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Blood Pressure Is Reduced and Insulin Sensitivity Increased in Glucose-Intolerant, Hypertensive Subjects after 15 Days of Consuming High-Polyphenol Dark Chocolate[1–3]

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Abstract

Flavanols from chocolate appear to increase nitric oxide bioavailability, protect vascular endothelium, and decrease cardiovascular disease (CVD) risk factors. We sought to test the effect of flavanol-rich dark chocolate (FRDC) on endothelial function, insulin sensitivity, β-cell function, and blood pressure (BP) in hypertensive patients with impaired glucose tolerance (IGT). After a run-in phase, 19 hypertensives with IGT (11 males, 8 females; 44.8 ± 8.0 y) were randomized to receive isocalorically either FRDC or flavanol-free white chocolate (FFWC) at 100 g/d for 15 d. After a wash-out period, patients were switched to the other treatment. Clinical and 24-h ambulatory BP was determined by sphygmometry and oscillometry, respectively, flow-mediated dilation (FMD), oral glucose tolerance test, serum cholesterol and C-reactive protein, and plasma homocysteine were evaluated after each treatment phase. FRDC but not FFWC ingestion decreased insulin resistance (homeostasis model assessment of insulin resistance; $P < 0.0001$) and increased insulin sensitivity (quantitative insulin sensitivity check index, insulin sensitivity index (ISI), ISI_0; $P < 0.05$) and β-cell function (corrected insulin response CIR_{120}; $P = 0.035$). Systolic (S) and diastolic (D) BP decreased ($P < 0.0001$) after FRDC (SBP, −3.82 ± 2.40 mm Hg; DBP, −3.92 ± 1.98 mm Hg; 24-h SBP, −4.52 ± 3.94 mm Hg; 24-h DBP, −4.17 ± 3.29 mm Hg) but not after FFWC. Further, FRDC increased FMD ($P < 0.0001$) and decreased total cholesterol (−6.5%; $P < 0.0001$), and LDL cholesterol (−7.5%; $P < 0.0001$). Changes in insulin sensitivity (Δ ISI − Δ FMD: $r = 0.510$, $P = 0.001$; Δ QUICKI − Δ FMD: $r = 0.502$, $P = 0.001$) and β-cell function (Δ CIR_{120} − Δ FMD: $r = 0.400$, $P = 0.012$) were directly correlated with increases in FMD and inversely correlated with decreases in BP (Δ ISI − Δ 24-h SBP: $r = -0.368$, $P = 0.022$; Δ ISI − Δ 24-h DBP $r = -0.384$, $P = 0.017$). Thus, FRDC ameliorated insulin sensitivity and β-cell function, decreased BP, and increased FMD in IGT hypertensive patients. These findings suggest flavanol-rich, low-energy cocoa food products may have a positive impact on CVD risk factors. J. Nutr. 138: 1671–1676, 2008.

Introduction

Several studies indicate fruits and vegetables as well as red wine, tea, and cocoa rich in polyphenols may reduce the risk of cardiovascular disease (CVD)[6] (1). Cocoa beans contain 6–8% polyphenols by dry weight and are particularly rich in monomeric (epicatechin and catechin) and oligomeric (procyanidin) flavanols (2). Interestingly, the Kuna Indian population of the San Blas islands of Panama is characterized by a low prevalence of atherosclerotic disease, type 2 diabetes, and arterial hypertension as well as an absence of age-related increases in blood pressure (BP) (3). These traits are not significantly genetic in nature, because they disappear after migration to urban areas on mainland Panama and subsequent changes in diet. McCullough

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[6] Abbreviations used: ABPM, ambulatory blood pressure monitoring; BP, blood pressure; CIR, corrected insulin response; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; EH, essential hypertension; FFWC, flavanol-free white chocolate; FMD, flow-mediated dilation; FRDC, flavanol-rich dark chocolate; HOMA-IR, homeostasis model assessment of insulin resistance; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure.

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et al. (3) have hypothesized the high intake of a traditional cocoa beverage may be partly responsible for the low incidence of CVD among the Kuna islanders. The biological plausibility of this relationship is based on the action of flavanols to increase the bioavailability of nitric oxide (NO) in endothelial cells via their antioxidant actions (4,5) and their capacity to activate vascular endothelial NO synthase (6).

Flavanol-rich chocolate beverages and bars have been found to augment endothelium-dependent vasorelaxation in healthy subjects (5,7,8) as well as in patients with hypertension (8) and coronary heart disease (9). Consumption of dark chocolate bars for 15 d has been reported to reduce systolic (S) BP in healthy subjects (10) as well as in young (8) and elderly (11) hypertensive patients. Studies of flavanol-rich cocoa also show an associated increase in endothelial NO bioavailability in healthy adults (5,7,12) and in patients with a high CVD risk profile (9).

Conditions of insulin resistance such as impaired glucose tolerance (IGT) or "prediabetes" (13) are characterized by high risk of CVD (14) and diabetes (15), decreased endothelial NO bioavailability with impaired endothelium-dependent vasorelaxation (16), and increased oxidative stress (17). Several studies have tried to improve insulin sensitivity in glucose-intolerant subjects with insulin sensitizers and appropriate lifestyle changes (18). In this context, we recently found an increase in insulin sensitivity after flavanol-rich but not after flavanol-free chocolate ingestion in healthy subjects (10) and hypertensive patients (8). Insulin sensitivity is partly dependent on NO bioavailability in endothelial cells (19), particularly in those with IGT (20). Thus, we examined the effect of flavanol-rich and flavanol-free chocolate bars on glucose and insulin responses to an oral glucose tolerance test (OGTT), endothelium-dependent vasorelaxation, clinical and 24-h ambulatory BP, and serum C-reactive protein (CRP) in adults with grade I essential hypertension (EH) also presenting with IGT.

Subjects and Methods

Patient selection. The study was conducted in 19 EH patients with IGT (11 males, 8 females, mean age = 44.8 ± 8.0 y) derived from 157 patients referred to The Division of Internal Medicine and Centre of Hypertension and Cardiovascular Prevention Outpatient Unit for screening due to hypertension and family history of diabetes, hypertension, hyper/dyslipoproteinemia or fasting plasma glucose ≥5.55 and <6.99 mmol/L. Exclusion criteria for the study included: clinically overt diabetes, BMI ≥30 kg/m^2, concomitant diseases, pregnancy, and use of any medications (including dietary supplements, steroids, and nonsteroidal antiinflammatory drugs). Also excluded were smokers and those with SBP ≥160 mm Hg and diastolic (D) BP ≥100 mm Hg. Echo-Doppler examinations of the limb and neck vessels excluded patients with atherosclerotic lesions. M-mode and B-mode echocardiograms excluded patients with cardiac abnormalities. The study was approved by the responsible Ethics Committee and all participants provided written informed consent.

Diagnosis of EH. The diagnosis of grade I EH was based on the criteria of the European Society of Hypertension/European Society of Cardiology (21). Before entering the study, BP and heart rate were measured after 10 min in a sitting position in a comfortable room of our Hypertension Outpatient Unit. According to entry criteria, SBP/DBP were >140/90 and <160/100 mm Hg on at least 4 visits performed at 1-wk intervals. On each visit, clinical BP was measured with a mercury sphygmomanometer and a stethoscope 4 times at 2-min intervals. The first BP reading was disregarded and the mean of the last 3 measurements recorded. On each occasion, the same physician, who was unaware of the study design, results, and purpose, always recorded BP. Secondary forms of hypertension were excluded by clinical examination and appropriate tests.

Diagnosis of IGT. The diagnosis of IGT was made by an OGTT with a 250- to 300-mL solution of 75 g anhydrous glucose. Plasma glucose after glucose loading was ≥7.77 and <11.1 mmol/L according to American Diabetes Association criteria (13).

Study design. After evaluation of eligibility criteria, IGT EH patients were instructed to maintain their usual diet except to refrain from flavanoid-rich foods and beverages (a detailed list was given to each participant), including wine as well as all other alcoholic beverages. All participants were encouraged to continue with their usual physical activity throughout the study period. Then, patients entered in a first 7-d, cocoa-free run-in phase. At the end of the run-in, subjects were randomly assigned to receive either 100 g flavanol-rich dark chocolate (FRDC) bars (Cuorenero Sugar Company) or 100 g flavanol-free white chocolate (FFWC) bars (Milka, Kraft Foods) over a period of 15 d. The chocolate was consumed each day in 2 half-bar doses at breakfast and lunch. The first dose was consumed at breakfast on d 1 and the last dose the day before the last visit on d 16. At the end of the first phase of intervention, patients entered a 2nd validated 7-d chocolate-free phase (8,10,11) and, after this period, were switched to the other treatment. Analysis of the FRDC using the Folin-Ciocalteu reaction revealed 1008 mg total phenols and using HPLC identified 110.9 mg epicatechin, 36.12 mg catechin, 2.5 mg quercetin, 0.03 mg kaempferol, and 0.2 mg isorhamnetin; the same 2 methods determined FFWC contained 0.13 g total phenols and 0.04 mg catechin. Energy, minerals, and other nutrient components of FRDC and FFWC are provided (**Supplemental Table 1**). Chocolate doses for each subject were rolled in aluminum foil and administered in dated, sequentially numbered, nontransparent boxes not labeled with regard to content. Involved physicians and staff were unaware of the group assignment. Patients did not receive information regarding the chocolate and were instructed not to disclose their assigned group to investigators. To avoid changes in body weight during the intervention, participants were carefully instructed how to make proportional reductions in energy from their habitual diet to substitute for that supplied by chocolates. Dieticians and physicians provided the subjects with individual diet counseling throughout the study. During the study period, the diet was assessed by daily food diaries and by measurement of body weight (8).

Assessment of insulin sensitivity and β-cell function. An OGTT was performed in each subject after the first 7-d run-in phase and then after both intervention phases following a 10- to 14-h overnight fast and ≥12 h from the last chocolate ingestion (13). Plasma glucose and insulin were assessed at baseline and then 30, 60, 90, 120, and 180 min after the glucose load. OGTT results were utilized for homeostasis model assessment of insulin resistance (HOMA-IR) (22), quantitative insulin sensitivity check index (QUICKI) (22), and insulin sensitivity index (ISI) described by Matsuda and DeFronzo (23). The fasting ISI ($ISI_0 = 10^4/I_0 \cdot G_0$) (24) and the 2-h corrected insulin response [$CIR_{120} = I_{120}/G_{120}$ ($G_{120} - 70$ mg/dL)], an index of β-cell function well correlated with the hyperinsulinemic-euglycemic clamp method (24–26), were calculated from the fasting and 2-h plasma glucose (G_0, G_{120}) and serum insulin concentrations (I_0, I_{120}).

Hematochemical assessment. The hematochemical assessment, including serum electrolytes, total cholesterol, HDL cholesterol, and triglycerides, was conducted by enzymatic methods (Abbott Diagnostics). LDL cholesterol was calculated by the Friedewald formula (27). Plasma homocysteine and uric acid (Abbott Diagnostics) and fibrinogen (IL Test fibrinogen C, Instrumentation Laboratory) were determined at the same time points as measures for insulin sensitivity. Before and after each study phase, serum was collected for high sensitivity analysis of CRP by enzyme-linked immunonephelometry (CRPLX Roche Diagnostics).

Endothelial function. Endothelium-dependent flow-mediated dilation (FMD) of the brachial artery was assessed after a 15-min rest period as previously described (8). The same investigator, unaware of the treatment assignments, conducted all examinations. 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-GP system (ESAOTE Biomedica) (8). The transducer was held at the same point throughout the scan by a stereotactic

clamp. End-diastolic frames (ECG-triggered) were acquired every second with a commercial software program (MovieBox-Studio v.9, Pinnacle Systems). Arterial flow velocity was obtained by pulsed Doppler signals at 70° to the vessel with the range gate (1.5 mm) in the center of the artery. A cuff was placed around the forearm just below the elbow. After a 1-min acquisition to measure basal diameter, the cuff was inflated for 5 min at 250 mm Hg and then deflated to induce reactive hyperemia. FMD was defined as the maximal dilation of the brachial artery induced by increased flow (8). Endothelium-independent vasodilation was achieved with 25 μg sublingual glyceryl trinitrate, a dose previously tested to obtain a dilation similar to FMD (8).

BP monitoring. The 24-h ambulatory BP monitoring (ABPM) was recorded by a noninvasive oscillometric device (Medical 90207–30; Spacelabs) after OGTT and FMD evaluations. BP was automatically recorded for 24 h after 15-min intervals during daytime (0600–2200) and 20-min intervals during nighttime (2200–0600). The mean daytime, nighttime, and 24-h BP were considered for statistical evaluation. Clinical BP was also measured and recorded by a standard mercury sphygmomanometer and stethoscope as described above.

Statistical analysis. Data were analyzed using Proc Mixed Procedure with subject treated as a random factor and treatment, sequence, and baseline as fixed factors. Multiple comparisons were performed by Tukey's honestly significant difference test. Differences were considered significant when $P < 0.05$. Data are expressed as means ± SD. Statistical analyses and power calculation were performed with SAS (SAS Institute; v.9.1.3, 2004). Calculation of statistical power was based on results obtained in our previous studies in similar populations using a chocolate bar containing ~500 mg polyphenols. Mean ambulatory BP decreased 6.5 mm Hg and the SD was 5.76 mm Hg. Based on these data, a difference of 4 mm Hg could be detected with a total of 19 patients ($\alpha < 0.05$ and power = 0.81). Spearman nonparametric correlation was used to evaluate correlations between the changing variables.

Results

Baseline characteristics. Baseline characteristics and laboratory results of the IGT EH patients showed a marked degree of insulin resistance, with high HOMA-IR and low QUICKI and ISI values (15,18,19). Per study inclusion criteria, none of the patients had Type 2 diabetes (**Supplemental Table 2**).

Insulin resistance and β-cell function. Consuming FRDC for 15 d decreased HOMA-IR (**Fig. 1***A*) and increased QUICKI (Fig. 1*B*) compared with baseline and FFWC values. No change was observed in HOMA-IR (Fig. 1*A*) or QUICKI (Fig. 1*B*) after consumption of FFWC. ISI increased compared with baseline (2.03 ± 0.55) and FFWC (1.99 ± 0.50) after FRDC ingestion (3.34 ± 1.33, $P = 0.0024$ vs. baseline; $P = 0.049$ vs. FFWC) but did not change after FFWC. Compared with baseline and FFWC values, FRDC consumption increased ISI_0 (Fig. 1*C*) and β-cell function as noted by the CIR_{120} (Fig. 1*D*), whereas FFWC did not affect either parameter (Fig. 1*C* and *D*, respectively). In contrast to FFWC, the FRDC consumption affected glucose and insulin responses to the OGTT ($P < 0.0001$ for treatment for both, respectively). The decrease in insulin resistance was inversely correlated with improvement in endothelial function (**Supplemental Table 3**) and directly correlated with the decrease in clinical and 24-h-monitored SBP and DBP (**Supplemental Table 4**). Consistent with this, the improvements in insulin sensitivity and β-cell function were directly correlated with the increase in FMD (Supplemental Table 3) and inversely correlated with the decrease in clinical and 24-h-monitored SBP and DBP (Supplemental Table 4).

Clinical and 24-h ABMP. Compared with baseline, clinical SBP (**Fig. 2***A*) and DBP (Fig. 2*B*) decreased after 15 d of FRDC. In contrast, neither clinical SBP (Fig. 2*A*) nor DPB (Fig. 2*B*) changed after FFWC. Similarly, ABMP was reduced after FRDC, but no significant changes from baseline were noted following FFWC (**Table 1**). After FRDC, subjects had lower 24-h, daytime, and nighttime SBP and DBP, whereas these measures did not differ from baseline after 15 d of FFWC (Table 1). Furthermore, clinical and monitored BP were significantly lower after FRDC with respect to the FFWC intervention (Fig. 2*A*,*B*; Table 1). Interestingly, the decrement in BP values was inversely correlated with the increase in FMD (**Supplemental Table 5**).

Endothelial function. FRDC ingestion significantly increased FMD (**Fig. 3***A*), whereas FFWC did not affect vascular reactivity (Fig. 3*B*). FMD values were also significantly higher after FRDC with respect to FFWC intervention phase (Fig. 3*A*,*B*). Baseline glyceryl trinitrate-induced vasodilation remained unchanged after both FRDC (from 8.40 ± 0.95 to 8.20 ± 1.34%) and FFWC (from 8.51 ± 0.80 to 8.31 ± 1.27%) intake.

Lipid profile. Compared with baseline, FRDC consumption decreased serum total cholesterol (−6.5%; $P < 0.0001$) and LDL cholesterol (−7.5%; $P < 0.0001$) but did not affect HDL cholesterol or triglycerides. The FFWC intervention did not affect the lipid profile. Compared with FFWC, FRDC intake decreased serum total cholesterol ($P = 0.007$ for treatment) and LDL cholesterol ($P = 0.041$ for treatment) levels.

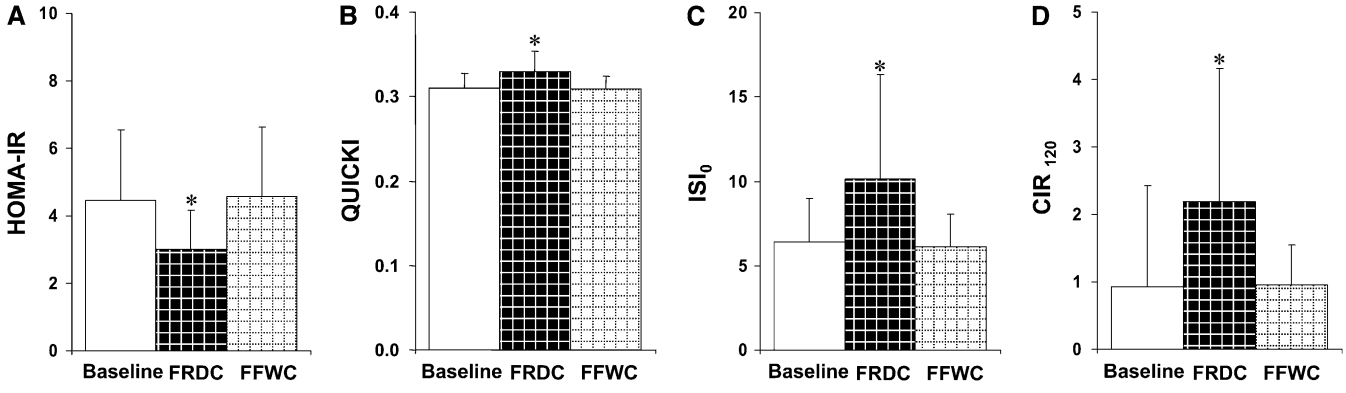

FIGURE 1 Effect of consuming FRDC and FFWC for 15 d on HOMA-IR (*A*), QUICKI (*B*), ISI_0 (*C*), and CIR_{120} (*D*) in IGT EH patients. Data are means ± SD, $n = 19$. *Different from baseline and FFWC, $P < 0.05$. Baseline data are pooled (mean) from 2 separate baselines.

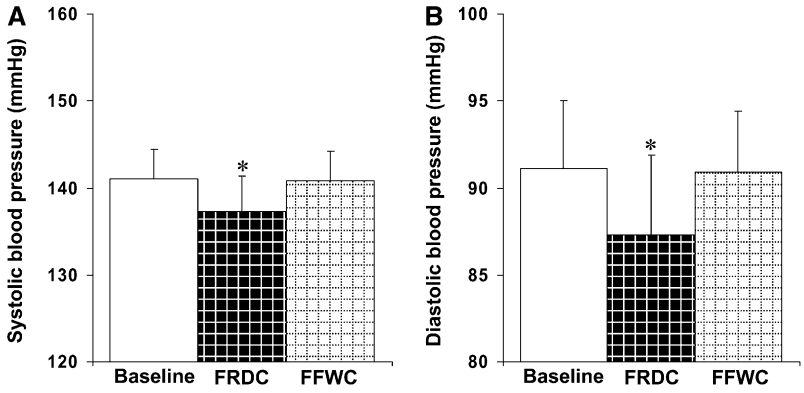

FIGURE 2 Effect of consuming FRDC and FFWC on clinical SBP (*A*) and DBP (*B*) in IGT EH patients. Data are means ± SD, $n = 19$. *Different from baseline and FFWC, $P < 0.05$. Baseline data are pooled (mean) from 2 separate baselines.

Other variables. Neither FRDC (from 3.8 ± 2.6 to 3.4 ± 3.3 mg/L) nor FFWC (from 3.7 ± 2.3 to 3.6 ± 1.4 mg/L) treatment affected serum CRP relative to baseline values. Further, no significant variation was observed in serum electrolytes, fibrinogen, homocysteine, or uric acid after FRDC or FFWC intake (data not shown). No variables influenced the effect of chocolate on HOMA-IR, QUICKI, ISI, ISI_0, CIR_{120}, ABPM, clinical BP, and FMD. The order of treatment did not affect any of the variables considered.

Discussion

Insulin resistance and IGT are associated with increased risk of type 2 diabetes and CVD. Hypertension, a well-established risk factor for CVD, has also been associated with IGT (14–16). This randomized, cross-over trial shows for the first time, to our knowledge, that FRDC is able to enhance insulin sensitivity and β-cell function, decrease BP, and increase FMD in EH patients with IGT but absent other risk factors for CVD and type 2 diabetes such as dyslipidemia, obesity, and smoking. These data also reveal that the changes in insulin sensitivity, β-cell function, and BP following FRDC are directly correlated with the amelioration of endothelial dysfunction.

Impairment of insulin sensitivity and vascular reactivity appear linked to CVD risk via a mutual dependence on the endothelial bioavailability of NO (28). For example, reactive oxygen species inactivate endothelium-derived NO in diabetic animals and humans and reduce vasodilatory responses (28). Insulin infusion under euglycemic glucose-clamp increases NO-dependent skeletal muscle blood flow and stimulates peripheral glucose transport, uptake, and disposal (29). Normal glucose tolerance is modulated via a balance between insulin secretion and insulin action maintained over a wide range of insulin sensitivity, with β-cells readily compensating for tissue insensitivity to the hormone (30). Nonetheless, before the presentation of IGT, declines in β-cell function may occur due to sustained hyperglycemia (30). In addition, hyperglycemia can increase oxidative stress status (17,28,29) and further promote insulin resistance and impair endothelium-dependent vasodilation (28,29).

Flavanols and related polyphenolic antioxidants may improve the insulin resistance by increasing the endothelial bioavailability of NO and decreasing the formation of reactive oxygen and nitrogen species. In vitro, polyphenols like resveratrol and silibinin inhibit IκB kinase and downregulate nuclear factor-κB (31), a redox-sensitive signal transduction pathway involved in the cascade of endothelial injury and in fat-induced insulin resistance (28,31). In vivo, silymarin, a flavonoid complex from milk thistle, improves glycemic control with a reduction in both fasting insulin and exogenous insulin requirements in type 2 diabetic patients with hepatic cirrhosis receiving insulin therapy (32). Observational studies suggest that generous intakes of apples and tea, foods rich in flavonoids, are associated with a reduced risk for type 2 diabetes (33). In a clinical trial of postmenopausal women, the isoflavone genistein was found to decrease fasting glucose and insulin and HOMA-IR (34). An antioxidant action of flavonoids on insulin resistance and vascular reactivity is suggested by studies with vitamin C (20,35). For example, Hirai et al. (20) found ascorbic acid infusion reversed blunted steady-state plasma glucose and FMD in nonsmokers with IGT as well as in glucose normotolerant smokers.

The effect of FRDC flavanols we observed is consistent with results from Schroeter et al. (4), who reported flavanol-rich cocoa improved FMD in conduit arteries and in microcirculation strongly correlated with the kinetics of increased NO species in plasma. These effects were also closely associated with circulating (−)-epicatechin and its metabolites, particularly epicatechin-7-*O*-glucuronide. The effects of cocoa flavanols were closely mimicked by pure (−)-epicatechin and abolished by the NO synthase inhibitor, L-N^G-mono-methyl-arginine. Flavanol-rich chocolate has also been found to increase FMD in reports by Fisher et al. (5,12) in healthy young and older subjects. Further, Heiss et al. (9,36) found drinking flavanol-rich chocolate increased FMD in patients with at least 1 CVD risk factor and reversed endothelial dysfunction in smokers in association with increased circulating pool of NO species (nitrite and nitrate).

Although in contrast to a few studies (5,7), our results of a beneficial effect of FRDC on BP in IGT EH patients are consistent with chocolate interventions in healthy subjects (10,37), EH patients (8), and elderly patients with isolated systolic hypertension (11). Our findings are also in agreement with

TABLE 1 24-h ABPM before and after 15-d treatment with FRDC or FFWC in IGT EH patients[1]

BP, *mm Hg*	FRDC		FFWC	
	Baseline	After	Baseline	After
24-h DBP ABPM	86.8 ± 3.7	82.6 ± 5.4*†	87.0 ± 3.5	87.0 ± 3.1
24-h SBP ABPM	134.6 ± 4.4	130.1 ± 5.0*†	133.8 ± 3.9	133.9 ± 3.6
DBP daytime ABPM	90.9 ± 4.0	86.5 ± 5.7*†	91 ± 3.5	90.8 ± 3.0
DBP nighttime ABPM	78.7 ± 4.4	74.5 ± 6.0*†	78.9 ± 4	79.2 ± 3.3
SBP daytime ABPM	139.5 ± 4.4	134.9 ± 5.3*†	138.5 ± 4.0	138.8 ± 4.1
SBP nighttime ABPM	124.9 ± 5.5	120.1 ± 5.4*†	124.2 ± 5.0	124.0 ± 3.3

[1] Data are means ± SD, $n = 19$. *Different from respective baseline, $P < 0.05$; †Different from FFWC at that time, $P < 0.05$.

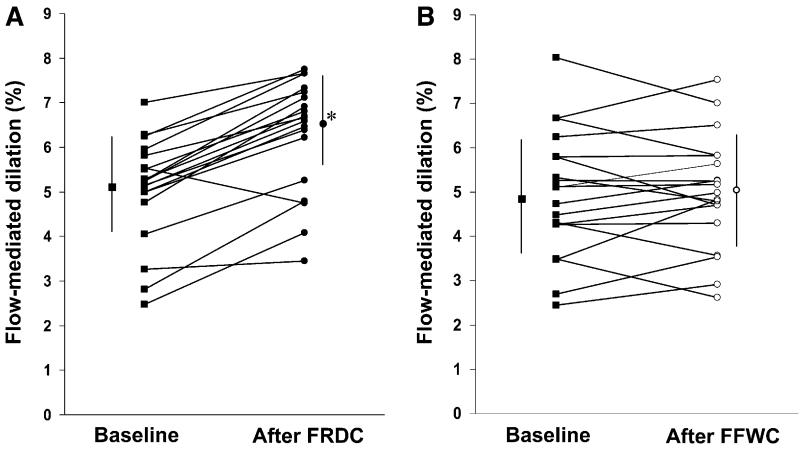

FIGURE 3 Effect of FRDC (*A*) and FFWC (*B*) on FMD in IGT EH patients. Data are means ± SD, $n = 19$. *Different from baseline and FFWC, $P < 0.05$.

observational data from the Zutphen Elderly Study (38), where intake of cocoa products was inversely associated with BP and 15-y CVD and all-cause mortality in elderly men. Further, although using a much lower dose, larger sample size, and longer duration, a recent report by Taubert et al. (39) was entirely consistent with our data. Taubert et al. (39) observed that only 6 g/d FRDC but not FFWC significantly reduced mean SBP (−2.9 mm Hg) and DBP (−1.9 mm Hg) and increased NO after 12–18 wk treatment in prehypertensive and grade 1 hypertensive patients.

The effect of cocoa on lipid profiles is equivocal (1), although our finding here of FRDC-induced reduction in serum total and LDL cholesterol is similar to our previous results (8) in EH patients and those of Fraga et al. (37) in healthy young people. Similarly, cocoa extracts have been found to have dose-dependent hypoglycemic and hypocholesterolemic actions in both normal and diabetic rats (40).

Study limitations. An inherent limitation of our study design was the inability to keep the participants unaware of the control and test items, a common problem with most whole food interventions. Further, we did not determine and correlate directly cocoa polyphenols and their metabolites in plasma or urine with NO and its actions, although this relationship has been previously characterized by Schroeter et al. (4). It is worth noting that additional mechanisms of action of cocoa flavanols have been suggested, e.g. via modulation of the renin-angiotensin system (41), and that other cocoa constituents like theobromine and caffeine (42) may also contribute partly to the observed changes in BP, glucose metabolism, and β-cell function. However, Baron et al. (43) found theobromine from dark chocolate had no hemodynamic or electrophysiologic effects in young adults (43). Fisher et al. (5) found the caffeine and theobromine content of flavanol-poor chocolate were almost identical to that of flavanol-rich chocolate, but only the FRDC affected vascular function and NO bioavailability in endothelial cells; so, methylxanthines appear unlikely to play a substantial role in the vascular responses to flavanol-rich cocoa (5). Thus, considering the robust nature of our randomized, single-blind, cross-over design together with independent evidence that epicatechin recapitulates the vascular effects of dark chocolate (4), it is plausible our study outcomes resulted from the increased intake of cocoa flavanols. Nonetheless, because our study was short term, had a small number of subjects, and employed an isocaloric protocol, larger and longer-term trials are still required to confirm and expand upon the potential role of flavonoid-rich chocolate in reducing CVD risk.

In conclusion, we observed a beneficial effect of short-term consumption of 100 g (2347 kJ) FRDC on vascular function, insulin sensitivity, and BP in a population of IGT EH patients. Importantly, the addition of any energy-dense food, like FRDC, to the diet always warrants caution because of its potential untoward influence on body weight. However, the report by Taubert et al. (39) that longer-term, daily intakes of only 6.3 g (126 kJ) FRDC can reduce BP and increase vasodilative NO suggests cocoa products can be reasonably incorporated into a dietary approach to lower CVD risk. These data also suggest a potential value to developing flavanol-rich, low-energy cocoa foods, beverages, and supplements.

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