

Dark Chocolate Intake Buffers Stress Reactivity in Humans

To the Editor: Dark chocolate consumption substantially lowers cardiovascular mortality due to the high content of polyphenolic flavonoids (1), but underlying mechanisms remain unclear. Psychosocial stress is a risk factor that supposedly promotes cardiovascular disease (CVD) by inducing hypothalamus-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS) stress responses implicated in the increase of CVD risk, either by direct effects or by inducing adverse changes in intermediate biological risk factors, or both (2). Animal studies suggest that flavonoid administration may protect from adverse stress effects by reducing stress responses including HPA axis activation (3). A human study in healthy men assessed endocrine stress reactivity after 6 weeks of consuming either flavonoid-containing tea or flavonoid-free placebo tea and found a faster decline of cortisol levels after a moderate mental stress task in the active tea group (4). Here, we investigate whether a single administration of dark chocolate buffers endocrine reactivity to acute psychosocial stress in healthy men and whether this effect relates to plasma levels of the flavonoid epicatechin. Moreover, we wanted to determine whether this effect would be peripheral (by measuring the adrenal gland hormones cortisol and epinephrine) or more central (by assessing adrenocorticotropic hormone [ACTH], norepinephrine, and cognitive stress appraisal).

We used a placebo-controlled, between-subject study design with healthy, medication-free, non-smoking men (20 to 50 years of age) who were age-matched assigned to the experimental dark chocolate group (n = 31) or the placebo control group (n = 34). The dark chocolate ("Noir 72%"; Chocolat Frey AG, Buchs/Aargau, Switzerland) contained 281 kcal and 125 mg of epicatechin per serving of 50 g. The optically identical placebo chocolate (310.5 kcal and 0 mg epicatechin per 50-g serving) was a flavonoid-free white chocolate that was dyed and flavored to match the color, appearance, and smell of the dark chocolate. After subjects ate a standardized breakfast at 10:00 AM, a venous catheter was inserted at 10:45 AM, followed 45 min later by the first saliva and blood sampling, with subsequent administration of 50 g of dark or placebo chocolate. Subjects underwent the psychosocial stressor 2 h after chocolate ingestion, when we expected plasma flavonoid levels to peak. We applied the Trier Social Stress Test (TSST), which combines a 3-min preparation phase after a short introduction, a 5-min mock job interview, and a 5-min mental arithmetic task in front of an audience.

As stress hormones secreted from the adrenal gland and thus in the periphery only we measured the HPA axis hormone cortisol (secreted by the adrenal cortex) and the SNS hormone epinephrine (secreted by the adrenal medulla). As hormones indicating a more central stress effect, we measured the HPA axis hormone ACTH secreted by the anterior pituitary and the SNS hormone norepinephrine released both as neurotransmitter from sympathetic nerve endings and to a lesser extent as stress hormone from the adrenal medulla. Saliva (Salivette; Sarstedt, Rommelsdorf, Germany) and

blood samples were collected before chocolate consumption and immediately before TSST. Additional saliva samples were collected immediately after and up to 60 min after stress cessation. Additional blood samples were obtained immediately and 10 min after TSST (epinephrine, norepinephrine, ACTH), as well as 60 min (ACTH) and 120 min (epicatechin) after stress cessation. Blood

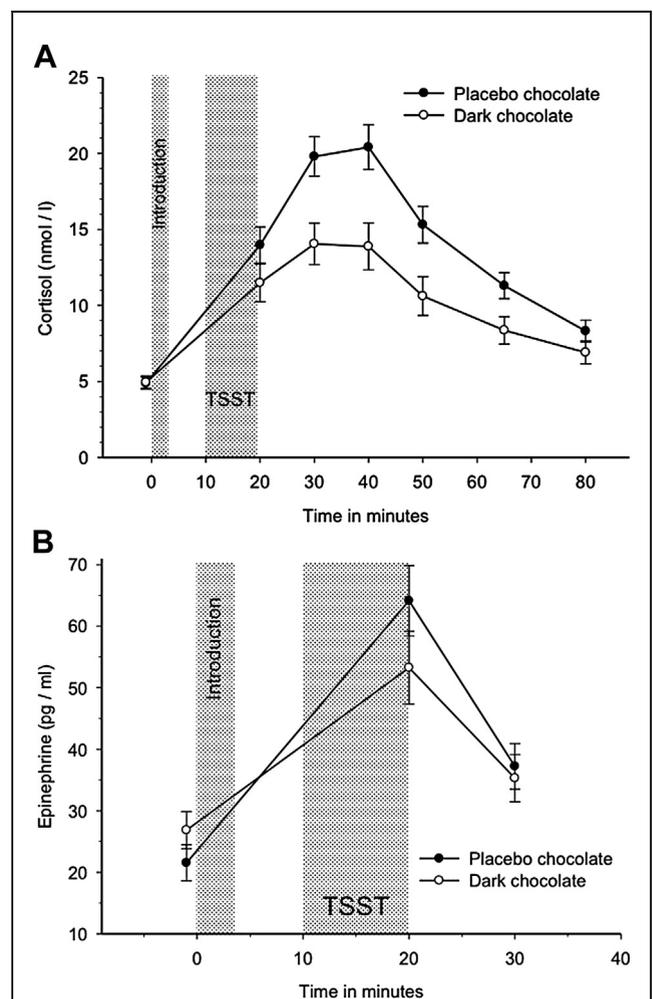


Figure 1

Physiological Reactivity to Psychosocial Stress (TSST) in the Dark Chocolate and the Placebo Chocolate Group

Values are means \pm SEM. General linear models with repeated measures of cortisol (A) and epinephrine (B) as dependent variables and chocolate group as the independent variable revealed that stress reactivity of both adrenal hormones was blunted in the dark chocolate group compared to the placebo group (cortisol: $F(2.5/154.8) = 7.47$, $p < 0.001$; epinephrine: $F(1.7/101.0) = 4.34$, $p = 0.021$). Baseline levels of the respective parameter were controlled.

was drawn into EDTA-coated monovettes (Sarstedt, Numbrecht, Germany) and immediately centrifuged for 10 min at 2000 × g and 4°C; plasma was stored at -80°C until analysis. Salivettes were stored at -20°C until biochemical analysis. Plasma ACTH concentrations were determined with a bead immunoassay (Human-Pituitary Bead Panel 1, Millipore, Zug, Switzerland) on a Guava EasyCyte flow cytometer (Millipore). Salivary cortisol was analyzed with a competitive chemiluminescence immunoassay (LIA, IBL, Hamburg, Germany). Plasma epinephrine, norepinephrine, and epicatechin levels were quantified by high-pressure liquid chromatography (HPLC) using electrochemical detection. Intra- and interassay variabilities were below 10%. As a psychological stress measure, we assessed anticipatory cognitive stress appraisal using the Primary Appraisal Secondary Appraisal (PASA) questionnaire.

Univariate analyses of variance (unit, dark chocolate group: mean ± SEM/placebo group: mean ± SEM) revealed that the groups significantly differed in epicatechin plasma levels before (ng/ml, 40.5 ± 2.9/<5, $p < 0.001$) and 120 min (ng/ml, 16.7 ± 1.1/<5, $p < 0.001$) after stress. Moreover, there were no group differences in stress hormone levels before chocolate consumption (cortisol: nmol/l, 10.1 ± 1.5/9.9 ± 1.2, $p = 0.90$; ACTH: pg/ml, 6.9 ± 1.7/8.6 ± 3.2, $p = 0.66$; epinephrine: pg/ml, 26.6 ± 3.6/19.9 ± 2.1, $p = 0.33$; norepinephrine: pg/ml, 397.4 ± 25.7/446.1 ± 33.9, $p = 0.26$), age (years, 34.5 ± 1.6/36.8 ± 1.5, $p = 0.30$), body mass index (BMI) (kg/m², 25.0 ± 0.8/25.2 ± 0.7, $p = 0.84$), mean arterial blood pressure (MAP) (mm Hg, 89.6 ± 1.8/91.3 ± 1.6, $p = 0.48$), or stress appraisal (PASA stress index, -0.79 ± 0.58/-0.45 ± 0.45, $p = 0.64$). To test whether dark chocolate consumption induced changes in stress hormone reactivity to acute psychosocial stress, we calculated general linear models with repeated measures while controlling for the pre-chocolate baseline of the respective stress hormone as covariate. Across all subjects, the TSST induced significant increases in cortisol, ACTH, epinephrine, and norepinephrine (all, $p < 0.001$). The dark chocolate group showed a significantly blunted cortisol (interaction group-by-stress $F(2.5/154.8) = 7.47$, $p < 0.001$, $\eta^2 = 0.108$, $f = 0.35$) (Fig. 1A) and epinephrine (interaction group-by-stress $F(1.7/101.0) = 4.34$, $p = 0.021$, $\eta^2 = 0.066$, $f = .27$) (Fig. 1B) reactivity to psychosocial stress compared to the placebo group. Additional controlling for age, BMI, and MAP did not significantly change these results (interaction group-by-stress cortisol: $F(2.6/155.6) = 6.59$, $p = 0.001$, $\eta^2 = 0.100$, $f = 0.33$; epinephrine: $F(1.8/101.8) = 4.06$, $p = 0.025$, $\eta^2 = 0.065$, $f = 0.26$). There were no group differences in terms of ACTH or norepinephrine stress reactivity (all, $p > 0.26$). To test whether epicatechin plasma levels prior to the TSST would predict subsequent physiological stress reactivity, we recalculated the previous general linear models but entered as independent variable pre-stress epicatechin plasma levels instead of group. Higher epicatechin plasma levels significantly related to lower stress reactivity of the adrenal gland hormones cortisol (interaction group-by-stress: $F(2.4/143.5) = 3.46$, $p = 0.027$, $\eta^2 = .054$, $f = 0.24$) and epinephrine (interaction group-by-stress $F(1.7/99.2) = 3.36$, $p = 0.047$, $\eta^2 = 0.053$, $f = 0.24$) across both subject groups, also independent of age, BMI, and MAP (interaction group-by-stress cortisol: $F(2.5/145.3) = 3.24$, $p = 0.032$, $\eta^2 = 0.053$, $f = .24$; epinephrine: $F(1.8/99.9) = 3.62$, $p = 0.036$, $\eta^2 = 0.060$, $f = 0.25$). There were no associations of epicatechin levels with ACTH or norepinephrine stress reactivity (all, $p > 0.27$).

Our findings indicate that acute flavonoid-rich dark chocolate intake buffers endocrine stress reactivity at the level of the adrenal gland, suggesting a peripheral stress-protective effect of dark chocolate consumption, particularly as in the dark chocolate group the unaffected ACTH stress response did not result in correspondingly high cortisol secretion. Although it is unclear whether epicatechin can access the human brain at levels sufficiently high to modify central nervous processes, inhibitory peripheral effects of dietary flavonoids on the biosynthesis and secretion of cortisol and catecholamines seem plausible (5). Strengths of our study include the use of a unique placebo chocolate and of a well-validated stressor. Future research is needed to determine mediating mechanisms, clinical relevance, long-term health consequences, and generalizability to chronic stress exposure and populations other than healthy men. The study is presented in more detail in the [Online Appendix](#).

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APPENDIX

The study is presented in more detail in the online version of this article.