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File: ■ Cocoa (*Theobroma cacao*)
■ Peripheral Artery Disease
■ Walking Autonomy

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RE: Dark Chocolate Intake Improves Walking Distance and Walking Time in Patients with Peripheral Artery Disease

Loffredo L, Perri L, Catasca E, et al. Dark chocolate acutely improves walking autonomy in patients with peripheral artery disease. *J Am Heart Assoc*. July 2014;3(4). pii: e001072. doi: 10.1161/JAHA.114.001072.

More than a fifth of adults older than 70 years are affected by peripheral arterial disease (PAD) in Western countries.[1] A major symptom of the disease is intermittent claudication (IC), pain caused by impaired blood flow to the limbs during physical exercise. Reduced blood flow in patients with PAD is the result of endothelial dysfunction, reduced glucose oxidation, accumulation of toxic metabolites, impaired nitric oxide (NO) generation, and/or oxidative stress. In an earlier study,[2] oxidative stress resulted in impaired walking distance autonomy (WDA), while inhibiting oxidative stress led to improved maximal walking distance (MWD). Polyphenol-rich cocoa (*Theobroma cacao*) has been associated with artery dilatation by reducing oxidative stress and increasing NO generation.[3,4] In particular, dark chocolate enhances artery dilatation by lowering the activation of NOX2, a subunit of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which has been shown to exert vasoconstrictor activity in both animals and humans. These authors conducted an interventional, crossover, single-blinded study to measure the acute effect of dark chocolate on WDA, artery dilatation, and NOX2-mediated oxidative stress in patients affected by moderate-to-severe PAD.

Specifically, the trial investigated the acute effect of 40 g chocolate (dark vs. milk) on MWD, maximal walking time (MWT), ankle brachial index (ABI) at rest and postexercise, flow-mediated dilatation (FMD), oxidative stress, and NO generation. Oxidative stress was assessed through blood levels of NOX-2 derivative peptide (sNOX2-dp; a marker of NOX2 activation) and isoprostanes. Serum levels of nitrite-nitrate (NOx) were used to evaluate NO generation.

The study, conducted between January 2012 and September 2013, included 20 patients with PAD with IC. At baseline, all patients underwent a full medical history and physical examination and answered a questionnaire about their fruit and vegetable intake. They were randomly assigned to receive either 40 g dark chocolate (≥85% cocoa) or milk

chocolate (≤35% cocoa) in a crossover design, with at least 1 week separating the 2 intervention phases. The modified Folin-Ciocalteu colorimetric method used to determine the polyphenol content of the chocolate revealed a significantly higher total and single polyphenol content in the dark compared with the milk chocolate (P<0.001).

On study visit days, fasting blood samples were collected, followed by the first ABI and FMD at rest. The patients then completed the first treadmill test, after which MWD and MWT were measured and postexercise ABI was performed. The patients consumed 40 g of dark or milk chocolate. Two hours later, blood samples were again collected to analyze oxidative stress markers and epicatechin levels, and a second ABI and FMD evaluation was conducted. After 20 minutes, each patient completed a second treadmill test to determine MWD and MWT, followed by another postexercise ABI.

Compared with baseline, no difference was observed 2 hours after milk chocolate consumption in serum epicatechin, its metabolite EC-3-O-methylether, or epigallocatechin-3-gallate (EGCG) levels; however, the levels of serum catechin increased significantly. Two hours after dark chocolate intake, serum levels of epicatechin and its metabolite EC-3-O-methylether, catechin, and EGCG increased compared with baseline values.

Compared with baseline, MWD and MWT increased after dark chocolate intake (P<0.001 for both) but not after milk chocolate intake. This is a novel finding, according to the authors, noting that it supports the hypothesis that polyphenol content may be responsible for this effect, as dark chocolate is richer in polyphenols than milk chocolate.[5]

In a within-group analysis, no significant effect on ABI at rest or after exercise was observed after dark or milk chocolate intake. The analysis of variance performed on the study data revealed a significant difference for treatments in FMD (P=0.003); sNOX2-dp release (P=0.04); serum 8-iso-prostaglandin F2α-III, an indicator of lipid peroxidation (P=0.018); MWD (P=0.01); MWT (P=0.006); and postexercise ABI (P=0.04).

Pairwise comparisons showed that sNOX2-dp (P<0.001) and serum isoprostanes (P=0.01) significantly decreased after dark chocolate consumption but not after milk chocolate intake. FMD (P<0.001) and NOx (P=0.001) increased after dark chocolate intake, but no changes were observed after milk chocolate intake.

Performing a multiple linear regression analysis using a forward selection, the authors report "that Δ of MWD was independently associated with Δ of MWT (P<0.001) and Δ of NOx (P=0.018)."

The authors conducted an accompanying in vitro study in which human umbilical vein endothelial cells (HUVECs) were cultured to analyze the effect of scalar doses of single polyphenols such as epicatechin, catechin, or EGCG or a mixture of those on HUVEC activation. They found that HUVECs incubated with a mixture of polyphenols significantly increased NO (P<0.001). Significant decreases were seen in levels of E-selectin (P<0.001) and soluble vascular adhesion molecule-1 (P<0.001) (both associated with cardiovascular disease risk).

The vasodilating effect of dark chocolate could be due to the antioxidant effect of its polyphenols, which has been documented in humans through reduction of oxidative

stress markers and an increase in its plasma antioxidant property. In this study, the patients who consumed dark chocolate experienced short-term changes in oxidative stress elicited by reduced serum isoprotanes, reduced NOX2 activity, and enhanced generation of NO. "These data may lead to speculation that the enhanced NO generation could be responsible for artery dilatation and eventually improve WDA," write the authors.

Referring to the study's limitations, the authors suggest that although the study is useful in understanding the mechanism of disease related to IC, the results are not transferable to clinical practice because of the small sample size and study design (single-blinded with no placebo group). Also, they say, only indirect evidence suggests that vasodilation is the mechanism behind the increase in walking autonomy; a direct analysis of peripheral circulation was not done.

"The results of this study suggest that short-term administration of dark chocolate improves walking autonomy with a mechanism involving its high content of polyphenols and perhaps mediated by an oxidative stress mechanism, which ultimately leads to enhanced NO generation." A longer duration of dark chocolate intake should be studied to assess whether it could be used to treat IC in patients with PAD.

—Shari Henson

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Referenced article can be found at http://jaha.ahajournals.org/content/3/4/e001072.long.