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ORIGINAL CONTRIBUTION

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Consumption of flavanol-rich cocoa acutely increases microcirculation in human skin

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Abstract *Background* Long term cocoa ingestion leads to an increased resistance against UV-induced erythema and a lowered transepidermal water loss. *Aim of the study* To investigate the acute effects of a single dose of cocoa rich in flavanols on dermal microcirculation. *Methods* In a crossover design study, 10 healthy women ingested a cocoa drink (100 ml) with high (329 mg) or low (27 mg) content of flavanols. The major flavanol monomer in both drinks was epicatechin, 61 mg in the high flavanol, and 6.6 mg in the low flavanol product per 100 ml. Dermal blood flow and oxygen saturation of hemoglobin were examined by laser Doppler flowmetry and spectroscopically at 1 mm skin depth at t = 0, 1, 2, 4, and 6 h. At the same time points, plasma levels of total epicatechin (free compound plus conjugates) were measured by means of HPLC. *Results* Subsequent to the intake of high flavanol cocoa, dermal blood flow was significantly increased by 1.7-fold at t = 2 h and oxygen saturation was elevated 1.8-fold. No statistically significant changes were found upon intake of low flavanol cocoa. Maximum plasma levels of total epicatechin were observed 1 h after ingestion of the high flavanol cocoa drink, 11.6 ± 7.4 nmol/l at baseline, and 62.9 ± 35.8 nmol/l at 1 h. No change of total epicatechin was found in the low flavanol group. *Conclusion* Flavanol-rich cocoa consumption acutely increases dermal blood flow and oxygen saturation.

Key words epicatechin – microcirculation – skin – human – flavonoids – blood flow

Introduction

Dietary constituents contribute to skin health, based on sufficient supply with macronutrients and micronutrients [1, 2]. Human and animal studies provide evidence that flavonoids, e.g., polyphenols of the catechin-type, such as (−)-epicatechin, are photopreventive agents [3]. (−)-Epicatechin and the stereoisomeric (+)-catechin are secondary plant constituents found in blueberries, cranberries, grapes, apples, red wine, tea, and cocoa [4]. They occur as monomeric flavan-3-ols or as polymers, called procyanidins. In a long-term intervention study with a high flavanol cocoa drink, we have recently shown that regular consumption of a cocoa beverage rich in flavanols increases cutaneous microcirculation and strengthens photo protective properties of the skin [5]. In that 12-week study, women who regularly consumed a flavanol rich cocoa beverage experienced a significant increase in blood flow in cutaneous and subcutaneous tissue, as well as improved resistance

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against UV-induced erythema. Also, skin structure and texture were affected by the regular consumption of a high flavanol cocoa drink. Density and hydration of the skin were elevated, whereas transepidermal water loss was lowered, as was skin roughness and scaling. These changes were not observed upon consumption of the macronutrient-matched low flavanol cocoa beverage.

The biochemical mechanisms responsible for the observed changes of skin performance and appearance are not completely known. Cocoa flavanols may enhance the antioxidant capacity of plasma, scavenging reactive oxygen, or nitrogen species, and chelate transition metal ions which participate in Fenton type reactions [6]. While the antioxidant properties of flavanols, including cocoa flavanols, have been investigated intensively, there is a growing body of in vitro and in vivo evidence supporting that cocoa flavanols may exert biological effects independent of their ability to function as antioxidants [7]. As an extension of our previous work demonstrating changes in skin quality and cutaneous blood flow following the consumption of flavanol rich cocoa for 12 weeks, the aim of the present study was to investigate if cocoa flavanols could exert acute effects on cutaneous microcirculation after a single dose.

Material and methods

Chemicals

(−)-Epicatechin and ß-glucuronidase/sulfatase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents and HPLC-grade solvents were obtained from Merck (Darmstadt, Germany). The cocoa powders high in flavanols (HF) or low in flavanols (LF) were provided by Mars Inc. (Hackettstown, NJ, USA); for details on the composition see Table 1. The powder was dissolved in 100 ml of hot water to obtain a dairy based cocoa drink.

Subjects and study design

Ten non-smoking adult female volunteers (18–65 years) with healthy, normal skin were included in the study. Exclusion criteria were: pregnancy and breast-feeding, smoking, intake of medication that might influence the outcome of the study, sunbathing or the use of sun-beds, intake of vitamin supplements, and diets comprising a change of normal eating habits. Volunteers were randomly assigned to either the high flavanol group (HF) or the low flavanol group (LF), after a washout period of 14 days crossover was performed. The drink was ingested after an overnight fast; volunteers were advised to refrain from the consumption of other flavanol-rich foods for 24 h prior testing.

Table 1 Composition of the cocoa powder used to prepare the high flavanol (HF) and low flavanol (LF) cocoa beverage

Parameter, unit	HF	LF
Energy, kJ	222	239
Total fat, g	1.0	1.0
Sodium, mg	60	140
Total carbohydrates, g	9.0	9.0
Fiber, g	4.0	4.0
Sugar, g	5.0	5.0
Protein, g	5.0	5.0
Caffeine, mg	10.6	12.3
Theobromine, mg	195	190
Total cocoa flavanols, mg	329	27
Epicatechin (monomer), mg	61.1	6.6
Catechin (monomer), mg	20.4	1.6

Units refer to the single dose applied in the experiment; 18 g of cocoa powder in 100 ml of water

In the HF group, a single dose of 329 mg of cocoa flavanols (the term 'cocoa flavanols' encompasses the flavanol monomers, specifically epicatechin and catechin, as well as the oligomers up through decamer formed from these monomers) was provided in the form of a beverage; the LF group received a macronutrient-matched cocoa drink providing 27 mg of flavanols. At baseline (t = 0 h) and 1, 2, 4, and 6 h after cocoa consumption, blood was collected into 10 ml S-Monovette-tubes (Sarstedt, Nuembrecht, Germany) containing potassium EDTA as the anticoagulant. Plasma was separated by centrifugation and stored at −80°C until analyses.

HPLC analysis of plasma epicatechin

Plasma samples were analyzed according to method Warden et al. [8] with modifications: 0.5 ml of plasma was mixed with 1.0 ml buffer (100 mM potassium phosphate, pH 7.4) and 20 μl glucuronidase/sulfatase (100, 000 and 7, 500 units/ml, respectively) and incubated at 37°C for 30 min to hydrolyse glucuronate and sulfate conjugates of epicatechin. For extraction of epicatechin 6 ml tert-butylmethyl ether was added, and the mixture was vortexed for 30 sec. After centrifugation (2 min, 4, 000 × g), 5 ml of the supernatant was transferred to a plastic tube. The organic solvent was evaporated under a stream of nitrogen and the residue was dissolved in 20 μl methanol/100 μl mobile phase for HPLC analyses.

Separation was performed on a reversed phase RP18 column (LiChrospher 100 RP-18e 5 μm 150 mm × 4.6 mm with a RP-18e guard column; Merck Hitachi) operated at 37°C (flow rate 1 ml/min) using 2 mM potassium phosphate buffer (pH 3) /16%

acetonitrile as mobile phase. The HPLC system consisted of a L-7100 pump equipped with a L-2200 autosampler, a 655A column oven (Merck Hitachi, Darmstadt, Germany). Detection was with an electrochemical detector (Coulochem Model 5100A, ESA Inc., Bedford, MA, USA) at + 550 mV. Quantification was with the external standard method.

Measurement of cutaneous microcirculation

Blood flow in skin and oxygen saturation of hemoglobin were determined with the Oxygen-To-See-system (O2C-system; Lea Instruments, Giessen, Germany) at 1 mm depth [9]. The measurements of blood flow and velocity are based on the Doppler effect; the frequency of light is shifted by a moving erythrocyte depending on its velocity. Hemoglobin amount and oxygen saturation were determined spectroscopically.

Calculations and statistical analysis

For the above-mentioned skin parameters, pre–post differences for all time points compared to baseline were calculated and analyzed descriptively. For the comparison of the two treatment groups, the pre–post differences were analyzed using the crossover analysis according to Koch.

Results

The composition of the HF and LF cocoa drinks was comparable except for the flavanol content (Table 1). A single dose of 329 mg flavanols was ingested with the HF beverage, only 27 mg of total flavanols was provided with the LF beverage. The dose of epicatechin consumed was 61.1 mg and 6.6 mg in the HF and LF groups, respectively.

Following consumption of the HF cocoa drink, an increase in blood flow was observed in cutaneous tissue (Fig. 1A). Compared to the baseline value, peripheral blood flow was significantly elevated about 1.7-fold at t = 2 h. Concomitantly, oxygen saturation of hemoglobin increased from 25 ± 6.3 % at t = 0 to 45 ± 10.7 % at t = 2 h. No significant change in either parameter was observed in the LF group. Blood flow velocity and hemoglobin concentration were not affected following the consumption of either the flavanol rich or flavanol poor cocoa beverage (data not shown).

At baseline, the concentration of total epicatechin in plasma (sum of free epicatechin plus enzyme cleaved glucuronate and sulfate conjugates) was similar in the HF (11.6 ± 7.4 nmol/l) and the LF group (9.5 ± 1.7 nmol/l). Plasma levels significantly rose after ingestion of a single dose of the HF cocoa drink, reaching a maximum of 62.9 ± 35.8 nmol/l at t = 1 h (Fig. 1B). The observed concentrations in plasma differed between individuals, with the maximum varying between 37 to 145 nmol/l. Total epicatechin levels decreased continuously thereafter and returned to almost baseline values at 6 h. No significant change in the plasma concentration of total epicatechin was detected in the LF group.

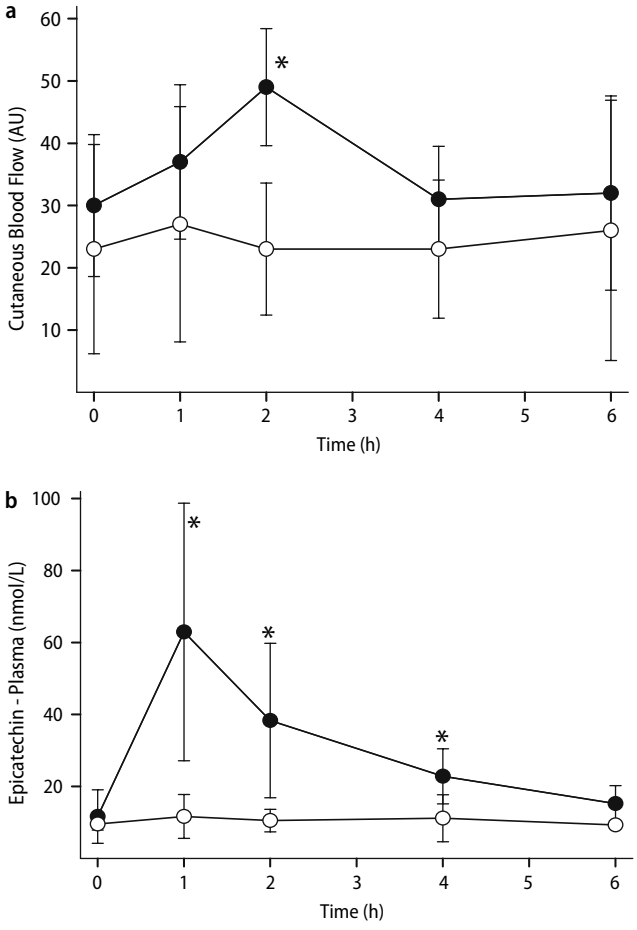

Fig. 1 Effect of high and low flavanol cocoa on cutaneous blood flow and plasma levels of total epicatechin (**A**) Cutaneous blood flow (1 mm depth) after ingestion of a single dose of high flavanol (•) or low flavanol (○) cocoa drink (n = 10). (**B**) Plasma levels of total epicatechin (free epicatechin plus glucuronate and sulfate conjugates) after ingestion of a single dose of high flavanol (•) or low flavanol (○) cocoa drink (n = 10). * significantly different from low flavanol group

Discussion

The present data demonstrates that the consumption of a cocoa drink rich in flavanols acutely increases

total plasma epicatechin concentration and cutaneous blood flow, with maximum effect observed at 1 h and 2 h after ingestion, respectively. Following the consumption of the low cocoa flavanol beverage, no increase in either cutaneous blood flow or total plasma epicatechin concentration was observed. Thus, the biological effect following the consumption of a dietary product is directly correlated to plasma levels of the bioactive compound.

The increase in cutaneous blood flow is consistent with previous work which has demonstrated the vasodilatory properties of cocoa rich in flavanols in humans [10]. Upon ingestion of flavanol-rich cocoa, an enhanced flow-mediated dilation of conduit arteries and an augmented microcirculation was measured [10, 11]. More recently, a study in smokers demonstrated improved flow-mediated vasodilation following the consumption of flavanol rich cocoa; this response was correlated with an increase in the concentration of flavanol metabolites in plasma [12]. While the specific active compounds in cocoa responsible for these effects remain to be fully elucidated, epicatechin as a vasoactive constituent was proven to mimic the effects in a human intervention study [11]. After ingestion of chemically pure (−)-epicatechin isolated from cocoa, the acute vascular effects of epicatechin were similar to those observed following the intake of flavanol-rich cocoa. Together, these studies support that specific flavanols in cocoa are at least in part responsible for the vasodilatory effects.

The mechanisms underlying the vasodilatory activity of cocoa flavanols are not yet fully understood; however, there is evidence from in vitro and in vivo studies that circulating NO-pools are affected. Increased levels of circulating NO compounds may contribute to vascular effects of flavanol-rich food [11–13] and may underlie the response of vascular function following the consumption of flavanol rich cocoa.

Cutaneous microcirculation influences thermoregulation, nutrient and oxygen delivery, and determines skin condition and appearance [1]. Long-term ingestion of cocoa flavanols affects parameters relevant for skin [5]. Short-term improvement of cutaneous blood flow may be of limited benefit for skin. However, we show in the present study that biological responses of this tissue can be obtained from diet-derived bioactive compounds.

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