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# Cocoa Reduces Blood Pressure and Insulin Resistance and Improves Endothelium-Dependent Vasodilation in Hypertensives

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**Abstract**—Consumption of flavanol-rich dark chocolate (DC) has been shown to decrease blood pressure (BP) and insulin resistance in healthy subjects, suggesting similar benefits in patients with essential hypertension (EH). Therefore, we tested the effect of DC on 24-hour ambulatory BP, flow-mediated dilation (FMD), and oral glucose tolerance tests (OGTTs) in patients with EH. After a 7-day chocolate-free run-in phase, 20 never-treated, grade I patients with EH (10 males;  $43.7 \pm 7.8$  years) were randomized to receive either 100 g per day DC (containing 88 mg flavanols) or 90 g per day flavanol-free white chocolate (WC) in an isocaloric manner for 15 days. After a second 7-day chocolate-free period, patients were crossed over to the other treatment. Noninvasive 24-hour ambulatory BP, FMD, OGTT, serum cholesterol, and markers of vascular inflammation were evaluated at the end of each treatment. The homeostasis model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and insulin sensitivity index (ISI) were calculated from OGTT values. Ambulatory BP decreased after DC (24-hour systolic BP  $-11.9 \pm 7.7$  mm Hg,  $P < 0.0001$ ; 24-hour diastolic BP  $-8.5 \pm 5.0$  mm Hg,  $P < 0.0001$ ) but not WC. DC but not WC decreased HOMA-IR ( $P < 0.0001$ ), but it improved QUICKI, ISI, and FMD. DC also decreased serum LDL cholesterol (from  $3.4 \pm 0.5$  to  $3.0 \pm 0.6$  mmol/L;  $P < 0.05$ ). In summary, DC decreased BP and serum LDL cholesterol, improved FMD, and ameliorated insulin sensitivity in hypertensives. These results suggest that, while balancing total calorie intake, flavanols from cocoa products may provide some cardiovascular benefit if included as part of a healthy diet for patients with EH. (*Hypertension*. 2005;46:398-405.)

**Key Words:** endothelium ■ insulin ■ hypertension, essential

Observational studies suggest dietary flavonoids decrease the risk of death from coronary heart disease,<sup>1</sup> cancer,<sup>1</sup> and stroke.<sup>2</sup> Flavonoid-rich foods include fruits and vegetables as well as tea, red wine, and chocolate.<sup>3</sup> The high flavonoid content, particularly in flavanols (ie, catechins) and their procyanidin oligomers, of these foods may contribute to some of their putative cardiovascular benefits.<sup>4,5</sup> The antioxidant protection afforded by flavonoids in the vascular endothelium may reduce the risk for atherosclerosis, including their action of inhibiting the oxidative conversion of NO to peroxynitrite.<sup>6</sup> Accordingly, cocoa flavonoids decreased oxidant-induced peroxynitrite production *in vitro*<sup>7</sup> and increased NO synthase (NOS) expression and NO-dependent vasorelaxation in rabbit aortic rings.<sup>8</sup> In healthy adults, drinking flavanol-rich cocoa increased NO-dependent vasorelaxation in finger arteries,<sup>9</sup> and eating flavanol-rich dark chocolate (DC) improved flow-mediated dilation (FMD) in brachial arteries in association with an increase in plasma epicatechin.<sup>10</sup>

Impaired NO-dependent vasorelaxation also contributes to a dysregulation of blood pressure (BP)<sup>11</sup> and a decrement of insulin-mediated glucose uptake.<sup>12</sup> In contrast, increased endothelial NOS expression and NO bioavailability ameliorate endothelial dysfunction, and thereby have the potential to decrease BP, increase insulin sensitivity, and slow down atherogenic processes. In this regard, the anthocyanin cyanidin-3-glucoside was able to increase NOS expression and NO bioavailability in vascular endothelial cells.<sup>13</sup> We recently demonstrated decrements in BP and increments in insulin sensitivity in healthy volunteers after 15 days of DC intake.<sup>14</sup> Thus, we studied patients with essential hypertension (EH) to evaluate the effects of flavanol-rich DC on 24-hour ambulatory BP monitoring (ABPM), endothelium-dependent vasorelaxation via FMD of the brachial artery, insulin sensitivity via oral glucose tolerance tests (OGTTs), and 2 serum biomarkers of vascular inflammation: high-sensitive C-reactive protein (hsCRP) and intercellular adhesion molecule-1 (ICAM-1).

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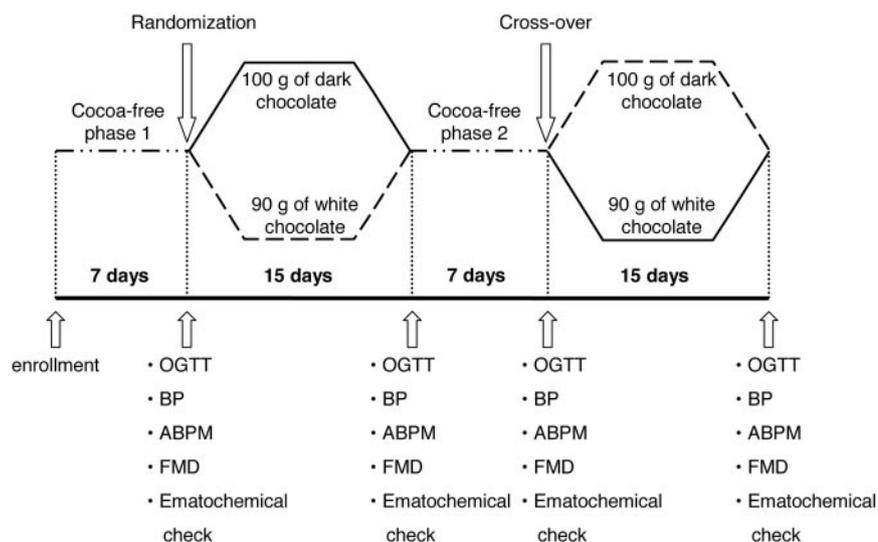
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**Figure 1.** Study design. After a cocoa-free run-in phase of 7 days, 20 never-treated essential hypertensives (10 males, 10 females, mean age  $43.65 \pm 7.8$  years) were randomly assigned to receive either 100-g DC bars containing 88 mg of flavanols or 90-g flavanol-free WC bars for 15 days. Successively, subjects entered a further cocoa-free wash-out phase of 7 days and then were crossed over to the other treatment. OGTTs were performed at the end of each period to calculate HOMA-IR, QUICKI, and ISI. ABPM, office BP, FMD, and hematochemical checks were performed at the same study phases. An identical protocol was used for evaluating controls.

## Methods

### Subject Selection

Twenty never-treated EH patients (10 males and 10 females; mean age  $43.65 \pm 7.8$  years) referred to our outpatient unit were recruited and tested in 2004. Entry criteria were: 25 to 60 years of age; no diabetes or impaired glucose tolerance;<sup>15,16</sup> systolic BP (SBP) between 140 to 159 mm Hg or diastolic BP (DBP) between 90 to 99 mm Hg; absence of macroproteinuria; body mass index between 18 and 27 kg/m<sup>2</sup> for males and 18 to 26 kg/m<sup>2</sup> for females; total serum cholesterol  $<6.1$  mmol/L; and serum triglyceride  $<1.7$  mmol/L. Exclusion criteria were pregnancy, concomitant diseases, and use of medications including dietary supplements. Smokers and consumers of wine or other alcoholic beverages were also excluded. Echo-Doppler examinations of limb and neck vessels excluded patients with atherosclerotic lesions. M-mode and B-mode echocardiograms excluded patients with cardiac abnormalities. The study was conducted according to the Declaration of Helsinki of the World Medical Association (Edinburgh revision, 2000).

### Diagnosis of EH

Grade I EH was diagnosed according to European Societies of Hypertension and Cardiology criteria.<sup>17</sup> For this purpose, before enrollment into the study, BP and heart rate were measured after 10 minutes in a seated position in a comfortable room. SBP/DBP for inclusion in the protocol were  $\geq 140/90$  and  $<160/100$  mm Hg on  $\geq 4$  visits performed at 1-week intervals. During each visit, BP was measured with a standard mercury sphygmomanometer and a stethoscope  $4 \times$  at 2-min intervals. The first BP reading was discarded and the average of the last 3 measurements recorded. On each occasion, BP was recorded by the same physician who was unaware of the study design, objectives, and results (ie, was not a member of the research team). Secondary hypertension was excluded by clinical examination and appropriate tests.

### Normotensive Control Group

Fifteen control subjects (7 males; mean age  $33.9 \pm 7.6$  years) who had participated in a related previous study<sup>14</sup> were recruited from the medical staff to serve as a normotensive reference group. General office BP and OGTT data from these subjects have been published recently.<sup>14</sup> These subjects had histories of normal SBP/DBP but were assessed in the outpatient unit on  $\geq 3$  occasions at 1-week intervals to confirm their values as  $<130/85$  mm Hg. Remaining entry criteria for this group were the same as those for the EH patients.

### Experimental Protocol

After evaluation of exclusion/inclusion criteria, EH subjects and controls were carefully instructed to maintain their usual diet but

asked to refrain from flavonoid-rich foods and beverages, including tea and wine; a list of these foods and beverages was given to each participant. All participants were asked to continue their usual physical activity throughout the study period and were found to comply with this instruction based on self-reports in daily physical activity diaries. Similar to the trial described by Taubert et al<sup>18</sup> on elderly patients with isolated systolic hypertension, our patients and reference group entered a 7-day run-in phase, which excluded all cocoa foods. At the end of the run-in period, both groups were assigned randomly to receive daily either 100-g DC bars (Ritter Sport Halbitter; Alfred Ritter GmbH & Co.) which, by our determination, contained 21.91 mg catechin, 65.97 mg epicatechin, 0.59 mg quercetin, 0.03 mg kaempferol, and 0.31 mg isorhamnetin, or 90-g flavanol-free white chocolate (WC) bars absent of any flavonoids (Milka; Kraft Foods) over a period of 15 days. DC and WC bars contained 480 kcal energy and similar amounts of cocoa butter, macronutrients, fiber, electrolytes, and vitamins.<sup>18</sup> At the end of the first phase of intervention, patients and controls entered a second 7-day chocolate-free phase. After this period, all participants were crossed over to the other treatment (Figure 1).<sup>18</sup> To avoid changes in body weight during the intervention, subjects were instructed how to substitute chocolate bars for foods of similar energy and macronutrient composition. The diet during the study was assessed by a diary of daily food intake and by daily measurement of body weight.<sup>18</sup>

### Assessment of Insulin Sensitivity

After the run-in phase and after both intervention phases, OGTTs using 75 g of D-glucose were performed according to standard procedures<sup>19,20</sup> after an overnight fast and  $\geq 12$  hours from the last chocolate intake. Plasma glucose and insulin were assessed at baseline (0 minutes) and 30, 60, 90, 120, and 180 minutes after the 75-g glucose load. OGTT results were used for the homeostasis model assessment of insulin resistance (HOMA-IR),<sup>19–21</sup> the quantitative insulin sensitivity check index (QUICKI),<sup>20</sup> and the insulin sensitivity index (ISI).<sup>22</sup>

### Hematochemical Assessment

A routine hematochemical assessment with serum electrolytes and lipid profile, including total cholesterol, HDL and LDL cholesterol, and triglyceride, was conducted at the same time periods as that for assessing insulin sensitivity.

### Endothelial Function

FMD of the brachial artery was assessed after a 15-minute rest period during scheduled visits (ie, after fasting [ $\geq 12$  hours from the last chocolate ingestion]). FMD was always determined by the same

physician, who was blinded to the study design and objectives (ie, was not a member of the research team), according to Ghiadoni et al.<sup>23</sup> Briefly, a B-mode scan of the right brachial artery was obtained in longitudinal section between 5 and 10 cm above the elbow using a 7.0-MHz linear array transducer and a standard MEGAS-platelet glycoprotein system (ESAOTE Biomedica) as described previously.<sup>24,25</sup> The transducer was held at the same point throughout the scan by a stereotactic clamp. End-diastolic frames (ECG-triggered) were acquired every second on a personal computer with the use of a commercial software program (MovieBox-Studio version 9; Pinnacle Systems GmbH). Arterial flow velocity was obtained by a pulsed Doppler signal at 70° to the vessel with the range gate (1.5 mm) at the center of the artery. A cuff was placed around the forearm just below the elbow. After 1-minute acquisition to measure basal diameter, the cuff was inflated for 5 minutes at 250 mm Hg and then deflated to induce reactive hyperemia. Endothelium-dependent vasodilation was considered the maximal dilation of the brachial artery induced by increased flow.<sup>23–25</sup> Endothelium-independent dilation was obtained by 25- $\mu$ g sublingual glyceryl trinitrate.<sup>23</sup>

### BP Monitoring

Before and after each study period, 24-hour ABPM was recorded by a noninvasive oscillometric device (Medical 90207-30; Spacelabs, Inc.). BP was recorded at 15-minute intervals (daytime 6 AM to 10 PM) or 20-minute intervals (nighttime 10 PM to 6 AM). The mean 24-hour, daytime, and nighttime BP were calculated for statistical evaluation. Before starting with ABPM, sitting BP was also measured and recorded by standard mercury sphygmomanometer and stethoscope, as described above.

### Biomarkers of Vascular Inflammation

Before and after each study phase, serum was also collected from all subjects for the determination of hsCRP (CRPLX Roche Diagnostics GmbH) and ICAM-1 (R & D Systems Inc.) by commercially available enzyme-linked immunonephelometric and immunoassay kits<sup>26,27</sup> according to manufacturer instructions.

### Chocolate Flavonoid Content

Fats were extracted from the DC and WC bars with hexane and the defatted material dissolved in an extraction buffer of acetone:water:acetic acid (70.0:29.5:0.5), centrifuged, and filtered. The extract was dried, dissolved in metaphosphoric acid, and injected into high-performance liquid chromatography (ESA CoulArray) for flavonoid determination according to Milbury.<sup>28</sup>

### Statistical Analysis

Continuous normally distributed data are expressed as mean $\pm$ SD. Differences in BP and metabolic indices between hypertensives and normotensives were analyzed by paired Student's *t* test. Within each treatment group, changes in insulin sensitivity indices, BP, hsCRP, and ICAM-1 from baseline were analyzed by 1-factor ANOVA. For multiple comparisons, data were analyzed with a 2-factor repeated-measures ANOVA with time and treatment as the 2 factors. Post hoc comparisons were performed by Tukey's honestly significant difference (HSD) test. Statistical analyses and power calculations were performed with SAS (2000; SAS Institute Inc.).

## Results

Baseline characteristics of the study participants are provided in Table 1. According to entry criteria, no subjects had abnormal glucose metabolism. In hypertensives, baseline HOMA-IR, QUICKI, and ISI were similar between those randomized to WC and DC interventions (HOMA-IR 2.8 $\pm$ 1.6 versus 2.8 $\pm$ 1.5,  $P=0.92$ ; QUICKI 0.3 $\pm$ 0.03 versus 0.3 $\pm$ 0.03,  $P=0.91$ ; ISI 3.9 $\pm$ 3.1 versus 3.9 $\pm$ 3.0,  $P=0.99$ , respectively). Baseline SBP/DBP were also similar between the randomized groups (142.3 $\pm$ 4.4 versus 142.3 $\pm$ 4.3 mm Hg,

**TABLE 1. General Characteristics of Essential Hypertensives (n=20; 10 Males, 10 Females; Age 43.6 $\pm$  7.8 Years) at Baseline and After 15 Days of DC or WC Consumption**

Characteristic	DC		WC	
	Before	After	Before	After
Body mass index (kg/m <sup>2</sup> )	25.4 $\pm$ 1.7	25.4 $\pm$ 1.7	25.4 $\pm$ 1.7	25.4 $\pm$ 1.7
Body weight (kg)	73.7 $\pm$ 9.2	73.7 $\pm$ 9.2	73.7 $\pm$ 9.2	73.7 $\pm$ 9.2
Total cholesterol (mmol/L)	5.4 $\pm$ 0.6	5.0 $\pm$ 0.7*	5.4 $\pm$ 0.6	5.4 $\pm$ 0.6
LDL cholesterol (mmol/L)	3.4 $\pm$ 0.5	3.0 $\pm$ 0.6†	3.4 $\pm$ 0.5	3.4 $\pm$ 0.5
HDL cholesterol (mmol/L)	1.4 $\pm$ 0.3	1.4 $\pm$ 0.3	1.4 $\pm$ 0.3	1.4 $\pm$ 0.3
Triglycerides (mmol/L)	1.3 $\pm$ 0.4	1.1 $\pm$ 0.4	1.3 $\pm$ 0.4	1.3 $\pm$ 0.4

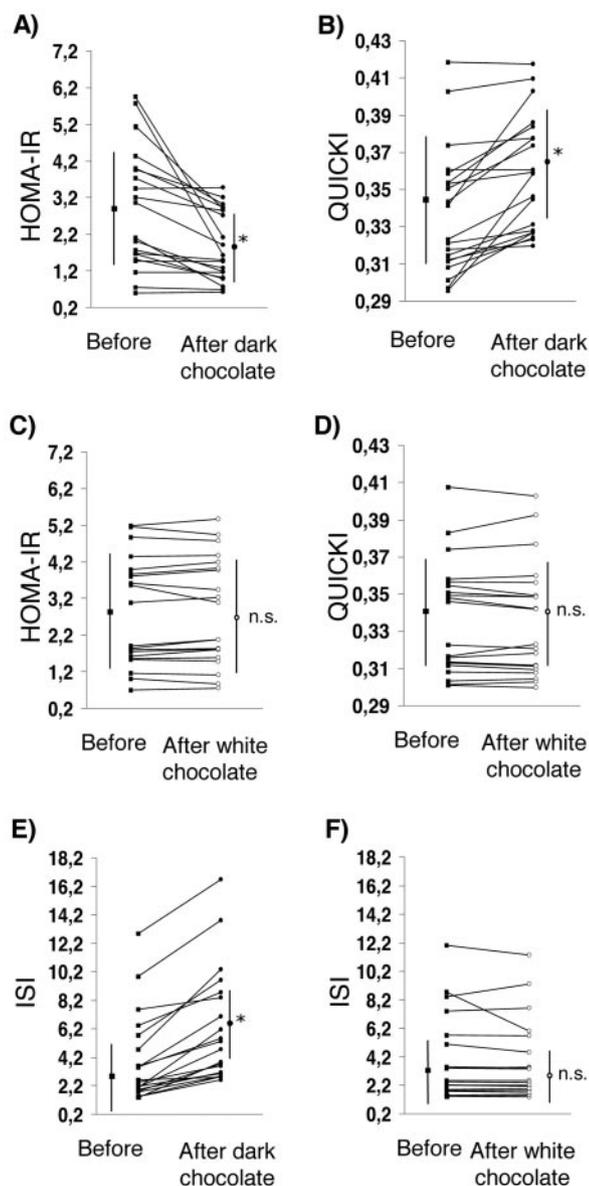
Data are given as mean $\pm$ SD.

\* $P=0.0003$  DC vs baseline and WC values; † $P<0.05$  DC vs WC and baseline values.

$P=0.97$ ; 91.6 $\pm$ 2.3 versus 90.8 $\pm$ 3.2 mm Hg,  $P=0.36$ , respectively).<sup>14</sup> Compared with controls, hypertensives had higher BP ( $P<0.0001$ ) and HOMA-IR ( $P=0.005$ ) and lower QUICKI ( $P=0.0007$ ) and ISI ( $P=0.01$ ).

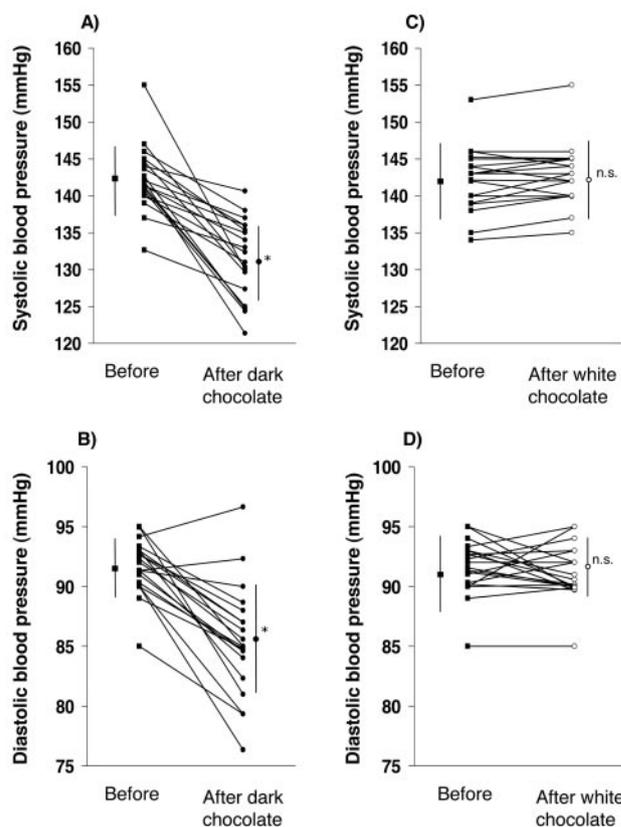
Compared with baseline, DC consumption by hypertensives lowered HOMA-IR ( $F=16.57$ ;  $P<0.0001$  by 1-factor ANOVA; Figure 2A) and raised QUICKI ( $F=29.37$ ;  $P<0.0001$ ; Figure 2B), whereas WC was ineffective (Figure 2C and 2D). Similarly, compared with baseline, ISI was higher after DC than WC ingestion ( $F=39.62$ ;  $P<0.0001$  by ANOVA; Figure 2E and 2F). Improvement in glucose and insulin responses during OGTTs was observed after DC but not WC ( $P<0.05$ ). With DC intervention, significant effects were noted in glucose responses for treatment ( $F=32.17$ ;  $P<0.0001$ ), time ( $F=72.48$ ;  $P<0.0001$ ), and treatment–time interactions ( $F=2.89$ ;  $P=0.0003$ ) with 2-factor repeated-measures ANOVA. Fasting glucose levels decreased after DC (from 4.7 $\pm$ 0.5 to 4.4 $\pm$ 0.4 mmol/L;  $P<0.0001$ ). No significant variations were observed with WC intervention in glucose responses and fasting glucose levels (from 4.7 $\pm$ 0.4 to 4.7 $\pm$ 0.3 mmol/L; NS). Fasting insulin levels decreased after DC (from 13.1 $\pm$ 6.7 to 9.3 $\pm$ 4.4 mUI/mL;  $P<0.0001$ ). Consistent relationships were noted after DC ingestion in insulin responses, with significant effects of treatment ( $F=33.55$ ;  $P<0.0001$ ), time ( $F=19.80$ ;  $P<0.0001$ ), and treatment–time interactions ( $F=4.47$ ;  $P<0.0001$ ) by 2-factor repeated-measures ANOVA. No significant variations were observed after WC for insulin responses and fasting insulin (from 13.1 $\pm$ 6.4 to 13.1 $\pm$ 6.5 mUI/mL; NS).

DC reduced SBP ( $-11.0\pm 6.3$  mm Hg;  $F=55.39$ ;  $P<0.0001$  versus baseline) and DBP in hypertensives ( $-6.2\pm 4.2$  mm Hg;  $F=17.35$ ;  $P<0.0001$  versus baseline) with results tested by 1-factor repeated-measures ANOVA (Figure 3A and 3B). SBP ( $-0.5\pm 1.6$  mm Hg; NS) and DBP ( $-0.3\pm 3.1$  mm Hg; NS) remained unchanged after WC (Figure 3C and 3D). ABPM results confirmed significant reductions after DC (24-hour SBP  $-11.9\pm 7.7$  mm Hg,  $F=33.78$ ,  $P<0.0001$ ; 24-hour DBP  $-8.5\pm 5.0$  mm Hg,  $F=38.80$ ,  $P<0.0001$  versus baseline) but not WC ingestion (24-hour SBP  $-0.9\pm 2.7$  mm Hg; 24-hour DBP  $-0.1\pm 2.5$  mm Hg; NS; Table 2).



**Figure 2.** Effects of DC (A, B, and E) and WC (C, D, and F) on HOMA-IR, QUICKI, and ISI in 20 never-treated essential hypertensive patients. In all panels, laterally to individual values, corresponding symbols indicate means, and vertical lines indicate SDs. Asterisks indicate significant differences between DC vs WC and baseline values as evaluated by Tukey's HSD test ( $P < 0.05$ ) when a 1-factor repeated-measures ANOVA showed a significant interaction for treatment.

Evaluating ABPM results for daytime and nighttime revealed a significant effect of treatment ( $P < 0.0001$ ) and time ( $P < 0.0001$ ) but not treatment–time interactions ( $P = 0.9901$  and  $P = 0.5$ , respectively) by 2-factor repeated-measures ANOVA. DC reduced SBP (daytime  $-12.0 \pm 7.3$  mm Hg,  $P < 0.05$ ; nighttime  $-11.9 \pm 11.6$  mm Hg,  $P < 0.05$  versus baseline) and DBP (daytime  $-7.8 \pm 5.2$  mm Hg,  $P < 0.05$ ; nighttime  $-10.2 \pm 6.3$  mm Hg,  $P < 0.05$  versus baseline), whereas WC did not (SBP daytime  $-0.7 \pm 3.6$  mm Hg, SBP nighttime  $-1.4 \pm 2.9$  mm Hg, NS; DBP daytime  $-0.2 \pm 2.9$  mm Hg, DBP nighttime  $-0 \pm 2.8$  mm Hg, NS; Table 2).



**Figure 3.** Effects of DC (A and B) or WC (C and D) on sitting SBP and DBP in 20 never-treated essential hypertensives. In both panels, laterally to individual values, corresponding symbols indicate means, and vertical lines indicate SDs. Asterisks indicate significant differences between DC vs WC and baseline values as evaluated by Tukey's HSD test ( $P < 0.05$ ) when a 1-factor repeated-measures ANOVA showed a significant interaction for treatment.

Responses of SBP and DBP (daytime and nighttime) to DC were similar in controls, with a significant effect of treatment ( $P < 0.0001$ ) and time ( $P < 0.0001$ ) by 2-factor repeated-measures ANOVA. In contrast, a significant treatment–time interaction ( $P = 0.001$ ) was found for SBP but not for DBP ( $P = 0.33$ ). DC intake reduced SBP (daytime  $-6.3 \pm 5.5$  mm Hg,  $P < 0.05$ ; nighttime  $-5.3 \pm 5.4$  mm Hg,  $P < 0.05$  versus baseline) and DBP (daytime  $-4.2 \pm 4.5$  mm Hg,

**TABLE 2. 24-Hour ABPM Data at Baseline and After 15 Days of DC or WC Ingestion in 20 Essential Hypertensives**

Characteristic	DC		WC	
	Before	After	Before	After
24-hour SBP ABPM	135.5 ± 5.8	123.6 ± 6.3*	135.6 ± 5.5	134.7 ± 4.7
24-hour DBP ABPM	88.0 ± 4.1	79.6 ± 5.4*	87.6 ± 4.3	87.5 ± 4.6
SBP daytime ABPM	141.3 ± 4.8	129.3 ± 5.7*	141.1 ± 5.4	140.4 ± 4.6
SBP nighttime ABPM	120.2 ± 11.6	108.7 ± 9.1*	120.9 ± 11	119.4 ± 10.2
DBP daytime ABPM	92.4 ± 3.8	84.6 ± 5.6*	91.8 ± 4.7	91.6 ± 4.7
DBP nighttime ABPM	76.2 ± 6.3	66 ± 7*	76.4 ± 6.1	76.4 ± 5.7

Data are given as mean ± SD.

\* $P < 0.0001$  DC vs WC and baseline values.

**TABLE 3. 24-Hour ABPM Data Obtained in 15 Control Subjects at Baseline and After DC or WC for 15 Days**

Characteristic	DC		WC	
	Before	After	Before	After
BP (mm Hg)				
24-hour SBP ABPM	109.3±8.4	102.7±6.4*	109.7±7.7	109.3±7.2
24-hour DBP ABPM	71.6±5.1	67.5±4.2*	71.6±5.2	71±4.8
SBP daytime ABPM	112.9±8.5	105.9±6.6*	113.2±7.9	112.7±7.6
SBP nighttime ABPM	99.8±8	94.5±6*	100.8±7.2	100.1±6.9
DBP daytime ABPM	74±5.7	69.8±4.5*	73.8±5.5	73.5±5.3
DBP nighttime ABPM	64.7±3.9	61.5±4*	65.2±3.9	64.3±3.9

Data are given as mean±SD.

\* $P<0.0001$  DC vs WC and baseline values.

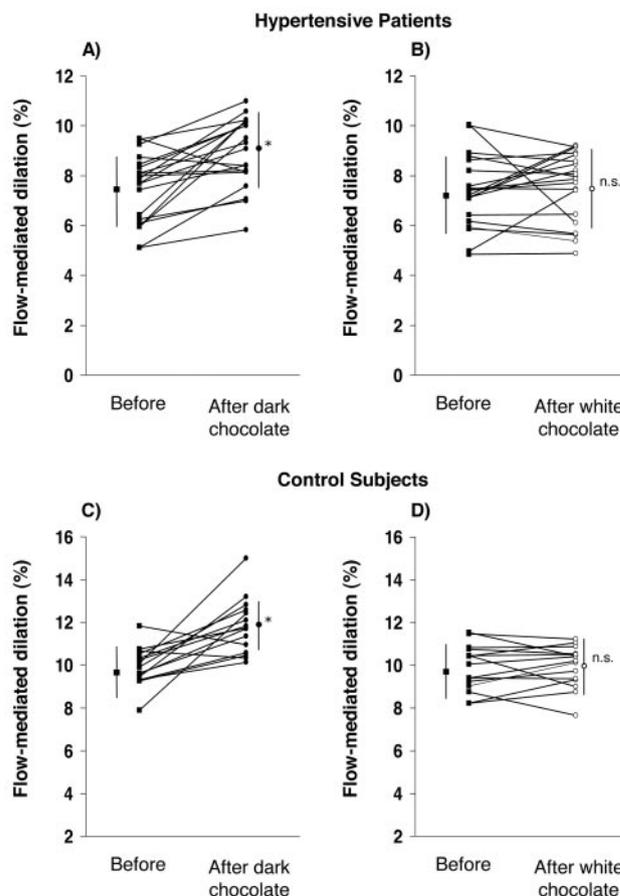
$P<0.05$ ; nighttime  $-3.1\pm3.8$  mm Hg,  $P<0.05$  versus baseline), whereas WC affected neither SBP (daytime  $-0.5\pm3.8$  mm Hg; nighttime  $-0.7\pm3.5$  mm Hg; NS) nor DBP (daytime  $-0.3\pm2.2$  mm Hg; nighttime  $-0.9\pm2.0$  mm Hg; NS; Table 3). As in hypertensives, DC reduced 24-hour SBP ( $-5.9\pm5.4$  mm Hg;  $F=15.21$ ;  $P<0.0001$  versus baseline) and 24-hour DBP ( $-4.1\pm4.1$  mm Hg;  $F=11.59$ ;  $P<0.0001$  versus baseline), whereas WC did not (24-hour SBP  $-0.5\pm3.7$  mm Hg; 24-hour DBP  $-0.6\pm2.1$  mm Hg; NS) when examined by 1-factor repeated-measures ANOVA (Table 3).

Baseline FMD was impaired in hypertensives compared with controls ( $7.4\pm1.4\%$  versus  $9.9\pm0.9\%$ , respectively;  $P<0.0001$ ) and increased in hypertensives almost to normal values after DC ( $8.9\pm1.4\%$ ;  $F=13.25$ ;  $P<0.0001$ ) but not WC ( $7.5\pm1.3\%$ ; Figure 4A and 4B). FMD also increased after DC ( $11.8\pm1.3\%$ ;  $F=19.86$ ;  $P<0.0001$  versus baseline) but not WC ( $10.1\pm0.9\%$ ; NS) in controls (Figure 4C and 4D).

Baseline glyceryl trinitrate-induced dilation did not differ between hypertensives ( $8.6\pm1.4\%$ ) and controls ( $9.0\pm1.2\%$ ) and remained unchanged after DC (hypertensives  $8.5\pm1.2\%$ , NS; normotensives  $9.2\pm1.3\%$ , NS) and WC (hypertensives  $8.7\pm1.7\%$ , NS; normotensives  $9.1\pm1.7\%$ , NS).

In hypertensives, serum total cholesterol ( $F=7.37$ ;  $P=0.0003$ ;  $P<0.05$  versus WC and baseline) and LDL cholesterol ( $F=2.94$ ;  $P=0.04$ ) but not triglycerides or HDL cholesterol levels decreased after DC but not WC (Table 1). Other variables including serum hsCRP and ICAM-1 levels remained unchanged after DC in hypertensives (hsCRP from  $0.37\pm0.39$  to  $0.33\pm0.39$  mg/dL, NS; ICAM-1 from  $144.9\pm6.5$  to  $144.2\pm6$   $\mu\text{g/L}$ , NS) and in normotensives (hsCRP from  $0.26\pm0.2$  to  $0.26\pm0.2$  mg/dL, NS; ICAM-1 from  $140.8\pm5.5$  to  $140.5\pm5$   $\mu\text{g/L}$ , NS) as well as after WC in hypertensives (hsCRP from  $0.35\pm0.31$  to  $0.36\pm0.31$  mg/dL, NS; ICAM-1 from  $144.9\pm6.2$  to  $144.8\pm6.3$   $\mu\text{g/L}$ , NS) and in normotensives (hsCRP from  $0.3\pm0.24$  to  $0.3\pm0.22$  mg/dL, NS; ICAM-1 from  $140.8\pm5.4$  to  $140.6\pm5.5$   $\mu\text{g/L}$ , NS).

No significant changes from baseline were observed in 24-hour urinary NaCl excretion in hypertensives after either DC (from  $128.3\pm36.8$  to  $123.1\pm31.8$  mmol/24 h; NS) or WC (from  $126.4\pm29.6$  to  $121.1\pm26.7$  mmol/24 h; NS). Similar findings were observed in normotensives with DC (from



**Figure 4.** Effects of DC and WC on brachial artery measurements by FMD in 20 never-treated essential hypertensives (A and B, respectively) and 15 controls (C and D, respectively). In both panels, laterally to individual values, corresponding symbols indicate means, and vertical lines indicate SDs. Asterisks indicate significant differences between DC vs WC and baseline values as evaluated by Tukey's HSD test ( $P<0.05$ ) when a 1-factor repeated-measures ANOVA showed a significant interaction for treatment.

$114.2\pm27.9$  to  $117\pm22.6$  mmol/24 h; NS) and WC (from  $116.5\pm25.1$  to  $112.6\pm18.9$  mmol/24 h; NS). Age, gender, and other variables did not influence the effect of chocolate on HOMA-IR, QUICKI, ISI, ABPM, and FMD values in either group.

## Discussion

The current study shows that consumption of flavanol-rich DC decreased daytime and nighttime BP, reduced insulin resistance, and improved NO-dependent vasorelaxation. These outcomes may be closely interrelated because impairment of NO-dependent vasorelaxation appears to contribute substantially to EH,<sup>11</sup> a disease often associated with insulin resistance and metabolic syndrome.<sup>29,30</sup> It is important to note that hypertension, and its association with atherosclerosis,<sup>31</sup> inflammation,<sup>31</sup> platelet activation,<sup>32</sup> and oxidative stress,<sup>33</sup> has been considered the most important cardiovascular risk factor worldwide.<sup>34</sup>

Our results in EH patients and normotensives support studies indicating flavanol intake can reduce BP in healthy,

young individuals<sup>14</sup> as well as geriatric patients with isolated systolic hypertension.<sup>18</sup> However, 24-hour BP reduction induced by DC in control subjects found here and also reported by Grassi et al<sup>14</sup> are in contrast to related interventions conducted by Fisher et al<sup>9</sup> and Engler et al.<sup>10</sup> These investigators did not observe any BP change in their healthy volunteers drinking a flavanol-rich chocolate beverage for 1 or 4 days<sup>9</sup> or eating DC bars for 2 weeks.<sup>10</sup> The absence of a reduction in BP in these 2 studies may be attributable to their shorter duration, use of different chocolate products, demographics or health status of the subjects, or other differences between the studies. Nevertheless, it is worthwhile to note that Engler et al<sup>10</sup> found a slight reduction in office SBP ( $-1.0 \pm 1.4$  mm Hg; NS) in their trial. Importantly, and in contrast with these studies, we used ABPM, a more robust determination of treatment-induced BP changes than office BP measurements.<sup>35</sup>

Consistent with our results, Fisher et al<sup>9</sup> and Engler et al<sup>10</sup> observed an increase in FMD with their chocolate interventions. Similarly, Heiss et al<sup>36</sup> found consumption of flavanol-rich chocolate increased FMD in patients with coronary artery disease, severe hypertension, or diabetes. In these patients, chocolate significantly increased plasma nitrite and nitrate, an integrated biomarker of NO and NO metabolism.<sup>37</sup> Thus, despite the potential of flavanols to induce prostacyclin production,<sup>38</sup> their capability to improve FMD may result from modulating NO status, potentially via increasing NOS<sup>39</sup> or inhibiting the NO conversion to peroxynitrite.<sup>6,14</sup> In vitro, flavanols have been reported to increase NOS expression<sup>39</sup> and activity<sup>8,39</sup> and prevent NO–superoxide anion reactions to form peroxynitrite.<sup>7</sup> In humans, drinking tea, a flavonoid-rich beverage, has been shown to improve FMD in the brachial arteries of hypertensives and normotensives with coronary artery disease.<sup>38</sup> Similarly, flavonoid-rich purple grape juice has been reported to increase FMD and decrease oxidative stress in patients with coronary artery disease.<sup>40</sup>

Insulin sensitivity is partly dependent on insulin-mediated NO release.<sup>41</sup> Thus, flavanols and other dietary antioxidants may decrease insulin resistance by ameliorating NO bioavailability. Consistent with this hypothesis, intravenous infusion of ascorbic acid to glucose intolerant subjects and smokers improved FMD and insulin sensitivity while reducing plasma thiobarbituric acid–reactive substances, an index of lipid peroxidation.<sup>42</sup> Similarly, Hirashima et al<sup>43</sup> reported that in patients with vasospastic angina, a condition associated with reductions in insulin sensitivity<sup>44</sup> and NO status,<sup>45</sup> ascorbic acid infusion normalized FMD and reduced insulin resistance.

CRP, an acute phase protein, is regulated by inflammatory cytokines and oxidants and decreases in response to therapies with agents possessing antioxidant properties.<sup>27</sup> However, flavanol-rich DC did not affect circulating hsCRP or ICAM-1 in our subjects. Similarly, Mathur et al,<sup>46</sup> although demonstrating the antioxidant action of cocoa on the susceptibility of LDL to oxidation in healthy adults, found no effect of cocoa on hsCRP. Also, using a less sensitive agglutination technique for the determination of CRP, Davidsson et al<sup>47</sup> found no change in plasma CRP in children provided iron-

fortified chocolate drinks.<sup>47</sup> Nonetheless, it is possible that the absence of an effect of flavanols on hsCRP or ICAM-1 may reflect an inadequate study dose or duration.

At variance with previous findings we obtained on normotensives,<sup>14</sup> a significant reduction of serum total and LDL cholesterol levels was observed in hypertensives after DC ingestion. A few studies have evaluated the effects of flavonoids contained in tea and cocoa on serum cholesterol.<sup>48</sup> Mursu et al<sup>49</sup> found that ingestion of DC bars for 15 days increased HDL cholesterol in healthy subjects without variations in total and LDL cholesterol. Recently, Fraga et al<sup>50</sup> showed significant decrements in serum total cholesterol ( $-11\%$ ) and LDL cholesterol levels ( $-15\%$ ) after short-term consumption of flavanol-rich chocolate in young subjects. Also, Davies et al<sup>51</sup> found significant decrements in serum total cholesterol ( $-6.5\%$ ) and LDL cholesterol ( $-11.1\%$ ), with unchanged HDL cholesterol levels after 3 weeks of black tea in hypercholesterolemic adults. Similarly, experimental data from rats showed a specific hypocholesterolemic effect of catechins.<sup>52</sup> Further, chocolate also contains linoleic and oleic acids, 2 fatty acids known to modulate cholesterol metabolism.<sup>53</sup> Therefore, although a study in smokers<sup>54</sup> and an early study in healthy subjects<sup>55</sup> showed no changes in serum total cholesterol and cholesterol fractions after black<sup>54,55</sup> or green tea,<sup>54</sup> our results are consistent with previous reports showing positive effects of flavonoids on serum lipid profiles.

In conclusion, our findings support a potentially beneficial action of chocolate flavanols on BP, vasorelaxation, and insulin sensitivity in essential hypertensives and suggest directions for further research in this area.<sup>56</sup> Interestingly, consumers of chocolate and other candies appear to have a lower mortality rate compared those who do not eat candy.<sup>57</sup> Nonetheless, it is important to note that the DC used in this and related studies differ markedly from the majority of commercially available cocoa or chocolate confectionery with very low flavanol content.<sup>56</sup> Further, caution is always warranted when considering dietary recommendations for foods high in fat and calories, especially for cardiovascular disease.

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