

Review

Polyphenols from Cocoa and Vascular Health—A Critical Review

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Abstract: Cocoa is a rich source of dietary polyphenols. *In vitro* as well as cell culture data indicate that cocoa polyphenols may exhibit antioxidant and anti-inflammatory, as well as anti-atherogenic activity. Several molecular targets (e.g., nuclear factor kappa B, endothelial nitric oxide synthase, angiotensin converting enzyme) have been recently identified which may partly explain potential beneficial cardiovascular effects of cocoa polyphenols. However cocoa polyphenol concentrations, as used in many cell culture studies, are not physiologically achievable. Bioavailability studies indicate that plasma concentrations of cocoa polyphenols following dietary intake are low and in the nanomolar range. Human studies regarding the effect of cocoa polyphenols on vascular health are often underpowered and lack a rigorous study design. If dietary cocoa polyphenol intake is due to chocolate its high energy content needs to be taken into account. In order to determine potential health benefits of cocoa polyphenols large scale, long term, randomized, placebo controlled studies, (ideally with a cross-over design) as well as prospective studies are warranted.

Keywords: cocoa; chocolate; polyphenols; cardiovascular disease; endothelial function

1. Introduction

There is experimental evidence suggesting that cocoa polyphenols may mediate beneficial effects on vascular health [1–3]. The present review critically evaluates studies on cocoa polyphenols and vascular health as conducted in cultured cells, laboratory rodents and humans over the last decade. First bioavailability studies with cocoa polyphenols are summarized. Secondly potential molecular targets of cocoa polyphenols are discussed. Thirdly an overview of the human studies on cocoa polyphenols and cardiovascular disease prevention is given. Finally merits and limitations of these studies are discussed and future challenges in cocoa polyphenol research are presented.

2. Bioavailability of Polyphenols in Cocoa

Cocoa beans (*Theobroma cacao*) have been used for a long period of time as major ingredient of cocoa and chocolate [4,5]. Cocoa is a rich source of polyphenols. Cocoa beans contain approximately 6-8% polyphenols by dry weight [6]. Polyphenols identified in cocoa beans and cocoa products comprise mainly catechins, flavonol glycosides, anthocyanins and procyanidins. As far as procyanidins are concerned, up to decamer ones have been identified in cocoa [7]. The chemical structure of selected cocoa polyphenols is given in Figure 1.

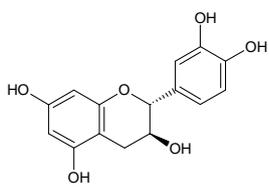
The biological activity of cocoa polyphenols largely depend on their bioavailability [8]. The bioavailability of cocoa polyphenols has been measured in several studies in humans (Table 1). Polyphenols were given as cocoa rich beverages or chocolate. Monomeric flavonoids as well as dimeric and trimeric procyanidins have been detected in human plasma after consumption. The peak plasma concentration of flavonols was determined 2-3 hours after ingestion [9,10]. Plasma concentrations of cocoa polyphenols were often in the nanomolar or low micromolar range. Donovan and coworkers demonstrated that commercial chocolate samples available around the world contain a predominance of the less bioavailable (-)-catechin enantiomer as compared to the (+)-catechin which is present in most other plant derived foods. This may explain the relatively low bioavailability of catechins from chocolate and cocoa-containing products [11]. The food matrix seems to be an important factor that may affect the bioavailability of cocoa polyphenols. Cocoa powder dissolved in milk, as one of the most common ways of cocoa powder consumption, did not change the bioavailability of cocoa powder flavonoids in healthy humans [12]. Furthermore, lipid and protein rich meals did not affect the bioavailability of cocoa polyphenols. However the uptake of flavonols in humans could be increased significantly by concurrent consumption of carbohydrates [13].

It is unclear if and to what extent higher oligomeric procyanidins from cocoa are absorbed. However, the biological activity of higher procyanidins may be at least partly attributed to their colonic breakdown products, including phenolic acids [14]. Data regarding the tissue distribution of cocoa polyphenols in laboratory rodents are currently missing.

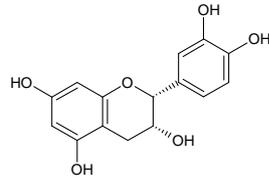
Within this review we mainly focus on potential cardiovascular health benefits of cocoa polyphenols. However it needs to be taken into account that cocoa beans contain also lipids, sterols, minerals and trace elements which may also affect vascular health as reviewed by Steinberg *et al.* [15].

Figure 1. Chemical structure of cocoa polyphenols.

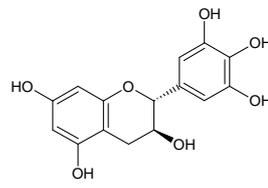
Catechins



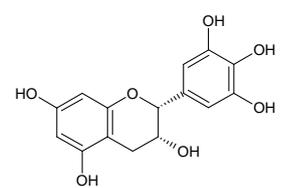
(+)-Catechin



(-)-Epicatechin

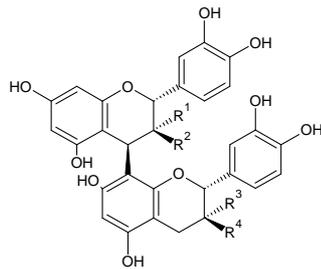


(+)-Gallocatechin

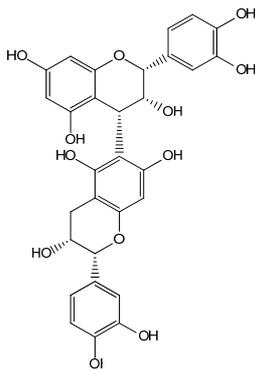


(-)-Epigallocatechin

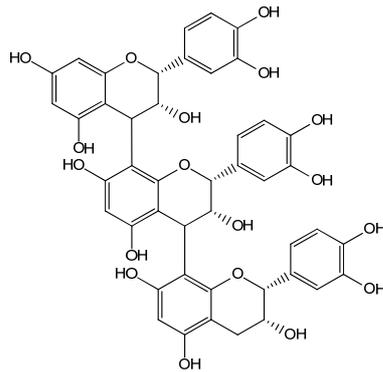
Procyanidins



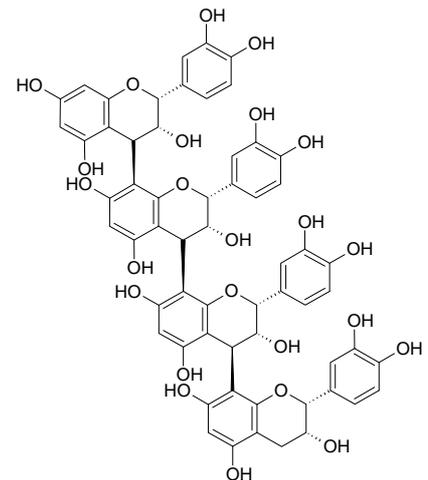
	R ¹	R ²	R ³	R ⁴
Procyanidin B1	OH	H	H	OH
Procyanidin B2	OH	H	OH	H
Procyanidin B3	H	OH	H	OH
Procyanidin B4	H	OH	OH	H



Procyanidin B5

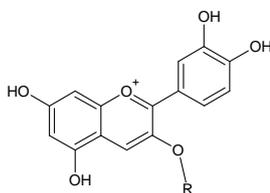


Procyanidin C1



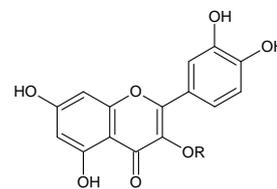
Procyanidin D

Anthocyanins



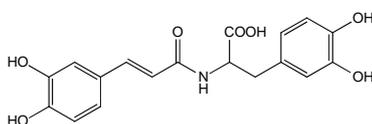
	R
Cyanidin-3-α-L-arabinosid	L-arabinose
Cyanidin-3-β-D-galactosid	D-galactose

Flavonol glycosides

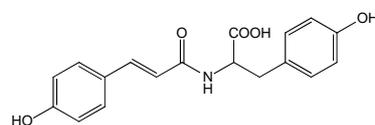


	R
Quercetin-3-O-α-D-arabinosid	D-arabinosid
Quercetin-3-O-β-D-glucopuranosid	D-glucopuranosid

Others



Clovamide



Dideoxyclovamide

Table 1. Human bioavailability studies on cocoa polyphenols.

No. Subjects	Age range (mean) (\pm SD)	BMI (kg/m ²) (\pm SD)	Dietary source of polyphenols	Polyphenol content	Plasma concentration	Reference
8	25–55 (40 \pm 15) (\pm SD)	23.94 \pm 2.35 (\pm SD)	Dark chocolate: 40 g 80 g	Epicatechin: 82 mg 164 mg	Epicatechin: 0.383 μ mol/L (t = 2 h) 0.7 μ mol/L (t = 2.57 h)	[16]
20	20–56	23.8 \pm 0.79 (\pm SEM)	Semi-sweet chocolate baking bits: 27 g 53 g 80 g	Total procyanidins (epicatechin): 186 (46) mg 365 (90) mg 551 (136) mg	Epicatechin (t = 2 h): 0.133 μ mol/L 0.258 μ mol/L 0.355 μ mol/L	[17]
13	26–49	23.2 \pm 1.2 (\pm SEM)	105 g semi-sweet chocolate baking bits (of which 80 g procyanidin-rich chocolate)	557 mg total procyanidins (137 mg epicatechin)	0.257 μ mol/L epicatechin (t = 2 h)	[10]
5	30–33 (31 \pm 1) (\pm SD)	22.5 \pm 1.3 (\pm SD)	96 g chocolate 66 g cocoa	Total polyphenols (epicatechin): 2.74 g (760 μ mol) 2.73 g (760 μ mol)	Total epicatechin (t = 2 h): 4.77 μ mol/L 4.92 μ mol/L	[18]
23	21–62	–	22 g cocoa powder + 16 g dark chocolate	466 mg total procyanidins (111 mg monomers)	0.036 nmol/L epicatechin (t = 2 h)	[19]
11	20–55 (39 \pm 5) (\pm SD)	24 \pm 3 (\pm SD)	37 g high- vs. low-procyanidin chocolate	Total procyanidins: 4 mg/g vs. 0.9 mg/g	0.212 μ mol/L vs. 0.011 μ mol/L epicatechin (t = 2 h)	[20]
16	22–49	–	300 mL cocoa beverage (18.75 g flavanol-rich cocoa powder)	897 mg total epicatechin & procyanidins	1.043 μ mol/L epicatechin (t=2 h)	[21]
5	23–34	–	Cocoa beverage (0.375 g cocoa/kg bw)	<i>Per g cocoa:</i> 12.2 mg monomers, 9.7 mg dimers, 28.2 mg procyanidins	0.041 μ mol/L dimer B2, 5.92 μ mol/L epicatechin, 0.16 μ mol/L catechin (t = 2 h)	[22]
6	23–39	23.1 \pm 0.7 (\pm SEM)	400 mL flavanol-rich cocoa beverage (37.5 g cocoa); 2 days	<i>Per g cocoa:</i> 12.2 mg monomers, 9.7 mg dimers, 20.2 mg procyanidins	0.08 μ mol/L dimer B2, 4.11 μ mol/L epicatechin, 0.4 μ mol/L catechin (t = 2 h)	[23]
18	–	–	25 g semi-sweet chocolate chips	220 mg flavanols & procyanidins	0.427 μ mol/L epicatechin (t = 2 h)	[24]
32	31–49 (40 \pm 9) (\pm SD)	26 \pm 4 (\pm SD)	Cocoa flavanol & procyanidin supplementation for 28 days	234 mg/d flavanols & procyanidins	0.116 μ mol/L epicatechin 0.091 nmol/L catechin (t = 28 d)	[25]

3. Putative Health Benefits of Polyphenols from Cocoa and Chocolate

3.1. Studies in Vitro and in Cultured Cells

In vitro studies, as well as studies in cultured cells, have identified several cellular and molecular targets by which cocoa polyphenols may mediate beneficial cardiovascular effects (Table 2). In fact it has been shown that cocoa polyphenols may prevent and/or inhibit the oxidation of LDL which is a key event in atherogenesis [26–30]. Furthermore cocoa polyphenols inhibit lipoxygenase activity *in vitro* [31]. However, comparing the 5-lipoxygenase-inhibitory effect of epicatechin (present in cocoa) with those of epigallocatechin-gallate (present in green tea) it was found that the latter flavonoid was at least one order of magnitude more potent [32]. There is experimental evidence from studies with rat liver microsomes that cocoa polyphenols may decrease NADPH-dependent lipid peroxidation and linoleic acid autoxidation [33].

Table 2. *In vitro* and cell-culture studies performed with cocoa polyphenols.

Cell type	Treatment	Outcomes	Reference
Human LDL	Cocoa powder extract (5 µmol/L GAE ^o) vs. pure catechin (5 µmol/L)	LDL oxidation ↓	[26]
Liposomes & human LDL	Cocoa catechin monomers & procyanidin fractions (0.1–10.0 µg/mL)	LDL oxidation ↓	[27]
LDL	220 mL cocoa drink (cocoa concentration: 1.5, 2.0, 2.5, 3.0, 3.5%)	LDL oxidation ↓ dose-dependent	[28]
LDL	Catechin, epicatechin, procyanidin B2, procyanidin C1, cinnamtannin A2 (0.125, 0.25, 0.5, 1.0, 2.0 µg/mL)	LDL oxidizability ↓	[29]
Human LDL & VLDL	Dark chocolate, cocoa, milk chocolate, hot cocoa mixes (126, 224, 52.2, 8.2 µmol/g total phenols)	Lag time of LDL & VLDL oxidation ↑	[30]
LDL & VLDL	Dark chocolate & cocoa powder (containing fat or defatted) vs. cocoa butter	LDL & VLDL oxidizability ↓	[49]
Rat liver microsomes	Cacao liquor	NADPH-dependent lipid peroxidation ↓ Linoleic acid autoxidation ↓	[33]
Recombinant human 5-LOX	Cocoa epicatechin & procyanidins (10 µmol/L)	5-LOX activity ↓ Proinflammatory mediators (LTB ₄ , LTC ₄ , LTD ₄) ↓	[32]
Isolated rabbit 15-LOX-1	Cocoa procyanidins (monomers through decamers; 2.9 mg/mL)	15-LOX-1 activity ↓ dose-dependent	[31]
Recombinant human platelet 12-LOX	Epicatechin & procyanidin decamer	12-LOX activity ↓ dose-dependent	
Human PBMC *	Cocoa procyanidins (monomers through decamers; 25 µg/mL)	IL-1β secretion ↑ IL-2 expression ↓ IL-4 expression & secretion ↓	[34]
Human PBMC *	Cocoa procyanidins (monomers through decamers; 25 µg/mL)	IL-1β transcription & secretion ↑ (pentamers-decamers) IL-1β transcription & secretion ↓ (monomers-tetramers)	[36]

Table 2. Cont.

Human PBMC *	Cocoa procyanidins (monomers through decamers; 25 µg/mL)	Secretory IL-4 ↑	[35]
Human PBMC *	Cocoa procyanidins (monomers through decamers; 25 µg/mL)	TNF-α secretion ↑	[37]
Human PBMC *	Cocoa procyanidins (monomers through decamers; 25 µg/mL)	IL-5 secretion ↑ (monomers-trimers) IL-5 secretion ↓ (hexamers-decamers)	[38]
Human PBMC ^o	Short- (monomers-pentamers) & long-chain (hexamers-decamers) flavanol fractions (20 µg/mL)	Inflammatory mediators (IL-1β, IL-6, IL-10, TNF-α) ↑	[53]
Whole blood	Purified trimeric & pentameric cocoa procyanidins (3, 10 µmol/L)	PAC-1 binding & P-selectin expression ↑ in unstimulated platelets Epinephrine-induced platelet activation ↓	[54]
Isolated rabbit aortic rings	Procyanidin-rich cocoa extracts	Endothelium-dependent relaxation ↑ NOS activity ↑	[45]
ACE from rabbit lung	Epicatechin, dimeric & hexameric procyanidins (0–500 µmol/L)	ACE activity ↓ molecular weight-dependent	[47]
Murine EL4BOU6 lymphocytes	Cocoa extract (5–80 µg/mL total polyphenols) vs. epicatechin (60–120 µg/mL total polyphenols)	IL-2 secretion ↓ IL-4 secretion ↑ T lymphocyte activation ↓	[41]
Murine RAW264.7 macrophages #	Cocoa extract (5–100 µg/mL total polyphenols) vs. epicatechin (60–120 µg/mL total polyphenols)	inducible NO ↓	[42]
Rat NR8383 macrophages #	Cocoa extract (10–50 µg/mL total polyphenols) vs. epicatechin (30–60 µg/mL total polyphenols)	Proinflammatory mediators (TNF-α, MCP-1, IL-1α, IL-6) ↓	
Jurkat T cells	Catechin, epicatechin & B-type oligomers (1.7–17.2 µmol/L)	PMA-induced NF-κB activation ↓ IL-2 expression & secretion ↓	[55]
VSMC	Cocoa procyanidins (0–100 µg/mL) & procyanidin B2 (0–100 µmol/L)	MMP-2 expression & activation ↓ VSMC invasion & migration ↓ MT1-MMP & MEK1 activities ↓	[46]
HUVEC	Epicatechin & flavanol metabolites mixture vs. control	Arginase-2 mRNA expression ↓ Arginase activity ↓ dose-dependent	[44]

GAE gallic acid equivalents, ^oPBMC, peripheral blood mononuclear cells; *, phytohemagglutinin (PHA)-stimulated; #, lipopolysaccharide (LPS)-stimulated; MCP-1, monocyte chemoattractant protein-1; [†], 12-O-tetradecanoylphorbol-13-acetate (TPA)-stimulated; LOX, lipoxygenase; COX-2, cyclooxygenase-2; VSMC, vascular smooth muscle cells; MMP, matrix metalloproteinase; MT1, membrane type-1; MEK, mitogen-activated protein kinase kinase; HUVEC, human umbilical endothelial cells.

Further studies in cultured cells suggest that cocoa polyphenols may exhibit anti-inflammatory activity by down regulating the production of pro-inflammatory cytokines including IL1β, IL2, IL4, IL6 and TNF-α [34–38]. Activated macrophages can generate large amounts of nitric oxide from L-arginine by the action of inducible NO Synthase (iNOS). Overproduction of NO by macrophages has been associated with chronic inflammation [39]. The transcription factor NF-κB, in cooperation with AP-1, coordinates the expression of iNOS and pro-inflammatory cytokines [40]. Cocoa polyphenols may decrease inducible nitric oxide production by inhibiting iNOS gene expression due to NFκB and

AP1 dependent signal transduction pathways [41,42]. Contrary other studies in cultured RAW264.7 murine macrophages indicate that oligomeric procyanidins such as procyanidin C2, as present in cocoa, exhibit pro-inflammatory activity [43]. Mao *et al.* have shown that the smaller fraction of cocoa polyphenols (monomer-tetramer) consistently decreased IL1 β expression in blood mononuclear cells, while the larger oligomers (pentamer-decamer) increased its expression [36].

Studies in endothelial cells suggest that cocoa polyphenols such as epicatechin inhibit arginase-2 mRNA expression and activity levels which in turn may result in a higher availability of the vaso-relaxing molecule nitric oxide in the vascular wall [44]. This finding is in line with studies using isolated rabbit aortic rings in which endothelium dependent relaxation and an increase in nitric oxide synthase has been observed. Karim and co-workers [45] studied the effect of polymeric procyanidins on endothelium dependent relaxation (EDR) in aortic rings from New Zealand White rabbits. Polymeric procyanidins (tetramers through decamers of catechins) caused EDR in aortic rings. Furthermore polymeric procyanidins significantly increased Ca²⁺ dependent NOS activity whereas monomers, dimers and trimers of catechins exhibited no such activity.

Cocoa procyanidins inhibit metalloproteinase-2 expression and activation in smooth muscle cells which may contribute to the anti-atherosclerotic effects of cocoa [46].

Interestingly cocoa polyphenols have also been shown to inhibit ACE activity [47] which may result in a decrease of blood pressure. ACE is centrally involved in the regulation of the rennin-angiotensin system. Angiotensin causes blood vessels to constrict resulting in increased blood pressure. Thus ACE inhibition is a therapeutic approach in blood pressure regulation. As observed for the purified ACE enzyme, ACE activity in kidney membrane was inhibited by 100 μ mol/L of dimer and hexamer epicatechin [47] a concentration which is not physiologically achievable.

Furthermore cocoa polyphenols circulate as conjugated metabolites whereas in most *in vitro* studies and studies in cultured cells non-conjugated cocoa polyphenols have been used. In this context cell culture studies using conjugated cocoa polyphenols are warranted. Furthermore the underlying mechanisms involved into the cellular uptake of cocoa polyphenols as well as its cellular concentrations and subcellular distribution need to be established.

3.2. Studies in Laboratory Animals

In vitro studies and studies in cultured cells are partly supported by studies in laboratory animals indicating that cocoa polyphenols may decrease LDL-oxidation, as well as other biomarkers of lipid peroxidation [4]. Furthermore studies in hypercholesterolemic rabbits [48] suggest that cocoa polyphenols may prolong LDL-oxidation lag time and decrease area of atherosclerotic lesions in the aorta (Table 4). Studies in hamsters report an increase in HDL as well as a decrease in LDL and triglyceride levels [49]. Furthermore cocoa procyanidins significantly reduced plasma cholesterol and increased steroid excretion in rats fed a high cholesterol diet [50]. In obese diabetic mice and rats a decrease in blood glucose as well as a decrease in 8-isoprostane levels has been observed [51]. However in other studies in rats no effect of cocoa polyphenols on biomarkers of lipid peroxidation in liver and heart has been reported [52].

The inhibition of arginase activity has been reported to improve endothelium vasodilating relaxation. In this context Schnorr *et al.* have found that cocoa flavonols lower vascular arginase

activity in rat kidney. Arginase competes with endothelial nitric oxide synthase for L-arginine as the substrate. Thus an inhibition of arginase activity can be associated with elevated endothelial NO levels [44]. Potential mechanisms by which cocoa polyphenols may affect vascular health are summarized in Table 3.

Table 3. Potential mechanisms by which polyphenols from cocoa may affect vascular health.

<ul style="list-style-type: none"> - Inhibition of LDL oxidation - Inhibition of lipoxygenase activity - Inhibition of inflammatory gene expression (e.g., IL1β, IL2, IL4, IL6, TNFα, iNOS) due to inhibition of NFκB and AP1 transcription factor activity - Inhibition of the expression of genes encoding for cell adhesion proteins, chemotactic factors and metalloproteinases - Increase in endothelial nitric oxide synthase activity - Inhibition of arginase activity - Inhibition of platelet aggregation - Increase in HDL and decrease in LDL and triglyceride levels
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Table 4. *In vivo* studies with cocoa polyphenols in laboratory animals.

Species	No. animals	Experimental duration (wk)	Treatment	Outcomes	Reference
Rats	5	–	Oral administration of 1 g cocoa powder/kg bw (7.8 mg epicatechin)	Lipid peroxides in plasma ↓ Oxidant-induced α -tocopherol consumption ↓	[4]
Rats	5–6	–	Gastric intubation with 100 mg cocoa extract	Plasma antioxidant capacity ↑ Erythrocyte hemolysis ↓	[56]
Rats	48	2	Diet containing by weight 0.5–2.0% flavanol- and procyanidin-rich cocoa (12.2 mg/g epicatechin, 2.8 mg/g catechin, 53.3 mg/g procyanidins)	Oxidative DNA damage in testes ↓ No effect on plasma F ₂ -isoprostane and TBARS	[52]
Hypercholesterolemic rabbits	12	24	Diet containing 10% cocoa powder (0.78 g total polyphenols)	TBARS in plasma ↓ No effects on plasma cholesterol, TG & phospholipids LDL oxidation lag time ↑ Area of atherosclerotic lesions in aorta ↓	[48]
Hamsters	27	10	Brownie (10 g vs. 1 g cocoa powder)	LDL & TG levels ↓ HDL ↑ dose-dependent LDL-oxidizability ↓	[49]
Obese-diabetic rats	40	4	Cocoa extract (600 mg/kg bw/d)	Blood glucose levels ↓ Plasma free fatty acids ↓ 8-isoprostane levels ↓ Superoxide dismutase activity ↑ No change in catalase activity	[51]
Rats	10	4	40 g cocoa powder per kg diet (11.0 mg/g epicatechin, 2.8 mg/g catechin, 43.0 mg/g procyanidins), vs. none	Renal arginase activity ↓	[44]

TG, triglycerides, TBARS: Thiobarbituric acid reactive substances.

3.3. Studies in Humans

There is evidence suggesting that cocoa polyphenols may also positively affect vascular health in humans. Several studies in humans suggest that cocoa polyphenols decrease LDL-oxidation [19,57,58]. Furthermore, an improvement of plasma antioxidative status due to cocoa polyphenols has been shown [9]. Some studies indicate an increase in plasma HDL-cholesterol [19,59,60], a decrease in plasma triglyceride and a decrease of biomarkers of lipid peroxidation such as TBARS [17,61] and F₂-Isoprostanes [62] following cocoa polyphenol consumption.

Importantly dietary cocoa polyphenols improve endothelial function by increasing vascular-NO-synthase activity [63–67]. This in turn may lead to a decrease in systolic and diastolic blood pressure [68–72]. The magnitude of blood pressure reduction due to cocoa polyphenols is often relatively low [73]. Blood pressure lowering effects were more evident in hypertensive than in normotensive volunteers [71,73]. However other studies did not report a beneficial effect of cocoa polyphenols on blood pressure, and flow mediated dilatation [63]. Balzer *et al.* [74] studied the effect of flavonol-containing cocoa on vascular function in medicated diabetic patients in a double-masked, randomized, controlled trial. The key observation was an acute effect and a later chronic effect of cocoa flavonols on endothelial function as evidenced by improvements in FMD. However, over a 6-week period, flavonol-rich cocoa did not modify vascular function in subjects with coronary artery disease. Furthermore no differences in soluble cellular adhesion molecule-concentration in plasma and forearm blood flow response to ischemia were evident [75].

Several studies report anti-aggregatory effects of cocoa polyphenols [25,61]. However the effects of cocoa polyphenols on platelet aggregation are rather modest and possibly large amounts need to be ingested to exhibit a similar anti-aggregatory effect as reported for aspirin [76]. Nevertheless in a recent study by Flammer and coworkers dark chocolate induced coronary vasodilation, improved coronary vascular function and decreased platelet adhesion 2 h after consumption of 40 g of dark chocolate containing 70% cocoa. These immediate effects were accompanied by a significant increase in plasma epicatechin levels [77].

Although there is experimental evidence that cocoa polyphenols may act as free radical scavengers *in vitro*, it is unlikely that cocoa polyphenols exhibit significant free radical scavenging activity *in vivo*. Cocoa polyphenols are present at low concentrations in the human plasma and mostly in the conjugated form [18]. Conjugation of flavonoids with glucuronic acid and sulphate blocks radical scavenging hydroxyl groups [78,79]. Furthermore the free radical scavenging activity of human plasma is mainly attributable to vitamin C, vitamin E, bilirubin and urate which occur in many times higher concentration as compared to cocoa polyphenols [80].

Hypertension is a leading risk of death in the world [87]. If a patient is diagnosed with high blood pressure anti-hypertensive drugs need to be prescribed. Functional food and their nutraceuticals may have a preventive potential, but they do not play a therapeutic role if the disease has already occurred. Although high flavonol cocoa polyphenols improved endothelial function, it did not improve the effects of exercise on body fat in obese adults [88]. From a nutritional point of view it is questionable whether one should increase the intake of dietary polyphenols due to cocoa-rich products such as chocolate, which is rich in fat and sugar and thereby high in energy. In the study by Taubert *et al.* consecutive daily doses of 100 g dark chocolate over two weeks increased the caloric intake by

480 kcal per day. Although systolic blood pressure was decreased by 5 mm Hg, in the long run these extra calories would probably lead to an increase in body weight and may counteract potential beneficial effects on vascular health [89]. Thus products low in sugar and fat should be preferred.

It needs to be taken into account that many human studies on cocoa polyphenols and vascular health as reported in the literature, were not placebo-controlled. Furthermore most studies regarding potential health benefits of cocoa polyphenols did include only small number of volunteers as summarized in Table 5. In fact many human intervention trials with cocoa polyphenols seem to be underpowered. Another problem with these studies is that rigorous study design is missing and compliance of the volunteers as well as plasma polyphenol concentrations have often not been reported. In order to determine health benefits of cocoa polyphenols large scale, long term randomised placebo-controlled studies (ideally with a cross-over design) are warranted [90]. Furthermore prospective studies on cocoa polyphenols need to be conducted in the future.

Table 5. Human intervention trials with cocoa and chocolate.

No. Subjects	Age range (mean)	BMI (kg/m ²)	Intervention	Polyphenol content	Outcomes	Reference
12	39 ± 4.0	–	Cocoa	–	LDL oxidation ↓	[57]
15	(32.5 ± 6.4)	21.7 ± 2.1	12 g cocoa powder x3/d for 2 weeks, vs. sugar	2610 mg total polyphenols/d (244 mg epicatechin)	LDL oxidation ↓ No change in plasma lipids or antioxidants Urinary excretion of epicatechin/metabolites ↑	[58]
23	21–62 (36)	–	22 g cocoa powder + 16 g dark chocolate/d for 4 weeks, vs. average American diet	466 mg procyanidins/d (111 mg monomers)	LDL oxidation ↓ Serum antioxidant capacity ↑ HDL cholesterol ↑	[19]
25	20–6 (32.4 ± 7.4)	24.4 ± 3.4	37 g dark chocolate & 31 g cocoa powder in a drink/d for 6 weeks, vs. none	651 mg total procyanidins/d (chocolate = 168 mg/d, cocoa = 483 mg/d)	LDL oxidizability ↓ No effect on inflammation markers, or plasma antioxidant capacity	[81]
45	19–49 (26)	21.5 ± 2.9 / 24.1 ± 3.5	75 g dark chocolate or high-phenolic dark chocolate for 3 weeks, vs. 75 g white chocolate	Total polyphenols (epicatechin): Dark = 274 (114) mg/d High = 418 (170) mg/d	HDL cholesterol ↑ Lipid peroxidation ↓ No change in plasma antioxidant capacity	[60]
25	(38 ± 1)	22.1 ± 0.4	26 g/d cocoa powder for 12 weeks	<i>Per 100 g:</i> 377 mg epicatechin, 135 mg catechin, 158 mg procyanidin B2, 96.1 mg procyanidin C1	LDL oxidation ↓ HDL-cholesterol ↑	[59]
20	20–56	23.8 ± 0.79	Semi-sweet chocolate baking bits (27, 53, 80 g), vs. none	Total procyanidins (epicatechin): 186 (46) mg 365 (90) mg 551 (136) mg	Plasma epicatechin ↑ dose-dependent Antioxidant capacity ↑ TBARS ↓	[17]
13	26–49	23.2 ± 1.2	105 g (of which 80 g chocolate) semi-sweet baking bits, vs. vanilla milk chips	557 mg total procyanidins (137 mg epicatechin)	Plasma epicatechin ↑ Total antioxidant capacity ↑ TBARS ↓	[10]
20	20–40	–	100 ml high- vs. low-flavanol cocoa drink	187 mg vs. 14 mg total monomers & oligomeric procyanidins	Plasma F2-isoprostanes ↓	[62]

Table 5. Cont.

12	25–35 (32.2 ± 1.0)	21.9 ± 0.4	100 g dark chocolate (with & without 200 mL milk), vs. 200 g milk chocolate	Not stated but FRAP values (147.4 µmol FE/100 g)	Plasma antioxidant capacity & epicatechin ↑, in absence of milk	[9]
30	24–49	–	Cocoa beverage (300 mL, 18.75 g procyanidin-rich cocoa powder), caffeinated beverage, or water	897 mg total epicatechin & oligomeric procyanidins	Platelet activation & function ↓	[61]
18	–	–	25 g semi-sweet chocolate chips, vs. none	220 mg flavanols & procyanidins	Plasma epicatechin ↑ Platelet function ↓	[24]
32	40 ± 9	26 ± 4	Cocoa flavanol/procyanidin tablets for 28 d, vs. placebo	234 mg flavanols & procyanidins/d	Platelet aggregation ↓ Plasma ascorbic acid ↑ No change in oxidation status markers Plasma epicatechin & catechin ↑	[25]
30	20–58 (30.6)	–	Dark (75% cocoa), vs. milk (20% cocoa) or white (no flavonoids) chocolate	–	Collagen-induced platelet aggregation ↓	[82]
27	18–72 (44 ± 3.4)	26.9 ± 0.9	High polyphenol vs. low flavanol cocoa drink (4x 230 mL/d for 4 d)	821 mg/d total flavanols (epicatechin, catechin & related oligomers)	Improved peripheral vasodilation	[63]
20	41 ± 14	25 ± 4	100 mL high polyphenol vs. low flavanol cocoa drink	176 mg total flavanols (70 mg monomers, 106 mg procyanidins)	NO bioactivity ↑ Arterial FMD ↑	[64]
10	–	–	200 mL high- vs. low-flavanol cocoa beverage	985 vs. 80.4 mg total flavanols	Erythrocyte arginase activity ↓	[44]
13	55–64	21.9 –26.2	100 g dark chocolate/d for 14 d, vs. 90 g white chocolate in hypertensive subjects	500 mg/d total polyphenols	Systolic & diastolic blood pressure ↓	[72]
15	(33.9 ± 7.6)	22.6 ± 3.0	100 g dark chocolate, vs. 90 g white chocolate for 15 d	500 mg total polyphenols	Insulin sensitivity ↑ Insulin resistance ↓ Systolic blood pressure ↓	[70]
28	–	–	105 g/d milk chocolate for 14 d, vs. cocoa butter chocolate; hypertensive subjects	168 mg/d flavanols (39 mg monomers, 126 mg polymers)	Diastolic & mean blood pressure ↓ LDL cholesterol ↓ Oxidative stress markers ↓	[69]
17	24–32 (28.9)	<27.0	100 g dark chocolate, vs. none	2.62 g procyanidins (0.54 g monomers & dimers, 0.76 g trimer-heptamers)	Improved endothelial function Vasodilation of brachial artery No change in blood pressure	[67]
20	(43.65 ± 7.8)	25.4 ± 1.7	100 g/d dark chocolate for 15 d, vs. 90 g white chocolate in hypertensive subjects	88 mg/d flavanols (22 mg catechin, 66 mg epicatechin)	Improved insulin sensitivity Systolic & diastolic blood pressure ↓ LDL cholesterol ↓ Improved FMD	[71]
11	(31 ± 1)	21.8 ± 0.8	100 mL high- vs. low-polyphenol cocoa drink	176–185 mg flavanols (70–74 mg monomers, 20–22 mg epicatechin, 106–111 mg procyanidins)	Circulating NO & FMD ↑	[65]
16	25–32	19–23	300 mL high- vs. low-flavanol cocoa drink	917 mg flavanols (19% epicatechin)	Circulating NO species ↑ FMD response of conduit arteries ↑ Microcirculation ↑	[66]
20	–	–	40 g dark chocolate, vs. white chocolate	Same brand as used for Vlachopoulos <i>et al.</i> (2005)	Improved FMD Platelet function ↓ Plasma total antioxidant status ↑	[83]

Table 5. Cont.

34	18–74 (47.9 ± 3.0)	28.0 ± 1.9/ 28.4 ± 1.3	High polyphenol cocoa drink 4x 230 mL/d for 4–6 d, vs. none	Per 100 mL: 9.2 mg epicatechin, 10.7 mg catechin, 69.3 mg flavanol oligomers (821 mg/d)	NO synthesis ↓ FMD ↑ Pulse wave amplitude ↑	[84]
40	61 ± 9	27.1 ± 3.9	48 g flavanol-rich chocolate bar + 18 g cocoa beverage/d, vs. placebo for 6 weeks in subjects with coronary artery disease	444 vs. 19.6 mg/d total flavanols (107 vs. 4.7 mg epicatechin)	No acute or chronic changes in FMD, systemic arterial compliance, forearm blood flow, soluble cellular adhesion molecules	[75]
32	57.7 ± 2.2/ 55.4 ± 1.7	24.9 ± 1.0/ 25.3 ± 0.8	High- vs. low-flavanol cocoa beverage for 6 weeks in hypercholesterolemic subjects	446 vs. 43 mg total flavanols	FMD ↑ Brachial artery hyperaemic blood flow ↑ VCAM-1 ↓	[85]
11	22–32 (27 ± 1)	22 ± 1	100 mL high-flavanol vs. low-phenolic cocoa drink x3/d for 1 week	Per 100 ml: 59 mg epicatechin, 15 mg catechin, 232 mg flavanol oligomers (918 mg/d procyanidins)	FMD ↑ No change in biomarkers of oxidative stress	[86]
45	30–75 (52.8 ± 11.0)	30.1 ± 3.3	74 g solid dark chocolate (22 g cocoa powder); 240 ml liquid cocoa (sugar-free vs. sugared)	821 mg total flavanols; 805.2 & 8.5 mg total flavanols	Improved FMD Systolic & diastolic blood pressure ↓	[68]

FE: Ferric equivalents; FMD: Flow-mediated dilation; FRAP: Ferric-reducing ability of plasma; TBARS: Thiobarbituric acid reactive substances.

All data refer to healthy subjects unless otherwise stated.

4. Conclusions

There is epidemiological evidence suggesting that consumption of fruits and vegetables appear to have a protective effect against coronary heart disease [91]. Furthermore, the intake of certain flavonoid rich food items may be inversely related to coronary heart disease [92,93]. However, only few epidemiological data are currently available for cocoa polyphenols. In a cohort of elderly men, cocoa intake was inversely associated with blood pressure and 15-year cardiovascular and all-cause mortality [94]. It is suggested that before drawing conclusions, confirmation by further observational and experimental studies on cocoa polyphenols is needed. Mink *et al.* [95] studied flavonoid intake and cardiovascular disease mortality in a prospective study in postmenopausal women. Individual foods associated with a reduction of cardiovascular disease risk included bran, apples and pears, red wine, grapefruit, strawberries and chocolate.

Hooper and coworkers have recently conducted a meta-analysis on flavonoids, flavonoid-rich foods and cardiovascular disease risk. One hundred thirty-three trials were included into this meta-analysis. Importantly chocolate increased FMD and reduced both systolic and diastolic blood pressure [96]. Janszky *et al.* [97] assessed the long-term effects of chocolate consumption amongst patients with established coronary heart disease in a population based cohort study comprising >1100 non diabetic patients. Chocolate consumption was associated with a significantly reduced cardiac mortality in patients surviving the first acute myocardial infarction.

In a recent review it has been stated that most cocoa polyphenol studies in humans over the last decade have been mainly funded by industrial sponsors. Thus there may be potential for research bias

[98]. Nevertheless several studies consistently reported beneficial effects of dietary polyphenols from cocoa on vascular health. However, it should be considered that the products used in controlled studies often contain much higher polyphenol contents than most of the commercially available products [94,99]. Since flavonols exhibit a bitter taste manufacturers have established processing techniques for cocoa which eliminate the bitterness together with the flavonoides [100]. As much as 90% of the flavonoids may be lost due cocoa processing [76]. Thus, it needs to be established whether the consumption of products with a lower polyphenol content are associated with any health benefits in humans. Furthermore the food industry is encouraged to label the flavonoid content on their cocoa derived products.

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