

# Cocoa consumption for 2 wk enhances insulin-mediated vasodilatation without improving blood pressure or insulin resistance in essential hypertension<sup>1–3</sup>

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## ABSTRACT

**Background:** Essential hypertension is characterized by reciprocal relations between endothelial dysfunction and insulin resistance. Cocoa flavanols stimulate production of the vasodilator nitric oxide from vascular endothelium.

**Objective:** The objective was to test the hypothesis that consumption of cocoa may simultaneously lower blood pressure, improve endothelial dysfunction, and ameliorate insulin resistance in subjects with essential hypertension.

**Design:** We conducted a randomized, placebo-controlled, double-blind, crossover trial of a flavanol-rich cocoa drink (150 mL twice a day, ≈900 mg flavanols/d) in individuals with essential hypertension ( $n = 20$ ). Antihypertensive medications were discontinued before study enrollment. After a 7-d cocoa-free run-in period, cocoa or flavanol-poor placebo (≈28 mg flavanols/d) treatment for 2 wk was followed by a 1-wk washout and then crossover to the other treatment arm. Blood pressure was measured thrice weekly. At baseline and after each treatment period, we assessed insulin sensitivity (hyperinsulinemic-isoglycemic glucose clamp) and insulin-stimulated changes in brachial artery diameter and forearm skeletal muscle capillary recruitment (Doppler ultrasound with or without microbubble contrast).

**Results:** Cocoa treatment for 2 wk increased insulin-stimulated changes in brachial artery diameter when compared with placebo [median percentage increase from baseline (25th–75th percentile): 8.3 (4.2–11.3) compared with 5.9 (–0.3 to 9.6);  $P < 0.04$ ]. Nevertheless, cocoa treatment did not significantly reduce blood pressure or improve insulin resistance and had no significant effects on skeletal muscle capillary recruitment, circulating plasma concentrations of adipocytokines, or endothelial adhesion molecules.

**Conclusions:** Daily consumption of flavanol-rich cocoa for 2 wk is not sufficient to reduce blood pressure or improve insulin resistance in human subjects with essential hypertension. This trial was registered at clinicaltrials.gov as NCT00099476. *Am J Clin Nutr* 2008;88:1685–96.

## INTRODUCTION

Hypertension and its underlying endothelial dysfunction are associated with disorders of metabolic dysregulation, including diabetes and obesity, due, in part, to reciprocal relations between endothelial dysfunction and insulin resistance (1, 2). In addition to metabolic actions of insulin, which directly promote glucose disposal, insulin stimulates the production of the vasodilator nitric oxide (NO) in the vascular endothelium, which leads to

increases in blood flow and capillary recruitment in skeletal muscle beds (3–7). This helps to couple regulation of glucose metabolism and blood flow (2, 8). Compounds that enhance insulin sensitivity (eg, thiazolidinediones) concurrently lower blood pressure in hypertension (9–12). Likewise, agents that improve endothelial dysfunction lower peripheral vascular resistance in hypertension (eg, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers) and simultaneously ameliorate insulin resistance (13–18).

Epidemiologic studies have shown that increased dietary intake of fruit, vegetables, and other flavonoid-rich foods, including green tea and cocoa, is associated with reduced cardiovascular morbidity and mortality (19–22). This may be due, in part, to the ability of flavanols to stimulate production of NO from vascular endothelium and to mimic metabolic actions of insulin by using signaling pathways partially overlapping with those regulating vasodilator actions of insulin (12, 23–25). Cocoa is rich in monomeric (epicatechin and catechin) and oligomeric (procyanidin) flavanols (26) and has been used for unproven medicinal purposes for hundreds of years (27). Cocoa flavanols acutely enhance vasodilation as well as flow-mediated dilation in the brachial artery of humans in an NO-dependent manner (28–30). Moreover, production of NO is linked to the ability of the green tea flavanol epigallocatechin gallate to lower blood pressure, improve endothelial dysfunction, ameliorate insulin resistance, and protect against myocardial ischemia and reperfusion injury in SHR rats (a rodent model of metabolic syndrome) (12). Taken together, these cellular, animal, and human studies suggest that the acute ability of flavanol-rich cocoa consumption to enhance NO-dependent vasodilation may potentially contribute to lowering peripheral vascular resistance, reducing blood pressure, and improving metabolic and vascular actions of insulin in humans. Indeed, 3 small clinical investigations suggest that consumption of dark chocolate rich in flavanols may improve endothelial function and lower blood pressure in

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subjects with hypertension (31–33). Moreover, short-term consumption of dark chocolate may also improve surrogate measures of insulin sensitivity in healthy subjects and in patients with essential hypertension (32, 34). However, all of these published studies investigating the effects of dark chocolate in hypertensive subjects suffer from small sample sizes, lack of adequate placebo controls with appropriate blinding, and use of indirect or surrogate measures of endothelial function and/or insulin resistance that are suboptimal for determining primary study outcomes. Thus, rigorous clinical investigations examining effects of cocoa consumption to simultaneously improve metabolic and vascular phenotypes in essential hypertension using state-of-the-art methods with robust placebo controls are lacking. In the present study, we evaluated the effects of cocoa consumption for 2 wk on hemodynamic and metabolic variables in subjects with essential hypertension by conducting a rigorous randomized, double-blind, placebo-controlled, crossover trial using state-of-the-art methods, including glucose clamp, brachial artery ultrasound, and ultrasound with microbubble contrast to evaluate changes in blood pressure, endothelial dysfunction, and insulin resistance.

## SUBJECTS AND METHODS

### Study design and subjects

This study was conducted exclusively at the National Institutes of Health (NIH) Clinical Center, Bethesda, MD. The study protocol was approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute, and all procedures followed were in accordance with institutional guidelines. Adults between 21 and 65 y of age, in good general health except for mild-to-moderate hypertension (systolic blood pressure of 140–170 mm Hg and/or diastolic blood pressure of 95–110 mm Hg without antihypertensive medication), and not taking any medication or nutritional supplements except for antihypertensive agents were recruited from the local community through newspaper advertisements. Subjects were specifically excluded from study enrollment if they were taking any medications other than antihypertensive drugs; if they were pregnant or had diabetes, liver disease, pulmonary disease, renal insufficiency, coronary heart disease, heart failure, peripheral vascular disease, coagulopathy, or any other severe systemic diseases; or if they were allergic to cocoa or perflutren lipid microspheres in microbubble contrast (Definity; Bristol Myers Squibb Medical Imaging Inc, North Billerica, MA). Subjects were also excluded if they had actively smoked within the past 2 y, were receiving treatment for or had a history of any form of cancer, or if they had positive blood tests for HIV, hepatitis B, or hepatitis C.

Of 65 individuals screened, 45 were deemed eligible for study run-in (**Figure 1**). Informed consent was obtained from each subject. During the initial 1 wk run-in period, study participants met with a dietitian and were instructed to maintain their usual physical activity and to begin a low-flavanol diet. All subjects were specifically provided with a list of foods rich in flavanols, which they were to avoid eating. In addition, antihypertensive medications, if taken, were discontinued. In some cases, the antihypertensive drug regimen was tapered off over the course of the run-in period for safety reasons. During the run-in period, and for the duration of the entire study, blood pressure was monitored thrice weekly in the NIH outpatient clinic. Blood pressure was measured in the dominant arm in the sitting position with a

standard sphygmomanometer with appropriately sized cuff. Blood pressure recorded was the mean of 3 successive readings 5 min apart (subjects were seated for  $\geq 15$  min before measurement). Participants whose blood pressure during the run-in phase did not meet the inclusion criteria were excluded from further study participation. Systolic or diastolic blood pressure measured at the screening visit (for participants not taking antihypertensive medication) or at the end of the run-in period (for participants taken off antihypertensive medication at the beginning of the run-in period) was used to determine study eligibility. In addition to monitoring blood pressure in the outpatient clinic, participants were required to self-monitor their blood pressure at home using a portable blood pressure-measuring device (OMRON Digital Blood Pressure Monitor, Bannockburn, IL). If a participant's blood pressure exceeded 160/100 mm Hg during measurements on 3 consecutive days or  $>170/110$  mm Hg on 3 determinations over a period of  $\geq 15$  min at any time, they were withdrawn from the study and appropriate antihypertensive therapy resumed. Of the 29 subjects who were randomly assigned, 20 subjects completed the entire study (**Figure 1**). Nine subjects failed to complete the entire study for a variety of reasons, including transient flank pain during administration of the microbubble contrast, allergic reaction to the microbubble contrast agent, family emergencies, other personal problems, difficulties with intravenous access, loss to follow-up, or exacerbation of chronic back pain.

After the 1-wk run-in period, enrolled subjects were randomly assigned in a double-blind fashion (block randomization by NIH Clinical Center Pharmacy) to the initial arm of the study: either a cocoa drink ( $\approx 450$  mg total flavanols twice a day) or a matching placebo drink ( $\approx 14$  mg total flavanols twice a day) for 2 wk. This was followed by a 1-wk washout period. Subjects were then crossed over to the other treatment arm for an additional 2 wk. Each enrolled subject underwent a hyperinsulinemic isoglycemic glucose clamp study and forearm vascular studies at baseline (at the end of 1-wk run-in period) and after each 2-wk treatment period. In addition, during the beginning of the study day (baseline and at the end of each 2-wk treatment period), blood pressure was measured and blood samples were drawn after the cocoa ( $\approx 450$  mg total flavanols) or placebo ( $\approx 14$  mg total flavanols) drink to estimate the pharmacokinetics of flavanols and their metabolites in plasma. A research nurse counted placebo or cocoa powder packets at the end of each treatment period to help monitor subject compliance. Study investigators and participants were blinded to treatment assignment, and assignment codes were not available to investigators until 20 participants completed the entire study and the database had been completed and secured. Participant blinding was assessed by a questionnaire administered at the end of 6 wk that asked patients to indicate which treatment they believed they received during each of the 2 phases (cocoa, placebo, or uncertain).

### Cocoa and Placebo Drink Preparations

Thirty one grams of cocoa or placebo beverage powder (CocoaPro; Mars Incorporated, Hackettstown, NJ) mixed in 150 mL warm water was consumed twice daily during the treatment phases of the study. The cocoa and placebo drinks were isocaloric and matched for fat, macronutrients, mineral content, theobromine, and caffeine (**Table 1**). In addition, the cocoa and placebo drinks were similar in color, taste, and packaging. Packets of



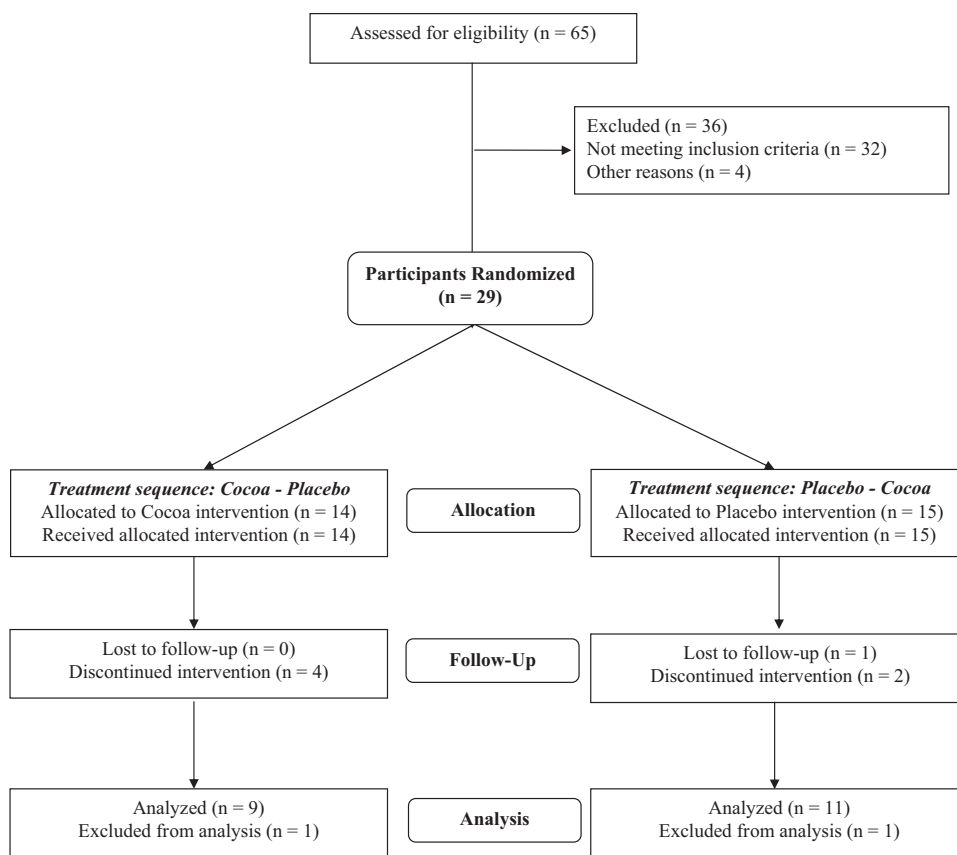


FIGURE 1. Participant flow throughout the trial.

cocoa and placebo powder were stored by the NIH Clinical Center pharmacy. To test the stability and variability of the cocoa and placebo powders, the polyphenol, macronutrient, and mineral contents were reassessed after the end of the study.

### Plasma flavanol and metabolite measurements and pharmacokinetics

Circulating plasma flavanols and their metabolites were measured after oral ingestion of the placebo drink ( $\approx 14$  mg total flavanols) or cocoa drink ( $\approx 450$  mg total flavanols) at the beginning of each glucose clamp study (at baseline and at the end of each 2-wk treatment period). After subjects were given the placebo or cocoa drink (time 0), peripheral blood samples were collected in EDTA-containing tubes containing ascorbate (1 mg/mL) at time 0, 0.5, 1, 2, and 3 h after oral administration. Plasma was obtained from blood samples by centrifugation and immediately frozen on dry ice for storage at  $-80^{\circ}\text{C}$ . Plasma samples were treated and analyzed by using methods described previously (29). Ingested flavanols in cocoa or placebo drinks are rapidly metabolized in phase I and II biotransformations to various *O*-glucuronidated, *O*-methylated, and *O*-sulfated forms (35, 36). Therefore, plasma samples were treated with  $\beta$ -glucuronidase according to procedures detailed previously (36). Resulting metabolites (epicatechin, catechin, 4'-*O*-methylcatechin, and 3'-*O*-methylcatechin) were separated and analyzed by using reversed-phase HPLC coupled with fluorescence detection as described previously (29). Concentrations of individual metabolites were quantified by using external calibration

curves generated with the use of authentic standards. The analytic chemists were blinded with respect to sample information until after the end of the study, when the database was completed and locked. Pharmacokinetic parameters for total plasma flavanols (sum of epicatechin, catechin, 4'-*O*-methylcatechin, and 3'-*O*-methylcatechin concentrations) were derived after oral dosing assuming first-order kinetics (37). The elimination constant,  $K_e$ , was estimated from the slope of the linear regression of log-transformed concentration values plotted versus time in the terminal phase, assuming first-order kinetics and instantaneous mixing. The apparent elimination half-time ( $t_{1/2}$ ) was calculated as  $t_{1/2} = \log(2)/K_e$  (assumes instantaneous mixing and no significant endogenous production). Time to peak plasma concentration ( $T_{\max}$ ) and peak plasma concentration ( $C_{\max}$ ) were estimated from the observed concentration versus time data assuming instantaneous mixing. The area under the curve to the last measurable concentration ( $\text{AUC}_{0-3\text{ h}}$ ) was calculated by using the trapezoidal rule for the observed values from 0 h to the last measured time point (3 h).

### Hyperinsulinemic isoglycemic glucose clamp

Insulin sensitivity was evaluated by glucose clamp as previously described (7, 38). The steady state period of the clamp was defined as a period of  $\geq 60$  min (1–2 h after the beginning of the insulin infusion) during which the CV for blood glucose, plasma insulin, and glucose infusion rate was  $< 5\%$ . The glucose clamp-derived index of insulin sensitivity ( $\text{SI}_{\text{Clamp}}$ ) was defined as  $M/(G \times \Delta I)$  corrected for body weight, where  $M$  is the steady state

**TABLE 1**

Composition of cocoa and placebo powders used to make the study drinks

Composition per dose (31 g)	Cocoa	Placebo
Energy (kcal)	119	117
Cocoa polyphenols (mg)	451	14
Epicatechin (mg)	87	1
Catechin (mg)	31	4
Procyanidins (mg)	338	8
Total fat (g)	1.4	1.5
Saturated fat (g)	0.8	0.8
Cholesterol (mg)	4.4	4.9
Carbohydrate (g)	17	17
Protein (g)	9.40	9.40
Fiber (g)	3.0	3.9
Caffeine (mg)	18.3	21.2
Theobromine (mg)	337	327
Sodium (mg)	105	155
Potassium (mg)	530	645
Calcium (mg)	244	241
Iron (mg)	1.9	2.9
Phosphorus (mg)	280	265
Magnesium (mg)	86	78
Zinc (mg)	1.6	1.6
Copper (mg)	0.4	0.4
Manganese (mg)	0.6	0.6

glucose infusion rate (mg/min),  $G$  is the steady state blood glucose concentrations (mg/dL), and  $\Delta I$  is the difference between basal and steady state plasma insulin concentrations ( $\mu\text{U/mL}$ ).

### Quantitative insulin-sensitivity check index

Quantitative insulin-sensitivity check index (QUICKI) was calculated as previously defined (38). QUICKI is calculated as  $1/[\log(I_0) + \log(G_0)]$ , where  $I_0$  is fasting insulin ( $\mu\text{U/mL}$ ) and  $G_0$  is fasting glucose (mg/dL). Because QUICKI is the reciprocal of the log-transformed product of fasting glucose and insulin, it is a dimensionless index without units.

### Brachial artery blood flow

Insulin is known to increase brachial artery diameter (BAD) and brachial artery blood flow (BAF). These vascular actions of insulin help to couple regulation of metabolic and hemodynamic homeostasis (1, 2). Therefore, as a measure of endothelial function relevant to insulin action, we assessed insulin-stimulated increases in BAD and BAF by using Doppler ultrasound. Measurements were performed at the beginning of each glucose clamp study (fasting insulin concentrations) and 2 h after initiation of the insulin infusion (during steady state hyperinsulinemia), as described previously (7). Briefly, the right brachial artery was visualized on the anterior aspect of the arm, 2–15 cm proximal to the antecubital fossa with a high-resolution ultrasound probe (HDI-5000 ultrasound machine with a 12-MHz linear array transducer; Philips Ultrasound, Bothell, WA). The position of the transducer on the arm was marked to facilitate visualization of the same portion of the artery throughout the study. BAD was measured from the anterior to the posterior “m” line (interface between media and adventitia) by using video calipers at end-diastole, coincident with the R wave on the electrocardiogram. BAF was estimated from Doppler flow velocity time integral (VTI), BAD, and HR measurements by using the equation  $\text{BAF} = \pi \times (\text{BAD}/2)^2 \times \text{VTI} \times \text{HR}$ . Insulin-induced

changes in BAD and BAF were expressed as a percentage of preclamp baseline values.

### Forearm skeletal muscle capillary recruitment

Insulin-stimulated capillary recruitment in the deep flexor muscles of the forearm was assessed by using microbubble contrast-enhanced ultrasonography as described previously by others (39). We compared estimates of microvascular blood flow before insulin infusion and at a period of steady state hyperinsulinemia during the glucose clamp. Immediately after each BAF determination, skeletal muscle capillary recruitment was estimated by using a power Doppler imaging technique. Ultrasound imaging of deep flexor muscles of the forearm was performed in a transaxial plane 5 cm distal to the antecubital fossa (P4-2 phased array transducer, HDI-5000; Phillips Ultrasound). Gain settings were kept constant throughout each study. A suspension of echogenic microbubbles with a similar size and rheology to red blood cells (Definity; Bristol Myers Squibb Medical Imaging Inc) was infused intravenously at a constant rate with an infusion pump (model A-99; Razel Industries, Stamford, CT). Two minutes after initiating the infusion of microbubbles (sufficient to achieve systemic steady state distribution of microbubbles), microbubbles were destroyed in a chosen region of interest (ROI) by using high-energy ultrasound (high mechanical index). Subsequently, a pulsing interval (time) versus video-intensity curve was generated to evaluate microbubble replenishment kinetics. This data reflects the refilling of microbubbles from outside the ROI where microbubble contrast agent is still intact. As the pulsing interval becomes longer, the acoustic intensity increases because of replenishment of microbubble contrast in the ROI. Images were recorded onto SVHS videotape and analyzed by using MCE software (University of Virginia). Plots of video contrast intensity ( $y$ ) versus pulsing interval ( $t$ ) were fit to the first-order exponential equation  $y = \alpha (1 - e^{-\beta t})$  (40). In this equation, the parameter  $\alpha$  represents the maximal signal intensity measured after complete refilling and is proportional to capillary blood volume (CBV) in the ROI. The parameter  $\beta$  is proportional to the initial capillary blood flow velocity (CFV). Capillary blood flow (CBF) was calculated as the product of CBV and CFV.

### Circulating endothelial adhesion molecules and adipocytokines

Soluble E-selectin, intercellular adhesion molecule 1 (sICAM-1), and vascular cell adhesion molecule 1 (sVCAM-1) were measured in serum in duplicate in the same assay by using the Human CVD Panel 1 Lincoplex kit (a multiplex assay kit based on Luminex xMAP technology; Linco Research, Inc, St. Charles, MI). The detection limits for these assays were 79, 9, and 16 pg/mL, respectively. The intraassay CVs were between 4.5% and 11.2%, and the interassay CVs were between 8.5% and 13.4%. Circulating adiponectin was also measured by using the Human CVD Panel 1 Lincoplex kit. The detection limit for this assay was 56 pg/mL, and the interassay and intraassay CVs were 16% and 9.2%, respectively. Plasma leptin, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) concentrations were measured in duplicate in the same assay from a fasting plasma sample with the use of the Human Cytokine Lincoplex kit (Linco Research, Inc, St. Charles, MI). The detection limits for these assays were 85, 0.14, 1.6, and 0.14 pg/mL, respectively. The intraassay CVs were

between 1.4% and 7.9%, and the interassay CVs were <20%. All assays were carried out on the same day to minimize assay variability.

### Laboratory assays

Routine assays for serum lipids, plasma glucose and insulin, and hemoglobin A<sub>1c</sub> were performed in the Department of Laboratory Medicine at the Clinical Center, NIH.

### Statistical analyses

Data from participants who completed all phases of the protocol ( $n = 20$ ) were analyzed according to a preestablished statistical analysis plan. Changes in blood pressure and changes in insulin sensitivity as measured by glucose clamp were prospectively designated as the primary endpoints for the study. All other comparisons were considered secondary. The presence of skewed data were evaluated by visual inspection of Q-Q plots, stem and leaf plots, or box plots and verified by the Shapiro-Wilk test for normal distribution. After testing data for normality, we used Student's paired  $t$  test or Wilcoxon's signed-rank test to evaluate differences between the outcome measures  $SI_{Clamp}$ , blood pressure, BAF, capillary recruitment, and adipocytokine concentration with treatment (cocoa compared with placebo). A one-sample sign test was used to assess the effect of hyperinsulinemia during the glucose clamp on vascular variables. The primary outcomes in this study were change in blood pressure and change in insulin sensitivity as measured by glucose clamp. Therefore, power analysis was calculated for these outcomes in this 2-treatment crossover study. On the basis of mean  $SI_{Clamp}$  values that we obtained for hypertensive subjects in previous studies using the glucose clamp method at an insulin infusion rate of 120 mU/m<sup>2</sup> · min ( $SI_{Clamp} = 3.70 \pm 0.19 \cdot 10^{-4} \text{ dL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}/(\mu\text{U/mL})$ ), a sample size of  $n = 20$  is sufficient to detect a 10% difference between the treatment and placebo in subjects with >90% power (41). A previous study reported that dark chocolate consumption (500 mg flavanols daily for 2 wk) reduced systolic blood pressure by  $5.1 \pm 2.4$  mm Hg (mean  $\pm$  SD) and diastolic blood pressure by  $1.8 \pm 2.0$  mm Hg in hypertensive subjects ( $n = 13$ ) (31). On the basis of this study, a sample size of 20 is sufficient to detect a 5-mm Hg change in systolic blood pressure with >90% power and a 2-mm Hg change in diastolic blood pressure with >90% power and a 2-sided  $\alpha = 0.05$ . To evaluate whether subjects were effectively blinded, Fisher's exact test was used to compare correct with incorrect treatment guesses by each subject at the end of the trial.  $P$  values were not adjusted for multiple comparisons, and  $P$  values <0.05 were considered to represent statistical significance. The statistical software programs SPSS (version 10.0; SPSS Inc, Chicago, IL) and SAS (version 9.0; SAS Institute Inc, Cary, NC) was used for the data analysis.

## RESULTS

### Baseline clinical characteristics of study subjects

Baseline clinical characteristics of the 20 hypertensive subjects who completed our entire study are reported in **Table 2**. The data shown in Table 2 were obtained on the morning of the first study day (after the run-in period was completed). Of the study completers, 13 were whites and 7 were African Americans. Of the 20 participants who completed all phases of the study, 6 were

**TABLE 2**

Baseline clinical characteristics of 20 study subjects who completed the entire study<sup>1</sup>

Clinical variables ( $n = 20$ )	Value ( $n = 8$ M, 12 F)
Age (y)	$51 \pm 1.5^2$
Sex (M/F)	8/12
BMI (kg/m <sup>2</sup> )	$33.2 \pm 1.4$
Systolic blood pressure (mm Hg)	$141 \pm 3$
Diastolic blood pressure (mm Hg)	$91 \pm 3$
Fasting plasma glucose (mg/dL)	$83 \pm 2$
Fasting plasma insulin ( $\mu\text{U/mL}$ )	$6.9 \pm 1.1$
$SI_{Clamp}$ [ $10^{-4} \text{ dL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}/(\mu\text{U/mL})$ ]	$5.14 \pm 0.33$
QUICKI	$0.383 \pm 0.011$
Hemoglobin A <sub>1c</sub> (%)	$5.56 \pm 0.09$
Total cholesterol (mg/dL)	$195 \pm 9$
LDL (mg/dL)	$132 \pm 11$
HDL (mg/dL)	$60 \pm 3$
Triglycerides (mg/dL)	$115 \pm 22$
Free fatty acid ( $\mu\text{Eq/L}$ )	$455 \pm 45$

<sup>1</sup>  $SI_{Clamp}$ , glucose clamp–derived index of insulin sensitivity; QUICKI, quantitative insulin-sensitivity check index.

<sup>2</sup>  $\bar{x} \pm \text{SEM}$  (all such values).

taking antihypertensive medications that were discontinued at the start of the run-in period. All of these participants met the blood pressure inclusion criteria at the end of the 1-wk run-in period; 14 were not treated with antihypertensive medication or had discontinued medication before the run-in or initial screening visit for reasons unrelated to the study. All of these participants met the blood pressure inclusion criteria at the screening visit (before the run-in). Of the 20 participants who completed the study, 11 participants met the systolic blood pressure inclusion criterion, 4 met the diastolic blood pressure inclusion criterion, and 5 met both criteria. All participants who completed this study had stage 1 hypertension according to JNC 7 criteria (stage 1: systolic blood pressure, 140–159 mm Hg; diastolic blood pressure, 90–99 mm Hg) (42) and were either obese or overweight. There were no significant differences between baseline characteristics of participants who initially received either placebo ( $n = 11$ ) or cocoa ( $n = 9$ ) for their first treatment phase (data not shown). At baseline, mean fasting plasma glucose and hemoglobin A<sub>1c</sub> concentrations were within the normal range. Similarly, mean fasting serum lipid profiles were normal at baseline. Mean fasting plasma insulin concentrations in our hypertensive subjects were slightly elevated when compared with concentrations from a historical cohort of healthy control subjects ( $n = 48$ ) studied in our previous investigations ( $6.9 \pm 1.1$  compared with  $5.25 \pm 0.51 \mu\text{U/mL}$ ;  $P = 0.06$ ) (7, 43). Consistent with these elevated fasting plasma insulin concentrations, the mean values for  $SI_{Clamp}$  in our hypertensive cohort were significantly lower than values obtained previously from our historical cohort of healthy control subjects ( $5.14 \pm 0.33$  compared with  $7.15 \pm 0.43 \times 10^{-4} \text{ dL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}/(\mu\text{U/mL})$ ;  $P < 0.003$ ) (7, 43). Thus, on average, our hypertensive subjects were insulin resistant compared with historical healthy control subjects.

With respect to baseline endothelial function (before treatment with either cocoa or placebo), hyperinsulinemia during the glucose clamp caused a significant increase in BAD (9% from baseline;  $P < 0.001$ ) and blood flow (138% from baseline;  $P <$

**TABLE 3**Vascular variables of the study subjects with essential hypertension who completed the entire study<sup>1</sup>

Vascular variables ( <i>n</i> = 20)	Baseline	Placebo drink	Cocoa drink	Difference (cocoa – placebo)	<i>P</i> value <sup>2</sup>
Brachial artery diameter (cm)					
Baseline	0.34 ± 0.02 <sup>3</sup>	0.365 ± 0.02	0.356 ± 0.02		
During steady state insulin infusion	0.37 ± 0.02	0.38 ± 0.02	0.38 ± 0.02		
Insulin-induced change from baseline (%)	9.1 ± 1.4	5.0 ± 1.3	8.1 ± 1.2	3.2 (0.4, 5.9) <sup>4</sup>	0.028
Brachial artery blood flow (mL/min)					
Baseline	52 ± 8	77 ± 22	72 ± 10		
During steady state insulin infusion	98 ± 14	104 ± 17	103 ± 18		
Insulin-induced change from baseline (%)	138 ± 41	90 ± 30	85 ± 35	–5 (–94, 85)	0.91
Capillary blood flow (arbitrary units)					
Baseline	0.45 ± 0.06	0.68 ± 0.23	0.51 ± 0.07		
During steady state insulin infusion	0.76 ± 0.09	0.67 ± 0.11	0.74 ± 0.09		
Insulin-induced change from baseline (%)	123 ± 35	309 ± 220	77 ± 24	–232 (–706, 242)	0.31

<sup>1</sup> Values are shown at baseline and after treatment with the placebo drink or the cocoa drink.<sup>2</sup> *P* values are for paired posttreatment comparisons (placebo compared with cocoa drink) with Student's paired *t* or Wilcoxon's signed-rank test.<sup>3</sup>  $\bar{x} \pm$  SEM (all such values).<sup>4</sup> Mean; 95% CI in parentheses for between-treatment differences (all such values).

0.001) (**Table 3**). In the skeletal muscle microvasculature, hyperinsulinemia tended to increase the initial skeletal muscle CFV (116% from baseline; *P* = 0.48) and significantly increased skeletal muscle capillary blood volume (71% from baseline; *P* < 0.05) so that the product, (skeletal muscle CBF) also increased significantly (123% from baseline; *P* < 0.01) (Table 3). When these vascular variables in our hypertensive subjects were compared with values obtained in a historical cohort of healthy normotensive control subjects (*n* = 20 for brachial artery studies, *n* = 10 for capillary studies) from one of our previous investigations (7), insulin-stimulated increases in BAD were not statistically different (9.1 ± 1.4 compared with 8.4 ± 1.4% from baseline) but insulin-stimulated CBF was lower (123 ± 35 compared with 202 ± 43% from baseline; *P* < 0.05). Thus, on average, our hypertensive subjects had endothelial dysfunction with respect to vasodilator action of insulin in nutritive capillaries when compared with historical healthy normotensive control subjects.

#### Effects of cocoa treatment on endothelial function in subjects with essential hypertension

We evaluated endothelial function with respect to vasodilator actions of insulin in the brachial artery and in skeletal muscle microvasculature in each study subject before and after 2-wk administration of cocoa or placebo. After 2 wk of treatment with the cocoa drink, we observed a significant increase in insulin-stimulated BAD when compared with placebo treatment in the same subjects (8.1 ± 1.2 compared with 5.0 ± 1.2% from baseline; *P* < 0.03) (Table 3). However, cocoa treatment did not significantly alter insulin-stimulated BAF when compared with placebo treatment (Table 3). Likewise, cocoa treatment did not alter insulin-stimulated skeletal muscle capillary recruitment when compared with placebo treatment (Table 3). When results were analyzed and compared between subgroups that started with cocoa treatment first (*n* = 9) compared with placebo treatment first (*n* = 11), we found no significant differences (data not shown). Thus, we could not detect any significant carryover effects from initial cocoa or placebo treatments with respect to endothelial function in our crossover study. Moreover, we did not observe any acute effects of a single dose of either cocoa or

placebo drink on any of the vascular actions of insulin that we evaluated (data not shown).

#### Effects of cocoa treatment on blood pressure and insulin resistance in subjects with essential hypertension

We evaluated blood pressure in each study subject before and after 2 wk of administration of cocoa or placebo. After 2 wk of treatment with the cocoa drink, we did not observe any significant differences in systolic or diastolic blood pressure when compared with placebo treatment in the same subjects (**Table 4**). Likewise, after 2 wk of treatment with the cocoa drink, we did not observe any significant differences in insulin sensitivity determined by glucose clamp or QUICKI when compared with placebo treatment in the same subjects (Table 4). Thus, insulin resistance in our subjects with essential hypertension was not improved by treatment with cocoa. Similarly, values for fasting plasma glucose, insulin, and lipids were unchanged by cocoa treatment when compared with placebo treatment (Table 4). When results were analyzed and compared between subgroups that started with the cocoa treatment first (*n* = 9) compared with placebo treatment first (*n* = 11), we did not find any significant differences (data not shown). Thus, we could not detect any significant carryover effects from initial cocoa or placebo treatments with respect to blood pressure, insulin sensitivity, or metabolic variables in our crossover study. Our data strongly suggest that consumption of flavanol-rich cocoa for 2 wk did not lower blood pressure or ameliorate insulin resistance in individuals with moderate hypertension.

#### Effects of cocoa treatment on circulating plasma adipocytokines and soluble adhesion molecules in subjects with essential hypertension

We measured circulating concentrations of plasma leptin, adiponectin, TNF- $\alpha$ , IL-6, MCP-1, sE-Selectin, sVCAM-1, and sICAM-1 in our study subjects at baseline and after each 2-wk treatment arm with cocoa or placebo. When compared with placebo treatment, cocoa treatment did not significantly alter the plasma concentrations of any of these circulating factors (**Table 5**).



**TABLE 4**Blood pressure and metabolic parameters for subjects with essential hypertension who completed entire study<sup>1</sup>

Clinical variables	Baseline	Placebo drink	Cocoa drink	Difference (cocoa – placebo)	<i>P</i> value <sup>2</sup>
BMI (kg/m <sup>2</sup> )	33.2 ± 1.4 <sup>3</sup>	32.9 ± 1.4	33.1 ± 1.4	0.1 (–0.2, 0.4) <sup>4</sup>	0.63
Systolic blood pressure (mm Hg)	141 ± 3	140 ± 2	139 ± 2	–1 (–4, 3)	0.74
Diastolic blood pressure (mm Hg)	91 ± 3	87 ± 2	88 ± 2	1 (–2, 4)	0.48
SI <sub>Clamp</sub> [10 <sup>–4</sup> dL · kg <sup>–1</sup> · min <sup>–1</sup> / (μU/mL)]	5.14 ± 0.32	5.08 ± 0.42	5.36 ± 0.58	0.29 (–0.54, 1.11)	0.48
QUICKI	0.383 ± 0.011	0.369 ± 0.010	0.365 ± 0.011	–0.004 (–0.018, 0.010)	0.54
Fasting plasma glucose (mg/dL)	83 ± 2	86.0 ± 3	88.0 ± 3	2 (–0.6, 5)	0.11
Fasting plasma insulin (μU/mL)	6.9 ± 1.1	8.6 ± 1.3	8.9 ± 1.5	0.29 (–1.49, 2.07)	0.73
Total cholesterol (mg/dL)	195 ± 9	182 ± 9	183 ± 9	1 (–8, 10)	0.78
LDL (mg/dL)	132 ± 11	121 ± 9	123 ± 10	2.9 (–7.7, 9.9)	0.48
HDL (mg/dL)	60 ± 3	53 ± 3	52 ± 3	–1 (–4, 2)	0.63
Triglycerides (mg/dL)	115 ± 22	109 ± 16	109 ± 20	0 (–19, 19)	0.99
Free fatty acid (μEq/L)	455 ± 45	555 ± 68	530 ± 47	–29 (–193, 137)	0.72

<sup>1</sup> *n* = 20. Values are shown at baseline and after treatment with the placebo drink or the cocoa drink.<sup>2</sup> *P* values are for paired posttreatment comparisons (placebo compared with cocoa drink) with Student's paired *t* or Wilcoxon's signed-rank test.<sup>3</sup>  $\bar{x} \pm$  SEM (all such values).<sup>4</sup> Mean; 95% CI in parentheses for between-treatment differences (all such values).

### Correlations between study variables

As expected, QUICKI (a surrogate index of insulin sensitivity that has been extensively validated in subjects with essential hypertension; 38, 41, 43) was significantly correlated with SI<sub>Clamp</sub>, a direct measure of insulin sensitivity ( $r = 0.524$ ,  $P = 0.01$ ). Plasma adiponectin concentrations were also positively correlated with SI<sub>Clamp</sub> ( $r = 0.463$ ,  $P = 0.03$ ). No significant correlations between insulin-induced changes in vascular variables and blood pressure, SI<sub>Clamp</sub>, circulating plasma concentrations of adipocytokines, or soluble adhesion molecules were noted. Moreover, there were no significant relations detectable between insulin sensitivity (SI<sub>Clamp</sub>) and plasma concentrations of leptin, cytokines (TNF- $\alpha$ , IL-6, and MCP-1), or soluble adhesion molecules (sE-Selectin, sVCAM-1, and sICAM-1).

### Pharmacokinetics of circulating plasma flavanols and metabolites

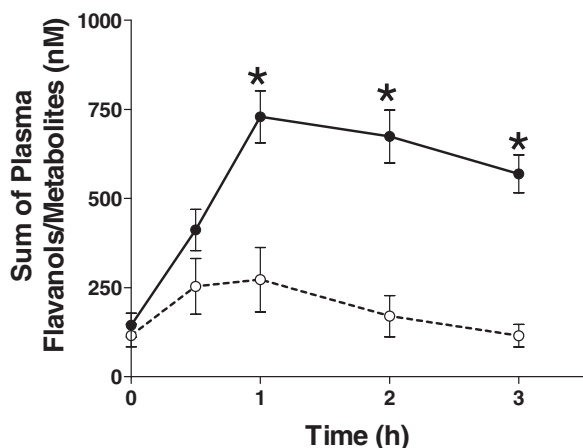
Circulating plasma flavanols and their metabolites were measured at study enrollment and after oral ingestion of a single dose

of cocoa (450 mg total flavanols) or placebo (14 mg total flavanols) drink at the beginning of each glucose clamp study (ie, at the end of the run-in period and at the end of each 2-wk treatment period). Baseline circulating total flavanol concentrations in plasma samples obtained at enrollment (beginning of the run-in period) were not significantly different from those in plasma samples obtained at the end of the 1-wk run-in period ( $134 \pm 49$  compared with  $125 \pm 42$  nmol/L;  $P > 0.53$ ). Pharmacokinetic parameters for total flavanols in plasma estimated from our oral dosing data (Figure 2) are reported in Table 6. As expected, ingestion of the cocoa drink resulted in significantly higher peak concentrations and area-under-the-curve measurements of circulating total flavanols (sum of epicatechin, catechin, 4'-*O*-methyl-catechin, and 3'-*O*-methyl-catechin concentrations) than did ingestion of the placebo drink (Figure 2, Table 6). The mean apparent elimination half-life for total flavanols was  $\approx 3.6$  h. This was derived from data where individual time-flavanol concentration series from both placebo and cocoa drinks were combined. Given this relatively short half-life, it is not surprising that

**TABLE 5**Circulating plasma concentrations of adipocytokines and vascular adhesion molecules in subjects with essential hypertension who completed entire study<sup>1</sup>

Circulating biomarkers	Baseline	Placebo drink	Cocoa drink	Difference (cocoa – placebo)	<i>P</i> value <sup>2</sup>
Leptin (ng/mL)	12.1 ± 2.8 <sup>3</sup>	13.4 ± 3.2	14.4 ± 3.3	0.9 (–1.2, 3.2) <sup>4</sup>	0.37
Adiponectin (μg/mL)	10.4 ± 1.7	11.6 ± 2.0	11.7 ± 1.6	0.1 (–1.2, 1.4)	0.91
TNF- $\alpha$ (pg/mL)	2.2 ± 0.4	2.4 ± 0.4	2.3 ± 0.3	–0.1 (–0.6, 0.5)	0.79
IL-6 (pg/mL)	1.9 ± 0.6	2.5 ± 0.7	2.7 ± 0.8	0.3 (–0.2, 0.7)	0.30
MCP-1 (pg/mL)	255 ± 35	259 ± 31	257 ± 31	–2 (–28, 25)	0.90
sE-Selectin (ng/mL)	66 ±	61 ± 10	61 ± 10	0.3 (–7.6, 8.2)	0.93
sVCAM-1 (ng/mL)	1577 ± 128	1648 ± 139	1622 ± 162	–26 (–195, 143)	0.74
sICAM-1 (ng/mL)	390 ± 32	398 ± 35	399 ± 42	2 (–36, 39)	0.92

<sup>1</sup> *n* = 20. Values are shown at baseline and after treatment with placebo drink or cocoa drink. TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; sE-selectin, soluble E-selectin; sVCAM-1, soluble vascular cell adhesion molecule-1; sICAM-1, soluble intercellular adhesion molecule-1.<sup>2</sup> *P* values are for paired posttreatment comparisons (placebo compared with cocoa drink) with Student's paired *t* or Wilcoxon's signed-rank test.<sup>3</sup>  $\bar{x} \pm$  SEM (all such values).<sup>4</sup> Mean; 95% CI in parentheses for between-treatment differences (all such values).



**FIGURE 2.** Mean ( $\pm$  SEM) circulating plasma flavanols and their metabolites after oral ingestion of a placebo drink ( $\approx 14$  mg total flavanols;  $\circ$ ) or cocoa drink ( $\approx 450$  mg total flavanols;  $\bullet$ ) in subjects ( $n = 20$ ) with essential hypertension. A comparison of the 2 curves with repeated-measures 2-factor ANOVA showed that the interaction term between treatment (placebo or cocoa drink) and sampling time was significant ( $P < 0.0001$ ). Therefore, pairwise comparison with the Bonferroni post hoc test was used to assess differences between treatments at each time interval. \*Significantly different from placebo,  $P < 0.001$ .

twice daily dosing with the cocoa drink for 2 wk did not result in any significant increase in steady state plasma flavanol concentrations ( $103 \pm 55$  compared with  $118 \pm 53$  nmol/L; before compared with after treatment:  $P = 0.73$ ,  $n = 9$ ; analysis of subjects who were initially randomly assigned to start on the cocoa treatment arm).

At the end of our study, only 20% of participants correctly identified their treatment assignment as cocoa in the first arm of the study, whereas 5% of participants correctly identified their treatment assignment as cocoa in the second arm of the study. These 2 results were not statistically different ( $P = 0.21$ ; Fisher's exact test). Moreover, these results indicate that our study subjects were appropriately blinded to their treatment regimen.

## DISCUSSION

Essential hypertension is linked to disorders of metabolic homeostasis, including type 2 diabetes. This is due, in part, to reciprocal relations between endothelial dysfunction and insulin resistance (1, 2, 44). Endothelial dysfunction often manifests as impaired endothelium-dependent vasodilator actions secondary to decreased bioavailability of NO. In particular, impaired

insulin-stimulated production of NO from the endothelium contributes to metabolic insulin resistance. This is because relative decreases in insulin-stimulated blood flow and capillary recruitment reduce delivery of insulin and metabolic substrates to skeletal muscle (2, 39, 45, 46). Thus, therapies that improve endothelial dysfunction are predicted to improve insulin resistance, whereas interventions that ameliorate insulin resistance may simultaneously improve endothelial dysfunction (1, 2, 9–18, 47, 48). Cross-sectional studies have found that cocoa intake is inversely related to blood pressure and cardiovascular mortality (49). Ingestion of flavanol-rich cocoa improves NO-dependent flow-mediated dilation in conduit arteries (28–30). Taken together, these studies suggest an intriguing rationale for beneficial cardiovascular and metabolic benefits of cocoa consumption (50). However, published studies to date do not convincingly address these issues. Therefore, in the present study, we conducted a rigorous randomized, double-blind, placebo-controlled, crossover trial to evaluate putative beneficial effects of cocoa consumption to lower blood pressure, ameliorate insulin resistance, and improve endothelial dysfunction in subjects with essential hypertension.

## Subjects with essential hypertension have endothelial dysfunction and insulin resistance

The 20 subjects who completed our entire study had stage 1 hypertension and obesity. Because endothelial dysfunction is a well-known characteristic of both essential hypertension and obesity (51, 52), it is not surprising that our subjects had impaired insulin-stimulated capillary recruitment in forearm skeletal muscle when compared with a historical cohort of normotensive healthy control subjects examined under similar conditions in our laboratory (7). Similarly, subjects with essential hypertension and obesity are generally insulin resistant (44, 53). Therefore, finding that our hypertensive cohort was insulin resistant when compared with a historical cohort of normotensive healthy control subjects previously studied in our laboratory (7, 38) was expected. Interestingly, despite the presence of insulin resistance, mean  $SI_{Clamp}$  values in the present study were higher than those observed in one of our previous studies of obese subjects with essential hypertension ( $5.14 \pm 0.33$  compared with  $3.39 \pm 0.25 \times 10^{-4}$  dL  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ /( $\mu$ U/mL);  $P < 0.001$ ). This may be due to the fact that the mean systolic blood pressure of the cohort in the present study was significantly lower than that of the cohort described in our previous study ( $141 \pm 3$  compared with  $152 \pm 2$  mm Hg;  $P < 0.003$ ) (41). Thus, milder hypertension is associated with milder insulin resistance.

**TABLE 6**  
Pharmacokinetic parameters of plasma flavanols and metabolites<sup>1</sup>

Parameters	Placebo drink ( $n = 31$ tests)	Cocoa drink ( $n = 29$ tests)	$P$ value <sup>2</sup>
$C_{max}$ (nmol/L)	$310 \pm 98^3$	$765 \pm 73$	$<0.001$
$T_{max}$ (h)	$0.77 \pm 0.16$	$1.43 \pm 0.12$	$<0.001$
$AUC_{0-3}$ (nmol/L $\cdot$ h)	$588 \pm 179$	$1754 \pm 177$	$<0.001$
Half-life (h) <sup>4</sup>	$\approx 3.6$		

<sup>1</sup> Pharmacokinetic parameters were determined after oral ingestion of the placebo drink ( $\approx 14$  mg total flavanols) or cocoa drink ( $\approx 450$  mg total flavanols).  $C_{max}$ , maximum plasma concentration;  $T_{max}$ , time to reach maximum concentration;  $AUC_{0-3h}$ , area under the curve (above baseline) from time 0 to 3 h.

<sup>2</sup>  $P$  values are for unpaired comparisons (placebo compared with cocoa drink) with Student's  $t$  test.

<sup>3</sup>  $\bar{x} \pm$  SEM of parameters determined by fitting data to first-order exponential decay (all such values).

<sup>4</sup> The mean half-life of plasma flavanols (sum) is the numerical average of individual half-lives determined from pharmacokinetic data from subjects consuming the placebo and cocoa drinks.

To reduce confounding factors, we excluded hypertensive subjects from our study if they had other medical conditions known to independently alter insulin sensitivity. In addition, rigorous eligibility criteria were enforced to eliminate medications or conditions with the potential to influence insulin sensitivity, blood pressure, or endothelial function. Moreover, frequent patient surveillance and coaching were used to maximize patient compliance with the treatment regimen and to minimize other changes in diet and lifestyle.

### Effects of cocoa treatment on endothelial function

We assessed endothelial function by measuring vasodilator responses to insulin in the brachial artery (a large conduit artery) as well as in nutritive microvascular beds of forearm skeletal muscle using sensitive ultrasound techniques. We chose to examine these 2 different types of vasculature because they have distinct, but related, physiologic functions relevant to regulation of both cardiovascular and metabolic homeostasis (1, 2, 54). We observed no significant acute effects of cocoa ingestion (450 mg total flavanols) on insulin-mediated vasodilation of the brachial artery or insulin-stimulated capillary recruitment. Previous studies in humans have shown that acute ingestion of flavanol-rich cocoa (821–1760 mg total flavanols) increases flow-mediated dilation (FMD) in conduit arteries (28–30). In contrast with insulin-mediated vasodilation, FMD is due to stimulation of NO production in response to shear stress on the endothelium induced by the rise in blood flow after a short period of ischemia. Insulin-induced vasodilation is also endothelium-dependent and NO-mediated. Although both stimuli activate endothelial nitric oxide synthase, they are regulated by distinct upstream signaling pathways (2). It is possible that cocoa consumption induces changes in FMD without acutely altering insulin-stimulated vasodilation or capillary recruitment. It is also possible that the dose of flavanols ( $\approx 450$  mg) we used to evaluate acute changes in endothelial function was not sufficiently high to cause a detectable effect. In our experimental paradigm it was not feasible to measure insulin-stimulated vasodilation of the brachial artery, insulin-stimulated capillary recruitment, and FMD all in a single study.

After cocoa treatment, we observed a small, but statistically significant, increase in insulin-stimulated vasodilation of the brachial artery when compared with placebo treatment. Changes in vessel diameter without accompanying changes in flow may represent a discrepant result. This assumes that all of the other hemodynamic factors remain unchanged (eg, blood pressure and cardiac output). BAF is a product of HR, blood flow velocity, and cross-sectional area of the vessel. In our study, insulin increased BAD, blood flow velocity, and BAF in subjects treated with either cocoa or placebo. However, when compared with placebo treatment, cocoa treatment significantly enhanced only insulin-induced increases in BAD but not in blood flow velocity or BAF. Although we were unable to detect significant changes in blood pressure, it is possible that changes in blood flow velocity or BAF that are below the limits of detection by our methods may account for an increase in BAD without a significant change in velocity or BAF. Because we did not measure cardiac output, it remains possible that there were changes in cardiac output induced by cocoa that may help to account for our findings. Alternatively, the increase in insulin-stimulated brachial artery vasodilation

that we observed after cocoa treatment may represent a false-positive result. Another consideration is that insulin dose-response experiments for forearm blood flow may have shown effects of cocoa that were not evident by using a single maximally stimulating dose of insulin. However, it was not feasible to perform these types of experiments in our investigational paradigm.

Large conduit arteries are less sensitive to vasodilator actions of insulin than are nutritive capillaries (2). Therefore, it is possible that cocoa treatment failed to increase insulin-stimulated capillary recruitment because capillary recruitment determined by hyperinsulinemia during the glucose clamp may have already been maximal. However, this seems less likely because insulin-stimulated capillary recruitment in our hypertensive cohort was significantly reduced when compared with an historical cohort of normotensive healthy control subjects. The ability of 2-wk cocoa treatment to increase insulin-stimulated BAD is consistent with the results of previous studies, which showed that 4-d or 1-wk treatment with flavanol-rich cocoa significantly increases FMD in conduit arteries (28, 55). We cannot rule out the possibility that a longer duration of cocoa treatment may also significantly alter insulin-mediated capillary recruitment. Although the salutary effect of cocoa treatment on endothelial function we observed was statistically significant, the clinical and/or biological significance of this finding remains uncertain.

### Effects of cocoa treatment on blood pressure and insulin resistance

The 2 prospectively designated primary outcomes of our cocoa intervention study were changes in blood pressure and changes in insulin resistance. Improvement in endothelial dysfunction manifested as an increase in insulin-stimulated dilation of the brachial artery might be expected to promote lowering of blood pressure and amelioration of insulin resistance (1, 2). We were unable to detect any significant lowering of systolic or diastolic blood pressure or any improvement in insulin resistance with cocoa treatment when compared with placebo treatment in the same subjects. These negative results, with respect to the study's primary outcomes, are extremely robust and are unlikely to result from a type II statistical error because our study was abundantly powered for these endpoints, we used state-of-the-art methods to assess blood pressure and insulin sensitivity (56), the effects of cocoa and placebo were evaluated in the same subjects in our double-blind crossover design, there was no evidence of a carryover effect of either cocoa or placebo in our crossover study, and we evaluated pharmacokinetics of cocoa flavanols (the putative active ingredient) after cocoa dosing during the study. Moreover, our cocoa and placebo packets ( $\approx 450$  mg and  $\approx 14$  mg total flavanols, respectively) were well-matched for calories, fat, macronutrients, mineral content, theobromine, caffeine, color, taste, and packaging. A survey conducted at the end of our study showed that subjects were appropriately blinded to their treatment regimen (cocoa compared with placebo). Thus, ascertainment and expectation bias was effectively eliminated in our double-blind study.

Antihypertensive therapies generally show a trend for a greater reduction in blood pressure in individuals with higher blood pressures (57–59). Although our study was abundantly powered for the stated primary endpoints, we cannot rule out the possibility that in patients with higher blood pressures, we may have observed significant effects of cocoa to improve our primary study outcomes.

Several previous studies have concluded that dark chocolate treatment (88, 168, or 500 mg flavanols daily for 2 wk) reduces blood pressure and/or improves insulin sensitivity in both hypertensive (31, 32) and normotensive (34, 60) healthy individuals when compared with white chocolate control. Another 18-wk intervention study with dark chocolate (30 mg flavanols/d) also found a beneficial effect on blood pressure in hypertensive subjects when compared with a white chocolate control (33). Thus, in previously published studies, the ability of dark chocolate or cocoa to lower blood pressure and improve insulin sensitivity in hypertensive or normotensive subjects is observed for a wide range of flavanol doses (30–500 mg/d) when the study design includes a white chocolate control. Many of these studies using a white chocolate control administered daily doses of cocoa flavanols that were well below the doses used in acute studies to elicit increases in flow-mediated dilation (28, 29, 55). Because neither investigators nor participants are properly blinded in studies using a white chocolate control, it is highly likely that ascertainment and/or expectation bias significantly influenced the outcomes of these studies. In addition, noncocoa polyphenol ingredients present in higher concentrations in dark chocolate than in white chocolate (eg, magnesium or theobromine) may have contributed to the observed reductions in blood pressure (61–63). Indeed, in double-blind studies using an appropriate placebo control, no significant effects of dark chocolate or cocoa treatment ( $\approx 400$  mg flavanols/d for 6 wk or  $\approx 260$  mg flavanols/d for 2 wk) to reduce blood pressure are observed in normotensive healthy subjects (64, 65).

Our present study was the first to rigorously evaluate the effects of cocoa treatment on blood pressure and insulin resistance in hypertensive subjects. The daily dose of flavanols used in our study ( $\approx 900$  mg/d) is well in excess of that used in previous dark chocolate or cocoa intervention studies examining blood pressure and is comparable with the high doses used in acute studies documenting improvements in flow-mediated dilation in conduit arteries with cocoa ingestion (28, 29, 55). Our placebo contained small amounts of cocoa flavanols ( $\approx 28$  mg/d) that may tend to minimize differences between cocoa and placebo treatment. However, this seems less likely because no significant differences in blood pressure or insulin resistance were observed when baseline values were compared with values after placebo treatment. Indeed, a recent placebo-controlled study showed that cocoa polyphenols ( $\approx 180$  mg flavanols/d) lowered blood pressure in hypercholesterolemic normotensive subjects after 5 wk of treatment (66). Studies examining the effects of cocoa treatment for longer than 2 wk on blood pressure in hypertensive subjects have not been reported. It remains possible that cocoa treatment may lower blood pressure or improve insulin resistance if treatment times are longer than 2 wk, if higher daily doses of flavanols are used, or if treatment is undertaken in subjects with more severe hypertension.

### Effects of cocoa treatment on biomarkers of endothelial dysfunction and insulin resistance

Acquired endothelial dysfunction and insulin resistance share common causes, including activation of proinflammatory signaling. Effective therapeutic interventions that improve blood pressure and ameliorate insulin resistance also often alter circulating plasma concentrations of relevant adipocytokines and cellular adhesion molecules that serve as both biomarkers and mediators of endothelial dysfunction and insulin resistance (2, 67).

Consistent with our negative results with respect to changes in blood pressure and insulin resistance, we observed no significant effects of cocoa treatment to alter circulating plasma concentrations of leptin, adiponectin, TNF- $\alpha$ , IL-6, MCP-1, sE-Selectin, sICAM-1, or sVCAM-1 when compared with placebo treatment.

### Pharmacokinetics of cocoa flavanols

On oral ingestion, cocoa flavanols are rapidly absorbed in the intestine and metabolized in phase I and II biotransformations to various *O*-glucuronidated, *O*-methylated, and *O*-sulfated forms (35, 36). Cocoa flavanol bioavailability may vary according to the food matrix used (eg, chocolate bars compared with drinks) (68). When our subjects were given a single dose of cocoa or placebo drink, we detected significant increases in plasma flavanol concentrations with peak concentrations at  $\approx 60$  min and a half-life of  $\approx 3.6$  h consistent with previous studies in humans (37, 69). Given this relatively short half-life, it is not surprising that twice-daily dosing did not result in a detectable increase in steady state concentrations of plasma flavanols after a 2-wk treatment period (at the end of either the first or the second arm of our crossover study). Moreover, the short half-life of plasma flavanols coupled with unchanged steady state concentrations after 2 wk of cocoa or placebo treatment is consistent with our inability to detect any significant carryover effects with respect to metabolic and hemodynamic parameters after the 1-wk wash-out period between treatment arms in our crossover study. Nevertheless, these findings, by themselves, do not rule out the possibility of beneficial cardiovascular and metabolic effects of long-term (ie,  $>2$  wk) chronic cocoa treatment. For example, repeated transient increases in plasma flavanol concentrations over periods  $>2$  wk may mediate changes in gene transcription or transient changes in endothelial function that have cumulative chronic beneficial consequences. Plasma concentrations of cocoa flavanols were not measured in previously published 2-wk intervention studies evaluating effects of cocoa on blood pressure in hypertensive subjects (31, 32). However, one recent study that evaluated effects of daily consumption of dark chocolate (30 mg flavanols) on blood pressure in hypertensive subjects over 18 wk also failed to find any significant changes in steady state concentrations of plasma flavanols (33). In our study, daily cocoa consumption ( $\approx 900$  mg flavanols) for 2 wk did not lower blood pressure or improve insulin resistance despite a small increase in insulin-stimulated brachial artery dilation in subjects with essential hypertension.

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The authors' responsibilities were as follows—RM: contributed to the data collection, data analysis, data interpretation, study supervision, and drafting of the manuscript; GH, TLK, and SKC: contributed to the data collection; RJK: contributed to the study design; and MJQ: contributed to the study design, study supervision, data analysis, and interpretation and critically revised the manuscript for important intellectual content. None of the authors had a personal or financial conflict of interest.

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