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Epicatechin ingested via cocoa products reduces blood pressure in humans: a nonlinear regression model with a Bayesian approach[1,2,3]

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Abstract

Background: Four meta-analyses of randomized controlled trials (RCTs) based on the classical random-effects model showed that cocoa consumption can reduce systolic blood pressure (SBP) and diastolic blood pressure (DBP). Because epicatechin is suggested to be responsible for the treatment effect, changes in blood pressure should depend on the dose of ingested epicatechin, which may explain the between-study differences.

Objective: The objective was to quantify the effect of epicatechin ingested via cocoa products on changes in SBP and DBP.

Design: A nonlinear meta-regression model was chosen to investigate the impact of the epicatechin dose on changes in SBP and DBP. A Bayesian approach using Markov chain Monte Carlo methods was applied for an appropriate treatment of the nonlinearity.

Results: Data from 16 RCTs on SBP and 15 RCTs on DBP were included. The dose of epicatechin ingested via cocoa products influenced the changes in SBP and DBP. The asymptotic limit for the reduction was estimated at −4.6 mm Hg (95% CI: −5.4, −3.9 mm Hg) for SBP and at −2.1 mm Hg (95% CI: −2.7, −1.6 mm Hg) for DBP. An intake of 25 mg epicatechin/d led to a mean reduction of −4.1 mm Hg (95% CI: −4.6, −3.6 mm Hg) in SBP and of −2.0 mm Hg (95% CI: −2.4, −1.5 mm Hg) in DBP.

Conclusions: Blood pressure reduction by consumption of cocoa products depends on the dose of ingested epicatechin, which explains most of the between-study differences in classical meta-analyses. Similar effects may be achieved by consumption of other foods that are also rich in epicatechin.

INTRODUCTION

Epidemiologic studies suggest that regular cocoa consumption may contribute to a reduced risk of development of hypertension, a well-known risk factor for cardiovascular disease (CVD)[4], which is the leading cause of death worldwide (1). In 2 cohort studies, the intake of cocoa products was inversely associated with systolic blood pressure (SBP) and diastolic blood pressure (DBP) (2) and with the prevalence of or mortality for CVD (3, 4). CVD is scarce in Kuna Indians who live in their indigenous islands in Panama and who have a traditionally high cocoa consumption (5). When Kunas emigrated to the urban areas of Panama City, their mean blood pressure (BP) increased, which may be related to changes in lifestyle and in dietary habits, including a reduction in consumption of cocoa-containing beverages by a factor of ~10 (6).

The protective effect of cocoa on BP is ascribed to flavanols that occur in cocoa as monomers (epicatechin and catechin), dimers, oligomers, and polymers, also called procyanidins (7). Cocoa flavanols are supposed to increase the bioavailability of nitric oxide, which can amplify the relaxation of smooth muscle and results in vasodilatation (8). Further potential mechanisms of cocoa flavanols

are the promotion of vasodilatory substances (eg, endothelium-derived hyperpolarizing factor, prostacyclin) and the inhibition of angiotensin-converting enzyme (7). After cocoa consumption, epicatechin in plasma seems to be primarily responsible for these effects because the bioavailability of catechin (9, 10) and procyanidins (10) is comparably low, and only epicatechin in plasma, not catechin, was related to the increase in flow-mediated dilatation (11). The administration of pure (−)-epicatechin increased the concentration of vasodilatory nitric oxide products and reduced vasoconstrictive endothelin-1 in plasma (12) and led to vasodilation, which did not occur after administration of pure (−)-catechin or (+)-catechin (9).

In the past decade, several randomized controlled trials (RCTs) were performed to evaluate the effect of cocoa product consumption on arterial BP. Three meta-analyses confirmed a reduction in BP by consumption of cocoa products (13–15). A further meta-analysis (16) published in 2010 found a significant reduction in BP in the prehypertensive/hypertensive subgroup after regular intake of chocolate or cocoa-containing beverages or tablets. In the normotensive subgroup, significant changes were not found. Statistical analyses were based on the classical random-effects model, which was implemented by using the Cochrane Review Manager (RevMan) (17). This model assumes that the heterogeneity across individual RCTs is caused by random effects.

According to Grassi et al (18), one reason for the different outcome in BP in single-intervention studies may be the variable dose of flavanols. This led to our hypothesis that the between-study differences resulted from different intakes of epicatechin, which has been shown to be the main flavanol in human plasma after cocoa consumption (19, 20).

The aim of our meta-regression analysis therefore was to investigate whether these between-study differences could be explained by a nonlinear regression model that considers the dose of epicatechin ingested.

METHODS

Literature search

We searched in MEDLINE via PubMed (http://www.ncbi.nlm.nih.gov/pubmed) for RCTs published until July 2011 that investigated the effects of daily consumption of flavanol-rich cocoa products on BP. "Cocoa" OR "cacao" OR "chocolate" were used as key words in combination with "blood pressure" AND "clinical trial" OR "clinical study" OR "intervention study." In addition, reference lists of published trials and reviews were checked. A literature search was performed by 2 independent investigators (SE and AR).

Selection of trials

RCTs were included in our meta-analysis if they met the following criteria:

1) Study control foods or drinks were low in or free of flavanols (eg, white chocolate, placebo drink).

2) BP was determined after an overnight fast (acute effects on BP, which may result from caffeine and theobromine, need to be excluded).

3) Numerical data on mean or median SBP or DBP and SDs, SEMs, 95% CIs, or *P* values for changes were given.

4) Data on epicatechin intake via cocoa were provided.

In 3 cases, missing data were obtained from the corresponding author (21–23) and in one case (24) from the meta-analysis of Ried et al (16). If data on the same study were published in different publications, only one article was considered.

Data extraction

Data on SBP and DBP (means, SDs, SEMs, 95% CIs, or *P* values) and on epicatechin intake via cocoa consumption were independently extracted by 2 investigators (SE and AR) by using a standard form. Extracted data also included study characteristics (authors, sample size, subject characteristics, type and period of

intervention, and study design). If available, data on ambulatory BP measurement (24-h values) were preferred to data on clinical BP (25–27).

Statistical analyses

The differences in BP changes between intervention and control groups were considered as a treatment effect, which was the primary outcome variable. Complete data (means and SDs) on treatment effect were provided only by Taubert et al (21) and Heiss et al (28). In 3 cases, the SDs of the treatment effect were calculated from the *P* values (27, 29, 30). In other cases, the SDs of the treatment effect were determined by those of the changes in values for the intervention and control groups. For crossover studies (25, 26, 28, 31, 32), a correlation (r = 0.68) between values obtained by treatment with cocoa and control products was assumed according to Desch et al (15). In 4 cases, all values needed at this stage were given (22, 23, 25, 33) (for hypertensive patients only in reference 25). In all other cases, means and SDs were calculated from the pre- and postconsumption values of the intervention control groups (26, 28, 31, 32, 34). A correlation between pre- and postconsumption data was assumed (r = 0.75), which reflects the correlation between pre- and postintervention BP of the placebo group (n = 90) in a previous study (35).

We investigated the dependency of BP change on the dose of epicatechin ingested via consumption of cocoa products. To describe the dependency, we chose a function approximately linear for small doses of epicatechin. This assumption is suggested by the finding of an in vitro study that epicatechin absorption is linear to epicatechin dose (36). The changes in BP, however, were limited. Therefore, we modified this function by introducing an asymptotic value K for large doses of epicatechin.

Our meta-regression model relates BP changes (y_i) to the dose of ingested epicatechin EE_i by the following equation:

$$y_i = KcEE_i/(K + cEE_i) + \epsilon_i \qquad (1)$$

where the error ϵ_i is assumed to be a random variable with a zero mean. For our analysis, the SDs of each random variable were taken from the results of individual trials. For small values of EE_i, the chosen regression curve shows an approximately linear decrease in BP cEE_i with an initial slope c.

To obtain posterior distributions that take into account the nonlinearity of the meta-regression model, we used Bayesian statistics in connection with Markov chain Monte Carlo methods. The Bayesian approach with a Markov chain Monte Carlo method provides samples for the variables c and K, leading to posterior probability distributions. We chose noninformative prior density distributions for c and K in the range of −10 to 0. Monte Carlo simulations were carried out with 4 chains, each with 20,000 iterations (37), 10,000 of which were used to obtain the posterior distributions of the variables. These distributions delivered credible intervals for coefficients c and K of BP reduction, which approximately correspond to CIs in classical (frequentistic) statistics. For better visualization of the variability, we smoothed the density function by using a Gaussian kernel.

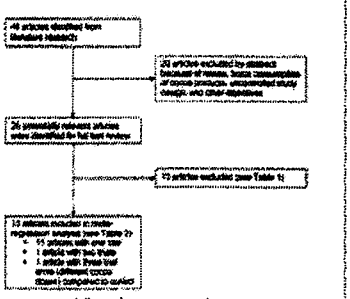
For our analysis, we used the package R2WinBUGS in the statistical programming language R in connection with OpenBugs (38), which is an open-source version of the simulation tools WinBUGS and Bugs (37).

RESULTS

Twenty-six potentially relevant articles that investigated the effect of daily cocoa consumption on BP in humans were identified from the literature search. As shown in **Figure 1**, 13 articles were excluded for the following reasons:

FIGURE 1.

Flowchart of trial selection process.



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1) A control group receiving a product free of or with traces of cocoa was missing (39, 40)

2) Postintervention BP was not measured after an overnight fast (41)

3) Data on mean or median SBP and DBP and on SDs, 95% CIs, SEMs, or *P* values were not available (42–44)

4) Data on epicatechin intake were missing (24, 45–49)

Two articles published data on BP of the same study with normotensive subjects (25, 50). In this case, the article that presented data on 24-h ambulatory BP (25) was selected for our analysis; the other one with data on clinical BP (50) was excluded. The characteristics of those studies excluded from our meta-regression analysis are shown in **Table 1**. Grassi et al (25) provided data on a further trial in hypertensive patients that was also included in our analysis. Davison et al (33) compared the dose-dependent effects of cocoa in 3 trial arms with a flavanol-free control group, which we considered separately. From Davison et al (23), only data on the nonexercise arm were included in our analysis.

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TABLE 1

Characteristics of randomized controlled trials excluded from our meta-analysis[1]

Sixteen trials from 13 publications (21–23, 25–34) were eventually included in our Bayesian meta-regression analysis (**Table 2**). Thus, 16 trials on SBP and 15 trials on DBP were considered. The regression curve characterized by the variables *K* and *c* shows the dependency of the reduction in SBP and DBP on the dose of epicatechin ingested via consumption of flavanol-rich cocoa (**Figure 2**). The variability is shown by 95% CIs and by probability density curves (**Figure 3**). The estimated asymptotic value *K* of the treatment effect was −4.6 mm Hg (95% CI: −5.4, −3.9 mm Hg) for SBP and −2.1 mm Hg (95% CI: −2.7, −1.6 mm Hg) for DBP (Figure 3). The coefficient *c* reflecting the initial slope of the curve was −2.5 mm Hg/mg for SBP (95% CI: −8.4, −0.6 mm Hg/mg). For DBP, the mean value for *c* was −4.3 mm Hg/mg (95% CI: −9.6, −0.2 mm Hg/mg) (Figure 3). The posterior density distribution of *c* cannot clearly be distinguished from the prior distribution, which is reflected by relatively broad credible intervals. The initial slope *c* determines the effect on BP only for very low doses of epicatechin. However, for a dose of 25 mg epicatechin, a mean reduction of −4.1 mm Hg in SBP (95% CI: −4.6, −3.6 mm Hg) and a mean reduction of −2.0 (95% CI: −2.4, −1.5 mm Hg) in DBP can be estimated (Figure 3). The widths of the credible intervals for an intake of 25 mg epicatechin are comparable to those of *K*.

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TABLE 2

Characteristics of randomized controlled trials included in the meta-regression analysis[1]

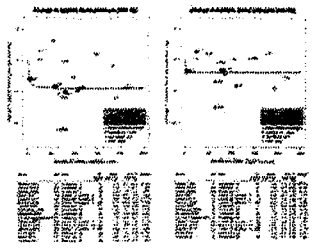

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FIGURE 2.

Regression curves showing the mean impact of the ingested epicatechin dose via cocoa consumption on changes in systolic and diastolic blood pressure. Results are based on a nonlinear regression model that was obtained with a Bayesian approach. Sixteen trials on systolic blood pressure and 15 trials on diastolic blood pressure were considered. The area of each diamond reflects the weight of the trial in our model, which is reciprocal to the variance given in each trial. Numbers in the upper part of the figure are reference numbers. The letter after the reference number marks different trials in the same publication. EC, epicatechin.

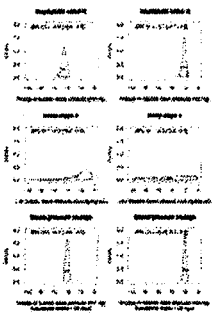

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FIGURE 3.

Posterior distribution of changes in systolic blood pressure (left panels) and diastolic blood pressure (right panels) dependent on the dose of epicatechin ingested with cocoa products. Results that included 95% credible intervals as a measure of uncertainty were obtained by Bayesian estimations with Markov chain Monte Carlo methods for the variable *K* (asymptotic value), for the initial slope *c* (to determine the changes in the treatment effect for low epicatechin doses), and for a daily intake of 25 mg epicatechin. Sixteen trials on systolic blood pressure and 15 trials on diastolic blood pressure were considered. The marked areas represent 95% credible intervals. CI, credible intervals; EC, epicatechin.

DISCUSSION

Our meta-regression analysis investigated the impact of the dose of the phytochemical epicatechin on changes in SBP and DBP by consumption of flavanol-rich cocoa products. Our analysis is a novel approach that considers the role of a nutrient by Bayesian statistics and includes a Markov chain Monte Carlo method that takes full account of the nonlinearity of the regression model.

Our results showed that the dose of ingested epicatechin influences the mean treatment effect (Figure 2). Moreover, the dose of ingested epicatechin explains in part the heterogeneity between the studies on changes in BP, because the 95% credible intervals are narrower in the nonlinear regression model (Figure 3) compared with the 95% CIs given in 2010 by Desch et al (15) (SBP: −5.87, −3.16 mm Hg; DBP: −3.87, −1.16 mm Hg) and Ried et al (16) (SBP: −5.08, −1.23 mm Hg; DBP: −3.35, −0.69 mm Hg). These meta-analyses had databases (10 and 15 RCTs, respectively) comparable to that of our study (16 RCTs). The much wider 95% CIs in the meta-analyses by Taubert et al (13) (SBP: −7.6, −1.8 mm Hg; DBP: −4.8, −0.8 mm Hg) and Hooper et al (14) (SBP: −9.55, −2.21 mm Hg; DBP: −5.77, −0.83 mm Hg) published in 2007 and 2008, respectively, may be explained by the fewer number of studies (n = 5 in both analyses) considered. Except for very low doses of epicatechin, our meta-regression model estimates the treatment effect more precisely than does the random-effects model as shown by the smaller credible intervals given by our model (Figure 3) compared with the CIs obtained by Desch et al (15) and Ried et al (16).

Ried et al (16) investigated the impact of the polyphenol dose ingested with cocoa on changes in BP by a meta-regression analysis but did not find an association with BP outcome. However, the dose of polyphenols reflects the sum of individual substances considered (flavanols such as catechin, epicatechin, several procyanidins, and further flavonoids or phenolic acids). Data on vasodilatory compounds with known bioavailability (eg, epicatechin and quercetin) (51) were not comparable between single trials. Because the concentration of quercetin in cocoa powder was ~1000 times lower than that of epicatechin (52), effects by quercetin intake with cocoa consumption on changes in BP are rather unlikely. Epicatechin, the major flavanol in human plasma after cocoa consumption (10, 19), may also originate from the degradation of procyanidins B2 and B5 (epicatechin dimers) (10). This finding has also been shown in studies with rats (53) and by perfusion models (54). Because the epicatechin dose in cocoa strongly correlates with the dose of epicatechin di-, tri-, and tetramers (R^2 = 0.989 for procyanidin B2, R^2 = 0.931 for procyanidin B5, R^2 = 0.956 for trimer C, and R^2 = 0.617 for tetramer D) (55), it is unlikely that the consideration of procyanidins in addition to epicatechin intake via cocoa would lead to other results due to multicollinearity. For these reasons, the dose of ingested epicatechin seems to be a suitable variable for regression analysis.

A strength of our analysis is the nonlinear regression function, which limits the effect on BP change and takes into account physiologic mechanisms to maintain BP in vivo. Furthermore, our meta-regression analysis used an adequate database because most of the included trials had a crossover design, were double-blind, or used a blinded operator for BP determination and had a high compliance with the consumption of test products (**Table 3**). In most trials, nonsmokers were investigated, and the consumption of flavanol-rich food was restricted. Hence, confounding effects on BP change by smoking (56) or by epicatechin intake from other sources are unlikely.

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TABLE 3

Studies conducted in subjects with normal blood pressure compared with studies in subjects with elevated blood pressure (prehypertension, hypertension)[1]

The statistical analysis gives only coarse estimates on BP change for relatively low epicatechin doses due to the few studies in which only small doses were ingested. Our analysis included data on 24-h ambulatory BP and on clinical BP. This was a compromise because data obtained by the same measurement method were not available. However, with regard to the relatively high correlation (r = 0.77) between clinical BP and 24-h ambulatory BP (57), this compromise should not have affected our results. A further limitation of our analysis results from the fact that only 4 out of 16 trials included in our meta-regression model examined subjects with normal BP (SPB <120 mm Hg and DBP <80 mm Hg) (58) (Table 3). For this reason, it is questionable if, from our results, BP change by epicatechin intake can be predicted for normotensive subjects with a similar precision. For these subjects, Ried et al (16) did not find a significant reduction of SBP and DBP by cocoa consumption in their meta-analysis.

It may be expected that a daily epicatechin intake of 25 mg via cocoa consumption can reduce blood pressure through an increased availability of nitric oxide because plasma nitroso species increased in plasma after administration of pure epicatechin (1 or 2 mg/kg body weight) (11) and after chocolate consumption that provided only 5.1 mg epicatechin (22). A 50% reduction in the activity of angiotensin-converting enzyme in vitro was observed for epicatechin concentrations of 1.7-3.4 mmol/L (59-61). The epicatechin concentration in human plasma after cocoa consumption reached only nanomolar and low micromolar concentrations (10, 62). Therefore, it is rather unlikely that an inhibition of angiotensin-converting enzyme in vivo explains BP reduction by epicatechin intake from cocoa consumption.

A reduction in SBP of 2 mm Hg was associated with a 10% lower stroke mortality and a 7% lower mortality from ischemic heart disease and other vascular diseases in middle-age subjects (63). It may be hypothesized that a mean reduction in SBP

of 4.1 mm Hg through intake of 25 mg epicatechin after cocoa consumption (Figure 3) will further decrease mortality rates.

Our results suggest that a daily dose of 25 mg epicatechin, which can be ingested by 25–30 g of commercially available cocoa-rich chocolate such as Ritter Sport Halbbitter (50% cocoa; Ritter) (Table 2) or by a flavanol-rich cocoa drink, leads to a significant reduction of −4.1 mm Hg in SBP (95% CI: −4.6, −3.6 mm Hg) and of −2.0 mm Hg (95% CI: −2.4, −1.5 mm Hg) in DBP (Figure 3). Because cocoa products have a relatively high energy density, cocoa consumption might favor obesity-related hypertension. This may compensate the BP-reducing effect of cocoa consumption. This raises the question whether BP can also be reduced by epicatechin intake from other foods such as apples and green tea. Apples provide 7–23 mg (64) and green tea 5–16 mg (65) epicatechin per serving size (125 g and 200 mL, respectively), depending on apple variety (64) and tea brand (65) and brewing conditions (66) but contain less energy per serving (or no energy) than do cocoa products. Previous meta-analyses of RCTs did not find any changes in SBP and DBP via regular consumption of black tea and/or green tea (13, 14). However, due to the low number of trials included by Taubert et al (13) (n = 5) and Hooper et al (14) (n = 4 for black tea and n = 2 for green tea, investigated separately), the effect of tea consumption on BP remains unclear. Nevertheless, BP-reducing effects by tea consumption would be plausible with regard to the results of mechanistic studies using different tea flavanols (51, 67). It should be emphasized that the synthesis of vasodilatory mediators such as nitric oxide and endothelium-derived hyperpolarizing factor is not restricted to epicatechin (51, 68). Thus, further flavanols in addition to epicatechin that are ingested with other foods might contribute to BP reduction in vivo, depending on the bioavailability of the flavanol (67).

In conclusion, our nonlinear meta-regression analysis showed that the dose of ingested epicatechin influences the reduction in SBP and DBP via regular cocoa consumption. Mean treatment effect can be estimated more precisely by our nonlinear regression model than by the random-effects model commonly used for meta-analysis. To achieve a reduction in BP, large amounts of chocolate or cocoa beverages, which are rich in energy and may thus favor obesity, a well-known risk factor for hypertension and for CVD, are not necessary. Similar meta-regression analysis should be performed to investigate whether epicatechin intake via sources other than cocoa confirm our results. Even if the BP-reducing effect by epicatechin ingestion is restricted to prehypertensive and hypertensive subjects, we have to be aware that these are the primary groups who may benefit from this measure for prevention of and therapy for hypertension.

Acknowledgments

The authors' responsibilities were as follows—SE: conceived this meta-analysis and drafted the manuscript with support from HPH; HPH: proposed the regression model with a Bayesian approach; SE and AR: searched independently for relevant studies, extracted independently relevant data by using a standard form, and cross-checked the results; PS: clarified the main purpose of the study; and SE and HPH: had primary responsibility for the design of this meta-analysis, for writing the manuscript, and for the final content. All of the authors read and approved the final manuscript. None of the authors had a conflict of interest.

Footnotes

4 Abbreviations used: BP, blood pressure; CVD, cardiovascular disease; DBP, diastolic blood pressure; RCT, randomized controlled trial; SBP, systolic blood pressure.

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