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Dark Chocolate may Improve NAFLD and Metabolic Syndrome by Reducing Oxidative Stress

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Nonalcoholic steatohepatitis (NASH) is the most prevalent chronic liver disease in the world (1). Therapeutic options for NASH are limited such that lifestyle modification remains the cornerstone of long-term therapy (2). Several nutraceuticals have been investigated in NASH; well known among these is the association between coffee consumption and fibrosis reduction in NASH (3). Poly-unsaturated fatty acids, and polyphenols such as silymarin, curcumin, and resveratrol have been studied in pre-clinical models and small scale trials demonstrating reduction in markers of liver injury, inflammation, apoptosis and oxidation (4). In a recent issue of the journal, *Loffredo et al* (5) have investigated the effects of dark chocolate consumption in patients with NASH.

Chocolate consumption, especially dark chocolate is associated with lowering of lipid peroxidation and thereby improving cardiovascular risk (6). The polyphenolic compounds present in dark chocolate including epicatechin, a known natural antioxidant, are implicated in many of the salutary effects of dark chocolate, partly via inhibition of nicotinamide adenine dinucleotide phosphate-oxidase (NOX) (7). Experimental models demonstrate that the mitochondrial oxidation of fatty acids is enhanced in nonalcoholic fatty liver disease (NAFLD), such that increased flux through the mitochondrial tricarboxylic cycle leads to an increase in reactive oxygen species (ROS) and induction of proinflammatory mediators; furthermore, the inhibition of this flux mitigates hepatic insulin resistance, ROS and inflammation (8). Therefore, inhibition of ROS signaling presents an attractive therapeutic opportunity (2).

In this single blind, crossover trial, 19 NASH subjects and 19 age, gender, and body mass index matched controls were randomized to 20 g dark chocolate or milk chocolate twice a day for 2 weeks, with a 1 week washout period in between. By self-monitoring, caloric intake was identical among subjects, and adjusted for chocolate-derived energy. NASH

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3. Both authors have approved the final version of this article

subjects had higher serum levels of soluble NOX2-derivative peptide (sNOX2-dp) and serum isoprostanes, both markers of oxidative signaling, compared to controls. Hepatic steatosis by ultrasonography and serum cytokeratin 18 fragment M30 (CK-18 M30), a marker of caspase activation were also elevated in NASH subjects. Following dark chocolate administration for 2 weeks there was a significant reduction in serum sNOX2-dp and isoprostane levels. This effect was not observed following milk chocolate administration. In keeping with this, there was a significantly greater increase in total polyphenols and an epicatechin metabolite following dark chocolate administration. This correlated, in the dark chocolate group, with a reduction in circulating CK-18 M30 levels; together, indicating a reduction in oxidative signaling and apoptosis. Innate immune cell-mediated inflammation and hepatocyte apoptosis are key pathogenic events in NASH. Indeed, in this study the cellular targets, i.e., hepatocytes vs. innate immune cells, or the subcellular pathways responsible for the reduction in liver injury and ROS production have not been dissected. Though dark chocolate led to a reduction in the sNOX2-dp levels, NOX2 is predominantly found on phagocytically active cells such as neutrophils and macrophages, though reported on hepatocytes and other liver cell types (9). Furthermore, the markers of oxidative stress and apoptosis utilized in this study remain non-specific, such that, we cannot convincingly conclude that ROS signaling and apoptosis were lowered in the liver following dark chocolate consumption.

Though this study provides preliminary data for the consideration of the nutraceutical cocoa in the armamentarium of NASH therapy, larger scale and long-term data with inclusion of clinically relevant endpoints, such as resolution of NASH or improvement in fibrosis, are needed before broader implementation of these findings. Recognizing the difficulties of conducting a double-blind trial of chocolate administration, cocoa extract in pill form would be amenable to ideal trial design, and this would be without the added caloric burden of chocolate consumption. Therefore, large, placebo-controlled, randomized controlled trials are needed to assess the efficacy of dark chocolate before making broad recommendations regarding its use as an anti-NASH therapy.

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