Central hemodynamics and arterial function
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Chapter 8b

Supplemental data to Effect on peripheral and central blood pressure of cocoa with natural or high dose theobromine: a randomised double-blind cross-over trial

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Hypertension 2010; 56: 839-46 online supplemental data
BIOAVAILABILITY STUDY

STUDY DESIGN

We recruited twelve healthy male volunteers (aged 22±2 yrs) by advertisement. After a three day polyphenolic poor diet (consumption of tea, wine and chocolate was not allowed) the subjects consumed in a fasting state an acidified milk based test drink with cocoa containing 500 mg of polyphenols. We draw blood before (t=0) and ½, 1, 1½, 2, 3, 4, 6 and 8 hours after consumption of the test product for kinetic profiles by determining plasma concentrations of epicatechin (EC), (-)-catechin (C), and the microbial products of catechins 5-(3,4-dihydroxyphenyl)-γ-valerolactone (V1) and 5-(3-methoxy-4-hydroxyphenyl)-γ-valerolactone (V2). Furthermore we collected 24-hours urine to measure accumulation of EC and C.

LABORATORY ANALYSIS

Plasma catechins were measured by high performance liquid chromatography–multiple reaction monitoring–mass spectrometry (HPLC-MRM-MS), urine accumulation of catechins was determined using gas chromatography–mass spectrometry (GC-MS). Sample preparation: To a 200 µl plasma or 24-h urine sample, 20 µl 10% ascorbic acid containing 0.1% EDTA and 20 µl 1.5 M NaOAc (pH 4.8), 10 ng internal standard (taxifolin / ethylgallate), and 500 units glucuronidase was added, mixed and incubated at 37 °C for 45 min. Then 300 µl water, 10 µl 2 N HCl and 1 ml EtOAc was added and vortexed for 30 sec, followed by centrifugation at 3000 x g for 10 min. The EtOAc fraction was collected and the extraction was repeated twice. All samples were analyzed by HPLC-MRM-MS using calibration standards from 0 to 500 ng/ml. Note that methylated forms of catechins will not be detected with this preparation.

RESULTS

Prior to consumption of the cocoa test product plasma concentrations of EC, C, V1 and V2 were virtually not detectable. Plasma concentrations of EC and C increased significantly, reaching peak values of 63 and 4.7 µg/L within one hour after consumption of the cocoa product (see supplemental figure S1). V1 and V2 increased more gradually, still rising 8 hours after test product consumption (54 and 2 µg/L at t=8 h). The measured EC concentrations are similar to the values reported in literature.1 Forty grams of chocolate, containing 892 mg polyphenols, increased EC plasma concentrations to max 111 µg/L two hours after consumption of the chocolate. Peak values of EC in the chocolate study may have shifted due to gradual stomach
emptying of the high fat and sugar product. In the present study, the rapid metabolization of the catechins was reflected in elevated urinary excretion of EC (165 mg in 24 h) and C (10 mg in 24 h) after consumption of the cocoa test product compared to a placebo product (1 and 2 mg, respectively).

WAVEFORM SEPARATION ANALYSIS

METHODS

We used a model of the human total arterial system as described previously, which is based on the original model published by Westerhof et al. In short, the model consists of 121 segments of artery. Each segment is based on Womersley’s oscillatory flow theory, and the wall material is viscoelastic. The local peripheries are modelled with Windkessels. With the model pressure and flow at any location in the arterial tree can be calculated from another location. We used radial pressure waves measured with applanation tonometry and calibrated with brachial blood pressure to derive aortic pressure and flow as calculated by the model for the mean baseline, placebo, NTC and TEC pressure waves. Backward and forward waves were separated with waveform analysis as described by Westerhof et al. Effects of higher PWV in the TEC group were not modelled.

Supplemental Figure S1 Plasma Concentrations of Catechin, Epicatechin and Valerolactone after Consumption of Acidified Milk Drinks with Cocoa Containing 500 mg Polyphenols.
Supplemental Figure S2 Total Pressure, Forward, Backward Waves after Intake of Test Product. The upper panel shows the central aortic pressure wave per treatment group, The lower panel shows forward and backward waves.

Supplemental Table S1 Nutritional Content of the Acidified Milk Based Test Drinks per 200 gram

<table>
<thead>
<tr>
<th>Content per dose</th>
<th>Placebo</th>
<th>NTC</th>
<th>TEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocoa powder*</td>
<td>g</td>
<td>0</td>
<td>0.36</td>
</tr>
<tr>
<td>Energy</td>
<td>kcal</td>
<td>72</td>
<td>84</td>
</tr>
<tr>
<td>Total fat</td>
<td>g</td>
<td>2.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>g</td>
<td>10.4</td>
<td>11.2</td>
</tr>
<tr>
<td>Protein</td>
<td>g</td>
<td>1.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Total Polyphenols</td>
<td>mg</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>Flavanols (1-10 units)†</td>
<td>mg</td>
<td>0</td>
<td>305</td>
</tr>
<tr>
<td>Catechin‡</td>
<td>mg</td>
<td>0</td>
<td>13.4</td>
</tr>
<tr>
<td>Epicatechin‡</td>
<td>mg</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Caffeine§</td>
<td>mg</td>
<td>0</td>
<td>10.4</td>
</tr>
<tr>
<td>Theobromine</td>
<td></td>
<td></td>
<td>mg</td>
</tr>
</tbody>
</table>

Abbreviations: NTC=natural dose theobromine cocoa, TEC=theobromine enriched cocoa. *ActicoaTM cocoa powder (Barry Callebaut, Belgium), †Gallic-acid equivalents using the Folin-Ciocalteu method and an acidified methanol extraction; ‡Measured by HPLC, §61% of total polyphenols, || Measured by H NMR
REFERENCES


4. Westerhof N, Noordergraaf A. Arterial viscoelasticity: a generalized model. Effect on input impedance 

   1971;31:776-781.

6. Stergiopulos N, Young DF, Rogge TR. Computer simulation of arterial flow with applications to arterial 