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Effects of sugar-sweetened and sugar-free cocoa on endothelial function in overweight adults

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ABSTRACT

Background: Studies of cocoa suggest an array of cardiovascular benefits; however, the effects of daily intake of sugar-free and sugar-sweetened cocoa beverages on endothelial function (EF) have yet to be established.
Methods: 44 adults (BMI 25–35 kg/m^2) participated in a randomized, controlled, crossover trial. Participants were randomly assigned to a treatment sequence: sugar-free cocoa beverage, sugar-sweetened cocoa beverage, and sugar-sweetened cocoa-free placebo. Treatments were administered daily for 6 weeks, with a 4-week washout period.
Results: Cocoa ingestion improved EF measured as flow-mediated dilation (FMD) compared to placebo (sugar-free cocoa: change, 2.4% [95% CI, 1.5 to 3.2] vs. −0.8% [95% CI, −1.9 to 0.3]; difference, 3.2% [95% CI, 1.8 to 4.6]; $p<0.001$ and sugar-sweetened cocoa: change, 1.5% [95% CI, 0.6 to 2.4] vs. −0.8% [95% CI, −1.9 to 0.3]; difference, 2.3% [95% CI, 0.9 to 3.7]; $p=0.002$). The magnitude of improvement in FMD after consumption of sugar-free versus sugar-sweetened cocoa was greater, but not significantly. Other biomarkers of cardiac risk did not change appreciably from baseline. BMI remained stable throughout the study.
Conclusions: Daily cocoa ingestion improves EF independently of other biomarkers of cardiac risk, and does not cause weight gain. Sugar-free preparations may further augment endothelial function.

1. Introduction

Studies of dark chocolate suggest an array of potential health benefits, including reduced inflammation, inhibition of atherogenesis, improved endothelial function, reduced thrombosis, and interference with cellular adhesion molecules [1–4]. The beneficial cardiovascular effects of dark chocolate are generally attributed to its high content of flavonoids. Studies suggest that flavanols (a subgroup of flavanoids) improve endothelial function via the induction of nitric oxide (NO) synthesis and the protection of NO bioactivity [5,6]. Flavanols are also associated with decreased blood clotting (via inhibition of platelet activation and aggregation) and act as antioxidants [7–12]. Moreover recent studies suggest cocoa flavanols increase the concentration of high-density lipoprotein (HDL), and modify the fatty acid composition of low-density lipoprotein (LDL) causing it to become resistant to oxidative damage [13,14].

While evidence indicates that cocoa improves endothelial function, it is possible that sugar may adversely affect cardiovascular risk by increasing blood glucose, lipid levels, and body weight [15]. In fact, one study reports impairment of endothelial function with acute glucose loading [16]. To our knowledge, no study thus far has compared the effects of daily ingestion of sugar-sweetened and sugar-free cocoa beverages on vascular function. The overall study of cocoa in beverage form as the delivery vehicle for the bioactive compounds found in dark chocolate has been limited. This study examines the impact of daily consumption of sugar-sweetened and sugar-free cocoa beverages on markers of cardiovascular risk, including endothelial function and body weight, in apparently healthy overweight adults. Overweight and obesity substantially increase the risk of morbidity from hypertension; dyslipidemia; type 2 diabetes; coronary heart disease; and stroke [17,18].

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2. Materials and methods

2.1. Design and randomization

The study was a randomized, double-blind crossover trial, designed to examine the effects of daily consumption of either a sugar-free cocoa (22 g/d cocoa), a sugar-sweetened cocoa (22 g/d cocoa and 91 g/d sugar) or placebo (no cocoa with 110 g/d sugar) beverage for a six-week period, on endothelial function, body weight, and risk factors for cardiovascular disease. The trial was conducted between August 2005 and May 2006 at the Yale-Griffin Prevention Research Center Vascular Laboratory. Forty-four participants were randomly assigned to one of the six possible sequences of sugar-free cocoa, sugar-sweetened cocoa, and placebo using a computer-generated random number sequence. Each cocoa beverage delivered 11 g of cocoa powder. Participants were asked to drink two cocoa beverages per day, to achieve a daily dose of 22 g of cocoa or placebo beverage with no cocoa. Participants adhered to each treatment for six weeks, separated by four-week washout periods. The ultrasonographer and the data analyst were unaware of the treatment assignment and participants were unaware of the composition of each treatment (Fig. 1).

Sample size, allowing for a 20% attrition rate, was calculated to detect a 3.5% difference in flow-mediated dilation (FMD) between cocoa and placebo with 80% power with a two-sided alpha set at 5% adjusted for three pair-wise comparisons.

The study was approved by Griffin Hospital Institutional Review Board and the Yale Human Investigation Committee. All participants provided written informed consent and were compensated for participating in the study.

2.2. Participants

Forty-four overweight, but otherwise healthy, men and women were recruited from the lower Naugatuck Valley, Connecticut through advertisements (Table 1). Inclusion criteria were (1) body mass index (BMI) between 25 and 35 kg/m^2, (2) waist circumference of $\geq$100 cm for men and $\geq$80 cm for women and (3) non-smoker. Participants with a current eating disorder, diagnosed coronary artery disease, diabetes mellitus, sleep apnea, pregnancy, insulin or glucose sensitizing medication use, restricted diet, allergy to cocoa or inability to comply with the protocol were excluded.

Eligible participants ($n=77$) were asked to complete a clinical screening examination consisting of height, weight, BMI, waist circumference, blood pressure, and fasting glucose and lipid panel.

2.3. Intervention

The daily treatment consisted of two hot cocoa beverages per day. The cocoa powder used in the study was a natural processed cocoa (not alkali treated) supplied by The Hershey Company (Table 2). Each test beverage contained 11 g of a natural cocoa powder (sugar-sweetened, sugar-free); the placebo beverage contained 0 g cocoa powder. Each powdered preparation was mixed in 8 oz of hot water. Participants were instructed to prepare and consume one beverage in the morning and one in the evening. Participants were instructed to maintain their usual physical activity and dietary habits and refrain from consuming flavonoid-rich foods for 24 h prior to each test day.

On the day of the brachial artery reactivity scans (BARS) test participants underwent endothelial function testing after an eight-hour overnight fast and then 2 h following each treatment.

Weight, height, waist circumference and blood pressure were assessed at each visit. Fasting blood glucose and blood lipid testing were conducted during each of the five visits pre- and postprandially. Blood samples were drawn, refrigerated and shipped overnight to the Department of Biobehavioral Health and Nutritional Sciences at Pennsylvania State University for oxidative LDL assay, lipid hydroperoxide assay and C-reactive protein testing.

Standard blood collection protocols were used with the addition of EDTA/plasma and BHT to extracted plasma. Participants were asked to complete a 3-day food diary for each of the six-week treatment phases as well as the four-week washout period to assess dietary patterns throughout the study period.

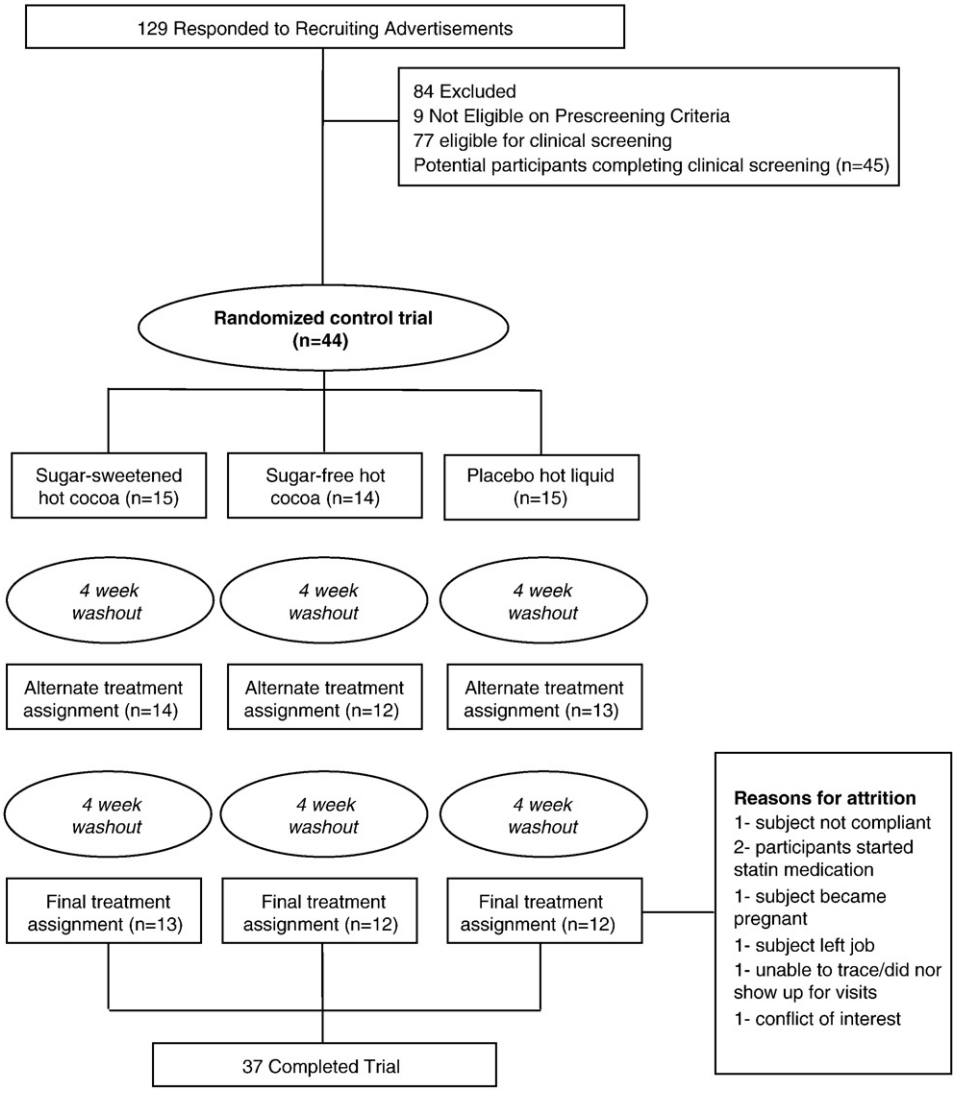

Fig. 1. Flow of participants through the trial.

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Table 1
Demographic characteristics and baseline values at the start of each treatment assignment.

Variable	Sugar-free cocoa ($n=38$)	Sugar-sweetened cocoa ($n=39$)	Placebo ($n=39$)
Age, *years, mean (SD)*	52.5 (10.4)	52.2 (11.0)	51.9 (10.8)
Gender, n (%)			
Male	6 (16)	6 (15)	6 (15)
Female	32 (84)	33 (85)	33 (85)
Body composition, mean (SD)			
Weight (lbs)	178.9 (23.9)	179.9 (24.1)	179.5 (23.2)
Body mass index, *kg/m²*	30.2 (3.4)	30.5 (3.4)	30.3 (3.4)
Waist circumference, *cm*	98.6 (7.4)	97.8 (7.7)	99.2 (8.4)
Blood Pressure, mean (SD)			
Systolic blood pressure, *mmHg*	122.8 (14.7)	123.8 (13.7)	123.6 (11.7)
Diastolic blood pressure, *mmHg*	68.6 (11.4)	68.3 (10.7)	67.3 (11.1)
Endothelial Function, mean (SD)			
Flow mediated dilation or FMD, *%*	6.4 (4.5)	6.5 (3.0)	6.0 (3.4)
Stimulus-adjusted response measure	0.06 (0.05)	0.06 (0.04)	0.05 (0.03)
Lipid Panel, mean (SD)			
Serum total cholesterol, *mg/dL*	197.5 (29.0)	197.8 (33.3)	195.5 (32.6)
Serum triglyceride, *mg/dL*	88.4 (26.0)	92.5 (33.1)	102.9 (38.2)
Serum high-density lipoprotein, *mg/dL*	59.7 (16.2)	59.8 (15.5)	56.8 (16.0)
Serum low-density lipoprotein, *mg/dL*	120.5 (27.2)	119.5 (31.3)	118.4 (32.5)
Total cholesterol/ HDL ratio	3.5 (0.9)	3.4 (0.8)	3.6 (1.0)
Serum Measures, mean (SD)			
Blood glucose level, *mg/dL*	91.9 (6.7)	91.9 (7.9)	92.7 (10.8)
LDL oxidation, *unit/L*	49.0 (13.9)	48.8 (15.9)	50.9 (12.9)
C-reactive protein,[a] *ng/mL*	868 (239, 4232)	1026 (167, 6796)	1196 (137, 12659)
Endothelin, [a]*pg/mL*	0.7 (0.0, 2.0)	0.6 (0.0, 5.0)	0.5 (0.0, 2.5)
Lipid hydroperoxide,[a] *μmol*	1.4 (0.1, 6.0)	1.5 (0.1, 8.3)	1.7 (0.2, 9.9)
Room temperature, *°F, mean (SD)*	73.8 (1.7)	73.9 (1.6)	73.5 (1.2)

[a] Values are median (minimum, maximum); for all variables, no significance (p-value > 0.05) difference observed between group (one-way ANOVA or χ^2).

Table 2
Composition of test products (amount consumed per day/two servings).

Content	Sugar-free cocoa	Sugared cocoa	Placebo cocoa
Weight*, *g*	23	115	126
Cocoa powder, *g*	22	22	0
Energy, *kcal*	90	460	500
Total fat, *g*	2	2	2
Carbohydrates, *g*	12	104	110
Sugar, g	0	91	110
Protein, *g*	6	6	8
Sodium, *mg*	110	110	410
Potassium, *mg*	334	334	512
Calcium, *mg*	33	33	314
Magnesium, *mg*	134	134	38
Catechin, *mg*	21	21	0
Epicatechin, *mg*	48	48	0
Procyanidin dimer, *mg*	92	92	0
Procyanidin trimer, *mg*	98	98	3
Procyanidin tetramer, *mg*	31	31	0
Procyanidin pentamer and hexamer, *mg*	55	55	6
Total procyanidins (total flavanols), *mg*	805	805	9
Theobromine, *mg*	436	436	0
Caffeine, *mg*	28	28	0

Energy and nutrient data of the tested products are provided by the Hershey Company
*refers to total product weight.

2.4. Endothelial function testing methodology

Endothelial function was assessed using the brachial artery reactivity study (BARS) methodology as described in published guidelines [19], enhanced by software for analysis of vessel diameter (Brachial Analysis Tools, Medical Imaging Applications). The BARS procedure is designed to measure flow-mediated dilatation (FMD) in the brachial artery as a percent of resting vessel diameter. Our methods have been published previously [20–22]. In brief, BARS was measured non-invasively in the right brachial artery using a high frequency ultrasound machine (Philips Medical Systems: Sonos 4500, Andover, MA). Participants were required to lie down (with an angled knee cushion) and rest in a quiet, temperature-controlled, softly lit room for at least 5 min before scanning. Measurement of vessel diameter and flow velocity was conducted by a vascular ultrasonographer, who was blinded to treatment.

2.5. Primary outcome

2.5.1. Endothelial function

Endothelial function was measured as FMD, i.e. the percent of change in brachial artery diameter from pre-cuff inflation to 60-s post-cuff release. Additionally, flow after cuff deflation within the first 15 s was used as an indicator of stimulus strength, hyperemic flow being the stimulus for endothelial reactivity. To account for potential variability in stimulus strength, FMD was divided by flow at 15 s post-cuff deflation to create a stimulus-adjusted response measure (SARM).

2.6. Secondary outcomes

2.6.1. Blood pressure

Blood pressure was determined using the Datascope Accutorr Plus automatic digital blood pressure device with the subject in a supine position after a 5 min period of rest. Both systolic and diastolic blood pressures were calculated as the mean value of two readings taken 5 min apart.

2.6.2. Lipid profile and fasting glucose

Serum lipids consisting of total cholesterol (TC), HDL, LDL, triglycerides and TC/HDL ratio were conducted at the Griffin Hospital laboratory using the VITROS Chemistry Analyzer (Abbott Laboratories, Abbott Park, Illinois) calorimetric method. Additionally, serum fasting blood glucose measures were taken during each visit followed by postprandial whole blood glucose measures.

2.6.3. 3-day food diary

The 3-day food diaries were analyzed using The Food Processor II — ESHA Research's basic nutrition and diet analysis software (version 7.0, ESHA Research, Salem, Oregon).

2.6.4. Serum endothelin-1

Serum endothelin-1 was quantified in human plasma (1 mL) post solid phase extraction (500 mg C18 Sep-Pak; Waters, MA) utilizing a human endothelin-1 enzyme immunometric assay kit (900-020; Assay Designs, MI) following manufacturer's instructions.

2.6.5. Serum C-reactive protein

Serum C-reactive protein (CRP) values were determined using a high sensitivity CRP (hsCRP) ELISA developed in the Pennsylvania State University GCRC Cytokine Core Laboratory. The test is intended for the quantitative determination of CRP in human serum.

2.6.6. Serum oxidized LDL

Serum oxidized LDL was determined utilizing a solid phase two-site enzyme immunoassay kit (Mercodia; Alpco Diagnostics, #0810114301) which was based on the direct sandwich technique. The ELISA quantifies circulating levels of endogenously oxidized LDL (native oxidized LDL).

2.6.7. Serum lipid hydroperoxide

The serum lipid hydroperoxide (LPO) assay was used to measure hydroperoxides in the isolated lipid-phase of the plasma, directly utilizing redox reactions with ferrous ions [23]. This assay involves the Cayman LPO kit (705003) which has been modified for higher throughput utilizing a 96-well plate reader. This method is based on the FOX assay but has been modified to produce higher reproducibility.

2.7. Statistical analysis

One-way ANOVA, including Duncan's multiple range tests, were used to assess baseline data for all outcome measures (i.e. endothelial function, blood pressure,

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anthropometric measures, lipid panel, and serum measures) and demographic variables between the three treatment assignments. Repeated measures ANOVA were used to assess differences in intra-individual responses across treatments. Paired *t*-tests were also used to assess mean changes of all outcome measures among participants by treatment assignment (i.e. sugar-free cocoa, sugar-sweetened cocoa, or placebo). The combined effect of independent variables (age, gender, race, and treatment sequence) and treatment assignment on all outcome measures was assessed with generalized linear modeling. Descriptive and exploratory analyses of all measured outcomes were carried out before embarking on modeling or hypothesis testing procedures. Normally distributed data were analyzed using parametric statistics, while non-normally distributed data were log transformed before applying parametric statistics. All analyses of endpoints were based on the intention-to-treat principle. Values presented in text and tables are means (95% CI). Data were analyzed using SAS software for Windows version 9.1 (SAS Institute Inc., 2001).

To assess the effect of different treatment assignments on outcome measures, we computed 95% confidence intervals (CI) for mean changes from baseline following each treatment assignment. Comparisons across treatment assignments were made using the 95% CI around the mean change of outcome measures from their baseline values. When the 95% CI of mean change of one treatment assignment was not included within the 95% CI of mean change of another treatment assignment (i.e. non-overlap), we considered the two treatment assignments significantly different at $p<0.05$. Mean change from baseline values of outcome measures after each treatment assignment was considered statistically significant when the 95% CI around the mean change did not include zero.

3. Results

3.1. Endothelial function

Consumption of the cocoa-containing treatment beverages (sugar-free cocoa and sugar-sweetened cocoa) for six weeks improved FMD compared to placebo ($p<0.01$) (Fig. 2). The magnitude of improvement in FMD after consumption of sugar-free cocoa was greater than after sugar-sweetened cocoa, but not significantly so ($p=0.15$) (Table 3).

Blood pressure did not improve with either active treatment as compared to placebo (sugar-free cocoa: systolic: $p=0.07$, diastolic: $p=0.50$; sugar-sweetened cocoa: systolic: $p=0.36$, diastolic: $p=0.49$) (Table 3).

3.2. Anthropometric measures

Body weight and BMI did not change as compared to placebo following ingestion of either sugar-sweetened or sugar-free cocoa over the course of the six week treatments. These findings persisted after controlling for age, gender and treatment assignment in multivariable models.

Waist circumference was reduced, but non-significantly, following ingestion of sugar-free cocoa or sugar-sweetened cocoa as compared to placebo ($p>0.05$).

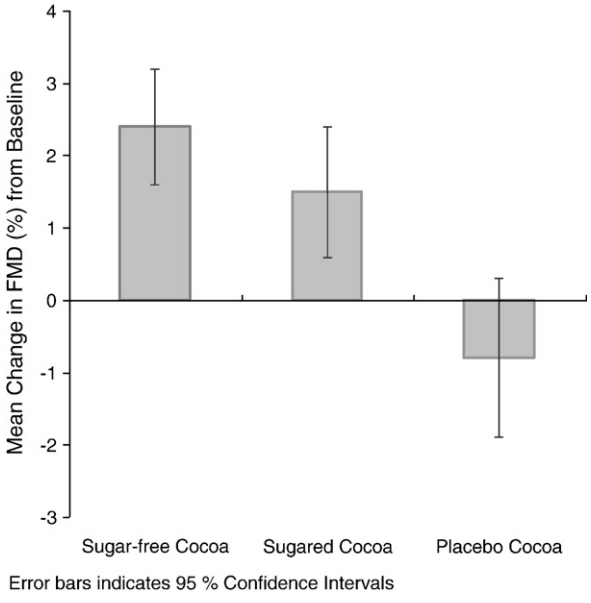

Fig. 2. Mean change in flow mediated dilatation after treatment assignment. Error bars indicate 95% confidence intervals.

3.3. Lipid profile

Serum total cholesterol to HDL ratio did not improve as compared to placebo following ingestion of sugar-free cocoa ($p=0.92$) or sugar-sweetened cocoa ($p=0.95$). Consumption of sugar-free cocoa or sugar-sweetened cocoa did not improve serum low density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride as compared to placebo (Table 3).

3.4. Other biomarkers

Serum fasting glucose, CRP, LDL oxidation, lipid hydroperoxide and endothelin did not change with either active treatment as compared to placebo (Table 3).

After adjustment for age, gender and treatment sequences, the adjusted and the unadjusted changes were similar for all outcome measures. The dietary pattern during each study phase is summarized in Table 4 and suggests that, aside from treatment assignment, dietary patterns remained stable throughout the study.

4. Discussion

To our knowledge, this is the first trial to examine the impact of sugar-free and sugar-sweetened cocoa consumption on endothelial function and body weight over a six-week period. The results of the study demonstrate that both sugar-free and sugar-sweetened cocoa significantly improve endothelial function compared to placebo without inducing weight gain. Eliminating sugar from the cocoa product seems to amplify the beneficial effects of cocoa on endothelial function, although this difference was not significant perhaps due to the limited number of participants in the trial. Sugar-free and sugar-sweetened cocoa did not have significant impact on blood pressure, lipid profile, CRP, LDL oxidation, lipid hydroperoxides, endothelin or body composition. The sugar-free cocoa treatment had a modest, favorable influence on waist circumference.

The observed impact of sugar-sweetened and sugar-free cocoa on endothelial function could be explained by the high content of flavanols in cocoa, which activate nitric oxide synthase (eNOS) [12,24]. This activation increases the production of nitric oxide (NO), which in turn leads to vascular dilation. One in vitro study found that polyphenolic compounds in red wine stimulated the expression and activity of the eNOS system, thereby leading to an increased production of vascular NO [25]. Additionally, evidence suggests that the acute ingestion of sugar may compromise endothelial function [16]. The larger improvement in endothelial function seen with sugar-free as compared to sugar-sweetened cocoa in the current study is consistent with this effect. Sugar may attenuate the benefit cocoa products confer on endothelial function, and thus products with no or lesser amounts of sugar may offer greater benefit. This area warrants further study.

Cocoa beverages in the current trial did not significantly impact biomarkers of antioxidant status and oxidative stress. These results are consistent with previous studies indicating that plasma antioxidant capacity remains unchanged acutely (2–6 h) and chronically (2–18 weeks) after consumption of high-flavonoid chocolate [1,26,27], yet other studies report significant increases in total plasma antioxidant capacity. 28]. This inconsistency may be related to non-standardized doses, forms, matrices and methods of analysis and requires further study. In addition, our results are consistent with prior studies suggesting that CRP levels do not change with cocoa or

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Table 3
Mean change in outcome measures from baseline.

Variable	Sugar-free cocoa Δ Mean (95% CI)	Sugar-sweetened cocoa Δ Mean (95% CI)	Placebo Δ Mean (95% CI)
Body composition			
Weight, *lbs*	0.0 (−0.3 to 0.4)	0.2 (−0.6 to 1.0)	0.6 (0.1 to 1.1)
Body mass index, *kg/m²*	0.0 (−0.1 to 0.1)	0.1 (−0.2 to 0.4)	0.2 (0.1 to 0.4)
Waist circumference, *cm*	−1.8 (−3.2 to −0.5)	−1.3 (−2.6 to 0.1)	−1.1 (−2.7 to 0.4)
Blood pressure			
Systolic blood pressure, *mmHg*	4.1 (0.8 to 7.3)	2.2 (−1.5 to 5.8)	−0.1 (−3.3 to 3.2)
Diastolic blood pressure, *mmHg*	−0.4 (−2.8 to 2.0)	−0.5 (−3.4 to 2.3)	0.8 (−1.7 to 3.2)
Endothelial function			
Flow mediated dilation, *%*	2.4 (1.5 to 3.2)	1.5 (0.6 to 2.4)	−0.8 (−1.9 to 0.3)
Stimulus-adjusted response measure	0.04 (0.00 to 0.07)	0.01 (−0.00 to 0.02)	−0.00 (−0.02 to 0.01)
Serum measures			
Blood glucose level, *mg/dL*	−0.9 (−3.5 to 1.7)	−2.0 (−5.5 to 1.5)	−8.2 (−14.2 to −2.2)
Serum total cholesterol, *mg/dL*	5.2 (2.4 to 8.1)	5.8 (3.5 to 8.2)	0.4 (−4.3 to 5.1)
Serum triglyceride, *mg/dL*	4.7 (1.9 to 7.4)	9.0 (4.9 to 13.1)	9.3 (4.7 to 13.8)
Serum high-density lipoprotein, *mg/dL*	2.4 (1.3 to 3.5)	3.3 (2.2 to 4.5)	1.4 (−0.2 to 2.9)
Serum low density lipoprotein, *mg/dL*	1.7 (−0.4 to 3.8)	0.8 (−1.5 to 3.1)	−2.6 (−6.9 to 1.7)
Total cholesterol/HDL ratio	−0.0 (−0.1 to 0.0)	−0.1 (−0.1 to −0.0)	0.0 (−0.1 to 0.0)
LDL oxidation, *unit/L*	−9.0 (−15.4 to −2.6)	−8.9(−14.1 to −3.6)	−11.7 (−19.2 to −4.2)
C-reactive protein, *ng/mL*	−542 (−1169 to 85)	−157 (−1010 to 697)	284 (−101 to 669)
Endothelin, *pg/ml*	0.6 (0.1 to 1.0)	0.1 (−0.1 to 0.4)	0.0 (−0.4 to 0.4)
Lipid hydroperoxide, *umol*	0.4 (−0.7 to 1.4)	−0.5 (−1.5 to 0.5)	−0.5 (−1.1 to 1.2)

Δ Mean = Mean change from baseline; CI = confidence interval.

dark chocolate ingestion. Grassi et al. reported no meaningful reduction in CRP in healthy adults with essential hypertension after ingesting dark chocolate for 15 days [29]. Mathur et al. demonstrated the antioxidant action of cocoa on the susceptibility of LDL to undergo oxidation in healthy adults, yet found no effect on hsCRP [30]. Also, using a less sensitive agglutination technique for the determination of CRP, Davidsson et al. found no change in plasma CRP in children provided iron-fortified chocolate drinks [31]. Therefore, it is possible that the absence of an effect of flavanols on CRP may reflect inadequate dose of cocoa, insufficient study duration or use of an apparently healthy subject population with relatively normal background inflammatory status [29].

Consumption of sugar-sweetened and sugar-free cocoa did not lead to any significant differences in body weight or BMI between the two groups, consistent with Engler et al [1]. In the current study, we did observe a decrease in waist circumference with consumption of

Table 4
Dietary pattern intake during treatment assignment.

Variable	Sugar-free cocoa, *n* = 32 Mean (95% CI)	Sugar-sweetened cocoa, *n* = 33 Mean (95% CI)	Placebo, *n* = 36 Mean (95% CI)
Energy, *kcal*	1779.3 (1599.6 to 1958.9)	1991.4 (1828.8 to 2154.0)	1697.6 (1511.8 to 1883.4)
Protein, *g*	77.8 (71.9 to 83.7)	77.0 (70.1 to 83.8)	83.4 (75.4 to 91.4)
Carbohydrate, *g*	212.7 (188.1 to 237.3)	274.0 (252.1 to 295.9)	297.4 (274.0 to 320.8)
Fiber, *g*	23.8 (21.3 to 26.3)	20.2 (18.1 to 22.2)	14.2 (12.3 to 16.1)
Fat, *g*	70.1 (59.9 to 80.3)	63.6 (56.1 to 71.1)	74.3 (64.7 to 83.9)
Saturated fat, *g*	23.0 (19.6 to 26.3)	20.9 (18.2 to 23.5)	24.0 (19.9 to 28.0)
Monounsaturated fat, *g*	15.0 (11.3 to 18.7)	14.6 (12.4 to 16.7)	16.3 (13.2 to 19.3)
Polyunsaturated fat, *g*	17.1 (14.9 to 19.3)	15.8 (13.4 to 18.1)	18.6 (17.0 to 20.2)
Cholesterol, *mg*	225.7 (192.3 to 259.0)	254.3 (205.9 to 302.7)	291.9 (240.4 to 343.5)
Water, *g*	1876.3 (1624.8 to 2127.8)	1632.4 (1433.2 to 1831.6)	1696.5 (1467.6 to 1925.4)
Vitamin A, *μg*	498.4 (323.5 to 673.3)	516.3 (381.2 to 651.4)	416.7 (314.3 to 519.1)
Vitamin B1, *mg*	1.1 (0.9 to 1.4)	1.1 (0.9 to 1.3)	1.0 (0.9 to 1.2)
Vitamin B2, *mg*	1.5 (1.2 to 1.9)	1.4 (12.2 to 17.2)	1.4 (1.2 to 1.6)
Vitamin B3, *mg*	15.1 (12.0 to 18.1)	14.7 (12.2 to 17.2)	14.3 (11.8 to 16.8)
Vitamin B6, *mg*	1.4 (1.0 to 1.7)	1.2 (0.9 to 1.4)	1.1 (0.9 to 1.3)
Vitamin B12, *μg*	3.7 (2.5 to 4.9)	2.9 (2.2 to 3.6)	3.5 (2.7 to 4.3)
Vitamin C, *mg*	82.3 (63.3 to 101.2)	68.3 (50.9 to 85.6)	65.9 (48.0 to 83.8)
Vitamin D, *μg*	3.9 (2.8 to 5.0)	4.5 (1.3 to 7.7)	3.7 (2.9 to 4.5)
Vitamin E, *mg*	6.4 (4.4 to 8.3)	4.9 (3.4 to 6.3)	5.4 (4.0 to 6.7)
Folate, *μg*	279.2 (210.0 to 348.4)	232.6 (183.0 to 282.2)	228.1 (182.6 to
Calcium, *mg*	713.0 (611.5 to 814.5)	711.7 (621.1 to 802.3)	965.0 (860.4 to 1069.6)
Iron, *mg*	17.0 (14.3 to 19.7)	17.6 (9.6 to 25.7)	12.8 (10.8 to 14.7)
Magnesium, *mg*	193.8 (165.0 to 222.6)	178.8 (149.4 to 208.2)	178.0 (150 to 205.9)
Phosphorous, *mg*	818.3 (692.7 to 943.9)	803.7 (684.2 to 923.3)	815.0 (715.9 to 914.0)
Potassium, *mg*	1843.4 (1581.2 to 2105.6)	1937.5 (1614.0 to 2261.0)	1831.1 (1828.8 to 2154.0)
Sodium, *mg*	3056.1 (2723.8 to 3388.3)	3073.9 (2721.9 to 3425.8)	3273.0 (2989.3 to 3556.6)
Zinc, *mg*	8.5 (6.3 to 10.7)	7.3 (6.0 to 8.7)	7.7 (6.2 to 9.1)
Alcohol, *g*	3.9 (1.5 to 6.4)	4.3 (0.3 to 8.3)	2.0 (0.0 to 4.0)
Caffeine, *mg*	111.7 (80.2 to 143.3)	101.1 (70.9 to 131.3)	103.2 (74.6 to 131.7)

CI = confidence intervals.

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sugar-free cocoa. Cocoa, both sugar-sweetened and sugar-free, offers a relatively low calorie delivery vehicle for the flavonoids and other nutrients found in cacao, and for the apparent cardiovascular benefits. Our study suggests that healthy, overweight adults can make cocoa ingestion part of their daily routine without adverse effects on body weight regulation, at least over the short term. The fact that many of our measured biomarkers of cardiac risk did not change significantly with cocoa ingestion while significant changes did occur with endothelial function, may be interpreted in several ways. The lack of influence on individual biomarkers might suggest lack of a cardioprotective effect. However, endothelial function is strongly and independently associated with cardiac risk [19]. Alternatively, endothelial function may reflect the aggregated, modest influences of a cardioprotective intervention on an array of biomarkers. Small improvements in lipids, blood pressure, and inflammatory cytokines might, when summed, translate into a significant cardiovascular benefit. The notion that endothelial function might represent this summative measure of cardiac risk is concordant with the findings in this trial. Larger effects on individual biomarkers might be seen with higher dosing, longer study duration, or in a population with greater baseline cardiac risk. Alternatively, the vascular endothelial mechanisms responsible for affecting FMD may require lower levels of flavanols than those required to affect other cardiovascular endpoints, suggesting a mechanism of action associated with gene regulation within the arteries themselves.

This study has several limitations. First, flavanols were not identified in the serum, therefore precluding associations between blood level, structure and affect. Moreover, the absorption efficiency of procyanidin polymers is questionable; therefore it is difficult to speculate whether the lack of effect on oxidative markers resulted from insufficient blood levels of the antioxidant flavanols. Second, the bioavailability of flavanols depends on other food constituents, and their interaction with the food matrix. Thus, our study results could be influenced by potential confounders such as the association between nutrient intake and their varying effects on flavanols. Additionally, endothelial and physiological responses could be skewed, secondary to unmeasured or inaccurately measured dietary intake data, changes in physical activity, noncompliance, vasoactive medication use, and genetic factors. Third, generalizability is limited due to the homogeneous population; the vast majority of enrolled participants were Caucasian women working and living in close geographic proximity.

In summary, the present trial demonstrates favorable effects of both sugar-sweetened and sugar-free daily cocoa consumption on endothelium-dependent vasodilatation. Beneficial effects on endothelial function were seen in the absence of significant effects on any single biomarker, and might represent the aggregation of modest, favorable effects on diverse indices of cardiac risk. This trial suggests, but does not conclusively establish, that the cardioprotective influence of cocoa ingestion is attenuated by the sugar content of cocoa-containing beverages, and accentuated by the removal of sugar. Finally, this trial shows that a sufficient dose of cocoa to influence vascular function can be incorporated into the daily diet of healthy adults without inducing weight gain over the short-term. Further study is warranted to assess optimal dosing (concentration, quantity, and frequency) and preparation (solid, liquid, sugar-sweetened, and sugar-free) of cocoa for cardiovascular benefit in the absence of untoward effects on either overall dietary quality or energy balance.

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The authors have certified that they comply with the Ethical publishing in the International Journal of Cardiology [32].

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