

ORIGINAL RESEARCH

Cocoa to Improve Walking Performance in Older People With Peripheral Artery Disease

The COCOA-PAD Pilot Randomized Clinical Trial

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RATIONALE: Cocoa and its major flavanol component, epicatechin, have therapeutic properties that may improve limb perfusion and increase calf muscle mitochondrial activity in people with lower extremity peripheral artery disease (PAD).

OBJECTIVE: In a phase II randomized clinical trial, to assess whether 6 months of cocoa improved walking performance in people with PAD, compared with placebo.

METHODS AND RESULTS: Six-month double-blind, randomized clinical trial in which participants with PAD were randomized to either cocoa beverage versus placebo beverage. The cocoa beverage contained 15 g of cocoa and 75 mg of epicatechin daily. The identical appearing placebo contained neither cocoa nor epicatechin. The 2 primary outcomes were 6-month change in 6-minute walk distance measured 2.5 hours after a study beverage at 6-month follow-up and 24 hours after a study beverage at 6-month follow-up, respectively. A 1-sided $P < 0.10$ was considered statistically significant. Of 44 PAD participants randomized (mean age, 72.3 years [± 7.1]; mean ankle brachial index, 0.66 [± 0.15]), 40 (91%) completed follow-up. Adjusting for smoking, race, and body mass index, cocoa improved 6-minute walk distance at 6-month follow-up by 42.6 m ([90% CI, +22.2 to + ∞] $P = 0.005$) at 2.5 hours after a final study beverage and by 18.0 m ([90% CI, -1.7 to + ∞] $P = 0.12$) at 24 hours after a study beverage, compared with placebo. In calf muscle biopsies, cocoa improved mitochondrial COX (cytochrome c oxidase) activity ($P = 0.013$), increased capillary density ($P = 0.014$), improved calf muscle perfusion ($P = 0.098$), and reduced central nuclei ($P = 0.033$), compared with placebo.

CONCLUSIONS: These preliminary results suggest a therapeutic effect of cocoa on walking performance in people with PAD. Further study is needed to definitively determine whether cocoa significantly improves walking performance in people with PAD.

CLINICAL TRIAL REGISTRATION: URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT02876887.

VISUAL OVERVIEW: An online [visual overview](#) is available for this article.

Key Words: clinical trial ■ intermittent claudication ■ mitochondria ■ muscles ■ peripheral artery disease

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People with lower extremity peripheral artery disease (PAD) have shorter 6-minute walk distance compared with people without PAD.^{1,2} Without an effective therapeutic intervention, people with PAD typically decline in walking performance over time.^{3–6} Cocoa flavanols, including (–) epicatechin, that are

present in dark chocolate have therapeutic properties that may improve walking performance in people with PAD. Preclinical evidence and preliminary evidence from humans without PAD suggest that cocoa may increase limb perfusion and improve skeletal muscle mitochondrial activity and muscle regeneration.^{7–13} In

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The online-only Data Supplement is available with this article at <https://www.ahajournals.org/doi/suppl/10.1161/CIRCRESAHA.119.315600>.

For Sources of Funding and Disclosures, see page 598.

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Circulation Research is available at www.ahajournals.org/journal/res

Novelty and Significance

What Is Known?

- People with lower extremity peripheral artery disease (PAD) have a faster decline in a 6-minute walk than people without PAD.
- Few therapies improve 6-minute walk or prevent decline in people with PAD.
- Preclinical and preliminary human evidence suggested that cocoa flavanols may increase limb perfusion, skeletal muscle regeneration, and mitochondrial activity.

What New Information Does This Article Contribute?

- Among 44 participants with PAD randomized to either a cocoa flavanol or placebo beverage for 6 months, cocoa flavanols significantly improved 6-minute walk distance at 6-month follow-up, compared with placebo.
- PAD participants randomized to cocoa flavanols had significant improvement in limb perfusion compared with placebo.
- PAD participants randomized to cocoa flavanols improved several skeletal muscle measures, including in mitochondrial activity and capillary abundance, compared with placebo.

Patients with PAD have poorer lower extremity perfusion, reduced calf muscle mitochondrial activity, and faster decline in walking performance than people without PAD. Few medical therapies improve walking ability in people with PAD. Preclinical and preliminary human evidence suggested that cocoa flavanols may increase limb perfusion, promote skeletal muscle regeneration, and improve mitochondrial activity. Therefore, in this pilot double-blinded clinical trial, 44 participants with PAD were randomized to either a cocoa flavanol beverage or placebo beverage taken 3× daily for 6 months. At 6-month follow-up, participants with PAD who were randomized to cocoa flavanols had significant improvement in 6-minute walk distance, compared with those randomized to placebo. Participants randomized to cocoa flavanols also had significant improvement in limb perfusion, calf muscle mitochondrial activity, and calf muscle capillary abundance, compared with those randomized to placebo. Further study with a larger sample size is needed to definitively assess the effects of cocoa flavanols on walking ability in people with PAD.

Nonstandard Abbreviations and Acronyms

ABI	ankle brachial index
BMI	body mass index
COX	cytochrome c oxidase
DAPI	4′6-diaminidino-2-phenylindole
eMyHC	embryonic myosin heavy chain
IC	intermittent claudication
PAD	peripheral artery disease
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator 1α

a single-blind, crossover pilot study of 20 people with PAD, one 40 g dose of dark chocolate (>85% cacao) increased maximal treadmill walking distance by 11% and increased serum nitrite/nitrate levels 2 hours after the dark chocolate was administered, while milk chocolate had no acute effect on treadmill walking distance or serum nitrite/nitrate.¹⁴ However, effects of chronic consumption of cocoa on walking ability in PAD are unknown.

The COCOA-PAD study was a double-blind, pilot randomized clinical trial designed to test the hypothesis that daily cocoa consumption for 6 months improves or prevents decline in 6-minute walk distance at 6-month follow-up, compared with placebo.

METHODS

Data supporting the findings of this study are available on reasonable request.

The study was a parallel-design, double-blind, randomized clinical trial performed at the Northwestern University in Chicago. Northwestern University Institutional Review Board approved the protocol. Participants gave written informed consent. Participants were randomized to either a flavanol-rich cocoa beverage or a similar appearing and tasting beverage without cocoa or flavanols (Online Table I). The first participant was randomized on January 18, 2017. Final follow-up occurred on October 30, 2018. Enrollment stopped when the target sample size was reached. Muscle samples were analyzed after follow-up visits and were completed on October 3, 2019.

Participant Identification

Participants were recruited using Chicago Transit Authority advertisements and through recruitment postcards mailed to people ≥60 years of age in the Chicago area. People who previously participated in research with the principal investigator (M.M.M.) and expressed interest in future research were contacted.

Inclusion and Exclusion Criteria

Inclusion criteria were age >60 years and the presence of PAD, defined as an ankle brachial index (ABI) ≤0.90 in either leg or vascular laboratory or angiographic evidence of PAD.^{2,3,5} The absence of classical symptoms of intermittent claudication (IC) was not an exclusion criterion because most people with PAD do not have IC symptoms.^{1–6,15}

People unwilling to give up major dietary sources of chocolate, allergic to chocolate, or unable to consume products manufactured on equipment processing nuts, egg, wheat, soy, or milk were excluded. People with major leg amputation, critical limb ischemia, wheel-chair confinement, walking aid use, walking impairment for a reason other than PAD, and significant visual or hearing impairment were excluded. Individuals on dialysis, those requiring oxygen, those with a major cardiovascular event, revascularization, or major surgery in the past 3 months, and those planning revascularization or major surgery in the next 6 months were excluded. People treated for cancer in the past 2 years were excluded unless prognosis was excellent. Those with a baseline 6-minute walk <500 or >1600 ft and those with a Mini-Mental Status Examination score <23 at baseline were excluded.^{5,6,15}

Interventions

Flavanol-rich cocoa and matching placebo beverages were manufactured by The Hershey Company and dispensed in packets. The study was double blinded. Dispensed packets looked identical except for labeling with A or B for each group. The packet contents in each group contained similar looking powder, and participants only saw the contents of their own assigned packets and did not have the opportunity to compare the packet contents. Throughout the trial, investigators, study staff, and the data management team were unaware of whether the cocoa intervention was group A or B. Participants were instructed to mix packet contents with water or milk and consume 3 packets daily. Dietary composition of each beverage is shown in Online Table I. To avoid weight gain from the beverage, participants were individually counseled on foods with similar caloric content to study beverages that could be eliminated during the study. Participants brought remaining unopened packets to monthly study visits, where investigators counted them. At the end of the study, The Hershey Company notified the data management team on which group (A or B) received cocoa and which received placebo.

Randomization

Participants were randomized to group A or B by the data management team, using a randomly permuted block method, stratified by baseline 6-minute walk distance. Randomization was performed separately for participants with baseline 6-minute walk distance <365.75 m (1200 ft) versus ≥367.65 m.

ABI Measurement

A hand-held Doppler probe (Pocket Dop II; Nicolet Biomedical, Inc, Golden, CO) was used to obtain systolic pressures twice in each brachial, dorsalis pedis, and posterior tibial artery using established methods.^{1,2} The ABI was calculated by dividing the mean of the dorsalis pedis and posterior tibial pressures in each leg by the mean of the 4 brachial pressures.^{1-3,16}

Medical History

Medical history, race, and demographics were obtained by a certified health interviewer using questionnaires administered to the participants as in previous randomized trials.^{5,6,15}

Leg Symptoms

Leg symptoms were characterized using the San Diego Claudication Questionnaire. IC was defined as exertional calf pain that did not begin at rest, caused the participant to stop walking, and resolved within 10 minutes of rest.¹⁻³ Participants without IC were either asymptomatic (ie, reported no exertional leg symptoms) or had leg symptoms not meeting criteria for IC.¹⁻³

Primary and Secondary Outcomes

Outcomes were measured before randomization and at 6-month follow-up by staff unaware of group assignment. To distinguish between acute versus chronic intervention effects, there were 2 primary outcomes: 6-month change in 6-minute walk distance, measured at 2.5 hours after a study beverage and at 24 hours after a study beverage was consumed.

Secondary outcomes were 6-month change in brachial artery flow-mediated dilation, measured both 2.5 and 24 hours after study beverage at 6-month follow-up and 6-month change in maximal and pain-free treadmill walking distance measured 48 hours after beverage. To obtain measures at 2.5, 24, and 48 hours after a beverage, at the 6-month follow-up time point, participants were instructed to discontinue their study beverage temporarily (at specific times to facilitate correct timing of follow-up measures) and resume the beverage for muscle biopsy and perfusion measurements.

Additional secondary outcomes were obtained at 6-month follow-up while participants were taking beverages daily and included calf muscle biopsy measures of PGC-1 α (peroxisome proliferator-activated receptor- γ coactivator 1 α), follistatin, and myostatin levels and citrate synthase, and COX (cytochrome c oxidase) activity. Exploratory outcomes were 6-month change in calf muscle perfusion and calf biopsy measures of satellite cell abundance, capillary density, centrally nucleated and eMyHC (embryonic myosin heavy chain)-expressing fiber abundance, and oxidative stress measures (nitrotyrosine and 4-hydroxynonenal; Online Table II).

Six-Minute Walk Test

The 6-minute walk has been well validated in people with PAD and simulates the type of walking typically performed in daily life (ie, corridor walking).^{1-6,17} Small, meaningful change for 6-minute walk distance has been defined as 12 to 20 m.^{17,18} Following a standardized protocol,^{1-3,17} participants walked up and down a 100-ft hallway for 6 minutes after instruction to cover as much distance as possible.

Treadmill Walking Performance

Maximal treadmill walking time and time to ischemic leg symptom onset were measured using the Gardner-Skinner protocol at baseline and 6-month follow-up.^{5,6,19}

Brachial Artery Flow-Mediated Dilation

Brachial artery flow-mediated dilation was measured in the proximal brachial artery (B mode and Doppler) after a 12-hour fast by Registered Diagnostic Cardiac Sonographers using a linear array vascular ultrasound transducer (Sequoia Model #256; frequency, 8 MHz; range,

5–8 MHz; Siemens Medical Solutions).^{5,6} A cuff proximal to the visualized brachial artery segment was inflated for 4 minutes at 50 mm Hg above systolic pressure. Brachial artery images were obtained 60 seconds after cuff deflation and interpreted by a single reader, blinded to group assignment, at the University of Wisconsin Atherosclerosis Imaging Research Program Core Laboratory.

Calf Muscle Perfusion

Arterial spin labeling with cardiovascular magnetic resonance imaging was used to measure changes in calf perfusion at 3 T between PAD participants receiving cocoa versus placebo.²⁰ A thigh cuff was inflated to 250 mm Hg in the leg with the lowest ABI and rapidly deflated after 5 minutes. Seven control-tagged image pairs were acquired over 60 seconds using pulsed arterial spin labeling pulse sequence with single-shot echo-planar imaging readouts (field of view, 200×200 mm; matrix, 64×64; repetition time, 4000 ms; echo time, 32 ms; slice thickness, 10 mm). Perfusion was measured and quantified on a Siemens Healthcare workstation by coinvestigator C.M.K.

Physical Activity

Free-living physical activity was acquired over 7 days with the ActiGraph accelerometer. The accelerometer was worn on the right hip and removed only for bathing or sleeping.^{17,21}

Calf Muscle Biopsy

An open-muscle biopsy at baseline was performed in the medial head of the gastrocnemius muscle. Anesthesia was achieved with subcutaneous lidocaine.^{6,22} Subcutaneous tissue was dissected, and ≈250 mg of muscle was removed and immediately prepared for freezing at −80°C. At 6-month follow-up, the biopsy was repeated, adjacent to the original biopsy, identifiable by the scar.

Mitochondrial Measures

For enzyme activity assays (citrate synthase and COX), whole muscle tissue homogenates were prepared and enzyme activity was measured as described.^{6,22–24} For immunoblotting (PGC-1α, follistatin, myostatin, nitrotyrosine, and 4-hydroxynonenal), whole muscle tissue homogenates were analyzed as described.^{6,22,24} Detailed methods are in the [Online Data Supplement](#).

Immunohistochemistry

Frozen 7-μm sections of muscle were processed and stained for detection of nuclei using 4′6-diaminidino-2-phenylindole (DAPI), satellite cells using anti-Pax7 (paired box protein pax-7), and fiber borders with anti-laminin as described,^{25,26} except that anti-eMyHC antibody was used. Validation of central nuclei was performed by detection of the nuclear lamina protein lamin A/C. Capillary density was determined using lectins *Ulex europaeus* and *Griffonia simplicifolia*. Whole cross section images were acquired on an AxioImager M1 microscope (Zeiss, Oberkochen, Germany) equipped with ZEN (v2.0) software. DAPI+ central fiber nuclei, Pax7+/DAPI+ satellite cells, eMyHC+ fibers, and lectin+ capillaries were expressed relative to fiber number (details in the [Online Data Supplement](#)).

Other Measures

Height and weight were measured at baseline and follow-up. Body mass index (BMI) was calculated as weight (kg)/(height [m])². Flavanol metabolites were measured as the structurally related (–)epicatechin metabolites and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone metabolites as described previously.^{27–29} Theobromine was measured via ultraviolet detection at 274 nm.

Sample Size Calculations

The sample size calculation was based on an anticipated drop-out rate of 10%.^{5,6} Using the SD of 52 m of change in 6-minute walk distance from prior trials,^{15,30} 20 participants per group provided 70% power to detect a difference of 0.58 SD, representing ≈30 m change in 6-minute walk between the cocoa group (n=20, respectively) and the placebo group (n=20), based on a 1-sided 2-sample *t* test with a significance level of 0.10. The level of statistical significance and power were selected because the trial was a pilot intended to collect preliminary data.

Statistical Analyses

Primary analyses were performed using the intention-to-treat principle. All participants were asked to return for follow-up testing, and data were analyzed according to their assigned group even if they discontinued the study beverage. For participants who declined to return for follow-up but remained alive, multiple imputation was performed using 20 imputations to account for missing data. In sensitivity analyses, analyses were repeated excluding participants who underwent lower extremity revascularization during follow-up and adjusting for beverage adherence, respectively. Baseline characteristics were summarized as means and SDs, frequencies, and percentages, as appropriate. χ^2 tests and *t* tests were used to compare continuous and categorical characteristics of participants between the 2 study groups, respectively. Two-sample 1-tailed *t* tests were used to compare changes in each outcome at 6-month follow-up between cocoa and placebo. A 1-sided *P* < 0.10 was considered statistically significant. Analyses were repeated using ANCOVA adjusting for baseline differences in characteristics of the intervention and control groups. Statistical analyses were performed using SAS, version 9.4.

RESULTS

Of 118 participants who provided written informed consent, 44 were randomized: 23 to cocoa and 21 to placebo (Figure 1). Of 74 who signed a consent but were not randomized, 58 had an ABI >0.90, 10 did not complete baseline testing or declined participation, 3 had a baseline 6-minute walk distance that met the exclusion criterion for this measure, 1 was scheduled for coronary revascularization after completing the baseline treadmill test, 1 had a Mini-Mental Status Examination score <23, and 1 had walking impairment not due to PAD. Forty of 44 randomized (91%) completed 1 or more 6-month follow-up measures reported here. Of these, 36 completed both 6-minute walk tests at 6-month follow-up, 1 completed the 6-minute walk

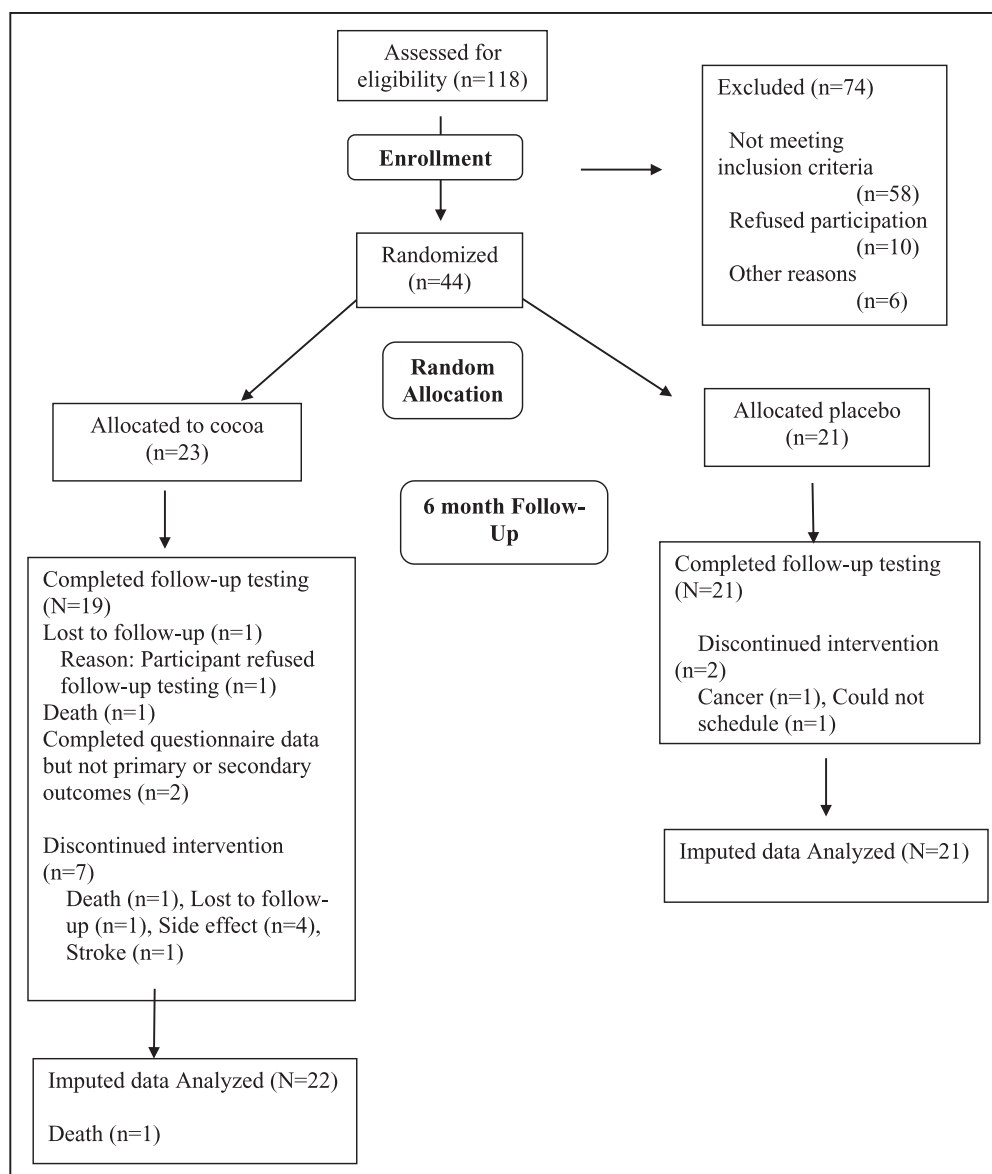


Figure 1. Overview of recruitment, randomization, and follow-up rates in the COCOA-PAD study.

at 2.5 hours but not at 24 hours after study beverage, and 3 completed the 6-minute walk at 24 hours but not at 2.5 hours after the beverage. Twenty-two participants underwent baseline muscle biopsy, but muscle tissue was only obtained for 21. Of these, 17 completed a follow-up biopsy. Of the 4 people who did not undergo follow-up muscle biopsy, 1 moved out of state, 1 had a stroke and could not return, 1 underwent the biopsy procedure but no muscle was obtained, and 1 repeatedly canceled his muscle biopsy appointment.

Participants randomized to cocoa had lower BMI and higher proportions of current smokers and blacks than those randomized to placebo (Table 1). Comparisons between the cocoa versus placebo groups adjusted for these baseline differences.

Beverage adherence rates were 68% and 80% in the cocoa and placebo groups, respectively, based on packet

counts. After excluding 7 participants who discontinued beverages but returned for follow-up, adherence was 80% and 87%, respectively. Six-month change in epicatechin metabolites and theobromine was significantly greater in participants randomized to cocoa compared with placebo, consistent with good adherence (Table 2).

Primary Outcome

In intention-to-treat analyses, adjusting for BMI, race, and smoking, participants randomized to cocoa improved 6-minute walk distance at 6-month follow-up by +42.6 m ($P=0.005$ [90% CI, +22.2 to +∞]) at the 2.5-hour time point and by +18.0 m ($P=0.12$ [90% CI, −1.7 to +∞]) at the 24-hour time point, compared with placebo (Table 3). Results became statistically significant according to our a priori definition of statistical significance at

Table 1. Baseline Characteristics of Participants by Group Assignment

Baseline Variable	Overall (N=44)	Trial Assignment	
		Cocoa Intervention (n=23)	Placebo (n=21)
Age, y; mean (SD)	72 (7)	71 (7)	73 (7)
Men, n (%)	29 (66)	15 (65)	14 (67)
Black, n (%)	31 (70)	19 (83)	12 (57)
ABI, mean (SD)	0.66 (0.15)	0.67 (0.14)	0.64 (0.15)
BMI, kg/m ² ; mean (SD)	29 (5)	27 (5)	31 (5)
Current smoker, n (%)	14 (32)	11 (48)	3 (14)
Former smoker, n (%)	24 (55)	9 (39)	15 (71)
Myocardial infarction, n (%)	6 (14)	4 (17)	2 (10)
Heart failure, n (%)	7 (16)	3 (13)	4 (19)
Stroke, n (%)	10 (23)	6 (26)	4 (19)
Angina, n (%)	7 (16)	4 (17)	3 (14)
Pulmonary disease, n (%)	11 (25)	4 (17)	7 (33)
Diabetes mellitus, n (%)	24 (55)	13 (57)	11 (52)
IC, n (%)	6 (14)	2 (9)	4 (19)
Leg pain other than claudication, n (%)	34 (77)	19 (83)	15 (71)
No exertional leg pain, n (%)	4 (9)	2 (9)	2 (10)
6-minute walk distance, m; mean (SD)	334 (81)	337 (75)	331 (88)
Maximal treadmill time, min; mean (SD)	9 (6)	9 (5)	10 (7)

ABI indicates ankle brachial index; BMI, body mass index; and IC, intermittent claudication.

the 24-hour time point when missing data were imputed (Table 3; +32.0 m; $P=0.040$ [90% CI, +8.6 to +∞]) at the 2.5-hour time point and +21.5 m ($P=0.084$ [90% CI, +1.5 to +∞]) at the 24-hour time point. Results became statistically significant at the 24-hour time point when analyses were repeated, excluding the 2 participants in the cocoa group who underwent lower extremity revascularization (+42.3 m, $P=0.006$ [90% CI, +21.4 to +∞]) at the 2.5-hour time point and +25.2 m, $P=0.058$ [90% CI, +4.8 to +∞] at the 24-hour time point). Results were

not substantially changed when analyses were repeated, adjusting for beverage adherence (+46.5 m, $P=0.002$ [90% CI, +26.6 to +∞] at the 2.5-hour time point and +18.9 m, $P=0.114$ [90% CI, −1.2 to +∞] at the 24-hour time point).

Secondary Outcomes

Cocoa had no statistically significant effect on change in treadmill walking time measured 48 hours after a study beverage or brachial artery flow-mediated dilation measured 2.5 and 24 hours after study beverage, compared with control (Table 4). Cocoa increased calf muscle biopsy COX activity compared with placebo but had no statistically significant effect on PGC-1 α , myostatin, or follistatin.

Exploratory Outcomes

Relative to placebo, cocoa reduced centrally located myofiber nuclei, increased capillary density, and increased calf muscle perfusion but did not significantly change measures of oxidative stress, eMyHC myofibers, or abundance of Pax7+ cells (Table 5; Figure 2A).

Post Hoc Analyses

To determine whether the centrally located myofiber nuclei were actually cytoplasmic chromatin fragments—a marker of senescent cells³¹—muscle sections were stained for lamin A/C, nuclear envelope proteins (Figure 2A and 2B). DAPI+DNA was surrounded by a nuclear envelope, verifying that these were central nuclei.

Adverse Events

There was no significant difference in BMI change between the cocoa versus placebo groups (26.6–26.4 versus 30.7–30.8 kg/m², respectively; mean difference, −0.20 kg/m² [95% CI, −0.82 to +0.41]; $P=0.50$). In the cocoa group, there was 1 death due to myocardial infarction, 2 hospitalizations for lower extremity

Table 2. Six-Month Change in Epicatechins, Valerolactones, and Theobromine by Group Assignment

	No.	Mean (SD)		Mean (90% CI)		
		Baseline	6-mo Follow-Up	Within-Group Change	Difference in Changes	P Value
Structurally related epicatechin metabolites, nM						
Cocoa	17	18.6 (22.2)	148.2 (106.4)	+129.6 (+80.8 to +178.3)	+113.1 (+63.7 to +162.4)	0.001
Placebo	19	15.7 (19.1)	32.3 (23.4)	+16.5 (+7.8 to +25.2)	...	...
γ-Valerolactone metabolites, nM						
Cocoa	17	23.7 (43.4)	69.0 (137.0)	+45.3 (−17.3 to +107.9)	+36.5 (−30.8 to +103.9)	0.36
Placebo	19	17.7 (41.9)	26.4 (54.4)	+8.7 (−18.9 to +36.4)	...	...
Theobromine, μM						
Cocoa	17	1.78 (1.49)	9.23 (8.60)	+7.45 (+3.98 to +10.92)	+6.86 (+2.66 to +11.07)	0.010
Placebo	19	2.86 (3.76)	3.44 (6.99)	+0.59 (−1.99 to +3.16)	...	...

Table 3. Adjusted Effects of Cocoa on Change in 6-min Walk Distance at 6-mo Follow-Up*

	No.	Mean (SD)		Mean (90% CI)		
		Baseline	6-mo Follow-Up	Within-Group Change	Difference in Changes	P Value
Intention-to-treat results						
6-mo change in 6-min walk distance at 2.5 h after the study beverage, m						
Cocoa	17	348.6 (74.2)	356.6 (64.0)	+18.4 (+0.34 to +36.5)	+42.6 (+22.2 to +∞)	0.005
Placebo	20†	337.3 (85.2)	322.0 (96.4)	−24.2 (−40.7 to −7.8)	...	...
6-mo change in 6-min walk distance at 24 h after the study beverage, m						
Cocoa	19	347.7 (74.3)	353.0 (76.9)	+15.1 (−1.9 to +32.1)	+18.0 (−1.7 to +∞)	0.12
Placebo	20†	329.1 (90.4)	335.4 (92.5)	−2.9 (−19.4 to +13.6)	...	...
Results imputed for missing data						
6-mo change in 6-min walk distance at 2.5 h after the study beverage, m						
Cocoa	22	338.9 (74.7)	337.7 (68.6)	+9.8 (−9.8 to +29.4)	+32.0 (+8.6 to +∞)	0.040
Placebo	21	330.7 (86.4)	320.0 (92.3)	−22.2 (−41.5 to −3.0)	...	...
6-mo change in 6-min walk distance at 24 h after the study beverage, m						
Cocoa	22	338.9 (74.7)	346.6 (74.2)	+17.2 (+0.50 to +34.0)	+21.5 (+1.5 to +∞)	0.084
Placebo	21	330.7 (86.4)	336.4 (88.3)	−4.3 (−20.7 to +12.1)	...	...

Baseline and 6-mo follow-up distances do not sum to the within-group change value due to adjustment for smoking, BMI, and race. BMI indicates body mass index.

*Data adjust for smoking, BMI, and race.

†Note that 1 participant randomized to placebo participated in the 2.5-h data collection but not the 24-h data collection, and 1 participant randomized to placebo participated in the 24-h data collection but not the 2.5-h data collection, resulting in differences in baseline values for the placebo group in the intention-to-treat analyses.

revascularizations, and 1 hospitalization each for ischemic stroke, foot infection, urinary difficulty, and depression. In the placebo group, there were 2 hospitalizations: 1 for atrial fibrillation and 1 for cough with chest pain. No serious adverse event was considered likely related to study participation.

DISCUSSION

In this pilot, double-blind, randomized clinical trial, participants randomized to cocoa for 6 months walked 42.6 m further in the 6-minute walk at 6-month follow-up, 2.5 hours after beverage consumption, and 18 m further at 6-month follow-up, 24 hours after beverage consumption, compared with those randomized to placebo. The difference in change at 6-month follow-up between the cocoa versus the placebo groups was statistically significant at the 2.5-hour time point but not at the 24-hour time point. Results did not substantially change but became statistically significant at the 24-hour time point when analyses were repeated using multiple imputation to account for missing data and when analyses were repeated, excluding the 2 participants randomized to cocoa who underwent revascularization during the trial. Among people with PAD, a small meaningful change in the 6-minute walk distance has been defined as 12.0 m, and a large meaningful change has been defined as 34.0 m.¹⁸ Cocoa significantly improved calf muscle perfusion and calf muscle COX enzyme activity, capillary density, and abundance of central nuclei, compared with placebo. The effects on calf muscle suggest a durable benefit from the cocoa beverage. To our knowledge, no

prior randomized trials have tested the effects of chronically administered cocoa on change in walking performance, calf muscle biopsy characteristics, or calf muscle perfusion in people with PAD. Results reported here are consistent with a recent 8-week randomized trial of 74 older men and women without PAD (mean age, 75.9), in which participants randomized to a cocoa flavanol beverage had a 35.7-m greater improvement in the 6-minute walk distance at 8-week follow-up, compared with those randomized to a placebo beverage ($P=0.02$).³²

A prior study of 20 participants with PAD demonstrated an acute effect of cocoa on treadmill walking time and serum nitrite levels in people with PAD.¹⁴ To our knowledge, no prior trials have reported the effects of chronic daily cocoa intake on change in walking performance in people with PAD. The current pilot trial was designed, in part, to test an acute (ie, immediate) effect versus chronic (ie, durable after the intervention is discontinued) effect of chronic cocoa on walking performance in PAD. Results reported here showing significant benefits of cocoa on calf muscle COX enzyme activity (an indicator of mitochondrial activity), capillary density, and central myofiber nuclei compared with placebo suggest that cocoa may have a durable beneficial effect on calf muscle in people with PAD, in addition to an acute effect reported previously.¹⁴ If daily cocoa has both an acute and chronic benefit in people with PAD, these combined benefits may explain the greater improvement in 6-minute walk distance at the 2.5-hour time point (which reflected both acute and chronic effects) than at the 24-hour time point (which reflected only chronic effects), relative to placebo.

Table 4. Adjusted Effects of Cocoa on Change in Secondary Outcomes at 6-mo Follow-Up*

	No.	Mean (SD)		Mean (90% CI)		
		Baseline	6-mo Follow-Up	Within-Group Change	Difference in Changes	P Value
6-mo change in brachial artery FMD 2.5 h after study beverage, %						
Cocoa	15	6.47 (3.25)	5.93 (2.69)	−0.65 (−2.08 to +0.78)	−1.28 (−2.94 to +∞)	0.84
Placebo	17	5.76 (3.34)	6.29 (4.57)	0.63 (−0.69 to +1.96)	...	...
6-mo change in brachial artery FMD 24 h after study beverage, %						
Cocoa	17	6.38 (3.34)	6.10 (3.85)	0.04 (−1.23 to +1.32)	+0.63 (−0.96 to +∞)	0.30
Placebo	15	6.03 (3.41)	5.81 (4.31)	−0.59 (−1.96 to +0.79)	...	...
6-mo change in pain-free treadmill walking time at 48 h after study beverage, min						
Cocoa	18	6.08 (4.28)	6.22 (3.81)	−0.07 (−1.13 to +1.00)	−0.46 (−1.70 to +∞)	0.68
Placebo	19	5.23 (3.38)	5.44 (3.20)	+0.39 (−0.64 to +1.43)	...	...
6-mo change in maximal treadmill walking time at 48 h after study beverage, min						
Cocoa	18	8.86 (4.86)	9.14 (4.43)	−0.03 (−1.16 to +1.10)	−0.31 (−1.60 to +∞)	0.62
Placebo	20	9.76 (7.40)	9.76 (8.57)	+0.28 (−0.79 to +1.34)	...	...
6-mo change in physical activity (activity counts)						
Cocoa	15	97812 (25410)	98551 (43057)	−1919 (−17743 to +13904)	+7062 (−10515 to +∞)	0.30
Placebo	19	89527 (54517)	78447 (40182)	−8981 (−22743 to +4780)	...	...
6-mo change in calf muscle biopsy abundance of PGC-1 α (arbitrary units)						
Cocoa	10	0.59 (0.22)	0.67 (0.29)	+0.09 (−0.09 to +0.28)	+0.05 (−0.21 to +∞)	0.40
Placebo	6	0.57 (0.28)	0.63 (0.53)	+0.04 (−0.21 to +0.29)	...	...
6-mo change in calf muscle biopsy abundance of myostatin (arbitrary units)						
Cocoa	10	1.27 (0.42)	1.52 (0.42)	+0.26 (+0.05 to +0.47)	+0.11 (−∞ to +0.40)	0.69
Placebo	6	1.25 (0.70)	1.43 (0.68)	+0.15 (−0.14 to +0.43)	...	...
6-mo change in calf muscle biopsy abundance of follistatin (arbitrary units)						
Cocoa	10	0.73 (0.46)	0.71 (0.29)	−0.03 (−0.20 to +0.15)	−0.12 (−0.37 to +∞)	0.74
Placebo	6	0.95 (0.63)	1.03 (0.60)	+0.09 (−0.15 to +0.34)	...	...
6-mo change in calf muscle biopsy citrate synthase activity, nmol/min per mg protein						
Cocoa	10	11.43 (4.73)	11.66 (4.24)	−0.25 (−3.85 to +3.36)	+0.86 (−4.12 to +∞)	0.41
Placebo	6	14.13 (4.75)	12.24 (5.45)	−1.10 (−5.98 to +3.77)	...	...
6-mo change in calf muscle biopsy COX activity, nmol/min per mg protein						
Cocoa	10	65.1 (64.3)	55.8 (32.8)	−4.6 (−37.2 to +28.0)	+85.5 (+40.4 to +∞)	0.013
Placebo	6	131.4 (95.4)	49.2 (26.4)	−90.0 (−134.1 to −46.0)	...	...

Baseline and 6-mo follow-up values do not sum to the within-group change value, due to adjustment for smoking, BMI, and race. Six-month outcomes obtained on study beverage unless otherwise specified. BMI indicates body mass index; COX, cytochrome c oxidase; FMD, flow-mediated dilation; and PGC-1 α , peroxisome proliferator-activated receptor- γ coactivator 1 α .

*Data adjust for smoking, BMI, and race.

In this trial, the 42.6-m improvement in 6-minute walk distance at the 6-month follow-up 2.5-hour time point in the cocoa group relative to placebo was due to a combination of improvement in the 6-minute walk distance in the cocoa intervention group (+18.4 m) and decline in the placebo group (−24.2 m). The decline in 6-minute walk distance among participants randomized to placebo at the 2.5-hour time point was consistent with the decline in 6-minute walk distance observed previously in longitudinal studies of people with PAD who were not randomized to an effective therapy.^{3–6,15,30} For example, prior randomized trials of people with PAD reported declines of −18.0, −15.0, and −11.4 m, in the control groups, respectively.^{15,30,33}

An unexpected finding was that among participants randomized to placebo, 6-minute walk distance declined

at 6-month follow-up by 24.2 m at the 2.5-hour time point compared with baseline but only declined by 2.9 m at the 24-hour time point compared with baseline. In contrast, among participants randomized to cocoa, 6-minute walk distance improved at 6-month follow-up by 18.4 m at the 2.5-hour time point compared with baseline and by 15.1 m at the 24-hour time point, compared with baseline. The greater benefit of cocoa relative to placebo at the 2.5-hour time point compared with the 24-hour time point may have been due, in part, to a learning effect between the 2.5- and the 24-hour time points for the 6-minute walk among participants randomized to placebo. It is possible that a learning effect between the 2.5- and 24-hour time points in the cocoa group was obscured by an acute effect of cocoa

Table 5. Adjusted Effects of Cocoa on Changes in Exploratory Outcomes at 6-mo Follow-Up*

	No.	Mean (SD)		Mean (90% CI)		
		Baseline	6-mo Follow-Up	Within-Group Change	Difference in Changes	P Value
MRI-measured calf muscle perfusion, mL/min per 100 g†						
Cocoa	13	2.91 (0.58)	2.93 (0.46)	+0.10 (−0.25 to +0.45)	+0.42 (+0.004 to +∞)	0.098
Placebo	13	3.01 (0.63)	2.78 (0.59)	−0.32 (−0.67 to +0.03)	...	...
Calf muscle biopsy measures						
#Pax7+/100 fibers						
Cocoa	11	11.87 (8.28)	14.85 (10.98)	+3.50 (−1.85 to +8.84)	+3.23 (−4.30 to +∞)	0.29
Placebo	6	18.28 (12.81)	19.50 (10.38)	+0.27 (−7.31 to +7.84)	...	...
Embryonic+fibers (percentage of total)						
Cocoa	11	0.16 (0.54)	0.04 (0.09)	−0.11 (−1.17 to +0.96)	−1.09 (−∞ to +0.42)	0.17
Placebo	6	0.48 (0.56)	1.49 (2.96)	+0.98 (−0.54 to +2.50)	...	...
Central nuclei (all percentage of total)						
Cocoa	11	9.29 (4.69)	9.59 (3.70)	+0.25 (−3.14, +3.63)	−7.10 (−∞ to −2.33)	0.033
Placebo	6	15.64 (9.04)	22.88 (5.95)	+7.34 (+2.55, +12.14)	...	...
Nitrotyrosine abundance (arbitrary units)						
Cocoa	10	1.00 (0.28)	1.14 (0.32)	+0.16 (+0.05 to +0.27)	+0.13 (−∞ to +0.28)	0.86
Placebo	6	0.99 (0.29)	1.05 (0.42)	+0.03 (−0.12 to +0.18)	...	...
4-HNE abundance (arbitrary units)						
Cocoa	10	1.07 (0.35)	1.10 (0.41)	−0.02 (−0.27 to +0.23)	−0.07 (−∞ to +0.28)	0.40
Placebo	6	1.03 (0.23)	0.99 (0.42)	+0.05 (−0.30 to +0.39)	...	...
Capillary density						
Cocoa	11	1.42 (0.76)	1.44 (0.83)	+0.07 (−0.08 to +0.21)	+0.38 (+0.17 to +∞)	0.014
Placebo	6	1.66 (0.15)	1.43 (0.22)	−0.31 (−0.52 to −0.11)	...	...

Baseline and 6-mo follow-up values do not sum to the within-group change value, due to adjustment for smoking, BMI, and race. 4-HNE indicates 4-hydroxynonenal; BMI, body mass index; MRI, magnetic resonance imaging; and Pax7, paired box protein pax-7.

*Data adjust for smoking, BMI, and race.

†Results are based on log-transformed data.

on 6-minute walk distance at the 2.5-hour time point. Specifically, the 6-minute walk distance performed 2.5 hours after cocoa ingestion in the cocoa group may have been enhanced by the acute benefits of cocoa at the 2.5-hour time point. The loss of this acute benefit from cocoa at the 24-hour time point in the cocoa group may have been compensated for by a learning effect in the cocoa group. Because the placebo had no acute effect on 6-minute walk distance at the 2.5-hour time point, a learning effect at 24 hours would be more apparent in the placebo group, since the 2.5-hour 6-minute walk distance was not enhanced by any acute effect 2.5 hours after the placebo drink. In support of this hypothesis, the mean net difference in 6-minute walk distance between the 2.5- and the 24-hour measurement was zero meters in the cocoa group and 13.0 m in the placebo group (Online Figures I and II). However, other possibilities include that the 6-minute walk distance measured at the 2.5-hour time point was influenced by a learning effect in both groups, since all participants had completed a baseline 6-minute walk 6 months previously or that those randomized to cocoa did not experience an acute benefit at the 2.5-hour time point but that instead a chronic benefit affected the 6-minute walk

distance at both the 2.5- and the 24-hour time points. While previous work suggested no learning effect of the 6-minute walk in people with PAD,¹⁷ to our knowledge, no studies have assessed whether a learning effect exists when participants with PAD perform two 6-minute walk tests 24 hours apart. It is also possible that fatigue from the 6-minute walk test 24 hours previously may have reduced the 6-minute walk distance achieved at the 24-hour 6-month time point in the cocoa group.

The higher rate of hospitalizations or death in the cocoa group compared with placebo was unexpected but may have been due to the substantially higher rate of current smoking among those randomized to cocoa compared with placebo (48% versus 14%). Of the 7 serious adverse events in the cocoa group, 4 were cardiovascular in nature. Of the 4 participants in the cocoa group with a cardiovascular event, 2 (50%) smoked cigarettes.

This study has limitations. First, this was a pilot study, and the sample size was small. Second, because of the small sample size, randomization resulted in imbalance of BMI, sex, and race. Results adjusted for these characteristics, but this adjustment may not have been sufficient to overcome potential confounding by baseline

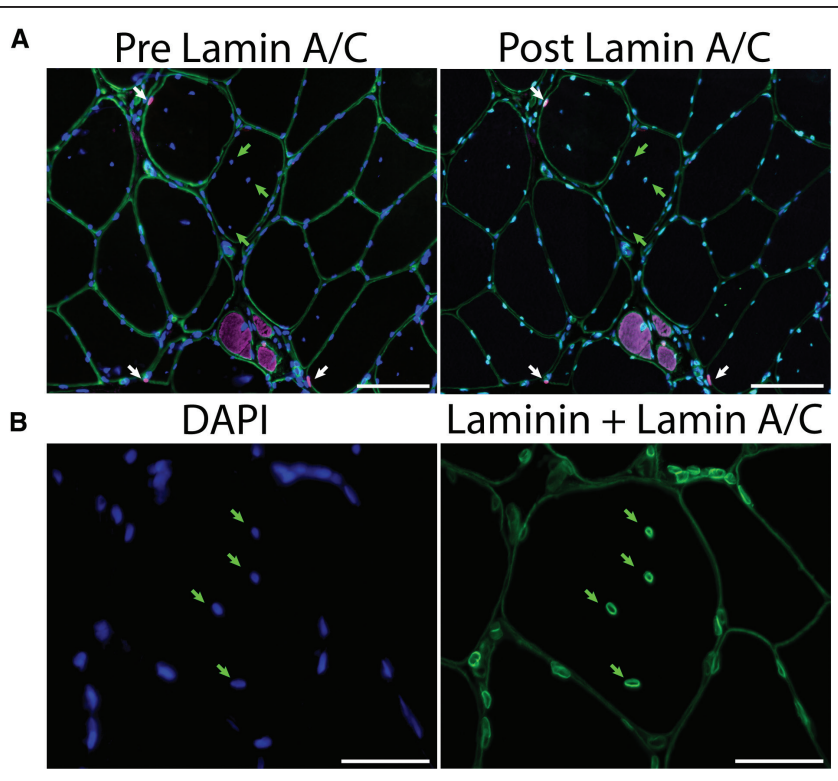


Figure 2. Immunohistochemistry of muscle from a participant with peripheral artery disease.

A, Left, Representative image showing red, Pax7+ (paired box protein pax-7) satellite cells (white arrows), 4′6-diaminidino-2-phenylindole (DAPI)+ (blue) labeled DNA, laminin (green) demarcating muscle fiber borders, and eMyHC (embryonic myosin heavy chain; pink) identifying small, regenerating myofibers. The abundance of centrally located DAPI+ myonuclei (green arrows) were changed with cocoa. **Right,** The same sample showing the nuclear envelope marker lamin A/C (bright green) surrounding each DAPI+ event. Image acquired at $\times 200$; scale bars=100 μm . **B,** Higher magnification showing the presence of lamin A/C nuclear envelope (bright green) surrounding each centrally located DAPI+ nucleus (green arrows). Laminin demarcating fiber borders is also shown (dim green). Image acquired at $\times 400$; scale bar=50 μm .

differences, resulting in residual confounding and bias toward the null. Third, there were multiple outcomes. It is possible that some significant findings were due to chance. Fourth, the study did not include people with PAD and an ABI >1.40 . There are no biologic reasons to anticipate that results would have differed in people with PAD and ABI >1.40 . Fifth, the absence of significant improvement in treadmill walking performance among participants randomized to cocoa may be because the treadmill test at 6-month follow-up was performed 48 hours after study beverages. Sixth, dietary data were not collected.

CONCLUSIONS

These preliminary results suggest a therapeutic effect of chronically administered cocoa on walking performance in people with PAD. Further study is needed to definitively determine whether cocoa significantly improves walking performance in people with PAD.

ARTICLE INFORMATION

Received June 24, 2019; revision received December 13, 2019; accepted January 9, 2020.

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Acknowledgments

We acknowledge Reedmond Y. Fong, MS, Department of Nutrition, University of California Davis (One Shields Av, Davis, CA 95616), and Javier I. Ottaviani, PhD, Mars, Incorporated (6885 Elm St, McLean, VA 22101), for performing plasma measures of epicatechin metabolites, valerolactones, and theobromine. Javier I. Ottaviani is employed by Mars, Inc—a company engaged in flavanol research and flavanol-related commercial activities.

Sources of Funding

This study was funded by the National Institute on Aging (R21-AG050897) and by the intramural office of the National Institute on Aging. This study was funded, in part, by the Office of Dietary Supplements, National Institutes of Health. The cocoa beverage and matching placebo were provided by The Hershey Company. Mars, Inc, performed measures of epicatechin metabolites, valerolactones, and theobromine.

Disclosures

M.M. McDermott receives research funding from Regeneron, for a project not related to the work in this paper. F. Villarreal is a cofounder and stockholder of Cardero Therapeutics, Inc. M.M. McDermott has received other research support from Helixmith, Reserveage, Chromadex, and ArtAssist. This other research support is not related to the current study. The other authors report no conflicts.

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