well as the thickness of the intestinal layer (Derrien, Belzer, & de Vos, 2017; Ottman et al., 2016).

Experimental studies have demonstrated bacterial colonization and modulation of the intestinal microbiota by dietary cocoa consumption (Massot-Cladera et al., 2014). A study in humans has evaluated the health benefits from consumption, for one month, of a drink with a high cocoa flavanol content (494 mg flavonols/day) compared with a low cocoa drink (23 mg cocoa flavanol/day). An increase in *Lactobacillus* and *Bifidobacterium* populations were subsequently observed, along with a decreases in the pathogenic *clostridia* count. In addition, reduction in triglycerides and C-reactive protein, levels have suggested a prebiotic benefit with flavanol-rich foods (Koh, 2003; Tzounis et al 2011). Janani et al. (2019) have observed that 90 children who received 30g of dark chocolate for 3 months presented with reduction in *S. Mutans* colony count, beneficial for oral health. Moreover, mice that received cocoa have shown a reduction in the *Firmicutes* and *Proteobacteria phyla* and increase in the number of bacteria from the *Cyanobacteria* spp e *Tenericutes*, associated with improved intestinal integrity (Nagalingam, Kao, & Young, 2011). A study in obese patients supplemented with 10g of dark chocolate for one month showed an increase in *Lactobacillus* associated with decrease corneocyte exfoliation (Wiese et al., 2019). Additionally, 30 healthy individuals supplemented with 40g of dark chocolate for two weeks showed a reduction in p-cresol sulfate, a toxin produced by the gut microbiota (Martin et al., 2009).

Suggest:

A range of studies in humans and in preclinical model organisms, have indicated that dietary intake of chocolate has a number of salutogenic effects resulting from modulation of gut microbiota diversity. These include improved gut inergrity, less inflammation….. (refs from above)

One potential sequale from these observations is that dark chocolate may decrease the levels of uremic toxins in CKD by reducing the degree of gut dysbiosis and improving gut barrier integrity; this merits further investigation. However, the most important focus for study of the effects of chocolate in CKD relates to associated cardiovascular (CV) complications. Few studies, however, have investigated chocolate as a nutritional strategy to attenuate risk factors for CV complications, such as inflammation, oxidative stress and endothelial dysfunction.

Chocolate and cardiovascular health

Cardiovascular diseases are considered the leading cause of death worldwide (Gaviria, Ramírez, Alzate, Contreras, & Jaramillo, 2020). Pertinent to this, a number of studies have demonstrated that endothelial function can be improved by consumption of dark chocolate (Balzer et al., 2008; Gammone et al., 2018; Heiss et al., 2005; Monahan et al., 2011; Schroeter et al., 2006; Rodriguez-Mateos et al, 2018). Cardiovascular disease and associated endothelial impairment, is related to the bioavailability of endothelial nitric oxide (NO) (Gammone et al., 2018). This can, in part, be mitigated by an increase in NO availability, though consumption of nutritionally derived flavonoids derived from cocoa (Ludovici et al., 2017). The flavonols present in cocoa increase the bioavailability of NO, triggering the activity of NO synthase (eNOS) and l-arginine by degradation of arginase. Mechanistically, epicatechin for example, does so thorugh induction of phospholipase C in the endothelial cell membrane, , which in turn releases Ca2 into the cytoplasm, thus forming the Ca2+/calmodulin-complex that leads to endothelial nitric oxide synthase activation, which increase NO synthesis and promote vasodilation. Thus, flavonols promote relaxation of the arteries by relaxing the hyperpolarizing factor of NO, thus causing vasodilation (Schini-Kerth, Auger, Étienne-Selloum, & Chataigneau, 2010; Kirch et al. 2014). Additionally, the flavonols present in cocoa inhibit platelet activation (Jumar & Schmieder, 2016). In support of this, epicatechin monomers have been postualted to be responsible for improvements in vascular function (via increased flow-mediated vasodilation) in healthy men (Rodriguez-Mateos et al, 2018). Treatment of human endothelial cells from umbilical veins (HUVEC) with cocoa extract has been reported to reduce angiotensin-converting enzyme (ACE) activity, which successfully translated to treatment of healthy individuals, who demonstrated a similar effect three hours after intake of 75g dark chocolate (Persson, Persson, Hägg, & Andersson, 2011). Epicatechin can also inhibit NADPH oxidase, consequently reducing the synthesis of superoxide anions ($O^{\bullet -}$) and formation of peroxynitrite reduing oxidative damage and , leading to NO availablity (Steffen et al. 2007).

Yuan et al. (2017) (Yuan, Li, Jin, & Lu, 2017) have performed a meta-analysis of 14 prospective studies and found an association between chocolate intake and lower risk

of coronary heart disease (CHD), stroke and diabetes, when chocolate consumption of 30g serving equivlents, was ≤6 servings / week,. Correspondingly, several studies have shown that (poly)phenolics help protect against atherosclerosis, hypertension, dyslipidemia and inflammation, in keeping with tehse observations (**Table 1).**

In toto, these findings indicate that the habitual consumption of dark chocolate seems to have cardioprotective effect in healthy individuals' due reduction of arterial pressure and improvement of the endothelium function, associated with reduction on oxidative stress (Al Sunni & Latif, 2014; Heiss et al., 2010; Taubert, 2008; Vlachopoulos et al., 2007 ;Balzer et al., 2008; Mellor et al. 2013). The introduction of this bioactive compound into the food routine can improve the coronary circulation, and consequently, reduce the chances of strokes and cardiovascular events (Paillard, 2014; Ried, Fakler, & Stocks, 2017).

Chocolate and central nervous effects

Chocolate consumption can provide psychological comfort (Bruinsma & Taren, 1999). The production of the neurotransmitter serotonin can be stimulated through consumption of chocolate and geenrate antidepressant effects (Borawska, 2006). Jackson et al., (2019) have evaluated the relationship between chocolate consumption and symptoms of depression, in a cross-sectional survey of 13,626 adults and found evidences that dark chocolate consumption was associated with reduced incidence of clinically relevant depressive symptoms. Conversely, the frequent desire for chocolate can trigger dependence on the product and the individual can enter into the process of "emotional feeding" (Bruinsma & Taren, 1999; Parker, Parker, & Brotchie, 2006; Rogers & Smit, 2000). The carving for chocolate can be explained by its organoleptic qualities resulting from its high sweetness content, as well as its high palatability, which simulates mesocorticolimbic circuitry, resulting in a pleasurable sensation (Berridge, Kringelbach 2016). Sørensen & Astrup (2011) have observed that healthy individuals who ate 100g of dark chocolate had a decreased desire to eat something sweet, savory or fatty, and they were more satiated and less hungry five hours after eating the chocolate.

In small animal studies, cocoa intake and flavonoid action promoted angiogenesis in the hippocampus and increase motor coordination (Cicvaric et al., 2018; Van Praag et

al., 2007). In humans, some studies have indicated a psychoactive property for chocolate in modulating stress and mood. Sunni & Latif (2014). In a study with three groups of supplementation (40g of dark chocolate, 40g of milk chocolate and 40g of white chocolate for two weeks), students who received dark, or milk chocolate, had stress reduced. Recently, Tsang et al., (2019) have studied salivary cortisol levels in 26 healthy individuals and their respective subjective mood states, after 4 weeks receiving 25g of high polyphenol dark chocolate, and observed a reduction in the total daily cortisol, morning cortisol, and the cortisol/cortisone ratio. These observations are in keeping with chocolate possessing significant psychoactive properties.

Methylxanthines, caffeine and theobromine are also components of chocolate which have been proposed to have psychoactive activity, affecting cognitive function and neuropsychologial actions, including learning and memory or blood flow promoting angiogenesis (Crichton, Elias, & Alkerwi, 2016). Consumption of chocolate can result in euphoria, resulting from stimulation of the neurotransmitter anandamide (a name derived from the Sanskrit word "ananda" meaning bliss). This is an endogenous cannabinoid naturally found in the human brain, that activates cannabinoid receptors, resulting in a euphoric state of heightened sensitivity and analgesia (Di Tomaso, Beltramo, & Piomelli, 1996).

Flavonoids are capable of promoting psychological comfort, tranquility and decreased fatigue in humans (Radin, Hayssen, & Walsh, 2007). Researchers have shown that cognitive activities, such as learning and memory, are stimulated by the action of flavonols, through mediation of key cellular signaling pathways, in particular the phosphoinosi-tide3-kinase (PI3-kinase/Akt), mitogen-activated protein (MAPK) and extracellular-signal-regulated (ERK) pathways. Through protein kinase inhibition, flavonoids modulate signal transduction transcription factors (Goyarzu et al., 2004) and promote the expression of brain derived neurotrophic factor (BDNF) that assists in neuronal survival related to memory and learning in the subventricular zone and hippocampus (D. H. Kim et al., 2006; Valente et al., 2009).

In toto, dark chocolate consumption affects the cardiac and central nervous systems as well as the microbiome. A range of indications for the use of chocolate as a therapeutic are listed in **Table 2** and include physical exercise, Alzheimer disease, autism, visual

performance. The majority of uses have shown some benefit, suggesting that this use of chocolate as a nutraceutical to mitigate the inflammatory and oxidative burden, and, cognitive alterations in CKD patients, is of some merit.

Chocolate in chronic kidney disease

Patients with CKD display an increasing prevalence of premature cardiovascular events. These are accompanied by a underpinning dysregulated ageing processes, typified by an elevated oxidative and inflammatory burden, mitochondrial dysfunction (Carrero et al., 2008; Dai, Schurgers, Shiels, & Stenvinkel, 2020; Kooman, Kotanko, Schols, Shiels, & Stenvinkel, 2014) as well as low bioavailability of NO and early vascular aging (Heitzer, Schlinzig, Krohn, Meinertz, & Münzel, 2001; Kooman et al., 2017; Meyer et al., 2010; Pedraza-Chaverri, Sánchez-Lozada, Osorio-Alonso, Tapia, & Scholze, 2016; Stenvinkel, 2006).

Cardiovascular complications in CKD could be mitigated via anti-oxidant and anti-inflammatory propreties of (poly)phenolics present in cocoa (Chidambaram et al., 2018; Mafra et al., 2018). Contributing to the improvement of blood pressure, inflammation and insulin resistance, flavonoids can decrease or prevent kidney damage associated with arterial hypertension, through the direct route of the renal parenchyma or by decreasing blood pressure (Schnorr et al., 2008; Vargas et al., 2018; Zoccali & Mallamaci, 2016).

Experimental studies have shown interesting results regarding oxidative stress and kidney structures after cocoa supplementation. An experimental study in male rats aged 12 weeks, supplemented with cacao polyphenol extract (CPE) for seven days after induction of hepatic-renal oxidative stress by carbon tetrachloride (CCI_4), showed a decrease in oxidative stress. Accordingly, reduced glutathione peroxidase activity was detected in the kidneys (Miyazawa et al., 2015). In Sprague Dawley rats, cocoa and other plant extracts used as a source of therapeutic flavonoids over a 6-week period, have been observed to decrease blood pressure via enhanced vasodilator and anti-oxidant activity (Paredes et al., 2018).

Pre-clinical studies in rats have indicated reno-protective activity for a range of cocoa derived (poly)phenolics, including flavanols. These have showed that nutraceuticals can reduce inflammatory and oxidative burden and decreased kidney

damage (Álvarez-Cilleros, López-Oliva, Goya, Martín, & Ramos, 2019) and the progression of renal failure (Papadimitriou, Peixoto, Silva, Lopes de Faria, & Lopes de Faria, 2014).

A limited number of studies with polyphenol interventions in CKD and a systematic review and meta-analysis show that hemodialysis (HD) patients who received supplementation with foods rich in (poly)phenolics, such as turmeric, pomegranate, cacao, grape, green tea and soy, showed clinical improvement, including improvement in diastolic blood pressure, triglycerides and myeloperoxidase (Marx et al., 2017).

To the best of our knowledge, the potential impact of regular dark chocolate intake on inflammation, oxidative stress, gut microbiota profile in CKD has not yet been investigated in prospective longitudinal examination. However, one single study has evaluated the effects of cocoa in HD patients. A randomized, double-blind, placebo-controlled study, enrolled 57 HD patients who ingested cocoa flavanol (CF)-rich drinks (900 mg CF/day) and compared these with a placebo group (Rassaf et al., (2016). Acute ingestion improved flow mediated dilation and after 30 days CF group presented an increase in baseline flow mediated dilation with reduced diastolic blood pressure. Additionally, since CF improved endothelial dysfunction during a HD session, flavonoids in cocoa deserve further studiesin CKD.

Conclusion

Evidence supports the use of cocoa as a nutraceutical agent to mitigate the effects of CKD. A growing body of literature has indicated that (poly)phenolics present in cocoa, provide a substrate for the growth of the normative core microbiome and engender the production of alkyl catechols that act as cytoprotective agonists. Since cocoa derived (poly)phenolics may reduce the inflammatory and oxidative burdens that accompany CKD, it may have direct impact on cardiovascular risk and neuro-psychiatric factors (**Figure 2**). However, many questions remain to be explored in this field: can regular intake of dark chocolate be prescribed in CKD without side effects? Can it modulate inflammatory processes? Many nutritional questions still remain to be answered with respect to the toxic uremic milieu and nutritional resources are not all globally accessible to CKD patients. This poses interesting challenges for public health and food scientists,

focusing on reformulation and health messaging (Mellor et al. 2018). Further studies on the use of cocoa as a nutraceutical to mitigate the effects of CKD are thus warranted. For chocolate lovers, positive results will confirm that this treat is divine and chocolate craving is justified.

References

Al Sunni, A., & Latif, R. (2014). Effects of Chocolate Intake on Perceived Stress : A Controlled Clinical Study. *International Journal of Health Sciences*, *8*(4), 397–406. https://doi.org/10.12816/0023996

Ali, F., Ismail, A., & Kersten, S. (2014). Molecular mechanisms underlying the potential antiobesity-related diseases effect of cocoa polyphenols. *Molecular Nutrition and Food Research*, *58*(1), 33–48. https://doi.org/10.1002/mnfr.201300277

All, S. (2020). *The Physiolo □□ of Taste*. 1–3.

Anhê, F. F., Roy, D., Pilon, G., Dudonné, S., Matamoros, S., Varin, T. V., … Marette, A. (2015). A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut*, *64*(6), 872–883. https://doi.org/10.1136/gutjnl-2014-307142

Aprotosoaie, A. C., Luca, S. V., & Miron, A. (2016). Flavor Chemistry of Cocoa and Cocoa Products-An Overview. *Comprehensive Reviews in Food Science and Food Safety*, *15*(1), 73–91. https://doi.org/10.1111/1541-4337.12180

Balzer, J., Rassaf, T., Heiss, C., Kleinbongard, P., Lauer, T., Merx, M., … Kelm, M. (2008). Sustained Benefits in Vascular Function Through Flavanol-Containing Cocoa in Medicated Diabetic Patients. A Double-Masked, Randomized, Controlled Trial. *Journal of the American College of Cardiology*, *51*(22), 2141–2149. https://doi.org/10.1016/j.jacc.2008.01.059

Berridge, K. C., Kringelbach, M. L., Arbor, A., & Hospital, W. (2016). Pleasure Systems of the Brain. *Neuron*, *86*(3), 646–664. https://doi.org/10.1016/j.neuron.2015.02.018.Pleasure

Bindels, L. B., Delzenne, N. M., Cani, P. D., & Walter, J. (2015). Opinion: Towards a more comprehensive concept for prebiotics. *Nature Reviews Gastroenterology and Hepatology*, *12*(5), 303–310. https://doi.org/10.1038/nrgastro.2015.47

Borawska, M. H. (2006). Mood food. *Chemical and Functional Properties of Food Components, Third Edition*, *170*(8), 427–437. https://doi.org/10.1038/nrn3842

Bruinsma, K., & Taren, D. L. (1999). Chocolate: Food or drug? *Journal of the American Dietetic Association*, Vol. 99, pp. 1249–1256. https://doi.org/10.1016/S0002-8223(99)00307-7

Cani, P. D., Everard, A., & Duparc, T. (2013). Gut microbiota, enteroendocrine functions and metabolism. *Current Opinion in Pharmacology*, *13*(6), 935–940. https://doi.org/10.1016/j.coph.2013.09.008

Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. *Free Radical Biology and Medicine*, *22*(5), 749–760.

https://doi.org/10.1016/S0891-5849(96)00351-6

Cards, G., Rentals, T., Us, S., Books, Y., Deals, T., Releases, N., … Macaroons, F. C. (2020). *Customers who viewed All About Chocolate : The Ultimate Resource for the World ' s Favorite Food also viewed All About Chocolate : The Ultimate Resource for the World ' s Favorite Food Product details Related video shorts (0) More about the author*. 3–5.

Carrero, J. J., Stenvinkel, P., Fellström, B., Qureshi, A. R., Lamb, K., Heimbürger, O., … Shiels, P. G. (2008). Telomere attrition is associated with inflammation, low fetuin-A levels and high mortality in prevalent haemodialysis patients. *Journal of Internal Medicine*, *263*(3), 302–312. https://doi.org/10.1111/j.1365-2796.2007.01890.x

Chidambaram, S. B., Bhat, A., Ray, B., Sugumar, M., Muthukumar, S. P., Manivasagam, T., … Sakharkar, M. K. (2018). Cocoa beans improve mitochondrial biogenesis via PPARγ/PGC1α dependent signalling pathway in MPP+ intoxicated human neuroblastoma cells (SH-SY5Y)†. *Nutritional Neuroscience*, *0*(0), 1–10. https://doi.org/10.1080/1028415X.2018.1521088

Christenson, A. J. (2007). *Sacred Book Of The Quiche Maya People*. 285. Retrieved from www.mesoweb.com/publications/

Cicvaric, A., Bulat, T., Bormann, D., Yang, J., Auer, B., Milenkovic, I., … Monje, F. J. (2018). Sustained consumption of cocoa-based dark chocolate enhances seizure-like events in the mouse hippocampus. *Food and Function*, *9*(3), 1532–1544. https://doi.org/10.1039/c7fo01668a

Cigarran Guldris, S., González Parra, E., & Cases Amenós, A. (2017). Microbiota intestinal en la enfermedad renal crónica. *Nefrologia*, *37*(1), 9–19. https://doi.org/10.1016/j.nefro.2016.05.008

Cordero-Herrera, I., Martín, M. A., Goya, L., & Ramos, S. (2015). Cocoa flavonoids protect hepatic cells against high-glucose-induced oxidative stress: Relevance of MAPKs. *Molecular Nutrition and Food Research*, *59*(4), 597–609. https://doi.org/10.1002/mnfr.201400492

Crichton, G. E., Elias, M. F., & Alkerwi, A. (2016). Chocolate intake is associated with better cognitive function: The Maine-Syracuse Longitudinal Study. *Appetite*, *100*, 126–132. https://doi.org/10.1016/j.appet.2016.02.010

Dai, L., Schurgers, L. J., Shiels, P. G., & Stenvinkel, P. (2020). Early vascular ageing in chronic kidney disease: impact of inflammation, vitamin K, senescence and genomic damage. *Nephrology Dialysis Transplantation*, 31–37. https://doi.org/10.1093/ndt/gfaa006

De, A. (2020). *B las o*. 1–25.

De Almeida Alvarenga, L., Borges, N. A., Moreira, L. D. S. G., Resende Teixeira, K. T., Carraro-Eduardo, J. C., Dai, L., … Mafra, D. (2019). Cranberries-potential benefits in patients with chronic kidney disease. *Food and Function*, *10*(6), 3103–3112. https://doi.org/10.1039/c9fo00375d

de Almeida Alvarenga, L., Leal, V. de O., Borges, N. A., Silva de Aguiar, A., Faxén-Irving, G., Stenvinkel, P., … Mafra, D. (2018). Curcumin - A promising nutritional strategy for chronic kidney disease patients. *Journal of Functional Foods*, *40*(December 2017), 715–721. https://doi.org/10.1016/j.jff.2017.12.015

Derrien, M., Belzer, C., & de Vos, W. M. (2017). Akkermansia muciniphila and its role in regulating host functions. *Microbial Pathogenesis*, *106*, 171–181. https://doi.org/10.1016/j.micpath.2016.02.005

Di Tomaso, E., Beltramo, M., & Piomelli, D. (1996). Brain cannabinoids in chocolate. *Nature*, *382*(6593), 677–678. https://doi.org/10.1038/382677a0

Digital, A., & Login, C. (2020). *of the*. 1–126.

Eggler, A. L., Gay, K. A., & Mesecar, A. D. (2008). Molecular mechanisms of natural products in chemoprevention: Induction of cytoprotective enzymes by Nrf2. *Molecular Nutrition and Food Research*, *52*(SUPPL. 1), 84–94. https://doi.org/10.1002/mnfr.200700249

Esgalhado, M., Kemp, J. A., Azevedo, R., Paiva, B. R., Stockler-Pinto, M. B., Dolenga, C. J., … Mafra, D. (2018). Could resistant starch supplementation improve inflammatory and oxidative stress biomarkers and uremic toxins levels in hemodialysis patients? A pilot randomized controlled trial. *Food and Function*, *9*(12), 6508–6516. https://doi.org/10.1039/c8fo01876f

Esgalhado, M., Kemp, J. A., Damasceno, N. R. T., Fouque, D., & Mafra, D. (2017). Short-chain fatty acids: A link between prebiotics and microbiota in chronic kidney disease. *Future Microbiology*, *12*(15), 1413–1425. https://doi.org/10.2217/fmb-2017-0059

Espín, J. C., González-Sarrías, A., & Tomás-Barberán, F. A. (2017). The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *Biochemical Pharmacology*, *139*, 82–93. https://doi.org/10.1016/j.bcp.2017.04.033

Favazza, A. R. (1984). The Anthropology of Medicine: From Culture to Method. *American Journal of Psychiatry*, *141*(8), 1007–1008. https://doi.org/10.1176/ajp.141.8.1007

Fisher, N. D. L., Hughes, M., Gerhard-Herman, M., & Hollenberg, N. K. (2003). Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *Journal of Hypertension*, *21*(12), 2281–2286. https://doi.org/10.1097/00004872-200312000-00016

Fraga, C. G., Croft, K. D., Kennedy, D. O., & Tomás-Barberán, F. A. (2019). The effects of polyphenols and other bioactives on human health. *Food and Function*, *10*(2), 514–528. https://doi.org/10.1039/c8fo01997e

Gammone, M. A., Efthymakis, K., Pluchinotta, F. R., Bergante, S., Tettamanti, G., Riccioni, G., & Orazio, N. D. (2018). Impact of chocolate on the cardiovascular health. *Frontiers in Bioscience - Landmark*, *23*(5), 852–864. https://doi.org/10.2741/4620

Gaviria, S., Ramírez, A., Alzate, M., Contreras, H., & Jaramillo, N. (2020). *Epidemiología del síndrome coronario agudo*. *39*(1), 49–57. https://doi.org/10.18566/medupb.v39n1.a08

Gonthier, M. P., Donovan, J. L., Texier, O., Felgines, C., Remesy, C., & Scalbert, A. (2003). Metabolism of dietary procyanidins in rats. *Free Radical Biology and Medicine*, *35*(8), 837–844. https://doi.org/10.1016/S0891-5849(03)00394-0

Goyarzu, P., Malin, D. H., Lau, F. C., Taglialatela, G., Moon, W. D., Jennings, R., … Joseph, J. A. (2004). Blueberry supplemented diet: Effects on object recognition memory and nuclear factor-kappa B levels in aged rats. *Nutritional Neuroscience*, *7*(2), 75–83. https://doi.org/10.1080/10284150410001710410

Guglielmetti S, Bernardi S, Del Bo' C, Cherubini A, Porrini M, Gargari G, et al. Effect of a polyphenol-rich dietary pattern on intestinal permeability and gut and blood microbiomics in older subjects: study protocol of the MaPLE randomised controlled trial. BMC Geriatr. 2020 Feb 26;20(1):77.

Heiss, C., Jahn, S., Taylor, M., Real, W. M., Angeli, F. S., Wong, M. L., … Yeghiazarians, Y. (2010). Improvement of endothelial function with dietary flavanols is associated with mobilization of circulating angiogenic cells in patients with coronary artery disease. *Journal of the American College of Cardiology*, *56*(3), 218–224. https://doi.org/10.1016/j.jacc.2010.03.039

Heiss, C., Kleinbongard, P., Dejam, A., Perré, S., Schroeter, H., Sies, H., & Kelm, M. (2005). Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *Journal of the American College of Cardiology*, *46*(7), 1276–1283. https://doi.org/10.1016/j.jacc.2005.06.055

Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T., & Münzel, T. (2001). Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*, *104*(22), 2673–2678. https://doi.org/10.1161/hc4601.099485

Hollenberg, N. K., Fisher, N. D. L., & McCullough, M. L. (2009). Flavanols, the Kuna, cocoa consumption, and nitric oxide. *Journal of the American Society of Hypertension*, *3*(2), 105–112. https://doi.org/10.1016/j.jash.2008.11.001

Huang, H. C., Nguyen, T., & Pickett, C. B. (2000). Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(23), 12475–12480. https://doi.org/10.1073/pnas.220418997

Istas G, Wood E, Le Sayec M, Rawlings C, Yoon J, Dandavate V, et al. Effects of aronia berry (poly)phenols on vascular function and gut microbiota: a double-blind randomized controlled trial in adult men. Am J Clin Nutr. 2019 Aug 1;110(2):316-329.

Jackson, S. E., Smith, L., Firth, J., Grabovac, I., Soysal, P., Koyanagi, A., … Yang, L. (2019). Is there a relationship between chocolate consumption and symptoms of depression? A cross-sectional survey of 13,626 US adults. *Depression and Anxiety*, *36*(10), 987–995. https://doi.org/10.1002/da.22950

Janani RG, Asokan S, Geetha Priya PR.Effect of Custom-made Probiotic Chocolates on Streptococcus mutans, Plaque pH, Salivary pH and Buffering Capacity in Children - A Randomised Controlled Trial. Oral Health Prev Dent. 2019;17(1):7-15.

Juárez López, J. (2012). La cocina infantil en recetarios de cocina, 1911-1950: Presencia y omisión. *Historia y Grafía*, (39), 147–166.

Jumar, A., & Schmieder, R. E. (2016). Cocoa Flavanol Cardiovascular Effects Beyond Blood Pressure Reduction. *Journal of Clinical Hypertension*, *18*(4), 352–358. https://doi.org/10.1111/jch.12715

Keen, C. L. (2001). Chocolate: Food as Medicine/Medicine as Food. *Journal of the American College of Nutrition*, *20*(September), 436S-439S. https://doi.org/10.1080/07315724.2001.10719181

Kim, D. H., Jeon, S. J., Son, K. H., Jung, J. W., Lee, S., Yoon, B. H., … Ryu, J. H. (2006). Effect of the flavonoid, oroxylin A, on transient cerebral hypoperfusion-induced memory impairment in mice. *Pharmacology Biochemistry and Behavior*, *85*(3), 658–668. https://doi.org/10.1016/j.pbb.2006.10.025

Kim, M. J., Ryu, G. R., Kang, J. H., Sim, S. S., Min, D. S., Rhie, D. J., … Jo, Y. H. (2004). Inhibitory effects of epicatechin on interleukin-1β-induced inducible nitric oxide synthase expression in RINm5F cells and rat pancreatic islets by down-regulation of NF-κB activation. *Biochemical Pharmacology*, *68*(9), 1775–1785. https://doi.org/10.1016/j.bcp.2004.06.031

Kitto, B. (1920). Cocoa and chocolate: Thier history from plantation to consumer. By A. W. Knapp. (London: Chapman and Hall, Ltd. 1920.) Price 12s. 6d. net. *Journal of the Society of Chemical Industry*, *39*(20), 368–368. https://doi.org/10.1002/jctb.5000392017

Kluknavsky, M., Balis, P., Puzserova, A., Radosinska, J., Berenyiova, A., Drobna, M., … Bernatova, I. (2016). (-)-Epicatechin Prevents Blood Pressure Increase and Reduces Locomotor Hyperactivity in Young Spontaneously Hypertensive Rats. *Oxidative Medicine and Cellular Longevity*, *2016*. https://doi.org/10.1155/2016/6949020

Koh, P. (2003). Middle East. (cover story). *Euromoney*, *34*(411), 88–93. https://doi.org/10.3945/ajcn.110.000075.Diet

Kooman, J. P., Dekker, M. J., Usvyat, L. A., Kotanko, P., van der Sande, F. M., Schalkwijk, C. G., … Stenvinkel, P. (2017). Inflammation and premature aging in advanced chronic kidney

disease. *American Journal of Physiology - Renal Physiology*, *313*(4), F938–F950. https://doi.org/10.1152/ajprenal.00256.2017

Kooman, J. P., Kotanko, P., Schols, A. M. W. J., Shiels, P. G., & Stenvinkel, P. (2014). Chronic kidney disease and premature ageing. *Nature Reviews Nephrology*, *10*(12), 732–742. https://doi.org/10.1038/nrneph.2014.185

Kumar, S., & Pandey, K., A. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *Farmacevtski Vestnik*, *58*(4), 145–148. https://doi.org/10.2307/j.ctt1w0ddx8.35

Ludovici, V., Barthelmes, J., Nägele, M. P., Enseleit, F., Ferri, C., Flammer, A. J., … Sudano, I. (2017). Cocoa, Blood Pressure, and Vascular Function. *Frontiers in Nutrition*, *4*(August), 1–12. https://doi.org/10.3389/fnut.2017.00036

Mafra, D., Borges, N., Alvarenga, L., Esgalhado, M., Cardozo, L., Lindholm, B., & Stenvinkel, P. (2019). Dietary components that may influence the disturbed gut microbiota in chronic kidney disease. *Nutrients*, *11*(3), 1–23. https://doi.org/10.3390/nu11030496

Mafra, D., Esgalhado, M., Borges, N. A., Cardozo, L. F. M. F., Stockler-Pinto, M. B., Craven, H., … Shiels, P. G. (2019). Methyl donor nutrients in chronic kidney disease: Impact on the epigenetic landscape. *Journal of Nutrition*, *149*(3), 372–380. https://doi.org/10.1093/jn/nxy289

Mafra, D., Gidlund, E. K., Borges, N. A., Magliano, D. C., Lindholm, B., Stenvinkel, P., & von Walden, F. (2018). Bioactive food and exercise in chronic kidney disease: Targeting the mitochondria. *European Journal of Clinical Investigation*, *48*(11), 0–2. https://doi.org/10.1111/eci.13020

Magrone, T., Russo, M. A., & Jirillo, E. (2017). Cocoa and dark chocolate polyphenols: From biology to clinical applications. *Frontiers in Immunology*, *8*(JUN), 1–13. https://doi.org/10.3389/fimmu.2017.00677

Maiorana, V. C. (2007). The true history of Middle Earth. *Evolutionary Theory*, *9*, 84. Retrieved from papers2://publication/uuid/7926FD29-BE7F-484E-A53F-B13F24520E02

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*, *79*(5), 727–747. https://doi.org/10.1093/ajcn/79.5.727

Marchesi, J. R., Adams, D. H., Fava, F., Hermes, G. D. A., Hirschfield, G. M., Hold, G., … Hart, A. (2016). The gut microbiota and host health: A new clinical frontier. *Gut*, *65*(2), 330–339. https://doi.org/10.1136/gutjnl-2015-309990

Marchesi, J. R., & Ravel, J. (2015). The vocabulary of microbiome research: a proposal. *Microbiome*, *3*(1), 1–3. https://doi.org/10.1186/s40168-015-0094-5

Martin, F. P. J., Rezzi, S., Peré-Trepat, E., Kamlage, B., Collino, S., Leibold, E., … Kochhar, S.

(2009). Metabolic effects of dark chocolate consumption on energy, gut microbiota, and stress-related metabolism in free-living subjects. *Journal of Proteome Research*, *8*(12), 5568–5579. https://doi.org/10.1021/pr900607v

Martins, I. C. V. S., Borges, N. A., Stenvinkel, P., Lindholm, B., Rogez, H., Pinheiro, M. C. N., … Mafra, D. (2018). The value of the Brazilian açai fruit as a therapeutic nutritional strategy for chronic kidney disease patients. *International Urology and Nephrology*, *50*(12), 2207–2220. https://doi.org/10.1007/s11255-018-1912-z

Marx, W., Kelly, J., Marshall, S., Nakos, S., Campbell, K., & Itsiopoulos, C. (2017). The effect of polyphenol-rich interventions on cardiovascular risk factors in haemodialysis: A systematic review and meta-analysis. *Nutrients*, *9*(12), 1–24. https://doi.org/10.3390/nu9121345

Marzocchella, L., Fantini, M., Benvenuto, M., Masuelli, L., Tresoldi, I., Modesti, A., & Bei, R. (2011). Dietary Flavonoids: Molecular Mechanisms of Action as Anti- Inflammatory Agents. *Recent Patents on Inflammation & Allergy Drug Discovery*, *5*(3), 200–220. https://doi.org/10.2174/187221311797264937

Massot-Cladera, M., Abril-Gil, M., Torres, S., Franch, À., Castell, M., & Pérez-Cano, F. J. (2014). Impact of cocoa polyphenol extracts on the immune system and microbiota in two strains of young rats. *British Journal of Nutrition*, *112*(12), 1944–1954. https://doi.org/10.1017/S0007114514003080

Mayorga-Gross, A. L., & Esquivel, P. (2019). Impact of cocoa products intake on plasma and urine metabolites: A review of targeted and non-targeted studies in humans. *Nutrients*, *11*(5). https://doi.org/10.3390/nu11051163

Mellor DD, Amund D, Georgousopoulou E, Naumovski N. Sugar and cocoa: sweet synergy or bitter antagonisms. Formulating cocoa and chocolate products for health: a narrative review. International Journal of Food Science & Technology, Volume53, Issue1, 2018 P 33-42.

Mellor, Duane D.; Sathyapalan, Thozhukat; Kilpatrick, Eric S.; et al. Diabetes and Chocolate: Friend or Foe? JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY Volume: 63 Issue: 45 Special Issue: SI Pages: 9910-9918 Published: NOV 18 2015

Mellor DD, Madden LA, Smith KA, Kilpatrick ES, Atkin SL.High-polyphenol chocolate reduces endothelial dysfunction and oxidative stress during acute transient hyperglycaemia in Type 2 diabetes: a pilot randomized controlled trial. Diabet Med. 2013 Apr;30(4):478-83.

Meyer, C., Heiss, C., Drexhage, C., Kehmeier, E. S., Balzer, J., Mühlfeld, A., … Rassaf, T. (2010). Hemodialysis-Induced Release of Hemoglobin Limits Nitric Oxide Bioavailability and Impairs Vascular Function. *Journal of the American College of Cardiology*, *55*(5), 454–459.

https://doi.org/10.1016/j.jacc.2009.07.068

Miyazawa, T., Suzuki, K., Nakagawa, K., Yamamoto, T., Miyazawa, T., Kimura, F., & Kamei, M. (2015). Carbon tetrachloride-induced hepatic and renal damages in rat: Inhibitory effects of cacao polyphenol. *Bioscience, Biotechnology and Biochemistry*, *79*(10), 1669–1675. https://doi.org/10.1080/09168451.2015.1039481

Monagas, M., Urpi-Sarda, M., Sánchez-Patán, F., Llorach, R., Garrido, I., Gómez-Cordovés, C., … Bartolomé, B. (2010). Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food and Function*, *1*(3), 233–253. https://doi.org/10.1039/c0fo00132e

Monahan, K. D., Feehan, R. P., Kunselman, A. R., Preston, A. G., Miller, D. L., & Lott, M. E. J. (2011). Dose-dependent increases in flow-mediated dilation following acute cocoa ingestion in healthy older adults. *Journal of Applied Physiology*, *111*(6), 1568–1574. https://doi.org/10.1152/japplphysiol.00865.2011

Montagna, M. T., Diella, G., Triggiano, F., Caponio, G. R., Giglio, O. De, Caggiano, G., … Portincasa, P. (n.d.). *Chocolate , " Food of the Gods ": History , Science , and Human Health*.

Nagalingam, N. A., Kao, J. Y., & Young, V. B. (2011). Microbial ecology of the murine gut associated with the development of dextran sodium sulfate-induced colitis. *Inflammatory Bowel Diseases*, *17*(4), 917–926. https://doi.org/10.1002/ibd.21462

Neyrinck, A. M., Etxeberria, U., Taminiau, B., Daube, G., Van Hul, M., Everard, A., … Delzenne, N. M. (2017). Rhubarb extract prevents hepatic inflammation induced by acute alcohol intake, an effect related to the modulation of the gut microbiota. *Molecular Nutrition and Food Research*, *61*(1), 1–12. https://doi.org/10.1002/mnfr.201500899

O'Toole PW, Shiels PG. The Role of the Microbiota in Sedentary Lifestyle Disorders and Ageing: Lessons From the Animal Kingdom J Intern Med, 287 (3), 271-282 Mar 2020.

Oteiza, P. I., Fraga, C. G., Mills, D. A., & Taft, D. H. (2018). Flavonoids and the gastrointestinal tract: Local and systemic effects. *Molecular Aspects of Medicine*, *61*(2017), 41–49. https://doi.org/10.1016/j.mam.2018.01.001

Ottman, N., HuuskonenL., L., Reunanen, J., Boeren, S., Klievink, J., Smidt, H., … De Vos, W. M. (2016). Characterization of outer membrane proteome of akkermansia muciniphila reveals sets of novel proteins exposed to the human intestine. *Frontiers in Microbiology*, *7*(JUL), 1–13. https://doi.org/10.3389/fmicb.2016.01157

Paillard, F. (2014). Effets du chocolat sur la physiologie et les pathologies cardiovasculaires. *Presse Medicale*, *43*(7–8), 848–851. https://doi.org/10.1016/j.lpm.2014.05.005

Papadimitriou, A., Peixoto, E. B. M. I., Silva, K. C., Lopes de Faria, J. M., & Lopes de Faria, J. B.

(2014). Increase in AMPK brought about by cocoa is renoprotective in experimental diabetes mellitus by reducing NOX4/TGFβ-1 signaling. *Journal of Nutritional Biochemistry*, *25*(7), 773–784. https://doi.org/10.1016/j.jnutbio.2014.03.010

Paredes, M. D., Romecín, P., Atucha, N. M., O'Valle, F., Castillo, J., Ortiz, M. C., & García-Estañ, J. (2018). Beneficial effects of different flavonoids on vascular and renal function in L-NAME hypertensive rats. *Nutrients*, *10*(4), 1–15. https://doi.org/10.3390/nu10040484

Parker, G., Parker, I., & Brotchie, H. (2006). Mood state effects of chocolate. *Journal of Affective Disorders*, *92*(2–3), 149–159. https://doi.org/10.1016/j.jad.2006.02.007

Pedraza-Chaverri, J., Sánchez-Lozada, L. G., Osorio-Alonso, H., Tapia, E., & Scholze, A. (2016). New Pathogenic Concepts and Therapeutic Approaches to Oxidative Stress in Chronic Kidney Disease. *Oxidative Medicine and Cellular Longevity*, *2016*. https://doi.org/10.1155/2016/6043601

Persson, I. A. L., Persson, K., Hägg, S., & Andersson, R. G. G. (2011). Effects of cocoa extract and dark chocolate on angiotensin-converting enzyme and nitric oxide in human endothelial cells and healthy volunteers-A nutrigenomics perspective. *Journal of Cardiovascular Pharmacology*, *57*(1), 44–50. https://doi.org/10.1097/FJC.0b013e3181fe62e3

Petyaev, I. M., & Bashmakov, Y. K. (2017). Dark Chocolate: Opportunity for an Alliance between Medical Science and the Food Industry? *Frontiers in Nutrition*, *4*(September). https://doi.org/10.3389/fnut.2017.00043

Physitian, T. A. (2020). *HUGHES, William.* 1–4.

Prakash, M., Basavaraj, B. V., & Chidambara Murthy, K. N. (2019). Biological functions of epicatechin: Plant cell to human cell health. *Journal of Functional Foods*, *52*(August 2018), 14–24. https://doi.org/10.1016/j.jff.2018.10.021

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … Zoetendal, E. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59–65. https://doi.org/10.1038/nature08821

Radin, D., Hayssen, G., & Walsh, J. (2007). Effects of Intentionally Enhanced Chocolate on Mood. *Explore: The Journal of Science and Healing*, *3*(5), 485–492. https://doi.org/10.1016/j.explore.2007.06.004

Rassaf, T., Rammos, C., Hendgen-Cotta, U. B., Heiss, C., Kleophas, W., Dellanna, F., … Kelm, M. (2016). Vasculoprotective effects of dietary cocoa flavanols in patients on hemodialysis: A double-blind, randomized, placebo-controlled trial. *Clinical Journal of the American Society of Nephrology*, *11*(1), 108–118. https://doi.org/10.2215/CJN.05560515

Ried, K., Fakler, P., & Stocks, N. P. (2017). Effect of cocoa on blood pressure. *Cochrane Database*

of Systematic Reviews, *2017*(4). https://doi.org/10.1002/14651858.CD008893.pub3

Rodriguez-Mateos A, Weber T, Skene SS, Ottaviani JI, Crozier A, Kelm M, Schroeter H, Heiss C. Assessing the respective contributions of dietary flavanol monomers and procyanidins in mediating cardiovascular effects in humans: randomized, controlled, double-masked intervention trial. Am J Clin Nutr. 2018 Dec 1;108(6):1229-1237.

Rodríguez-Ramiro, I., Ramos, S., Bravo, L., Goya, L., & Martín, M. Á. (2012). Procyanidin B2 induces Nrf2 translocation and glutathione S-transferase P1 expression via ERKs and p38-MAPK pathways and protect human colonic cells against oxidative stress. *European Journal of Nutrition*, *51*(7), 881–892. https://doi.org/10.1007/s00394-011-0269-1

Rogers, P. J., & Smit, H. J. (2000). Food craving and food "addiction": A critical review of the evidence from a biopsychosocial perspective. *Pharmacology Biochemistry and Behavior*, *66*(1), 3–14. https://doi.org/10.1016/S0091-3057(00)00197-0

Roopchand, D. E., Carmody, R. N., Kuhn, P., Moskal, K., Rojas-Silva, P., Turnbaugh, P. J., & Raskin, I. (2015). *Dietary polyphenols promote growth of the gut bacterium Akkermansia muciniphila and attenuate high fat diet-induced metabolic syndrome. (Running title: Microbes provide the missing link between dietary polyphenols and metabolic disease)*.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, *45*(4), 287–306. https://doi.org/10.1080/1040869059096

Schini-Kerth, V. B., Auger, C., Étienne-Selloum, N., & Chataigneau, T. (2010). Polyphenol-induced endothelium-dependent relaxations. Role of NO and EDHF. *Advances in Pharmacology*, *60*(C), 133–175. https://doi.org/10.1016/B978-0-12-385061-4.00006-4

Schnorr, O., Brossette, T., Momma, T. Y., Kleinbongard, P., Keen, C. L., Schroeter, H., & Sies, H. (2008). Cocoa flavanols lower vascular arginase activity in human endothelial cells in vitro and in erythrocytes in vivo. *Archives of Biochemistry and Biophysics*, *476*(2), 211–215. https://doi.org/10.1016/j.abb.2008.02.040

Schroeter, H., Heiss, C., Balzer, J., Kleinbongard, P., Keen, C. L., Hollenberg, N. K., … Kelm, M. (2006). *参考文献⑤Epicatechin mediates beneficial effects of.pdf*. *103*(4).

Seem, S. A., Yuan, Y. V., & Tou, J. C. (2019). Chocolate and chocolate constituents influence bone health and osteoporosis risk. *Nutrition*, *65*, 74–84. https://doi.org/10.1016/j.nut.2019.02.011

Senger, D. R., Li, D., Jaminet, S. C., & Cao, S. (2016). Activation of the Nrf2 cell defense pathway by ancient foods: Disease prevention by important molecules and microbes lost from the modern western diet. *PLoS ONE*, *11*(2), 1–40. https://doi.org/10.1371/journal.pone.0148042

Shiels, P. G., Buchanan, S., Selman, C., & Stenvinkel, P. (2019). Allostatic load and ageing: Linking the microbiome and nutrition with age-related health. *Biochemical Society Transactions*, *47*(4), 1165–1172. https://doi.org/10.1042/BST20190110

Simenhoff, M. L., Saukkonen, J. J., Burke, F., Wesson, L. G., Schaedler, R. W., & Gordon, S. J. (1978). Bacterial populations of the small intestine in uremia. *Nephron*, *22*(1–3), 63–68. https://doi.org/10.1159/000181424

Sørensen, L. B., & Astrup, A. (2011). Eating dark and milk chocolate: A randomized crossover study of effects on appetite and energy intake. *Nutrition and Diabetes*, *1*(1), 1–6. https://doi.org/10.1038/nutd.2011.17

Stenvinkel, P. (2006). Inflammation in end-stage renal disease: The hidden enemy. *Nephrology*, *11*(1), 36–41. https://doi.org/10.1111/j.1440-1797.2006.00541.x

Stenvinkel, P., Meyer, C. J., Block, G. A., Chertow, G. M., & Shiels, P. G. (2019). Understanding the role of the cytoprotective transcription factor nuclear factor erythroid 2–related factor 2—lessons from evolution, the animal kingdom and rare progeroid syndromes. *Nephrology Dialysis Transplantation*, 1–10. https://doi.org/10.1093/ndt/gfz120

Strid, H., Simrén, M., Stotzer, P. O., Ringström, G., Abrahamsson, H., & Björnsson, E. S. (2003). Patients with chronic renal failure have abnormal small intestinal motility and a high prevalence of small intestinal bacterial overgrowth. *Digestion*, *67*(3), 129–137. https://doi.org/10.1159/000071292

Taubert, D. (2008). Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide. *Southern Medical Journal*, *101*(7), 670. https://doi.org/10.1097/SMJ.0b013e318179a317

Tomás-Barberán, F. A., & Espín, J. C. (2019). Effect of Food Structure and Processing on (Poly)phenol–Gut Microbiota Interactions and the Effects on Human Health. *Annual Review of Food Science and Technology*, *10*(1), 221–238. https://doi.org/10.1146/annurev-food-032818-121615

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 Jan;93(1):62-72.

Tomás-Barberán, F. A., Selma, M. V., & Espín, J. C. (2016). Interactions of gut microbiota with dietary polyphenols and consequences to human health. *Current Opinion in Clinical Nutrition and Metabolic Care*, *19*(6), 471–476. https://doi.org/10.1097/MCO.0000000000000314

USDA. United State Department of agriculture. https://reedir.arsnet.usda.gov/codesearchwebapp/(S(xpc0hhhqikpmgmm0mow45btb))/measures.aspx?id=91705010. Accessed April 8, 2020.

Valente, T., Hidalgo, J., Bolea, I., Ramirez, B., Anglés, N., Reguant, J., … Unzeta, M. (2009). A diet enriched in polyphenols and polyunsaturated fatty acids, LMN diet, induces neurogenesis in the subventricular zone and hippocampus of adult mouse brain. *Journal of Alzheimer's Disease*, *18*(4), 849–865. https://doi.org/10.3233/JAD-2009-1188

Vallabhapurapu, S., & Karin, M. (2009). Regulation and Function of NF-κB Transcription Factors in the Immune System. *Annual Review of Immunology*, *27*(1), 693–733. https://doi.org/10.1146/annurev.immunol.021908.132641

Van Praag, H., Lucero, M. J., Yeo, G. W., Stecker, K., Heivand, N., Zhao, C., … Gage, F. H. (2007). Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice. *Journal of Neuroscience*, *27*(22), 5869–5878. https://doi.org/10.1523/JNEUROSCI.0914-07.2007

Vargas, F., Romecín, P., García-Guillén, A. I., Wangesteen, R., Vargas-Tendero, P., Paredes, M. D., … García-Estañ, J. (2018). Flavonoids in kidney health and disease. *Frontiers in Physiology*, *9*(APR), 1–12. https://doi.org/10.3389/fphys.2018.00394

Verna, R. (2013). The history and science of chocolate. *Malaysian Journal of Pathology*, *35*(2), 111–121.

Vlachopoulos, C. V., Alexopoulos, N. A., Aznaouridis, K. A., Ioakeimidis, N. C., Dima, I. A., Dagre, A., … Stefanadis, C. I. (2007). Relation of Habitual Cocoa Consumption to Aortic Stiffness and Wave Reflections, and to Central Hemodynamics in Healthy Individuals. *American Journal of Cardiology*, *99*(10), 1473–1475. https://doi.org/10.1016/j.amjcard.2006.12.081

Wang, Y., Feltham, B. A., Suh, M., & Jones, P. J. H. (2019). Cocoa flavanols and blood pressure reduction: Is there enough evidence to support a health claim in the United States? *Trends in Food Science and Technology*, *83*(November 2018), 203–210. https://doi.org/10.1016/j.tifs.2018.11.023

Wiese, M., Bashmakov, Y., Chalyk, N., Nielsen, D. S., Krych, Ł., Kot, W., … Petyaev, I. (2019). Prebiotic Effect of Lycopene and Dark Chocolate on Gut Microbiome with Systemic Changes in Liver Metabolism, Skeletal Muscles and Skin in Moderately Obese Persons. *BioMed Research International*, *2019*. https://doi.org/10.1155/2019/4625279

Williamson, G. (2009). Bioavailability and health effects of cocoa polyphenols. *Inflammopharmacology*, *17*(2), 111. https://doi.org/10.1007/s10787-008-8030-y

Wollgast, J., & Anklam, E. (2000). Review on polyphenols in Theobroma cacao: Changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International*, *33*(6), 423–447. https://doi.org/10.1016/S0963-

9969(00)00068-5

Yuan, S., Li, X., Jin, Y., & Lu, J. (2017). Chocolate consumption and risk of coronary heart disease, stroke, and diabetes: A meta-analysis of prospective studies. *Nutrients*, *9*(7). https://doi.org/10.3390/nu9070688

Zoccali, C., & Mallamaci, F. (2016). Cocoa flavanols: A magic potion for protecting the endothelium in kidney failure? *Clinical Journal of the American Society of Nephrology*, *11*(1), 9–11. https://doi.org/10.2215/CJN.12141115

Zugravu, C., & Otelea, M. R. (2019). Dark chocolate: To eat or not to eat? A review. *Journal of AOAC International*, *102*(5), 1388–1396. https://doi.org/10.5740/jaoacint.19-0132

Senger DR, Li D, Jaminet S-C, Cao S (2016) Activation of the Nrf2 Cell Defense Pathway by Ancient Foods: Disease Prevention by Important Molecules and Microbes Lost from the Modern Western Diet. PLoS ONE 11(2): e0148042. doi:10.1371/journal.pone.0148042

Kirch, Natalie & Ellinger, Sabine. (2014). Cocoa Flavanols and cardioprotective Effects What Flavonoids can make a Contribution to Vascular Health?. Ernährungs Umschau. 61. M482-M489. 10.4455/eu.2014.025.

SteffenY, ScheweT, SiesH. (2007)(-)-Epicatechin elevates nitric oxide in endothelial cells via in- hibition of NADPH oxidase. Biochem Biophys Res Commun 359(3): 828–833.

Li T, Li F, Liu X, Liu J, Li D. Synergistic anti-inflammatory effects of quercetin and catechin via inhibiting activation of TLR4-MyD88-mediated NF-κB and MAPK signaling pathways. Phytother Res. 2019 Mar;33(3):756-767.

Prince PD , Rodríguez Lanzi C , Fraga CG , Galleano M. Dietary (-)-epicatechin affects NF-κB activation and NADPH oxidases in the kidney cortex of high-fructose-fed rats. Food Funct. 2019 Jan 22;10(1):26-32.