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Cocoa flavanols and cardioprotective effects

Which flavanols may contribute to cardiovascular health?

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Summary

Regular cocoa consumption decreases blood pressure, improves vascular elasticity, and exerts beneficial effects on carbohydrate and lipid metabolism. These effects are attributed to flavanols. However, the question which flavanols in cocoa may be responsible for these effects has not been clarified yet. The flavanols' bioavailability differs strongly: procyanidins are rarely bioavailable; catechin is bioavailable, but much less than epicatechin. Only the administration of isolated epicatechin, but not catechin, enhanced vascular elasticity in rats. Regression analyses have shown that blood pressure reduction depends on epicatechin intake with the cocoa product and improvement in vascular elasticity on the concentration of epicatechin in plasma, but not on those of catechin. Therefore, epicatechin seems to be responsible for the cardioprotective effects of cocoa. This should be investigated by intervention studies administrating isolated epicatechin and other flavanols in doses that can be ingested with cocoa products.

Keywords: cocoa, flavanols, epictechin, phytochemicals, cardiovascular effects

Introduction

Studies on the cardioprotective effect of cocoa

Meta-analyses of controlled intervention studies have shown a reduction in blood pressure [1–4] and an increase in flow-mediated dilatation (FMD), which is an indicator of vascular elasticity [3, 4]. Moreover, a decline in LDL-cholesterol [4, 5], triglycerides and insulin in serum as well as an improvement in insulin sensitivity [3, 4] was ascertained. The meta-analysis of Shrime et al. showed the same effects whether or not studies with industrial support were considered [4], while Hooper et al. found only evidence for an increase in FMD [3]. The cardioprotective effects of cocoa are attributed to flavanols, in particular to epicatechin [6]. After a bolus administration (one-time administration) of a flavanol-rich cocoa drink to healthy volunteers, the increase in FMD was dependent on the concentration of epicatechin and its metabolite epicatechin-7-O-glucuronide in plasma [7]. A meta-regression analysis of intervention studies showed that the extent of blood pressure reduction depends on the intake of epicatechin ingested by regular consumption of cocoa products. The confidence intervals for blood pressure reduction were determined with Bayesian statistics by the use of a Markov chain Monte Carlo method [8]. The confidence intervals were considerably smaller than in meta-analyses based on the classical Cochrane model [1–3, 9–11], which assumes that differences between studies are random (◆ Table 1). 75 % of the studies included in the meta-regression analysis investigated volunteers with elevated blood pressure and the remaining studies investigated normotensive participants [8].

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Flavanols in cocoa

Cocoa flavanols occur as monomers (catechin, epicatechin) as well as procyanidins. The latter are di-, tri- and oligomers of catechin and/or epicatechin [12] (◆ Figure 1). In unroasted cocoa beans, epicatechin represents the largest fraction (about $^2/_3$) of the flavanol monomers and dimers. By roasting the cocoa beans, (–)-epicatechin partly epimerizes to (–)-catechin. Nevertheless, (–)-epicatechin remains the main monomer in the roasted cocoa bean [13] and in cocoa products [14]. In cocoa products, epicatechin is almost exclusi-

Glossary

Bayesian statistics: In Bayesian statistics, prior knowledge (prior probabilities) and conditional probabilities (likelihood) are taken into account for statements on probabilities for events. In particular, specific advantages can be obtained if data from different sources are evaluated. The influence of prior distributions on the results decreases with an increasing number of data sets.

Flow-mediated dilatation: Vascular elasticity is determined as flow-mediated dilatation (FMD) via ultrasound of the brachial artery. FMD reflects the ability of arteries to respond to shear stress with dilatation. FMD is mediated by nitric oxide which is synthesized by endothelial nitric oxide synthase. FMD is a marker of vascular function. A disorder of the vascular function is involved in the pathogenesis of arteriosclerosis, hypertension and heart failure.

Markov chain Monte Carlo methods: By Markov chain Monte Carlo methods, it is possible to generate samples of random variables which can be used to get estimates of uncertainty, e.g., confidence intervals. This helps with non-linear models in which the probability density of dependent variables (e.g., effect of epicatechin on blood pressure reduction) cannot be described by analytical methods. Physiological processes often show non-linear dependencies with probability density distributions, which can be analyzed by Markov chain Monte Carlo methods.

Stereoisomers: Catechin and epicatechin are stereoisomers with the same elemental formula, but a different steric configuration of the hydroxyl group. Both can occur in two enantiomeric forms, ((+)-catechin and (–)-catechin as well as (+)-epicatechin and (–)-epicatechin) which are mirror images of each other. Consequently, four stereochemically different flavanols exist with the same elemental formula (◆ Figure 1).

vely found as (–)-epicatechin [15, 16]. For the content of flavanols in cocoa products applies: procyanidins >> (–)-epicatechin > (–)-catechin >> (+)-catechin.

In the meantime, cocoa products with extraordinarily high flavanol content are available from different manufacturers. In September 2013, the European Food Safety Authority (EFSA) approved the health claim of a big manufacturer of cocoa products [17]. Accordingly, this company has the right to promote products manufactured by a special process with the statement: "Cocoa flavanols help maintain the elasticity of blood vessels, which contributes to normal blood flow". This health claim is only allowed for cocoa products which provide at least 200 mg cocoa flavanols per day [18].

Which flavanols are responsible for the cardioprotective effects?

Since cocoa flavanols are a very heterogeneous substance group, the question arises which flavanols from cocoa may affect vascular health. Is the bioavailability similar for all cocoa flavanols? Are there any findings on the effects and mechanisms of individual flavanols on vascular function? This review presents the current knowledge on the bioavailability of single cocoa flavanols and on their effect on cardiovascular parameters.

Current state of research

Bioavailability of different cocoa flavanols

Bioavailability reflects the fraction of

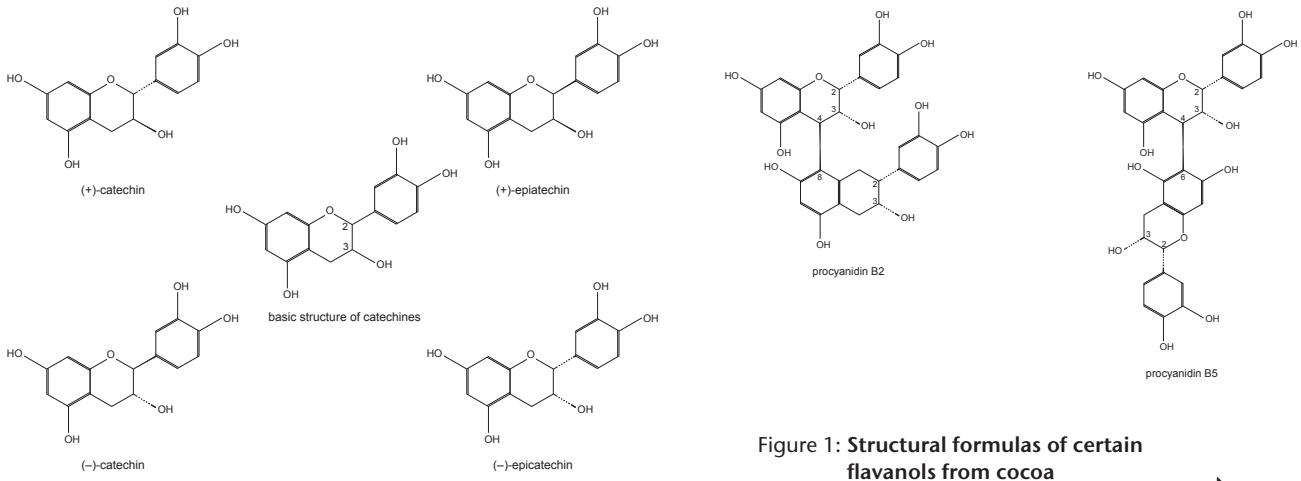

Figure 1: **Structural formulas of certain flavanols from cocoa**

Meta-analyses	Individual studies considered (n)	Variables	ΔRR sys (mmHg) Mean [95 %-KI]	ΔRR dia (mmHg) Mean [95 %-KI]
Cochrane model				
Taubert et al. 2007	5	–	-4.7 [-7.6; -1.8]	-2.8 [-4.8; -0.8]
Hooper et al. 2008	5	–	-5.88 [-9.55; -2.21]	-3.30 [-5.77; -0.83]
Desch et al. 2009	10	–	-4.52 [-5.87; -3.16]	-2.52 [-3.87; -1.16]
Ried et al. 2010	15	–	-3.16 [-5.08; -1.23]	-2.02 [-3.35; -0.69]
	5[a]		-5.02 [-7.99; -2.05]	-2.73 [-4.89; -0.58]
	10[b]		ns	ns
Ried et al. 2012	20	–	-2.77 [-4.72; -0.82]	-2.20 [-3.46; -0.93]
	5[a]	–	-3.99 [-7.02; -0.97]	ns
	k.A.[b]	–	ns	ns
Hooper et al. 2012	k.A.	–	ns	-1.60 [-2.77; -0.43]
Regression model				
Ellinger et al. 2012	16	K	-4.6 [-5.4; -3.9]	-2.1 [-2.7; -1.6]
		25 mg EC	-4.1 [-4.6; -3.6]	-2.0 [-2.4; -1.5]

Table 1: **Mean changes in blood pressure to be expected after regular consumption of cocoa products – results from meta-analyses of controlled intervention studies**
[a] Subgroup analysis for trials investigating subjects with prehypertension or hypertension (blood pressure ≥ 140/80 mmHg),
[b] subgroup analysis for trials with normotensive subjects (blood pressure < 140/80 mmHg)
95%-CI: 95%-confidence interval; ΔRR sys/dia: Changes in systolic and diastolic blood pressure, respectively; EC: epicatechin; K: asymptotic limit; n.d.: no data available; ns: not significant

an administrated nutrient that is accessible for the organism. It depends on absorption, metabolism, storage in the body, and on excretion [19].

The mechanisms involved in the absorption of cocoa flavanols were examined by using in vitro models with a monolayer of Caco-2 cells. Mono-, di- and trimers were transported through the paracellular route and their permeability was comparable [20, 21], whereas procyanidins with a degree of polymerization ≥ 4 were not able to pass the Caco-2-monolayer [20].

Two hours after bolus administration of a water-based cocoa drink prepared with 38 g [22] and 26 g [23] cocoa, the epicatechin concentration in plasma increased to 6 and 4 µM, respectively. Catechin concentration reached 0.4 µM [22] and 0.2 µM [23], respectively. Procyanidin B2 was detected in concentrations of 0.08 µM [22] and 0.04 µM [23]. The consumption of 40 g and 80 g dark chocolate led to a maximum concentration of epicatechin of 0.35 µM after 2.0 h and 0.68 µM after 2.6 h, respectively. The area under the curve was almost twice as high after consuming 80 g compared to 40 g dark chocolate [24]. A low-flavanol cocoa drink enriched with procyanidins (di- to decamers) led to a small increase in epicatechin metabolites in plasma 2 h after consumption, which was attributed to the epicatechin contained in traces in the cocoa drink. However, this increase was smaller than that obtained after administration of the low-flavanol drink enriched with flavanol monomers. Consequently, a breakdown of procyanidins to flavanol monomers and a subsequent rise of those monomers in plasma can be excluded [25].

In a randomized, double-blind, placebo-controlled study, the plasma level of (–)-catechin achieved 2 h after administration of isolated flavanol stereoisomers (1.5 mg/kg body weight (BW)) was three times lower than those of (+)-catechin and (+)-epicatechin, and six times lower than the plasma level of (–)-epicatechin [26]. Consequently, the order of bioavailability of cocoa flavanols is: (–)-epicatechin >> (+)-catechin = (+)-epicatechin > (–)-catechin >> procyanidin B2.

Metabolism of flavanols

Holt et al. [23], Steinberg et al. [22], and Ottaviani et al. [25–27] determined the concentration of flavanol monomers as the sum of free, sulfated, and glucuronidated monomers. After consumption of a cocoa drink enriched with epicatechin, only traces of free epicatechin (4 nM) were detected in plasma; after bolus administration of 100 g dark chocolate, free epicatechin was not detectable at all [27].

(–)-Epicatechin is metabolized in the intestinal mucosa and in the liver [28]. In addition to sulfated and glucuronidated metabolites, methylated derivates were found in plasma, among them methyl sulfates and methyl glucuronides [26, 28]. These metabolites differ in all pharmacokinetic parameters such as maximum plasma concentration, time to achieve maximum concentration, area under the curve, and elimination half-life [28].

In humans, epicatechin and its metabolites are primarily excreted by urine. Ottaviani et al. detected 90 % of orally administered (–)-epicatechin within 8 h [26] in urine. Tomas-Barberán et al., however, found the highest renal excretion after 24 h which indicates the existence of an enterohepatic circulation [29]. This assumption is supported by the detection of epicatechin metabolites in the bile after intestinal administration of 50 mg isolated (–)-epicatechin [28].

Effects of isolated flavanols on vascular function

The intravenous application of 100 nM (–)-epicatechin, dissolved in saline solution, improved vascular elasticity in rats, while (+)-epicatechin, (+)-catechin, (–)-catechin, and the saline solution itself had no effect. The authors calculated plasma concentrations of about 30 nM to be achieved. In this study, an effect on blood pressure could not be detected [26]. The efficacy of (–)-epicatechin on vascular function was also examined in humans. In healthy volunteers, bolus administration of an aqueous solution providing 1 and 2 mg (–)-epicatechin/kg BW, respectively, increased FMD and reactive hyperemia in comparison to water. Similar changes were also observed after consumption of a flavanol-rich cocoa drink providing equal amounts of epicatechin. This cross-over study was carried out with three volunteers [7].

Mechanisms of epicatechin

This raises the question which mechanisms could mediate the changes in blood pressure and in vascular elasticity.

In hypertensive rats with diminished activity of endothelial nitric oxide synthase (eNOS), the administration of 304 mg epicatechin/kg BW/day for four days increased the availability of vasodilatory nitric oxide (NO) and decreased blood pressure in a dose-dependent manner. The supplementation of epicatechin reduced lipid peroxidation and the ratio of oxidized to reduced glutathione compared to untreated rats, which indicates antioxidative effects. Additionally, an activation of eNOS was found. Therefore, an increased NO availability is ascribed to an enhanced NO synthesis and a reduced breakdown of NO [30]. Similar effects were shown by two further studies with hypertensive rats: the supplementation of (–)-epicatechin in doses of 250 mg/kg BW/day [31] and 10 mg/kg BW/day [32], respectively, decreased systolic blood pressure [31, 32] and lipid peroxidation [32], while an increase in NOS activity in the aorta was found [31]. Furthermore, the administration of (–)-epicatechin reduced the concentration of the vasoconstrictor endothelin-1 in plasma and inhibited the NADPH oxidase activity in the aorta in which the transcription of nuclear factor-E2-related factor 2 (Nrf2) was increased. Nrf2 is a transcription factor regulating the expression of ROS-detoxifying genes. Besides, an enhanced expression of Nrf2-dependent genes was observed [32].

Similar findings on the mechanisms of epicatechin originate from a randomized, placebo-controlled cross-sover study performed with 12 he-

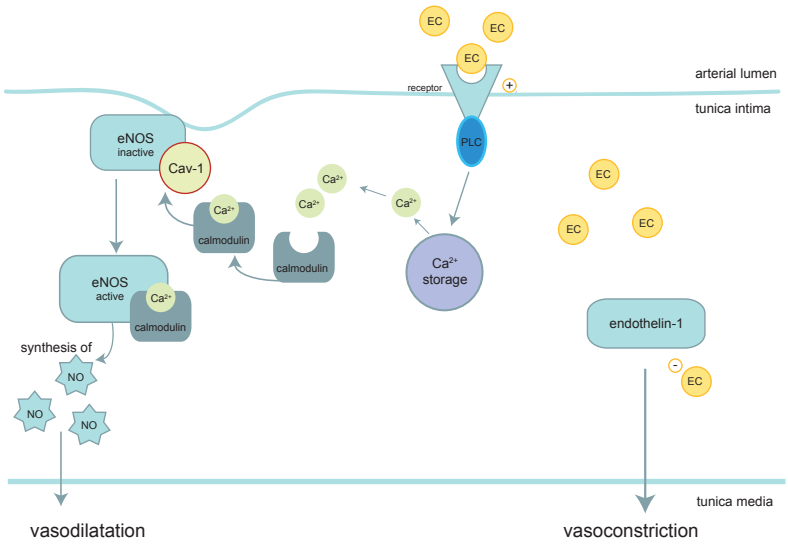

Figure 2: **Effect of epicatechin on the endothelial nitric oxide synthase**
Cav-1: Caveolin-1; EC: epicatechin; eNOS: endothelial nitric oxide synthase; NO: nitric oxide; PLC: phospholipase C

althy volunteers. Bolus administration of an aqueous solution of 200 mg isolated (–)-epicatechin increased the concentration of NO derivates in plasma and urine. The concentration of endothelin-1 decreased simultaneously in plasma, but not in urine. The levels of F2-isoprostanes, an indicator for lipid peroxidation, remained unchanged in plasma and urine [33].

In the meantime, several mechanisms at molecular level are discussed to mediate the vasoprotective effects of epicatechin. Epicatechin stimulates signal cascades inducing anti-inflammatory and vasodilatory effects. These effects are based on the modulation of cellular redox state and on specific interactions between epicatechin and elements of the signal cascade, e.g., an inhibition of the TNF-α-receptor, an activation of protein kinases, a reduced inactivation of phosphatases, and a stimulation of Nrf2. Besides, an inhibition of both NADPH oxidase and nuclear factor-κB (NF-κB), a stimulation of eNOS, and an increase in intracellular concentration of Ca^{2+} was found [34, 35]. Some of the underlying mechanisms and effects are explained below.

Stimulation of eNOS

In its inactive state, eNOS is bound to caveolin-1 at the cytoplasmatic side of the cell membrane (◆ Figure 2) [36]. An increase in intracellular calcium concentration activates calmodulin and leads to the formation of the Ca^{2+}/calmodulin-complex which displaces caveolin-1 from eNOS. This leads to an activation of eNOS [37]. Epicatechin stimulates phospholipase C, which induces the release of Ca^{2+} from intracellular storages. Eventually, eNOS is activated due to the formed Ca^{2+}/calmodulin-complex [36].

Inhibition of NADPH oxidase

NADPH oxidase catalyzes the synthesis of superoxide anions ($O_2^{\cdot-}$), which react with NO to peroxynitrite, thus reducing the availability of NO (◆ Figure 3). Peroxynitrite may isomerize to nitrate or may induce the oxidation and nitration of proteins, lipids, and DNA. In vitro, 5 µM (–)-epicatechin reduce the formation of peroxynitrite about 50 % by scavenging $O_2^{\cdot-}$. Furthermore, NADPH oxidase is inhibited about 50 % concentrentions of 11.5 and 20.7 µM of 3'- und 4'-O-methyl-(–)-epicatechin. This decreases the production of peroxynitrite and thereby increases NO availability [38].

Inhibition of NF-κB

NF-κB stimulates the transcription of several genes which favor atherogenesis. These include genes which encode for adhesion molecules, pro-inflammatory cytokines as well as for growth factors. The latter stimulate the migration and/or proliferation of smooth muscle cells [39]. In some cell-lines, the activation of NF-κB depends on oxidation status and can be inhibited by antioxidants [40].

In Jurkat T-cells, 8.6 µM epicatechin inhibited the activation of IκB-kinases, thus suppressing the phosphorylation of IκB and the release of NF-κB from NF-κB-IκB-complex. Additionally, the binding of NF-κB to promotors as well as the interleukin-2 synthesis were inhibited [41]. In Hodgkin lymphoma cells, 25 and 50 µM epicatechin suppressed the translocation of NF-κB from the cytosol to the nucleus and the binding of NF-κB to the promoter, leading to a reduced transcription of NF-κB-dependent genes [42]. Epicatechin can affect the activation of NF-κB at different stages in the signal cascade: directly by interaction with elements of the signal cascade and indirectly by antioxidative effects (◆ Figure 4) [41, 42]. In a human study, a reduced activation of NF-κB could also be found after bolus administration of 40 g cocoa powder [43].

Intake of epicatechin by cocoa consumption

If epicatechin is essential for the cardioprotective effects of cocoa, which amounts of cocoa products have to be consumed to achieve these desirable effects? Precise information about this is currently not available because the amount of epicatechin depends on the content in the cocoa bean and on further cocoa processing [13, 44]. The cocoa fraction in dark chocolate is not an appropriate indicator for the epicatechin content as the correlation between these

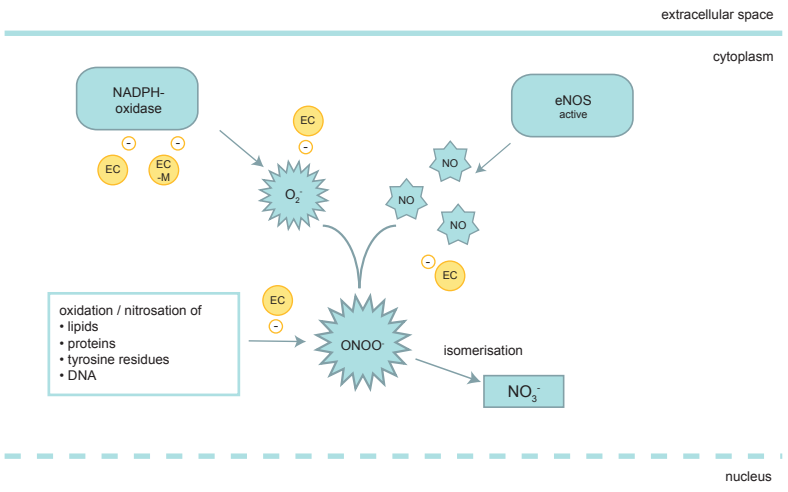

Figure 3: **Effect of epicatechin on NADPH oxidase activity**
EC: epicatechin; EC-M: epicatechin metabolites; eNOS: endothelial nitric oxide synthase; NO_3: nitrate; NO: nitric oxide; $O_2^{\cdot-}$: superoxide anion; $ONOO^-$: peroxynitrite

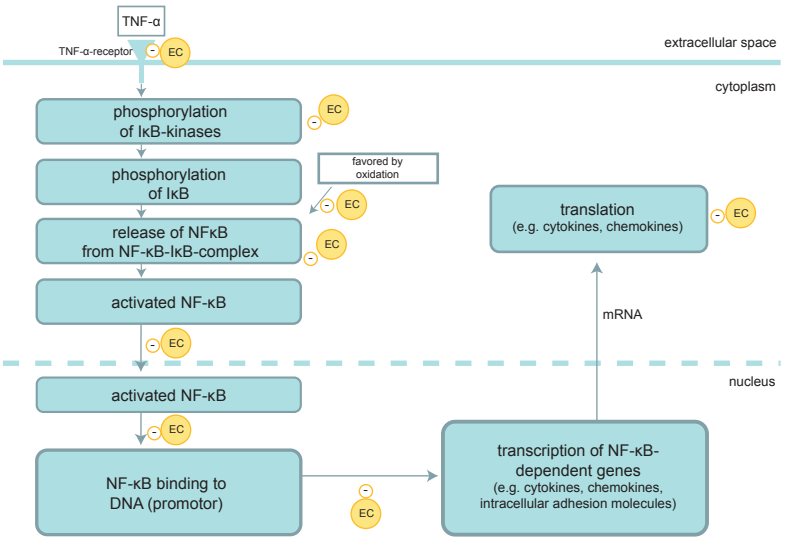

Figure 4: **Effect of epicatechin on NF-κB-signal cascade**
EC: epicatechin; IκB: inhibitor of NF-κB; NF-κB: *nuclear factor*-κB; TNF-**α:** tumor necrosis factor alpha

two factors is very low (R^2 = 0.4) [45]. The Directive 2000/36/EC of the European Parliament and Council does not prescribe the labeling of health-promoting ingredients in cocoa products. Hence, data on the epicatechin content of commercially available cocoa products originates only from studies. For example, four pieces of a semi-sweet chocolate from a German manufacturer used in a study provided 25 mg epicatechin [46]. The same amount of epicatechin could be ingested by lower amounts of the above mentioned flavanol-rich cocoa products. This is desirable due to the high energy density of cocoa products. 10 g chocolate (2 pieces) or 2.5 g cocoa powder (½ tablespoon) of these products provide 200 mg cocoa flavanols, among them 18 mg and 18.6 mg epicatechin, respectively (personal communication). These servings can be easily included in an isocaloric diet.

Discussion

The results of studies investigating the bioavailability of cocoa flavanols and the effects of isolated cocoa flavanols suggest that epicatechin is essential for the effect of cocoa consumption on vascular health. In concentrations of 17.2 μM, catechin and procyanidin B2 inhibit the binding activity of NF-κB in vitro [41], which may contribute to the vasoprotection by anti-inflammatory effects. However, after cocoa consumption, plasma concentrations were only 0.08 [22] and 0.04 μM for procyanidin B2 [23], and 0.4 [22] and 0.2 μM for catechin [23].

In mice, a high fat feed enriched with flavanol-oligomers improved postprandial glucose tolerance and insulin sensitivity much more than feed enriched with flavanol mono- or polymers or with cocoa extract. Therefore, it is assumed that oligomers improve the postprandial carbohydrate metabolism by local mechanisms in the intestine, e.g., by inhibiting the digestion and the absorption of carbohydrates [47]. Human intervention studies have shown that non-absorbed cocoa flavanols are degraded by the microbiota of the colon to lower molecular phenolic acids [48] as well as to γ-valerolactones [25] which can be absorbed. However, it has not been investigated yet, whether these metabolites may contribute to vascular health.

Molecular targets with key roles in inflammatory and vasodilatory processes can be modulated by epicatechin in concentrations that can be achieved in plasma after cocoa consumption. In plasma, the major fraction of epicatechin occurs as metabolites; epicatechin itself is not [28] or only in traces [27] detectable. Most in vitro studies did not investigate metabolites of epicatechin [36, 37, 41, 42]. Steffen et al. [38] found an inhibition of NADPH oxidase in vitro by 3′- and 4′-O-methylepicatechin in concentrations which can be achieved after cocoa consumption (IC50 11 μM and 21 μM, respectively). In humans, an inhibition of NF-κB after cocoa consumption was observed as well [43].

Administration of 10 mg epicatechin/kg BW to rats led to an inhibition of Nrf2, NADPH oxidase, endothelin-1, and lipid peroxidation in plasma [32]. This dose was 28-times higher than that ingested by humans with an epicatechin-rich diet [12]. After bolus administration of 200 mg isolated (–)-epicatechin, Loke et al. [33] observed changes in different mediators that can explain directly (increase of NO-derivates, decrease of endothelin-1) or indirectly (inhibition of lipid peroxidation) the reduction in blood pressure and vasodilatory effects. Loke et al. postulate that 200 mg (–)-epicatechin can be ingested with a flavonoid-rich diet. With an average intake of epicatechin of about 20 to 25 mg/day, Great Britain, Umeå/Sweden and Varese/Italy are characterized by a high intake in flavanol monomers in Europe [12]. Hence, the dose of 200 mg epicatechin supplied by Loke et al. [33] is rather a pharmacological than a nutritional dose. It still needs to be clarified if epicatechin in nutritive doses that can be consumed with a flavanol-rich diet may also exert vasoprotective effects.

With regard to a mean blood pressure reduction of 4.2 mmHg systolic expected after an intake of 25 mg epicatechin by cocoa products [8], the question about the efficacy of nutritive doses of epicatechin is primarily of interest in the prevention of hypertension. Although the effect will be too low to normalize a pathologically increased blood pressure, the consumption of flavanol-rich cocoa products may support the pharmacological treatment of hypertension in patients with a normal body weight.

Conclusion

There are several indications that (–)-epicatechin is essential for the vasoprotective effects of cocoa products. To prove this hypothesis, randomized, double-blind, placebo-controlled intervention studies are needed. These should administrate isolated flavanols in nutritive doses which can be ingested by usual consumption of cocoa products. On this basis, it may be possible to determine the amount of a cocoa product with known flavanol composition which is needed to obtain beneficial effects on vascular health. This is necessary as the intake of cocoa products can only be recommended to a limited extent due to the high energy density and the high amounts of sucrose and saturated fatty acids..

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