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File: ■ Dark Chocolate (*Theobroma cacao*)
■ Gut Microbiota
■ Metabonomics

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RE: Daily Dark Chocolate Consumption Modifies the Metabolism of Healthy Humans

Martin F-PJ, Rezzi S, Peré-Trepat E, et al. Metabolic effects of dark chocolate consumption on energy, gut microbiota, and stress-related metabolism in free-living subjects. *J Proteome Res*. October 7, 2009: [epub ahead of print] doi: 10.1021/pr900607v

The metabolic phenotype of humans is influenced by dietary preferences, lifestyle characteristics, and genetics, and these factors help determine health status and the likelihood of disease development. Dietary preferences are influenced by many biological and behavioral processes, which integrate factors such as satiety, psychological perception, and the metabolic effects of foods. One of the greatest challenges in the field of nutrition is the classification of metabolic interactions between complex food matrices in an effort to understand their role in human disease processes.

Several studies have demonstrated the potential health implications of the various constituents of dark chocolate (*Theobroma cacao*), but rarely of chocolate as a "whole product." Cocoa is rich in flavonoids (e.g., epicatechin, catechin, and their oligomers), which have been shown to improve insulin sensitivity and glucose tolerance and to have cardiovascular health benefits, i.e., reductions in blood pressure, improvements in endothelial function, and decreases in thrombosis, oxidation, and inflammation. Cocoa also contains theobromine, which reduces blood pressure; phenylethylamine, a neurotransmitter; and *N*-oleoyl- and *N*-linoleoyl-ethanolamine, which slows the breakdown rate of the brain neurotransmitter anandamide. Despite evidence of the health benefits of cocoa consumption, the mechanisms of action of the bioactive components of cocoa at the molecular level are poorly understood. The objective of the present study was to use metabonomics (the metabolic response of living systems to pathophysiologic stresses or genetic modification) to evaluate the response of free-living adults to daily chocolate consumption.

Thirty healthy men (n = 11) and women (n = 19) aged 18-35 years were enrolled in this randomized parallel study, which was conducted by TNO Quality of Life (the Netherlands). The participants were requested to avoid consuming chocolate and chocolate-containing products for 8 days before the study began and then were divided into either a low-anxiety or a high-anxiety group based on their responses to a validated psychological questionnaire—the State-Trait

Anxiety Inventory test. Both groups consumed 20 g of chocolate (Noir Intense; 74% cocoa solids; Nestlé; Amsterdam, Netherlands) twice daily for 2 weeks as a midmorning and midafternoon snack for a total daily dose of 40 g. Fasting blood samples and morning spot urine samples were collected on day 1 (before intervention) and on days 8 and 15 (after intervention). The plasma and urine samples were subjected to nuclear magnetic resonance (NMR)- and mass spectrometry (MS)-based metabonomic analyses to determine global changes in metabolic profiles. Partial least squares (PLS) and orthogonal PLS discriminant analyses were used for classification purposes.

NMR and MS analyses detected a wide range of amino acids, organic acids, ketone bodies, sugars, osmolytes, saturated and unsaturated fatty acids, and triglycerides in plasma samples and of ketone bodies, organic acids, amino acids, and aromatic metabolites in urine samples. NMR and MS data indicated statistically significant time-dependent changes after dark chocolate consumption, e.g., a reduction in the urinary excretion of the stress hormones cortisol and catecholamines and partial normalization of stress-related differences in indicators of energy metabolism (glycine, *trans*-aconitate, proline, citrate, and β-alanine) and in the levels of hippurate and *p*-cresol sulfate from microbial metabolism in the gut. MS-based metabolic profiling showed that the metabolic effects induced by chocolate consumption were statistically significant only in subjects who were classified with high-anxiety. Compared with low-anxiety subjects, MS-based data showed a pre-intervention metabolic signature of higher urinary concentrations of glycine, 3-methoxytyrosine, β-alanine, proline, 3,4-dihydroxyphenylalanine, and adrenaline, and lower levels of *p*-cresol sulfate and aconitate in high-anxiety subjects. Compared with low-anxiety subjects, NMR-based data showed higher urinary excretions of hippurate, glycine, and citrate and lower levels of methyl-succinate and trans-aconitate in the high-anxiety subjects. In plasma, high-anxiety subjects tended to have higher levels of choline and lower levels of glycine and glutamine than in low-anxiety subjects. These levels in high-anxiety subjects showed a trend toward low-anxiety levels, a normalization of metabolic profiles, following chocolate consumption of 2 weeks.

The metabolic profiles in urine and plasma indicated greater interindividual differences than intraindividual differences, which point to the strong influence of lifestyle and genetic factors on individual metabolic phenotypes. The authors conclude that the results of this study provide "strong evidence" that the daily consumption of 40 g of dark chocolate for 2 weeks is sufficient to modify the metabolism of free-living and healthy human subjects, as per variation in both the host and gut microbial metabolism. These metabolic changes have "potential long-term consequences on human health within only 2 weeks treatment" as evidenced by the reduction in stress hormone levels and in the normalization of systemic stress metabolic signatures. Thus, even subtle changes in dietary patterns will likely effect changes in the metabolic status of free-living persons that may be associated with long-term health consequences.

—Brenda Milot, ELS