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Sustained Benefits in Vascular Function Through Flavanol-Containing Cocoa in Medicated Diabetic Patients

A Double-Masked, Randomized, Controlled Trial

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Objectives Our goal was to test feasibility and efficacy of a dietary intervention based on daily intake of flavanol-containing cocoa for improving vascular function of medicated diabetic patients.

Background Even in fully medicated diabetic patients, overall prognosis is unfavorable due to deteriorated cardiovascular function. Based on epidemiological data, diets rich in flavanols are associated with a reduced cardiovascular risk.

Methods In a feasibility study with 10 diabetic patients, we assessed vascular function as flow-mediated dilation (FMD) of the brachial artery, plasma levels of flavanol metabolites, and tolerability after an acute, single-dose ingestion of cocoa, containing increasing concentrations of flavanols (75, 371, and 963 mg). In a subsequent efficacy study, changes in vascular function in 41 medicated diabetic patients were assessed after a 30-day, thrice-daily dietary intervention with either flavanol-rich cocoa (321 mg flavanols per dose) or a nutrient-matched control (25 mg flavanols per dose). Both studies were undertaken in a randomized, double-masked fashion. Primary and secondary outcome measures included changes in FMD and plasma flavanol metabolites, respectively.

Results A single ingestion of flavanol-containing cocoa was dose-dependently associated with significant acute increases in circulating flavanols and FMD (at 2 h: from 3.7 ± 0.2% to 5.5 ± 0.4%, $p < 0.001$). A 30-day, thrice-daily consumption of flavanol-containing cocoa increased baseline FMD by 30% ($p < 0.0001$), while acute increases of FMD upon ingestion of flavanol-containing cocoa continued to be manifest throughout the study. Treatment was well tolerated without evidence of tachyphylaxia. Endothelium-independent responses, blood pressure, heart rate, and glycemic control were unaffected.

Conclusions Diets rich in flavanols reverse vascular dysfunction in diabetes, highlighting therapeutic potentials in cardiovascular disease. (J Am Coll Cardiol 2008;51:2141–9) © 2008 by the American College of Cardiology Foundation

The prevalence of type 2 diabetes mellitus is rising worldwide, accompanied by an increasing risk of cardiovascular disease and mortality (1). Intense pharmacologic treatment regiments are necessary, but often remain inadequate to prevent incidence and complications of type 2 diabetes mellitus (2). Observational studies have shown that physical activity, weight loss, and diet can prevent diabetes and its complications (3). Primary and secondary prevention of type 2 diabetes and its complications by life style modifications is highly desirable.

See page 2150

Diet is a major lifestyle factor that can greatly influence the incidence and the progression of chronic diseases, such as cancer, cardiovascular disease, and diabetes (1). Recently, flavanols, a subgroup of plant-derived phytochemicals called flavonoids, have gained increasing attention, as epidemiological investigations revealed an inverse correlation between the dietary intake of flavanols and the mortality of

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Abbreviations and Acronyms

FMD = flow-mediated dilation
GTN = glycerol trinitrate
NO = nitric oxide

cardiovascular disease (4), and the incidence of diabetes (5). In the context of human nutrition, flavanols are found in fruit, vegetables, tea, red wine (6), and especially high concentrations can be present in cocoa and cocoa products (7). Dietary interventions with flavanol-containing cocoa products in humans indicate beneficial effects of flavanols on low-density lipoprotein oxidation (8), platelet aggregation (9), insulin sensitivity (10), endothelial function (11), and blood pressure (12).

Controlled trials evaluating longer-term effects of flavanol-containing cocoas on endothelial function in diabetic patients are missing. The aim of this double-masked, randomized, controlled study was to examine the vasculoprotective impact of daily flavanol-containing cocoa intake in diabetic patients.

Methods

A *feasibility study* was performed as proof-of-concept and dose-finding, and to assess safety and tolerability. In addition, the effect size of flow-mediated dilation (FMD) was measured in order to calculate appropriate sample sizes for subsequent investigations. In a consecutive *efficacy study*, the effects of daily flavanol-containing cocoa intake on vascular function were investigated. Both studies were double-masked, randomized, and controlled for macro- and micro-nutrient content in the flavanol-containing cocoa and its control.

Participants. Volunteers of both genders, between 50 and 80 years of age, with established and stably-treated type 2 diabetes mellitus for at least 5 years were screened. Eligibility was confirmed according to the guidelines of the American Diabetes Association (13). Other cardiovascular risk factors and pre-existing cardiovascular disease did not preclude from participation in the study. Exclusion criteria were congestive heart failure with a cardiac ejection fraction of <30%, malignancies, chronic kidney disease with a glomerular filtration rate of <30 ml/min, severe cardiac arrhythmias, and inflammation (C-reactive protein >5 mg/l). All volunteers were either life-long nonsmokers or reported smoking abstinence of at least 5 years before study inclusion. Volunteers participating in the *feasibility study* were asked to refrain from foods already known to have a high content of flavanols, such as tea, red wine, certain vegetables, and cocoa products (6). Participants enrolled for the *efficacy study* were allowed to continue their normal, daily lifestyle and eating habits. All volunteers fasted overnight and refrained from their prescribed medication for 12 h before and until completion of the studies in the morning on each study day.

Study design. For the *feasibility study*, diabetic patients were randomized in a double-masked, 3-period cross-over design, and consumed either a cocoa drink that provided a control (75 mg of flavanols), medium (371 mg of flavanols), or high (963 mg of flavanols) dose of flavanols on 3 different occasions. Eligible patients were assigned to the control, medium, and high flavanol dose by permuted block randomization with blocks of length 3 stratified by gender. The *efficacy study* was undertaken using a randomized, double-masked, parallel-group design, and eligible diabetic patients were allocated to a treatment group (321 mg of flavanols per dose; 3 doses per day), or a control group (25 mg of flavanols per dose; 3 doses per day). Each group ingested a single dose of either treatment or control thrice daily over a period of 30 days. Volunteers were assigned to the control or the treatment group using permuted block randomization with blocks of lengths 4 stratified by gender. To conceal allocations from investigators, instructed staff at an independent site, not involved in this investigation, generated and maintained the randomization lists.

Study protocols were approved by the institutional review boards. All volunteers were informed about the study, and gave written informed consent to be included. Before intake of the flavanol-containing cocoa, baseline measurements of FMD of the brachial artery, plasma flavanol metabolites, arterial blood pressure, and heart rate were obtained. For the *feasibility study*, FMD measurements were repeated at 1, 2, 3, 4, and 6 h after ingestion of the cocoa drink (Fig. 1A). Based on previous reports demonstrating that flavanol metabolites reach a maximum plasma level around 2 h after ingestion (14,15), a blood sample was also taken at the 2 h time point to determine plasma flavanol concentrations. Study days were separated by at least 3 days to avoid potential carry-over effects. For the *efficacy study*, measurements of baseline FMD, blood pressure, heart rate, clinical routine, and plasma flavanol metabolites were obtained on day 0 before intake of the first cocoa drink. To assess acute effects, the latter measurements were repeated on the same day, 2 h after ingestion of the control (25 mg of flavanols) or the treatment (321 mg of flavanols) cocoa drink. Volunteers were then asked to ingest the cocoa drinks thrice daily. For the evaluation of long-term effects, the same study protocol was repeatedly applied on days 8 and 30 (Fig. 1B). Adherence to the study protocol was confirmed by weekly telephone inquiries, and by collecting the returned empty cocoa sachets at each follow-up visit.

Flavanol-containing test materials. The cocoa drinks were supplied as dry cocoa beverage mix by Mars Incorporated (Hackettstown, New Jersey). The flavanol-containing cocoa drinks were made using CocoaPro cocoa powder. All cocoa drinks were standardized for their flavanol content and profile, and closely matched for equal macro-nutrients, micro-nutrients, caloric load, theobromine, and caffeine content (Table 1). All cocoa drinks were similar in taste and supplied in individual, opaque sachets that were labeled with an anonymized 3-digit identifier code. All sachets contained 18 g of cocoa beverage mix, of which 54% (w/w) consisted of nonfat milk powder. A sachet either contained 25 mg (control) or 321 mg of total flavanols. Total flavanol amounts referenced here are defined as the sum of all monomeric flavanols and their oligomeric derivatives (dimers to decamers, i.e., 2 to 10 monomeric subunits). The monomeric flavanol fraction, 18%