Effect of Dark Chocolate on Arterial Function in Healthy Individuals

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Background: Epidemiologic studies suggest that high flavonoid intake confers a benefit on cardiovascular outcome. Endothelial function, arterial stiffness, and wave reflections are important determinants of cardiovascular performance and are predictors of cardiovascular risk.

Methods: The effect of flavonoid-rich dark chocolate (100 g) on endothelial function, aortic stiffness, wave reflections, and oxidant status were studied for 3 h in 17 young healthy volunteers according to a randomized, single-blind, sham procedure-controlled, cross-over protocol. Flow-mediated dilation (FMD) of the brachial artery, aortic augmentation index (AIx), and carotid-femoral pulse wave velocity (PWV) were used as measures of endothelial function, wave reflections, and aortic stiffness, respectively. Plasma oxidant status was evaluated with measurement of plasma malondialdehyde (MDA) and total antioxidant capacity (TAC).

Results: Chocolate led to a significant increase in resting and hyperemic brachial artery diameter throughout the study (maximum increase by 0.15 mm and 0.18 mm, respectively, $P < .001$ for both). The FMD increased significantly at 60 min (absolute increase 1.43%, $P < .05$). The AIx was significantly decreased with chocolate throughout the study (maximum absolute decrease 7.8%, $P < .001$), indicating a decrease in wave reflections, whereas PWV did not change to a significant extent. Plasma MDA and TAC did not change after chocolate, indicating no alterations in plasma oxidant status.

Conclusions: Our study shows for the first time that consumption of dark chocolate acutely decreases wave reflections, that it does not affect aortic stiffness, and that it may exert a beneficial effect on endothelial function in healthy adults. Chocolate consumption may exert a protective effect on the cardiovascular system; further studies are warranted to assess any long-term effects. Am J Hypertens 2005;18:785–791 © 2005 American Journal of Hypertension, Ltd.

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emia, or family history of premature vascular disease. They were clinically well and were taking no regular cardiovascular medications or antioxidant vitamin supplementation. Subjects abstained from caffeine and ethanol intake for at least 12 h and from flavonoid-containing food for at least 24 h before each session. Female subjects were examined during the follicular phase of menstrual cycle, and none were using oral contraceptives. The study protocol was approved by our Institutional Research Ethics Committee, and all subjects gave written informed consent.

Study Design

The study was carried out using a randomized, single-blind (operator), sham procedure–controlled, cross-over design. Subjects were studied on 2 separate days: 1 day involving chocolate consumption and 1 day sham-eating (ie, chewing). All measurements were performed in the afternoon between 3 and 7 pm on 2 separate days, in a quiet, temperature-controlled room at 23°C, while the subjects had fasted for at least 8 h. After subjects rested for 20 min in the supine position, baseline measurements for evaluation of endothelial function and arterial elastic properties were taken. The subjects were then randomized to either eat 100 g of a commercially available, procyanidin-rich dark chocolate (74% cocoa, Noir Intense, Nestlé, Vevey, Switzerland) and to drink 250 mL of water, or to sham-eat (ie, chew) and to drink 250 mL of water. Thereafter, measurements were repeated at 30, 60, 90, 120, 150, and 180 min. Venous blood for assessment of markers of plasma oxidant status was drawn into Vacutainer tubes containing ethylenediaminetetraacetate (EDTA), at baseline and 180 min after chocolate or control intake.

The chocolate used in our study contained the following procyanidins, as measured using a modified normal-phase, high-performance liquid chromatography (HPLC) method, coupled with on-line mass spectrometry: total procyanidins 2.62 g per 100 g of chocolate (procyanidin monomers plus dimers 0.54 g/100 g, trimers through heptamers 0.76 g/100 g and the remainder oligomers of greater molecular weight).

Evaluation of Endothelial Function

Flow-mediated dilation (FMD) is predominantly dependent on nitric oxide (NO) release by the endothelium, and it can be used as an estimate of endothelial function. Resting and hyperemic arterial diameters and flows and FMD of the conduit brachial artery were determined by using a high-resolution, linear-array ultrasonic transducer of 7.5 to 10.5 MHz (Sonos 5500, Hewlett-Packard, Andover, MA), as previously described.9–11 The brachial artery was scanned in the longitudinal plane, above the antecubital fossa. Reactive hyperemia was then induced by inflating a forearm occlusive cuff at suprasystolic levels for 4.5 min. Brachial artery was continuously scanned from 30 sec before to 90 sec after cuff deflation. Hyperemic velocity was assessed by a Doppler signal obtained within the first 15 sec after cuff deflation. Then, 10 min after the 180-min cuff deflation, endothelium-independent, nitrate-induced dilation was measured after delivering a single (0.4-mg) dose of nitroglycerin spray that was sublingually administered.

All scans were performed by the same examiner throughout the study. Images were recorded on super-VHS videotape; from there they were digitally produced and measured off-line by the same observer, who was blinded to the image sequence and the randomization assignment. Three cardiac cycles were analyzed, and measurements were averaged. Hyperemic artery diameter was measured 50 to 60 sec after cuff release. The FMD was calculated as the percent increase in brachial artery diameter during hyperemia compared with the resting value.

Evaluation of Aortic Elastic Properties

The pulse travels at a higher velocity in a stiffer aorta and vice versa. Carotid–femoral pulse wave velocity (PWV), an established index of aortic stiffness,2,3,12,13 was calculated from measurements of pulse transit time and the distance traveled between two recording sites (pulse wave velocity = distance [meters] / transit time [sec]) using a validated noninvasive device (Complior, Artech Medical, Pantin, France), which allows on-line pulse wave recording and automatic calculation of pulse wave velocity.14 Two different pulse waves were obtained simultaneously at two sites (at the base of the neck for the common carotid and over the right femoral artery) with two transducers. The distance was defined as follows: (distance from the suprasternal notch to femoral artery) − (distance from carotid artery to the suprasternal notch).

Measurement of Wave Reflection Indices

Augmentation index (AIx) of the central (aortic) pressure waveform was measured as an index of wave reflections.2,4,15–18 The AIx (defined as augmented pressure divided by pulse pressure and expressed as a percentage) is a composite measure of the magnitude of wave reflections and arterial stiffness, which affects timing of wave reflections. Larger values of AIx indicate increased wave reflections from the periphery or earlier return of the reflected wave as a result of increased pulse wave velocity (due to increased arterial stiffness) and vice versa. Because AIx is influenced by changes in heart rate, it was also accordingly corrected.19 Augmentation index was measured by using a validated, commercially available system (SphygmoCor, AtCor Medical, Sydney, Australia) which uses the principle of applanation tonometry and appropriate acquisition and analysis software for both noninvasive recording and analysis of the arterial pulse. The technique has been previously described in detail. In brief, from radial artery recordings, the central (aortic) arterial pressure was derived with the use of a generalized transfer function, which has been shown to give an accurate esti-
mate of the central arterial pressure waveform and its characteristics. Waveforms of radial pressure were calibrated according to sphygmomanometric systolic and diastolic pressure measured in the brachial artery, as the pressure pulse amplification between the brachial and the radial artery is practically negligible.2

### Assessment of Plasma Oxidant Status

Immediately after acquisition of venous blood, plasma was separated by centrifugation (3000 g at 4°C for 15 min) and stored at −80°C until analysis. Plasma oxidant status was evaluated with two methods, described below.

The plasma total antioxidant capacity (TAC) was determined colorimetrically (ImAnOx; Immundiagnostik AG, Bensheim, Germany). Plasma samples were assayed for their ability to eliminate a certain amount of exogenously provided hydrogen peroxide (H₂O₂) in a defined time period. The difference between applied and measured H₂O₂ is proportional to the reactivity of the antioxidants in the sample and represents its TAC. Values for lower detection limit and the intra- and interassay coefficient of variation were 130 μmol/L, 0.9% to 2.3%, and 1.63% to 2.43%, respectively.

Circulating malondialdehyde (MDA) concentration, which is one of the most reliable methods of evaluating lipid peroxidation (that is, a marker of oxidative stress) was measured as previously described.20 The MDA concentration was determined spectrophotometrically and expressed as μmol/L (Colorimetric Assay for Lipid Peroxidation, Oxford Biomedical Research, Oxford, MI). Measurements of each group were performed in triplicate, and the standard deviation was less than ±10%.

### Statistical Analysis

Numeric data are expressed as the mean ± SEM. All variables were tested for homogeneity of variance and normal distribution, before any statistical analysis was applied. Baseline parameters and endothelium-independent, nitrate-induced dilation values between the two sessions, as well as TAC and MDA within each session, were compared using the Student t test for paired measures. To evaluate the composite effect of the chocolate versus placebo over time on the variables of interest, an overall 7 × 2 analysis of variance (ANOVA) for repeated measures was performed (7 periods [baseline, 30, 60, 90, 120, 150 and 180 min] × 2 interventions [dark chocolate versus sham-eating]). A repeated-measures ANOVA was also performed to detect significant changes in variables over time within the two sessions separately. A value of $P < .05$ was considered to be statistically significant. Data analysis was performed using the SPSS statistical package for Windows, version 10.0 (SPSS Inc., Chicago, IL).

### Results

#### Baseline Characteristics

There were no differences in any baseline characteristic between the chocolate consumption and control sessions (Table 1).

#### Changes After Chocolate or Control Sessions

The effect of chocolate on each variable is best described as change in the response of each variable, where the
Response is defined as net chocolate minus control values at each time point. Accordingly, P values refer to repeated-measures ANOVA significance between the chocolate consumption and the control session throughout the study.

### Brachial Artery Study

Resting brachial artery diameter increased with chocolate throughout the study period (by 0.15 mm at 90 min and steadily increased thereafter, P < .001) (Fig. 1). Hyperemic brachial artery diameter also increased with chocolate consumption throughout the study period (by 0.15 mm at 60 min, with the response reaching 0.18 mm at 180 mm, P < .001) (Fig. 1). The FMD showed a trend to increase with chocolate consumption, and this increase was significant at 60 min (by an absolute value of 1.43%, P < .05), as shown in Fig. 1. Responses are also shown in Fig. 1.

Endothelium-independent, nitrate-induced dilation was decreased with chocolate (12.8 ± 1.1 v 14.9 ± 0.7 % for the chocolate consumption and control sessions, respectively, P < .05). Nonetheless, resting brachial artery diameter at the time of nitroglycerin administration was significantly increased in the chocolate session as compared with control session (3.97 ± 0.15 v 3.78 ± 0.13 mm, respectively, P < .001).

Resting brachial artery flow increased with chocolate consumption (by 54.0 mL/min at 90 min, P < .01), whereas there was no significant change regarding either hyperemic brachial artery flow or the percentage of reactive hyperemia.

### Heart Rate, Blood Pressure, Aortic Stiffness, and Wave Reflections

Heart rate increased significantly with chocolate consumption (maximum at 180 min, by 8.4 beats/min P < .005). Both the peripheral and central systolic, diastolic, and pulse pressures did not change significantly (P = NS) throughout the study. Concerning wave reflections indices, both augmented pressure and AIX were significantly decreased with chocolate consumption (maximum at 180 min, by 3.2 mm Hg and 10.3% [absolute value], respectively, P < .001 for both) (Fig. 2). The decrease of AIX remained significant (by 7.8% [absolute value], P < .001), even after correction for changes in heart rate (Fig. 2).

The PWV showed a stepwise decrease from 90 min onward, reaching a response of −0.28 m/sec at 180 min; however, this decrease was not significant.

### Plasma Oxidant Status

Neither plasma TAC nor MDA values changed significantly during the chocolate consumption or control session, indicating no alteration in the oxidant status of the subjects (Fig. 3).

### Discussion

This is the first study to demonstrate that, in healthy individuals, dark chocolate has an acute potent dilating effect on muscular arteries such as the brachial artery; that it decreases wave reflections; and that it does not affect stiffness of large, elastic-type arteries such as the aorta. Furthermore, our data indicate that consumption of dark
chocolate may exert a beneficial effect of endothelial function. These effects are not mediated through an improvement in antioxidant status.

Mechanisms

The decrease in wave reflections should be attributed to the decrease in the amount of the incident wave reflected at peripheral sites and not to the delayed return of this reflected wave, as there was no difference in pulse wave velocity. Thus, the predominant mechanism appears to be dilation of small and medium-sized peripheral arteries and arterioles.

Our results indicate that consumption of dark chocolate improves endothelial function. It is known that vasodilator responses of arteries decrease as their diameter increases.\(^9\,10\,21\) In our study, brachial artery response to hyperemia increased despite the fact that the artery dilated under resting conditions. Our results are in agreement with a recent study that showed that a single dose of cocoa drink rich in flavonoids reversed endothelial dysfunction in patients with either coronary artery disease or at least one cardiovascular risk factor.\(^7\) Our results are also in line with the study of Fisher et al, which showed that flavanol-rich cocoa increased pulsatile blood volume in healthy individuals in a NO-dependent manner.\(^8\)

An important finding of our study is that, contrary to our initial hypothesis and to the logical inference, these effects on arterial function are not mediated by a beneficial effect on antioxidant status, as there was no increase in plasma antioxidant capacity and there was a neutral effect of chocolate on lipid peroxidation and oxidative stress, at least within the time frame of the study. Although there is a consensus about the effect of several cocoa products in increasing plasma flavonoids, evidence concerning the resultant changes in total antioxidant capacity is conflicting.\(^22\,24\) Our results are in agreement with previous studies showing minimal increase\(^24\) or no increase\(^23\) in antioxidant capacity after ingestion of a cocoa beverage. Furthermore, our results are in line with a recent study showing no decrease in wave reflections after infusion of antioxidant ascorbic acid.\(^25\)

The dilatory effect of chocolate under resting conditions (dilation of brachial artery, decrease in wave reflections) can be attributed to improved NO bioavailability, prostacyclin increase, direct effect on chocolate in smooth muscle cells, or activation of central mechanisms. Further studies are warranted to clarify this issue. With regard to the effect of chocolate in response to stimuli such as the increase in flow, this could be attributed to improved NO bioavailability secondary to increased NO synthesis. Indeed, Karim et al\(^26\) have shown that cocoa oligomeric procyanidins induce endothelium-dependent relaxation in rabbit aortic rings in vitro, by activating endothelial nitric oxide synthase. Moreover, in the same study, investigators observed that monomeric procyanidins found in cocoa did
not exhibit the same relaxant response, nor did they activate NO synthase, even though their antioxidant capacity is well documented. Thus, the findings of Karim et al suggest that the beneficial effect of cocoa flavonoids on endothelium is not primarily a function of their antioxidant activity, and this is consistent with our results. Furthermore, our results could also be attributed to increased endothelial production of prostanoids. Dark chocolate may increase plasma prostacyclin content through modulation of the endothelial cell eicosanoid system pathway by inhibiting cyclooxygenase. This was shown in a recent study and is consistent with the ability of dark chocolate to decrease platelet aggregation in healthy individuals.

We studied dark chocolate because it is more beneficial in terms of flavonoid yield than milk chocolate, as the addition of milk in the manufacturing process inhibits the absorption of epicatechin into the bloodstream. Moreover, dark chocolate is formulated with a higher percentage of cocoa bean liquor, thus containing greater amounts of flavonoids.

**Clinical Implications**

Our study may have important clinical implications. Indeed, a growing body of evidence focuses on the potential for dietary flavonoids to decrease cardiovascular risk. Our findings provide a mechanism according to which chocolate and dietary flavonoids in general may exert a protective effect on the cardiovascular system. Wave reflections are important determinants of coronary blood flow and cardiovascular performance. Enhanced wave reflections as well as their pathophysiologic manifestations, ie, increased systolic pressure and pulse pressure (and especially central pulse pressure) have been identified as independent prognosticators of cardiovascular morbidity and mortality. Underlying mechanisms include impaired left ventricular performance, compromised coronary flow, and disrupted arterial integrity.

Furthermore, endothelial dysfunction is an important early event in atherogenesis in subjects with risk factors for atherosclerosis, which precedes arterial plaque formation. It also comprises a common feature among several states leading to atherosclerosis, such as hypertension, diabetes, hyperhomocysteinemia, and cigarette smoking. Impaired peripheral arterial endothelial function has been associated with abnormal coronary vasomotion and increased future cardiovascular risk.

The vascular actions of chocolate observed in this work could comprise the pathophysiologic background of the antihypertensive effect of chocolate in subjects with isolated systolic hypertension suggested by a recent study.

**Study Limitations and Comments**

This study refers to healthy young subjects, and further research is needed to confirm our results in other population groups. An important issue is also whether these acute findings can be extrapolated to long-term impact, and this investigation can serve as a stimulus for further research.

Chocolate contains substances such as theobromine and caffeine (in small quantities) that theoretically could have influenced our results. Although the effect of theobromine is unknown, we have previously shown that caffeine increases aortic stiffness and wave reflections. Accordingly, the observed changes could be the net result of the beneficial effect of flavonoids and the adverse effect of caffeine.

Our control arm (sham-eating) is not a direct control for the effect of cocoa flavonoids, for which a chocolate devoid of flavanols would be more appropriate. However, our study aimed at the assessment of the effects of dark chocolate as a whole, mimicking real-life consumption. Some of the vascular effects of cocoa flavonoids per se on arterial performance have already been well documented in other studies.

Endothelium-independent, nitrate-induced percent dilation was decreased with chocolate. However, most likely this does not represent a true impairment of functional integrity of smooth muscle cells but, rather, is associated with the already increased resting diameter of the vessel at the time of nitroglycerine administration.

In conclusion, our study shows for the first time that dark chocolate acutely dilates muscular arteries, decreases wave reflections, and may improve endothelial function in healthy humans. These effects do not seem to be mediated through an improvement in antioxidant status. Thus, chocolate consumption may exert a protective effect on the cardiovascular system, and further studies are warranted to assess any long-term effects. Our results suggest that studies conducted on arterial function should control for flavonoid intake.

**References**


