Epicatechin, procyanidins, cocoa, and appetite: a randomized controlled trial[1,2]

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ABSTRACT

Background: In 2 randomized controlled trials, it was reported that dark chocolate acutely decreased appetite in human subjects, but the authors did not assess the types or concentrations of cocoa compounds that are needed. Other studies have suggested that the cocoa compounds epicatechin and procyanidins may be involved.

Objective: We sought to test the hypotheses that, compared with placebo (an alkalized cocoa mixture containing essentially no epicatechin or procyanidins), the following beverages cause a decrease in appetite: *1*) a nonalkalized cocoa mixture; *2*) epicatechin plus placebo; and *3*) procyanidins plus placebo. We measured the concentrations of cocoa compounds in all beverages.

Design: We used a 4-way randomized, crossover, placebo-controlled trial that was balanced for period and carryover effects in 28 healthy, young-adult men. We also conducted a smaller (n = 14), parallel, secondary randomized trial in which we explored the effects of higher doses of epicatechin and procyanidins. Our primary measure of appetite was ad libitum pizza intake 150 min after beverage ingestion. We used a linear mixed-model analysis.

Results: Intakes of beverages with the nonalkalized cocoa mixture that contained 0.6 mg epicatechin, 0.2 mg catechin, and 2.9 mg monomer-decamer procyanidins/kg body weight did not decrease pizza intake significantly (P = 0.29) compared with intake of the placebo. In the smaller secondary trial, a combination of epicatechin and the nonalkalized cocoa mixture that contained 1.6 mg epicatechin/kg body weight significantly decreased pizza intake by 18.7% (P = 0.04).

Conclusions: Our nonalkalized cocoa mixture was associated with an acute decrease in food intake only after being supplemented with epicatechin. It is possible that epicatechin at a dose of >1.6 mg/kg body weight, alone or in concert with appropriate catalytic cocoa compounds, may be useful for helping people control their food intakes. This trial was registered at clinicaltrials.gov as NCT02408289. *Am J Clin Nutr* 2016;104:613–9.

Keywords: appetite, cocoa compounds, epicatechin, food intake, procyanidins, visual analog scales, weight loss

INTRODUCTION

Obesity has been shown to be the second most important lifestyle mortality risk factor in the United States (1). The growth in adiposity over the past few decades has occurred despite the availability of a wide variety of weight-loss techniques including exercise, diet, pharmaceutical drugs, and bariatric surgery (2).

Accordingly, there has been considerable interest in the results of several recent experiments that found that cocoa inhibited fat accumulation and weight gain in rodents (e.g., references 3–5), and in 2 randomized placebo-controlled trials that showed that dark chocolate can decrease appetite in human subjects. The first of those 2 studies, study by Sorensen and Astrup (6), included 16 male participants and used the amount of an ad libitum meal that was consumed 2 h after intake of chocolate as the primary measure of appetite. Compared with milk chocolate with low amounts of cocoa compounds, dark chocolate decreased food and energy intakes by 8% and promoted higher satiety scores over a period of 5 h. The second study by Akyol et al. (7) included 25 subjects and also used the amount of an ad libitum meal eaten as the primary measure of appetite. Akyol et al. (7) showed that, compared with milk chocolate, dark chocolate decreased food intake by 19.5% 4 h after intake of the chocolate but did not alter satiety scores. Neither study assessed the amounts of cocoa compounds that were used to achieve the observed decreases in appetite. In addition, the studies did not identify the responsible cocoa compounds.

Several studies have suggested that epicatechin and procyanidins, which are phytochemicals in the flavanol subclass of cocoa flavonoids that, in turn, are polyphenols (8), might be partly responsible for cocoa's potential to help people control their body weights. Hughes et al. (9) showed a significantly lower increase in body weight in women in the highest quintile of epicatechin intake in a prospective epidemiologic study that involved 4280 Dutch adults. Gutiérrez-Salmeán et al. (10) showed that a daily 1-mg dose of epicatechin/kg significantly decreased food intake in rats fed a high-fat diet. Dorenkott et al. (11) showed that supplementation of a 12-wk high-fat diet with the oligomeric fraction

[1] Supported jointly by the Professional Staff Congress and the City University of New York (PSC-CUNY research award A/C 66522-0044). The Hershey Company provided the cocoa samples and data on the compounds in the samples.

[2] Supplemental Table 1 and Supplemental Figures 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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of procyanidins decreased the gain in weight and fat mass in male mice.

The objectives of our study were to conduct a randomized, 4-way, crossover, placebo-controlled trial to assess the dose of cocoa compounds needed to decrease appetite in humans and to investigate whether epicatechin or procyanidins are responsible for the effect. We planned to test the following primary hypotheses: *1*) Our nonalkalized cocoa mixture would decrease appetite more than would our alkalized cocoa mixture (placebo). *2*) Epicatechin plus the placebo would decrease appetite more than would the placebo. *3*) Procyanidins plus the placebo would decrease appetite more than would the placebo. We also conducted a smaller secondary parallel trial to explore the effects of higher doses of epicatechin and procyanidins. The hypotheses were that *1*) epicatechin plus our nonalkalized cocoa mixture would decrease appetite more than would the placebo and that *2*) procyanidins plus our nonalkalized cocoa mixture would decrease appetite more than would the placebo.

METHODS

Participants

We recruited 30 healthy, young-adult male Brooklyn College students as participants. Students who met our exclusion and inclusion criteria and signed an informed consent were invited to participate.

Inclusion criteria were as follows: nonsmokers, BMI (in kg/m^2) between 18.5 and 30, stable weight (a gain or loss of $<$5% in the past 6 mo), moderate alcohol users ($<$2 drinks/d), mentally and physically healthy, and willing to consume pizza and cocoa beverages. Exclusion criteria were as follows: being regular, frequent drinkers of coffee, tea, or sodas that contained caffeine ($>$1 serving/d); participating in regular, frequent vigorous physical activity; using a medication that could affect appetite; being allergic to chocolate, cocoa, or pizza; attempting to gain or lose weight; and being interested in registering for courses taught by the principal investigator. Women were excluded to avoid possible effects of menstrual hormones on appetite.

Between December 2015 and February 2016, we solicited participants via campus e-mail, radio, website, Facebook page, posters on bulletin boards, face-to-face solicitations, and informational sessions (**Figure 1**). There were 203 students who sent us queries. We sent the students a screening questionnaire (SurveyMonkey) to assess compliance with our inclusion and exclusion criteria. We invited 61 students who appeared to be qualified to sign informed consents, and 47 of the students did so. We invited 32 eligible students to enter the run-in session, and 30 students entered the randomized laboratory sessions that were conducted between 3 March 2015 and 1 May 2015.

Participation requirements

Participants attended a run-in session and 4 randomized laboratory sessions that were spaced ≥1 wk apart to help achieve an appropriate washout of the effects of the beverage ingested at the previous laboratory session and to minimize bias that was due to participants being able to recall the amount of pizza that they ate at the previous session.

During each laboratory session, participants were required to remain seated and were not allowed to consume any foods or liquids that were not supplied by the researchers. Subjects were allowed to read, do homework, play board or card games, use the toilet, or talk about matters other than those that could affect their appetites. All participants who attended the randomized laboratory sessions complied with these requirements.

Between laboratory sessions, participation requirements were as follows: to eat similar quantities of foods that contained constituents from the same food groups and with similar amounts of macronutrients during the same periods of the day after midnight of the day before each laboratory session; to avoid chocolate or cocoa beverages, tea, coffee, or other caffeinated drinks or tobacco or nicotine products for the duration of the study; and to refrain from exercise and use of alcohol or psychotropic drugs during the 48 h before laboratory sessions. Compliance was assessed at the start of each laboratory session with the use of a behavioral, health, and dietary-recall questionnaire. Participants who arrived $>$10 min late at a laboratory session or who reported an illness, stress, or noncompliance with participation requirements were required to postpone the laboratory session. Participants complied with all of these requirements except that one participant had one laboratory session postponed because of late arrival.

Assessment of appetite

We used 2 indicators of appetite. Our main indicator was the amount (in grams) of pizza eaten ad libitum by participants 150 min after ingestion of the beverage at the start of the laboratory session. When we planned the study, the research team taste tested several

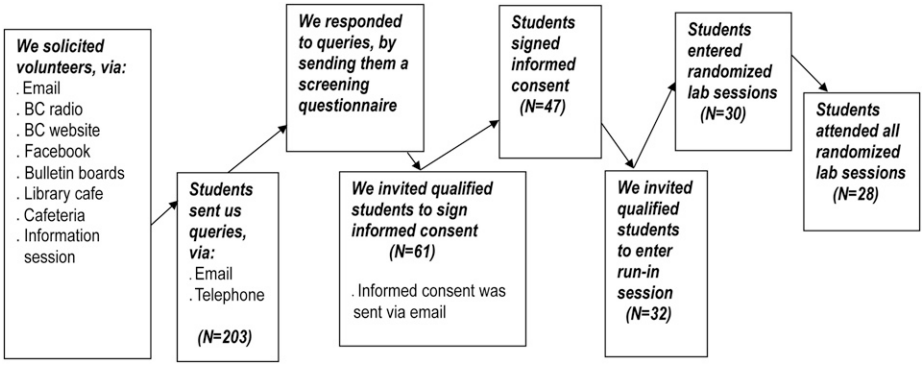

FIGURE 1 Participant flow diagram. BC, Brooklyn College.

different brands of frozen pizza and decided to serve DiGiorno's Original Rising Crust Four Cheese pizza (Nestlé USA Inc.). According to the manufacturer's data, each pizza weighed 798 g and contained 1860 kcal, 60 g fat, 30 g saturated fat, 120 mg cholesterol, 4620 mg Na, 12 g dietary fiber, 30 g sugar, 234 g carbohydrate, and 96 g protein. The macronutrient constituents (29.0% of kilocalories from fat, 50.3% of kilocalories from carbohydrate, and 20.5% of kilocalories from protein) were within the acceptable ranges issued by the board of the Institute of Medicine (12). The pizza was baked in toaster ovens (model 31103A; Hamilton Beach) and served at a temperature of 38–46°C. Each serving was one-half of a pizza that was cut into pieces of different sizes and shapes to decrease participant awareness of the amount consumed. Participants were asked to eat ad libitum and were given as many servings as desired. They were also given one 236.6-mL (8-fl oz) bottle of water at room temperature, which they were required to finish. All participants complied with these requirements. Pizza servings were weighed before and after consumption, and the before-after difference was our main indicator of appetite.

For our secondary indicator of appetite, we asked participants to perform visual analog scale (VAS) ratings of perceived hunger, emptiness, satiety, and fullness (13) 10 min before beverage ingestion (baseline) and 15, 30, 60, 90, 120, and 150 min after beverage ingestion. All participants complied. These time points were selected on the basis of a previous study that showed that plasma concentrations of cocoa catechins reached a maximum 2–2.6 h after ingestion, and for cocoa procyanidins, the maximum was reached 2 h after ingestion (14). VAS ratings ranged from 0 to 100. For instance, to rate fullness, participants were asked "How full do you feel?" A rating of 0 indicated "Not at all full"' and 100 corresponded to "totally full.''

Cocoa beverages

The alkalized cocoa (treated with an alkali solution) and nonalkalized mixtures were obtained in packets from Hershey Co. Concentrations of calories, macronutrients, and some cocoa compounds were provided by the company (**Table 1**, **Supplemental Table 1**). Concentrations of epicatechin and catechin were assessed with the use of liquid chromatography–atmospheric-pressure chemical ionization-spectrometry (15), and concentrations of flavanol dimers through decamer polymers were assessed with the use of liquid chromatography–mass spectrometry (**Supplemental Figures 1** and **2**) (16). The 2 forms of cocoa contained very similar doses of macronutrients and other constituents that could have potentially affected appetite. Epicatechin was purchased in powder form (item ASB-00005125–025, 96.2% purity; Chromadex). We purchased procyanidins as powder in capsules [Flavay, The Original (pine bark and grape seed extract) from Healthy Source]. Cocoa mixtures and compounds were dissolved in hot water to form the beverages that were served in mugs at a temperature of 40–46°C. Each participant had the same mug at each laboratory session, and the volume of each beverage was proportional to the participant's body weight (2.96 mL/kg). Participants all complied with the requirement that they ingest all contents in their mugs. Participants and research staff were blinded to the beverage contents. The principal investigator and data analyst was not blinded to the beverage contents.

To test our primary hypotheses, participants were randomly assigned to sequences of 4 beverages as follows: *1*) a nonalkalized cocoa mixture (that contained 0.6 mg epicatechin, 0.2 mg catechin, 2.9 mg dimer-decamer procyanidins, and 3.7 mg flavanols/kg body weight; flavanols were defined as epicatechin, catechin, and dimer-decamer procyanidins); *2*) an alkalized cocoa mixture (a placebo that contained close to 0 mg/kg of the previously listed compounds) (Supplemental Table 1); *3*) epicatechin (that contained 1.0 mg epicatechin/kg) plus the placebo; and *4*) procyanidins (that contained 6.6 mg procyanidins/kg) plus the placebo. The random assignment was balanced for carryover and period effects with the use of an orthogonal Latin square (17).

In the secondary small, parallel trial that explored the effects of higher doses of epicatechin and procyanidins, we gave epicatechin plus the nonalkalized cocoa mixture (1.6 mg epicatechin/kg) and procyanidins plus the nonalkalized cocoa mixture (10.3 mg procyanidins/kg) each to 7 randomly selected participants before the randomized laboratory sessions. We were only able to reject the null hypothesis of no appetite suppression for epicatechin. Therefore, we gave epicatechin plus the nonalkalized cocoa mixture to another randomly selected 7 participants after the completion of the randomized laboratory sessions.

The 7 participants who had been given epicatechin plus the nonalkalized cocoa mixture before the randomized laboratory sessions were excluded from the random selection of subjects who were to be given epicatechin plus the nonalkalized cocoa mixture after the laboratory sessions. All 14 participants were given the placebo during the laboratory sessions so that 50% of them were given epicatechin plus the nonalkalized cocoa mixture before the placebo and the other 50% were give the placebo before the epicatechin plus the nonalkalized cocoa mixture. Hence, the test of the effects of epicatechin plus the nonalkalized cocoa mixture involved 14 assessments that were balanced for period and carryover effects. With these data, we tested 3 secondary hypotheses that a mix of epicatechin plus the nonalkalized cocoa mixture (1.6 mg epicatechin/kg) would decrease appetite more than would *1*) the placebo (close to 0 mg epicatechin/kg), *2*) a combination of epicatechin plus the placebo (1.0 mg epicatechin/kg), and *3*) the nonalkalized cocoa mixture (0.6 mg epicatechin/kg). All beverages used for testing the secondary hypotheses were matched for doses of macronutrients and other constituents that could potentially affect appetite (Table 1).

Statistical methods

Our statistical power calculation for testing our first main hypothesis was based on the results in the trial of Sorensen and Astrup (6) because their experimental design was similar to ours. We made the following assumptions: *1*) we required a power of 0.80 to detect a 10% difference in pizza intake between the nonalkalized and alkalized cocoa mixtures with $\alpha = 0.05$; *2*) on the basis of the results of Sorensen and Astrup (6), a 10% difference would be 179 kcal, and the SD for each of the 2 means would be 215 kcal; *3*) of our initial 30 participants, 25 subjects would complete the trial; and *4*) we would use the Dunnet-Hsu test to adjust for multiple comparisons in our post hoc analyses. With the use of the method of Cohen (18), our statistical power was 0.83. We considered this estimate to be conservative because

TABLE 1
Doses of compounds served per kilogram of body weight in tested beverages[1]

Constituent	Nonalkalized cocoa mixture	Alkalized cocoa mixture	Nonalkalized cocoa mixture plus epicatechin	Nonalkalized cocoa mixture plus procyanidins	Alkalized cocoa mixture plus epicatechin	Alkalized cocoa mixture plus procyanidins
Calories, kcal	1.4	1.4	1.4	1.4	1.4	1.4
Total fat, g	0.0	0.0	0.0	0.0	0.0	0.0
Carbohydrates, g	0.2	0.2	0.2	0.2	0.2	0.2
Dietary fiber, g	0.0	0.0	0.0	0.0	0.0	0.0
Sugar, g	0.2	0.2	0.2	0.2	0.2	0.2
Protein, g	0.1	0.1	0.1	0.1	0.1	0.1
Magnesium, mg	0.9	1.0	0.9	0.9	1.0	1.0
Potassium, mg	7.3	7.9	7.3	7.3	7.9	7.9
Iron, mg	0.1	0.1	0.1	0.1	0.1	0.1
Calcium, mg	3.9	4.0	3.9	3.9	4.0	4.0
Caffeine, mg	0.3	0.1	0.3	0.3	0.1	0.1
Theobromine, mg	2.6	2.6	2.6	2.6	2.6	2.6
Flavanols,[2] mg	3.7	0.0	4.7	0.0	1.0	0.0
Epicatechin, mg	0.6	0.0	1.6	0.0	1.0	0.0
Catechin, mg	0.2	0.0	0.2	0.2	0.0	0.0
Procyanidins, mg	6.6	0.1	6.6	10.3	0.1	3.8
Procyanidins, dimers-decamers, mg	2.9	0.0	2.9	NA	0.0	NA

[1]Cocoa mixtures and data on constituent concentrations in alkalized and nonalkalized mixtures were provided by the Hershey Co. Epicatechin was purchased in powder form from Chromadex. Procyanidins were purchased as powder in capsules (Flavay, The Original) from Healthy Source and were derived from pine bark and grape seeds. NA, not applicable.

[2]Defined as epicatechin, catechin, and dimer-decamer procyanidins.

we did not account for the correlation between the pairing of observations in our crossover protocol.

Linear mixed models were used to test hypotheses. The Akaike information criterion and the Bayes information criterion were our indicators of a model fit (19). Likelihood ratio tests were used to assess the heterogeneity of residual variance, and type 3 *F* tests, on the basis of a residual maximum-likelihood estimation, were used to select fixed factors (19). The participant number, which was a categorical variable, was entered as a fixed factor rather than a random factor because our design was balanced (17). A first-order carryover variable (20) was created and entered as a categorical fixed factor to assess the effects of the previous laboratory session on appetite during the current session. Similarly, the laboratory session number was entered as a categorical fixed factor to test for period effects (20). Conditional Studentized residuals were used to check conformance with the model assumptions and to perform influence diagnostics.

The model with pizza intake as a dependent variable had the participant number and beverage as categorical fixed factors and the baseline VAS satiety rating as a continuous variable baseline covariate. The model accommodated heterogeneous residual variances across types of beverages with a variance-component covariance matrix structure. To help satisfy the normality assumption, the pizza-intake variable was inverted.

We calculated the AUC between 0 and 150 min for each of the 4 VAS measures with the use of the trapezoidal rule (21). We used pizza-intake and AUC data to assess the effects of the different beverages on appetite and the VAS data to examine changes in appetite over time. The model with the AUC as dependent variable exhibited homogeneous residual variance and contained the following 5 fixed factors: 4 categorical variables (beverage, participant number, laboratory-session number, and carryover indicator) and one continuous variable (baseline covariate). The AUC and the baseline covariate were derived from the same measure of perceived appetite. The model with the VAS rating as a dependent variable contained the following 7 fixed factors: 3 categorical variables (beverage, participant number, and time), one continuous variable (baseline covariate), and 3 cross-products (beverage crossed with time, participant number crossed with time, and baseline covariate crossed with time). The model contained a compound symmetry residual covariance structure in different categories of beverages and homogeneous residual variance. The VAS rating and baseline covariate were derived from the same measure of perceived appetite.

A 2-sided 5% level was used in all significance tests. Tests of secondary hypotheses were adjusted for multiple comparisons with the use of the Dunning-Hsu test. Linear mixed-model analyses were conducted with SAS software (version 9.4; SAS Institute Inc.). Other analyses were performed with IBM SPSS Statistics software (version 22; IBM Corp.).

Ethics

Approval to conduct the study was obtained from the City University of New York's Human Research Protections Program (no. 688347–3), and the study was conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983. Each participant was given $30 after each laboratory session. To be able to appropriately respond to adverse effects, *1*) there was a health clinic and emergency medical services system on campus; *2*) at the start of each laboratory session, participants answered questions concerning adverse effects; and *3*) participants were asked to immediately contact the principal investigator in the event of any untoward potential side effect when not in the laboratory. No side effects of consequence were reported. This trial was registered at clinicaltrials.gov as NCT02408289.

RESULTS

Of the original 30 participants, 2 subjects dropped out before the start of laboratory sessions, which left 28 subjects who attended all laboratory sessions. The mean ± SD age was 22.7 ± 3.9 y (range: 18–32 y), height was 1.73 ± 0.06 m (range: 1.60–1.88 m), weight was 70.0 ± 7.9 kg (range: 54.0–83.5 kg), BMI was 23.3 ± 2.4 (range: 20.4–29.6), waist circumference was 81.4 ± 7.6 cm (range: 68.6–104.1 cm), hip circumference was 100.3 ± 5.7 cm (range: 88.9–111.8 cm), and waist-to-hip ratio was 0.81 ± 0.05 (range: 0.71–0.93).

The pizza-data results (**Table 2**) showed that the nonalkalized cocoa mixture plus epicatechin, which contained the highest dose of epicatechin (1.6 mg epicatechin/kg), decreased pizza intake significantly and 18.7% more than with the alkalized-cocoa mixture and showed a trend toward ($P = 0.06$) decreasing pizza intake 16.7% more than with the beverage that contained epicatechin plus the placebo (1.0 mg epicatechin/kg). There were no other significant beverage effects for the pizza-intake data.

An analysis of the VAS data yielded no significant beverage-by-time interactions but did show significant changes over time as exhibited for satiety in **Figure 2**. The results of the hypothesis tests with the use of the VAS AUC data were different for different measures of perceived appetite. Hunger and emptiness yielded similar conclusions for all 6 hypothesis tests. Satiety and hunger yielded similar conclusions in 3 of 6 tests as did hunger and fullness. Satiety and fullness yielded similar conclusions in 2 of 6 tests.

Similarly, an agreement between the results of the hypothesis tests with the use of the VAS AUC data and pizza-intake data depended on the VAS measure of perceived appetite and the 2 beverages being compared. For instance, *1*) for the significant decrease in pizza intake for epicatechin plus the nonalkalized cocoa mixture compared with the placebo, each of the 4 VAS AUC measures showed different mean ± SE results [hunger: +39 ± 135 ($P = 0.78$); emptiness: −82 ± 122 ($P = 0.50$), satiety: +216 ± 135 ($P = 0.11$); and fullness: −468 ± 145 ($P = 0.001$)]; and *2*) for the nonsignificant differences in pizza-intake results for epicatechin plus placebo compared with placebo, all 4 VAS measures showed significant increases in appetite that differed in magnitude [hunger: +552 ± 109 ($P < 0.0001$), emptiness: +1020 ± 96 ($P < 0.0001$); satiety: −395 ± 107 ($P = 0.0002$); and fullness: −893 ± 114 ($P < 0.0001$)].

DISCUSSION

In our randomized, crossover, controlled trial involving 28 healthy, young-adult participants, a nonalkalized cocoa mixture that contained 0.6 mg epicatechin, 0.2 mg catechin, 2.9 mg dimer-decamer procyanidins, and 3.7 mg flavanols/kg body weight did not cause a significant acute decrease in pizza intake compared with the effect of the alkalized cocoa-mixture placebo that contained close to 0 mg/kg of these compounds. Our random assignment was balanced for period and carryover effects. In a secondary smaller ($n = 14$) randomized trial, which was also balanced for period and carryover effects, we observed a potentially more important result. The nonalkalized cocoa mixture supplemented with epicatechin significantly decreased ad libitum pizza intake by 18.7% 150 min after beverage ingestion. The supplemented beverage contained 1.6 mg epicatechin/kg (0.6 mg/kg from the nonalkalized cocoa mixture and 1.0 mg/kg from the epicatechin supplement). This beverage also caused a trend toward a significant decrease in pizza intake of 16.7% ($P = 0.06$) compared with the effect of a combination of epicatechin and placebo (1.0 mg epicatechin/kg). Compared with the placebo, neither the nonalkalized cocoa mixture alone nor the combination of epicatechin and placebo yielded a significant decrease in pizza intake.

The appetite suppression we observed may have been due to the high dose of epicatechin acting alone. This possibility was supported by a recent finding by Gutiérrez-Salmeán et al. (10)

TABLE 2
Differences in pizza intake 150 min after ingestion of different cocoa beverages[1]

Beverages compared	Epicatechin doses compared,[2] mg/kg	*n*	Difference in pizza intake,[3] g (%)	*P*
Tests of main hypotheses				
Nonalkalized cocoa mixture compared with alkalized cocoa mixture (placebo)[4]	0.6 compared with 0.0	28	−29.5 ± 25.6 (−6.0)	0.29
Epicatechin plus placebo compared with placebo	1.0 compared with 0.0	28	−11.6 ± 23.8 (−2.4)	0.73
Procyanidins plus placebo compared with placebo	0.0 compared with 0.0	28	−25.2 ± 25.7 (−5.1)	0.23
Tests of secondary hypotheses				
Epicatechin plus nonalkalized cocoa mixture compared with placebo	1.6 compared with 0.0	14	−91.3 ± 42.5 (−18.7)	0.04
Epicatechin plus nonalkalized cocoa mixture compared with epicatechin plus placebo	1.6 compared with 1.0	14	−79.7 ± 39.4 (−16.7)	0.06
Epicatechin plus nonalkalized cocoa mixture compared with nonalkalized cocoa mixture	1.6 compared with 0.6	14	−61.8 ± 40.5 (−13.5)	0.20

[1]Linear mixed-model analysis was used to analyze the data. The Dunnet-Hsu test was used to adjust for multiple comparisons in the tests of secondary analyses.

[2]Epicatechin doses are expressed as mg epicatechin/kg body weight.

[3]Differences are expressed as means ± SEs with percentages in parentheses. Values were calculated from the following least-squares means from the linear mixed-model analysis: alkalized cocoa mixture, 488.85 g; nonalkalized cocoa mixture, 459.37 g; epicatechin plus placebo, 477.30 g; procyanidins plus placebo, 463.69 g; and epicatechin plus nonalkalized cocoa mixture, 397.55 g.

[4]Alkalized cocoa is cocoa treated with an alkali solution.

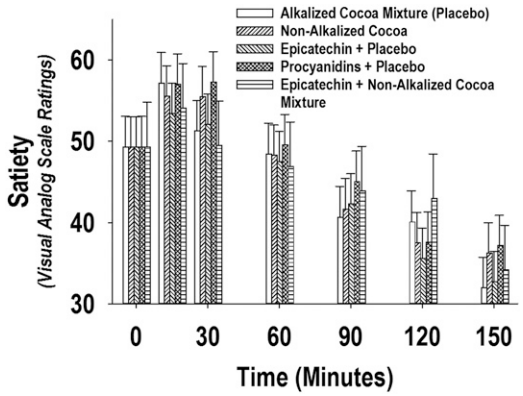

FIGURE 2 Mean satiety compared with the time after ingestion of beverages. Beverages were ingested at time = 0 min. Linear mixed models were used for analyses. Satiety showed no significant beverage effects, only significant decreases with increasing time. $n = 28$ for all beverages except for the epicatechin plus nonalkalized cocoa mixture, for which $n = 14$. Error bars represent 95% CIs.

in a study in which they gave male Wistar rats a daily dose of 1 mg epicatechin/kg that significantly decreased food intake and body weight in rats that had previously gained weight from consumption of a high-fat diet for 5 wk. Alternatively, the appetite suppression we observed may have been due to the high dose of epicatechin acting in concert with one or more catalyst compounds in the nonalkalized cocoa mixture, although we are not aware of the existence of such compounds. Our epicatechin plus nonalkalized cocoa mixture, which contained 1.6 mg epicatechin/kg, showed a trend toward decreasing appetite compared with the effect of epicatechin combined with the placebo, which contained 1.0 mg epicatechin/kg, but showed no significant decrease in appetite compared with the effect of the nonalkalized cocoa mixture that contained 0.6 mg epicatechin/kg. Considering that the alkalized cocoa-mixture placebo contained essentially no catechin or procyanidins (Table 1), these results suggests that either *1*) nonepicatechin catalyst cocoa compounds, such as possibly catechin or one or more procyanidins, are needed for appetite suppression, or *2*) one or more nonepicatechin cocoa compounds are also able to suppress appetite. We gave catechin and procyanidins to participants at doses of 0.2 and 6.6 mg/kg, respectively, in the nonalkalized cocoa mixture, which did not significantly decrease pizza intake. Hence, if catechins and procyanidins are able to suppress appetite, the doses of these compounds or of their needed catalysts in the nonalkalized cocoa mixture were not high enough in our trial. Procyanidins, when added to our cocoa mixtures, produced no significant decrease in food intake whether *1*) they were added to the placebo (3.8 mg procyanidins/kg) for the testing of our third primary hypothesis or *2*) they were added to our nonalkalized cocoa mixture (10.4 mg procyanidins/kg) in a small ($n = 7$) secondary randomized trial. This latter result should be regarded as tentative because the sample was small and the analysis was not balanced for period or carryover effects. Also, the procyanidins that we added to our cocoa mixtures were derived from pine bark and grape seeds, and thus, they may have different effects than cocoa procyanidins. It is also possible that a difference in taste between our alkalized and nonalkalized cocoa mixtures was responsible for some of the differences that we observed in pizza intake. Additional research is needed to determine whether one or more nonepicatechin catalysts or cocoa compounds are involved in the suppression of appetite and to establish dose-response relations.

Our results suggest that a cocoa beverage may be better suited than dark chocolate for appetite suppression. According to Langer et al. (22), 100 g 70% cocoa nonalkalized dark chocolate contains ~80 mg epicatechin. Assuming that the dose of 1.61 mg epicatechin/kg, which produced our significant result, is required and that the dark chocolate contains adequate concentrations of appropriate catalysts, a 70-kg young-adult male would need to eat ~140 g (~5 oz) of this chocolate, which would contain ~840 kcal (23). In comparison, only 98 kcal would be in the 31.5 g (1.1 oz) of our nonalkalized cocoa mixture supplemented with epicatechin that would be needed to deliver a dose of 1.61 mg epicatechin/kg to this person. The high caloric density of dark chocolate might explain why Koli et al. (24) did not observe a decrease in body weight in human subjects who ate dark chocolate instead of habitual snacks for 8 wk in a randomized trial. It is also possible that a dose of our nonalkalized cocoa mixture that would be large enough to deliver 1.6 mg epicatechin/kg might be able to suppress appetite. For a 70-kg young man, this dose would be 80 g (2.8 oz), which would contain 248 kcal energy. We did not test this possibility.

It seems likely that the reason our nonalkalized cocoa mixture did not significantly decrease appetite, whereas the dark chocolate in the 2 previous similar trials (6, 7) did, is that the dark chocolate in the previous trials provided higher doses of cocoa compounds, such as epicatechin, procyanidins, or catechin, than did those in our nonalkalized cocoa mixture. Note that the participants in an 18-wk randomized weight-loss trial by Nickols-Richardson et al. (25), who were given a daily flavanol dose of 3.2 mg/kg in dark chocolate and a cocoa combination, did not lose more weight than their placebo participants. This result is consonant with our finding that the flavanol dose of 3.7 mg/kg in our nonalkalized cocoa mixture did not suppress appetite more than our alkalized cocoa mixture placebo.

The specific biological mechanisms that underlies the potential for cocoa to suppress appetite are currently unknown. One human trial by Massolt et al. (26) suggested that the incretin hormone ghrelin might be involved. Massolt et al. (26) showed that subjects who smelled 85% cocoa chocolate experienced an acute increase in satiety and a concomitant decrease in plasma ghrelin concentrations. Because plasma ghrelin has been shown to increase food intake (27), the findings Massolt et al. (26) suggest that ghrelin may be involved in biological pathways that make it easier for people who smell cocoa compounds to control their food intakes.

One of the limitations of our trial is our small sample size. A larger sample could have yielded a larger number of significant effects. In addition, we did not have any female participants. It is possible that responses to cocoa or appetite may differ by sex. Another limitation is that our potentially most important finding was the result of a secondary smaller trial. However, this trial was randomized and balanced for period and carryover effects, and we adjusted the results for multiple-comparison testing. An additional limitation is that our VAS ratings did not agree with our pizza-intake measurements. We were unable to explain this discrepancy. Sorensen and Astrup (6) showed that their VAS ratings were in agreement with their ad libitum food-intake data, but Akyol et al. (7) did not. It is possible that we may have shown agreement if we had collected VAS ratings for >150 min.

Also, although we used a dietary recall questionnaire to check the compliance of participants with our requirement that they eat the same foods in the same quantities before each laboratory session, the use of a written diet diary the day before each session would have yielded a more reliable check. Finally, our pizza-intake measures of appetite would have been more convincing had they been coupled with assessments of concentrations of satiety and hunger hormones such as ghrelin or polypeptide YY. Our trial also has some strengths. First, our randomization scheme was well balanced so as to account for period and carryover effects. Second, we provided measurements of the doses of epicatechin, procyanidins, catechins, and flavanols, which we gave to our participants, and thus, our results should be a meaningful contribution to future research on doses and types of cocoa compounds for helping people avoid gaining excess weight.

In conclusion, our randomized placebo-controlled trial involving healthy, young-adult men showed that a nonalkalized cocoa mixture was able to cause a significant acute decrease in food intake only after being supplemented with epicatechin. Compared with the placebo of an alkalized cocoa mixture, the supplemented cocoa mix, with a 1.6-mg/kg dose of epicatechin, significantly decreased ad libitum pizza intake by 18.7% 150 min after beverage ingestion. This effect may be solely due to the action of epicatechin or it may require the presence of other catalytic cocoa compounds. It is also possible that cocoa compounds other than epicatechin are able to acutely suppress appetite. Additional research is needed.

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