Flavonoid-Rich Cocoa Consumption Affects Multiple Cardiovascular Risk Factors in a Meta-Analysis of Short-Term Studies[1]

Mark G. Shrime,[2,3,9*] Scott R. Bauer,[2,8,9] Anna C. McDonald,[2,4] Nubaha H. Chowdhury,[2,5] Cordelia E. M. Coltart,[2,6] and Eric L. Ding[7,8*]

[2]Harvard School of Public Health, Boston, MA; [3]Harvard Interfaculty Initiative in Health Policy, Boston, MA; [4]University of North Carolina School of Medicine, Chapel Hill, NC; [5]Stony Brook University School of Medicine, New York, NY; [6]WHO, Geneva, Switzerland; [7]Harvard School of Public Health, Department of Nutrition, Boston, MA; and [8]Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA

Abstract

A growing body of evidence suggests that the consumption of foods rich in polyphenolic compounds, particularly cocoa, may have cardioprotective effects. No review, however, has yet examined the effect of flavonoid-rich cocoa (FRC) on all major cardiovascular risk factors or has examined potential dose-response relationships for these effects. A systematic review and meta-analysis of randomized, controlled trials was performed to evaluate the effect of FRC on cardiovascular risk factors and to assess a dose-response relationship. Inclusion and exclusion criteria as well as dependent and independent variables were determined a priori. Data were collected for: blood pressure, pulse, total cholesterol, HDL cholesterol, LDL cholesterol, TG, BMI, C-reactive protein, flow-mediated vascular dilation (FMD), fasting glucose, fasting insulin, serum isoprostane, and insulin sensitivity/resistance indices. Twenty-four papers, with 1106 participants, met the criteria for final analysis. In response to FRC consumption, systolic blood pressure decreased by 1.63 mm Hg ($P = 0.033$), LDL cholesterol decreased by 0.077 mmol/L ($P = 0.038$), and HDL cholesterol increased by 0.046 mmol/L ($P = 0.037$), whereas total cholesterol, TG, and C-reactive protein remained the same. Moreover, insulin resistance decreased (HOMA-IR: –0.94 points; $P < 0.001$), whereas FMD increased (1.53%; $P < 0.001$). A nonlinear dose-response relationship was found between FRC and FMD ($P = 0.004$), with maximum effect observed at a flavonoid dose of 500 mg/d; a similar relationship may exist with HDL cholesterol levels ($P = 0.06$). FRC consumption significantly improves blood pressure, insulin resistance, lipid profiles, and FMD. These short-term benefits warrant larger long-term investigations into the cardioprotective role of FRC.   J. Nutr. 141: 1982–1988, 2011.

Introduction

The Kuna, an indigenous group of approximately 50,000 people who live predominantly on small islands off the coast of Panama, are virtually free of hypertension and cardiovascular disease. Kuna who migrate to nearby Panama City, however, lose this advantage, a loss that cannot be attributed to changes in salt intake (1) or stress (2). The Kuna who live on the Caribbean archipelago, however, consume a striking amount of natural cocoa drinks, whereas those who migrate to the mainland do not (1).

The case of the Kuna is but one piece in a growing body of evidence that foods rich in plant-derived polyphenolic compounds may have cardioprotective effects. Three recent meta-analyses have examined these hypotheses: Taubert et al. (3) examined blood pressure in response to cocoa and tea con-

sumption. Hooper et al. (4) looked at dietary flavonoids in general (not specifically cocoa) and their effects on cardiovascular risk. And, most recently, Desch et al. (5) studied the effects of cocoa products, specifically on blood pressure. Although all found improvement in their examined measures, they all faced major limitations. The former two did not separate cocoa from other dietary flavonoids, and only Hooper et al. (4) looked at cardiovascular outcomes other than SBP[10]. Until ours, no review has, to our knowledge, examined the role of FRC specifically on all major cardiovascular risk factors, and no study has yet examined whether a dose-response curve exists for FRC and cardiovascular risk factors.

In its natural state, cocoa is high in flavonoids, exhibiting higher antioxidant capacity than almost any other known food

[10] Abbreviations used: CRP, C-reactive protein; DBP, diastolic blood pressure; FMD, flow-mediated vascular dilation; FRC, flavonoid-rich cocoa; ISI, insulin-sensitivity index; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure.

(6). However, processing alkalinizes the cocoa and strips it of much of this salutary effect (7). Because certain cocoa formulations avoid this and are rich in flavonoids, and because flavonoids may exert a cardioprotective effect that extends beyond their antihypertensive properties, we undertook a systematic review and meta-analysis of randomized, controlled trials performed in adults to examine the effects of FRC on multiple markers of cardiovascular health and to determine whether a dose-response curve exists between FRC intake and these outcomes.

Materials and Methods

Search strategy, study selection, and quality assessment. MEDLINE, EMBASE, The Cochrane Controlled Clinical Trials Register, and the clinicaltrials.gov Web site were searched to January 2011 for randomized, controlled trials examining the effect of FRC on markers of cardiovascular risk and outcomes. The search strategies utilized both indexing [e.g., MeSH (medical subject headings) or EmTree] terms and free text to search for synonyms of cocoa, chocolate, and randomized trial within the databases. Articles were not automatically excluded based on language or date of publication. For MEDLINE, the following search strategy was used: (cacao[MESH] OR cacao[tiab] OR cacaos [tiab] OR cocoa[tiab] OR cocoas[tiab] OR cocao[tiab] OR chocolate [tiab] OR chocolates[tiab]) AND (randomized controlled trial[publication type] OR (randomized[tiab] AND controlled[tiab] AND trial[tiab]) OR clinical trial*[tiab]). Similar search strategies were used for the other databases. Initially, all randomized controlled trials using cocoa and indexed within the above databases were collected regardless of outcome. Additional studies were identified from the reference lists of retrieved articles. Titles and abstracts were reviewed by two independent reviewers (S.B., A.M.) to assess for inclusion. When assessment by title and abstract was insufficient, the full text of the article was obtained. Full texts articles were reviewed for inclusion by at least two authors (M.S., N.C., C.C., S.B) independently. Disagreements were resolved by group discussion.

Articles were excluded if they: presented no evidence of randomization; did not use cocoa as an intervention; were nonhuman studies; randomized to multifactorial interventions from which the cocoa intervention could not be extracted; followed patients for fewer than 2 wk; did not measure flavonoid levels, report relevant outcomes, or include a control arm; or were review articles. Assessment of quality included allocation concealment (adequate, inadequate, or unclear), participant masking, researcher masking, outcome assessor masking, and reported industry funding (4).

Data extraction. A data extraction form was designed a priori. Two authors (S.B., M.S.) reviewed the included papers to determine which variables to extract, after which the form was designed by one of the authors (N.C.) and piloted. Extraction was performed by all authors independently. Extraction data were then reviewed by one author (M.S.) to ensure uniformity. When data were not available, one author (M.S.) attempted to contact the study authors. Two attempts were made for each study that required contact.

In addition to the study-level quality measures mentioned above, the following data points were extracted from each study: author name; publication year; study design; population characteristics; type of control; sample size; flavonoid dose in intervention and control arms; mean age of patients in each arm; total flavonoid, flavonol, and flavan-3-ol doses; total catechin/epicatechin dose; energy load of each arm; fat and sugar content of each arm; presence of milk; route of administration; and study duration.

When available, the following outcome variables were extracted: SBP, DBP, pulse, total cholesterol, HDL cholesterol, LDL cholesterol, TG, CRP, BMI, FMD, fasting glucose and insulin levels, serum isoprostane level, QUICKI (an index of insulin sensitivity in diabetic, obese, and hypertensive patients) (8), ISI, and HOMA-IR, the latter two of which are also useful in the detection of metabolic syndrome (9).

Data synthesis. For each continuous outcome variable, baseline and final mean values with SD, for both intervention and control groups, as well as the mean within-arm change and its SD were extracted or calculated. For parallel-group trials, the treatment effect was defined as the mean difference of final mean values between groups, assuming similar baseline levels. If the difference between the mean values of the intervention and control arms at baseline was statistically significant, these data were evaluated using the mean difference within study arms. When not reported, SD for mean change was either calculated from CI or P values or estimated based on the SD of the outcome variable at the end of treatment.

For crossover trials, the treatment effect was defined as the mean within-subject difference of the treatments, assuming no carry-over or period effect. The standard error, when not explicitly reported, was calculated from the SD or CI. When necessary, the separate means and SD of the intervention and control arms were extracted, providing a conservative estimate of the variance (10). As with parallel study designs, when SD were not reported, they were calculated from CI or P values or conservatively estimated based on the SD of the outcome variable at the end of treatment.

Meta-analysis was performed in STATA v.11 (StataCorp), using the Dersimonian and Laird random-effects model (11). Results were considered statistically significant when $P \leq 0.05$. Outcome variables with data from fewer than three studies were analyzed but were not considered for interpretation, and subgroup analysis was performed only when data from at least five studies were available. I^2 was used to assess the magnitude of heterogeneity. I^2 was considered potentially significant at a threshold of $P \leq 0.1$. Meta-regression, as a random-effects, weighted, linear regression of dose difference compared with mean outcome difference in the studies, was performed to determine the existence of a dose-response curve and for subgroup analyses to assess potential effect modifications. Subgroup analyses were performed to assess effect modification by age, milk background, fat background, population characteristics, study quality, and study duration. Sensitivity analyses, determined a priori, were also performed to test the robustness of results. Funnel plots, tested using the methods of Egger et al. (12) and Begg (13), were constructed to assess for publication bias and trim-and-fill analysis was performed to determine the estimated effect of unpublished studies. This study was performed in compliance with the 2009 PRISMA guidelines (14).

RESULTS

Our initial literature search returned 346 papers. Based on title or abstract, 279 were excluded (69 review articles and 210 for meeting at least one other exclusion criterion). The full text of the remaining 67 was reviewed. Forty-seven of these met at least one exclusion criterion and were therefore not included in the final analysis. At the conclusion of the study, a follow-up literature search was undertaken through January 2011, which returned an additional 53 studies. The full text of 6 of these was pulled for review; 4 more (15–18) were included in the final analysis (**Supplemental Fig. 1**). Twenty-four studies (15–38) were included in the final analysis, covering 1106 participants (**Table 1**).

Twenty-two studies (15–18,20–25,27–38) measured the impact of FRC on blood pressure or pulse and 20 (15,17,19,22–38) on lipid profiles or BMI. Ten studies (17,24–26,30,31,33–36) examined outcomes related to insulin resistance or metabolic syndrome, 8 studies (20,22,30–32,34,36,37) measured levels of circulating inflammatory mediators, and 8 (16,22,25,26,28,33,34,37) reported FMD.

Eleven studies (20,22,26–33,38) were randomized, placebo-controlled trials; 12 (15–17,19,21,23–25,34–37) used a crossover design. One study (18) used a placebo-controlled crossover design. To maintain consistency, this study was treated as a crossover study between placebo and non-theobromine–

TABLE 1 Characteristics of included trials[1]

Study name	Design	Population	Age, y (int/cont)	Control type	Flavonoid type	Route of administration	Control dose, mg/d	Total flavonoid dose, mg/d	Study size, n	Duration, d	Outcomes
Allen (15)	Crossover	HL	45.9/45.9	LFC	Polyphenols	Bar	0	360	88	28	SBP, DBP, TC, HDL, LDL, TG
Balzer (31)	Placebo-controlled	Diabetic	63.1/64.4	LFC	E	Drink	75	963	41	30	P, TC, HDL, LDL, TG, BMI, FMD, CRP, Gluc
Crews (32)	Placebo-controlled	Healthy volunteers	68.8/68.7	LFC	Pro	Bar + drink	7.4	377.35	90	42	SBP, DBP, P, TC, HDL, LDL, TG, CRP, BMI
Davison (33)	Placebo-controlled	Overweight volunteers	45.3/44.4	LFC	Flavan-3-ols	Drink	18	451	23	84	SBP, DBP, P, TC, HDL, LDL, TG, BMI, FMD, HOMA-IR, Gluc, Ins
Engler (22)	Placebo-controlled	Healthy volunteers	31.8/32.5	LFC	Pro	Bar	0	259	21	14	SBP, DBP, TC, HDL, LDL, TG, BMI, FMD, IP
Farouque (26)	Placebo-controlled	CAD	61/61	LFC	E	Bar + drink	19.6	444	38	42	TC, HDL, LDL, TG, Gluc, FMD
Fraga (23)	Crossover	Healthy males	18/18	White chocolate	Pro, E, C	Bar	5	168	56	14	SBP, DBP, P, TC, HDL, LDL, TG
Grassi (24)	Crossover	Healthy volunteers	33.9/33.9	White chocolate	Phen	Bar	0	500	30	15	SBP, DBP, P, TC, HDL, LDL, TG, BMI, HOMA-IR, QUICKI, ISI
Grassi (25)	Crossover	HTN	43.7/43.7	White chocolate	C, E, Q, K, I	Bar	0	87.8	40	15	SBP, DBP, TC, HDL, LDL, TG, BMI, FMD, HOMA-IR, QUICKI, ISI, Gluc, Ins
Grassi (34)	Crossover	Glucose intolerant, HTN	44.8/44.8	White chocolate	E, C, Q, K, I, Phen	Bar	0	1080	38	15	SBP, DBP, TC, HDL, LDL, TG, CRP, FMD, HOMA-IR, QUICKI, ISI
Heiss (16)	Crossover	CAD	64/64	LFC	Pro, E, C	Drink	18	750	32	28	SBP, FMD
Mellor (17)	Crossover	Diabetic	68/68	LFC	E	Bar	2	16.6	24	56	SBP, DBP, TC, HDL, LDL, TG, HOMA-IR, Gluc, Ins
Monagas (36)	Crossover	High-risk volunteers	69.7/69.7	Skim milk	Pro, E, C	Drink	0	93.02	84	28	SBP, DBP, P, TC, HDL, LDL, TG, BMI, CRP, Gluc
Muniyappa (35)	Crossover	HTN	51/51	LFC	Pro, E, C, Phen	Drink	14	781	40	14	SBP, DBP, TC, HDL, LDL, TG, BMI, QUICKI, Gluc, Ins
Murphy (20)	Placebo-controlled	Healthy volunteers	40/47	Placebo	Flavan-3-ols	Tablet	6	234	28	28	SBP, DBP, P, BMI, IP
Njike (37)	Crossover	Overweight volunteers	52.5/51.9	LFC	Flavan-3-ols	Drink	9	805	74	42	SBP, DBP, TC, HDL, LDL, TG, CRP, BMI, FMD
Polagruto (27)	Placebo-controlled	Healthy volunteers	49/56	LFC	Sterol esters	Bar	10.4	127.9	67	42	SBP, DBP, P, TC, HDL, LDL, TG, BMI
Ried (38)	Placebo-controlled	Healthy volunteers	48.8/57.9	Placebo capsule	Polyphenols	Bar	0	750	21	56	SBP, DBP, BMI
Shiina (29)	Placebo-controlled	Healthy men	29.3/30.3	White chocolate	N/A	Bar	0	550	39	14	SBP, DBP, P, TC, HDL, LDL, TG
Taubert (21)	Crossover	HTN	60/60	White chocolate	Phen	Bar	0	500	26	14	SBP, DBP, P
Taubert (30)	Placebo-controlled	HTN	63.4/63.7	White chocolate	Pro, E, C, flavonols	Bar	0	26.7	44	126	SBP, DBP, P, TC, HDL, LDL, TG, BMI, IP, Gluc
van den Bogaard (18)	Placebo-controlled crossover	HTN	62/62	Placebo drink	Pro, E, C, flavonols	Drink	0	500	84	31	SBP, DBP,P
Wan (19)	Crossover	Healthy volunteers	36/36	Average American diet	Pro	Bar + drink	0	466	46	28	TC, HDL, LDL, TG
Wang-Polagruto (28)	Placebo-controlled	HL, post-menopause	57.7/55.4	LFC	Flava-3-ols	Drink	43	446	32	42	SBP, DBP, P, TC, HDL, LDL, TG, BMI, FMD

[1] C, catechin; cont, controls; CRP, C-reactive protein; DBP, diastolic blood pressure; E, epicatechin; FMD, flow-mediated vascular dilation; Gluc, fasting glucose; HL, hyperlipidemia; HOMA-IR, homeostatic model assessment of insulin resistance; HTN, hypertension; I, isorhamnetin; Ins, fasting insulin; int, intervention; IP, isoprostane; ISI, insulin-sensitivity index; K, kaempferol; LFC, low-flavonoid cocoa; P, pulse; Phen, phenols; Poly, polyphenols; Pro, proanthrocyanidin; Q, quercitin; QUICKI, quantitative insulin-sensitivity check index; SBP, systolic blood pressure; TC, total cholesterol.

enhanced chocolate consumption. The control group was given white chocolate in 7 studies (21,23–25,29,30,34) and low-flavonoid–containing chocolate in 12 studies (15–17,22,26–28,31–33,35,37). The remaining 5 studies (18–20,36,38) used various controls. In 12 studies (15,17,21–25,27,29,30,34,38), the intervention and control were administered as a bar, in 8 (16,18,28,31,33,35–37) as a drink, in 3 (19,26,32) as a bar and a drink, and in one (20) as a tablet.

Nine studies (19,20,22–24,27,29,32,36,38) used normal, healthy patients. Eight studies (16,18,21,25,26,30,34,35) examined patients with hypertension and/or coronary artery disease, 4 (17,31,34,36) examined diabetic patients, and 4 (15,28,33,37) patients who were either overweight or hyperlipidemic.

Study quality varied. Of the studies with adequate reporting, allocation concealment occurred adequately in 8 studies (17,18,22,30–32,35,38) and inadequately in 2 (27,28). Fifteen studies blinded patients (15–18,20,22,26–28,31–33,35–37), 18 blinded researchers (15–18,20,22,26,27,29,30–38), and 18 blinded outcome assessors (15–18,20–22,24,27,30–38). Thirteen (15–18,20,22,27,31–33,35–37) blinded all 3. In 9 studies (15,16,18,19,23,27,28,31,37), at least one author was employed by a chocolate company. In one additional study (35), the cocoa industry was involved in some of the data analysis. Two studies (21,25) did not report any information with respect to financial conflicts of interest (**Supplemental Table 1**).

Intention-to-treat analysis

Blood pressure and pulse. Twenty studies (15–18,20–25,28–30,32–38) had information on SBP (**Fig. 1**). There was significant heterogeneity among these studies ($I^2 = 82.7$; $P < 0.001$). Consumption of FRC for at least 2 wk decreased SBP by 1.63 mm Hg (95% CI = 0.13, 3.12; $P = 0.033$). DBP and pulse were not affected (all nonsignificant results can be found in **Supplemental Figs. 2–9**).

Insulin resistance and metabolic syndrome. Five studies (17,24,25,33,34) examined HOMA-IR (**Supplemental Fig. 10**) without significant heterogeneity ($I^2 = 33.6$; $P = 0.197$). HOMA-IR decreased by 0.94 points (95% CI = 0.59, 1.29; $P < 0.001$) with the consumption of FRC. ISI was examined by 3 studies (24,25,34) without significant heterogeneity ($I^2 = 48.9$; $P = 0.14$). FRC consumption significantly increased ISI by 4.95 points (95% CI = 2.80, 7.10; $P < 0.001$) (**Supplemental Fig. 11**). QUICKI and fasting glucose levels did not change significantly with the consumption of FRC. There were not enough data available to analyze fasting insulin levels.

FMD and inflammation. Nine studies (16,22,25,26,28,31, 33,34,37) examined the impact of FRC intake on FMD. Heterogeneity was significant among these studies ($I^2 = 76.5$; $P < 0.001$); FRC increased FMD by 1.53% (95% CI = 0.67, 2.40; $P < 0.001$) (**Supplemental Fig. 12**). Circulating CRP levels did not change and insufficient data were available for the evaluation of isoprostane levels.

Lipid profiles and BMI. Nineteen studies (15,17,19,22–37) examined the impact of FRC on lipid profiles. Heterogeneity was significant with all outcomes ($P < 0.001$ for each). Consumption of FRC increased HDL cholesterol by 0.0463 mmol/L (95% CI = 0.0028, 0.089; $P = 0.037$) (**Fig. 2**) and decreased LDL cholesterol by 0.077 mmol/L (95% CI = 0.0044, 0.149; $P = 0.038$) (**Supplemental Fig. 13**), whereas total cholesterol and TG levels were unchanged. Similarly, no significant difference was seen in BMI with FRC intake in the
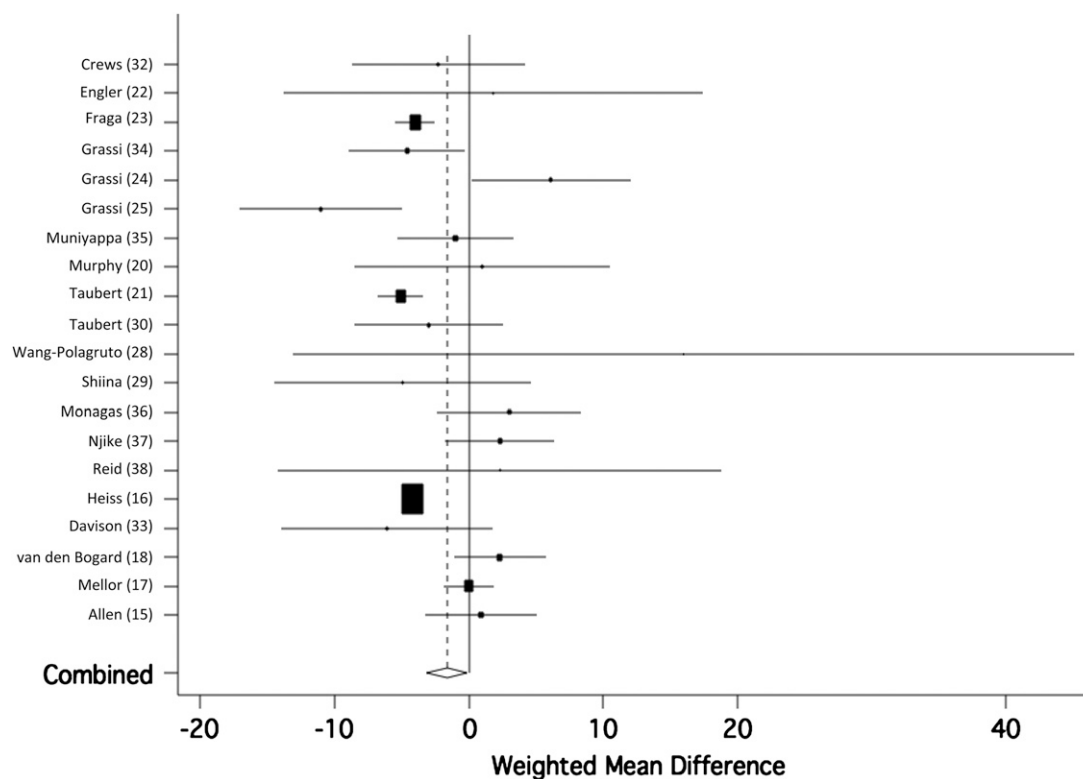

FIGURE 1 Forest plot for the effect of consumption of FRC on SBP. Weighted mean differences are reported. The weight of each study is represented by the size of the box; variance is represented by the length of the horizontal line. FRC, flavonoid-rich cocoa; SBP, systolic blood pressure.

11 studies that evaluated this outcome (20,22,24,25,30–33,35–37).

Dose-response curve. We performed a meta-regression to determine whether a dose-response relationship existed between FRC consumption and any outcome measure. Enough data were available to perform this regression for SBP, DBP, pulse, total cholesterol, HDL cholesterol, LDL cholesterol, TG, FMD, fasting glucose, and BMI.

Linear regression showed no statistically significant association between the total polyphenol dose and any outcome. However, a quadratic relationship was seen between total polyphenol dose and FMD, with FMD increasing as the dose approached 500 mg and decreasing at higher doses (*P*-nonlinearity = 0.004). A similar, almost significant, pattern was seen with HDL cholesterol (*P* = 0.06). Fat did not modify the dose response seen with FMD, but information on fat content was limited. Meta-regression examining the relationship between the combined dose of epicatechin, catechin, and procyanidins and outcomes of interest found no significant relationship.

Subgroup and sensitivity analysis

Subgroup analysis was performed by population characteristics (healthy vs. not healthy), study duration, study type, mean age of participants, fat content (for lipid outcomes), and milk consumption.

The impact of FRC consumption on both total cholesterol and LDL cholesterol was significantly modified by the participant age (*P* for effect modification = 0.026 and 0.002, respectively): patients older than 50 y did not gain the same benefit as younger patients. Similarly, study duration modified effects; decreases in LDL cholesterol (*P* = 0.015) and total cholesterol (*P* = 0.024) were seen in studies <4 wk in duration

and increases in HDL cholesterol (*P* = 0.04) in studies of longer duration.

Lipid levels were also sensitive to fat consumption: LDL cholesterol and total cholesterol decreased in studies with at least 6 g of fat in the intervention (*P* = 0.001 and 0.006, respectively), whereas HDL cholesterol levels only increased in studies with lower fat content (*P* = 0.016). TG levels were not modified by fat consumption and no significant association existed between fat content and study quality or participant age. In the presence of milk, the beneficial impact of cocoa on FMD was attenuated (*P* = 0.042). There was no significant difference between energy consumption in the control and intervention arms in studies that provided this information.

Finally, we stratified by study characteristics. No effect modification was seen by baseline participant health, publication date, or industry involvement on any outcome.

Publication bias

We utilized funnel plots, combined with Egger (12) and Begg (13) statistical analyses of these plots, to assess for publication bias. There was evidence of statistically significant publication bias for only SBP (*P* < 0.001) (**Supplemental Fig. 14**). In addition, trim-and-fill analysis was performed for all outcomes. Linear extrapolation of missing studies changed our outcomes for SBP, DBP, HDL cholesterol, TG, and FMD. In most cases, this analysis strengthened the effect of high-flavonoid cocoa consumption; in the case of HDL cholesterol, statistical significance was lost with the addition of these extrapolated studies.

DISCUSSION

In a meta-analysis of 24 randomized, controlled studies, with a total of 1106 participants, FRC had multiple salutary effects on
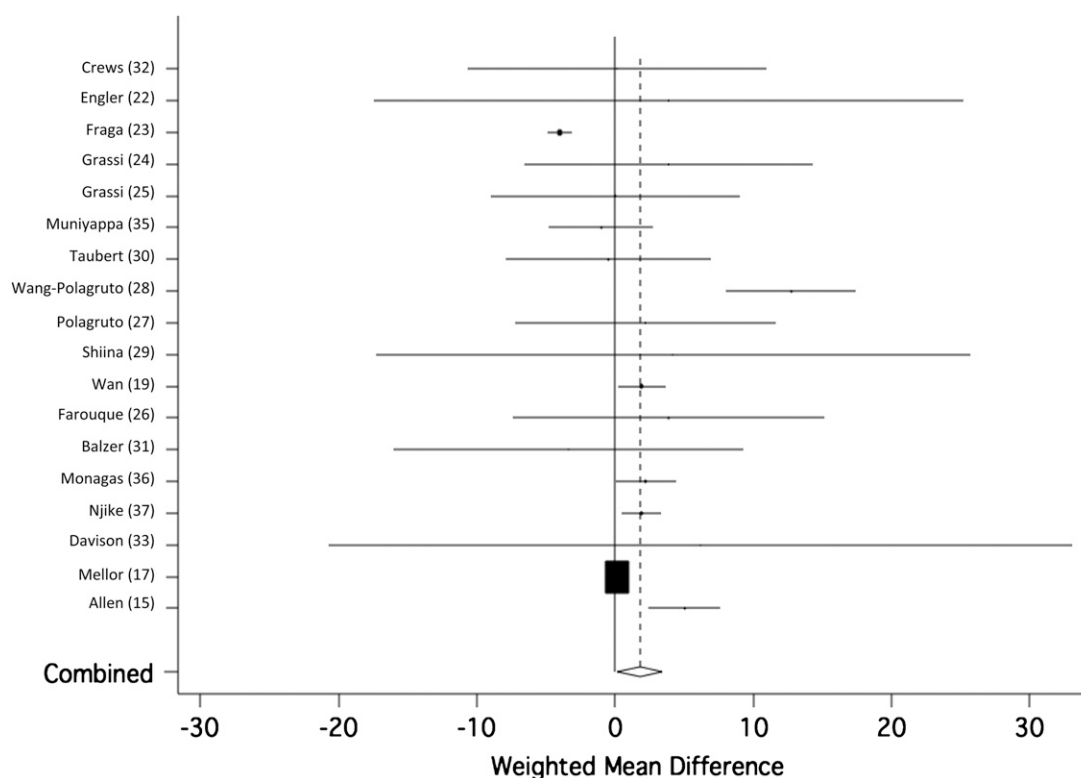

FIGURE 2 Forest plot for the effect of consumption of FRC on HDL cholesterol levels. Weighted mean differences are reported. The weight of each study is represented by the size of the box; variance is represented by the length of the horizontal line. FRC, flavonoid-rich cocoa.

cardiovascular health. SBP, LDL cholesterol levels, and insulin resistance all decreased in response to short-term consumption of FRC. HDL cholesterol levels and FMD concomitantly increased. BMI and total cholesterol, TG, fasting glucose, and CRP levels all remained constant, which is consistent with other studies on the role of cocoa in inflammation (39).

These findings suggest a beneficial role for cocoa-flavonoids in cardiovascular and metabolic health. For example, FMD, an indicator of endothelium-driven vasodilation, decreases in patients with atherosclerotic disease (40); this decrease reverses itself after the initiation of medical therapy. In our analysis, the consumption of FRC significantly improved FMD, supporting the conclusion that FRC consumption may be beneficial in patients with atherosclerotic disease.

In addition, at least some of these salutary effects appear to be dose dependent: FMD and to a lesser degree HDL cholesterol levels increase as the total flavonoid daily dose approaches 500 mg and decrease again above this threshold. The beneficial effects seen in this meta-analysis, however, seem to be mitigated by a number of study characteristics: HDL cholesterol increases in longer term trials with low fat consumption, whereas LDL cholesterol and total cholesterol decrease in shorter term studies, in patients younger than 50 y, and in trials with higher fat consumption. These findings are consistent with other studies that suggest that cocoa consumption may blunt some of the detrimental metabolic effects of a lipid challenge (41). In addition, extrapolation of potentially unpublished studies effaces the significant effect of FRC on HDL cholesterol levels.

These sensitivity analyses should be interpreted with caution: the differences reported are seen at the study level. Because we do not have individual patient data, extrapolation from study-level variability to mechanistic, patient-level recommendations is impossible.

The dose-dependent effect of FRC on FMD, and potentially HDL cholesterol, lends credence to the hypothesis that the observed association is causative. The presence of milk attenuated the impact of FRC on FMD, independent of fat content, which appeared to have a separate effect on lipid profiles. It should be noted that, although the chocolate consumed by the intervention and control group was frequently provided by confectionary companies, it was not always specifically formulated for the study itself. In at least one study (23), M&Ms were used.

Although neither BMI nor fasting glucose changed with the consumption of FRC, this should not be interpreted as an indication that cocoa consumption will not increase either. The short-term nature of the studies as well as the fact that intervention and control arms were matched for energy and sugar content precludes meaningful assessment of changes in BMI or glucose levels.

To our knowledge, this study represents the largest systematic review of the impact of FRC consumption on all major cardio-vascular risk factors and is the first to explore the possibility of a dose-response curve. Unlike other meta-analyses, which have either focused on multiple dietary sources of flavonoids (3,4) or on single cardiovascular outcomes (5), we specifically limited the interventions in this study to FRC and examined its impact on several possible cardiovascular and metabolic outcomes. Stringent assessments of industry involvement and study characteristics were undertaken and conservative statistical assumptions were made, significantly decreasing the chance that our findings are spurious.

Despite these strengths, several weaknesses exist. First, there is significant heterogeneity among our studies. Although we

have specifically examined sources of this heterogeneity in our subgroup analyses and meta-regression, there remains the possibility that other sources of heterogeneity exist. Second, this systematic review relies on study-level data; we did not have access to patient-level data. Thus, patient-level conclusions cannot be made. Finally, only short-term consumption of FRC was examined (studies ranged from 2 to 18 wk); generalizability to long-term cocoa consumption is impossible.

These limitations notwithstanding, this opens the door to more research. Large, long-term randomized, controlled studies examining FRC and insulin resistance, metabolic syndrome, and clinically significant cardiovascular outcomes are warranted.

In conclusion, the consumption of FRC significantly improves blood pressure, circulating lipid levels, insulin resistance, and FMD, the latter in a dose-dependent manner, without exerting an effect on circulating CRP levels or TG levels. Further study is warranted to determine whether these findings translate to an improvement in adverse cardiovascular outcomes.

Literature Cited

1. McCullough ML, Chevaux K, Jackson L, Preston M, Martinez G, Schmitz HH, Coletti C, Campos H, Hollenberg NK. Hypertension, the Kuna, and the epidemiology of flavonoids. J Cardiovasc Pharmacol. 2006;47:S103–9.

2. Hollenberg NK, Mohres E, Meinking T, Preston M, Crespo B, Rivera A, Jackson L, Martinez G, Loken WM. Stress and blood pressure in Kuna Amerinds. J Clin Hypertens (Greenwich). 2005;7:714–20.

3. Taubert D, Roesen R, Schönig E. Effect of cocoa and tea intake on blood pressure: a meta-analysis. Arch Intern Med. 2007;167:626–34.

4. Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, Ryder JJ, Hall WL, Cassidy A. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2008;88:38–50.

5. Desch S, Schmidt J, Kobler D, Sonnabend M, Eitel I, Sareban M, Rahimi K, Schuler G, Thiele H. Effect of cocoa products on blood pressure: systematic review and meta-analysis. Am J Hypertens. 2010;23:97–103.

6. Williams S, Tamburic S, Lally C. Eating chocolate can significantly protect the skin from UV light. J Cosmet Dermatol. 2009;8:169–73.

7. Andres-Lacueva C, Monagas M, Khan N, Izquierdo-Pulido M, Urpi-Sarda M, Permanyer J, Lamuela-Raventos RM. Flavanol and flavonol contents of cocoa powder products: influence of the manufacturing process. J Agric Food Chem. 2008;56:3111–7.

8. Chen H, Sullivan G, Yue LQ, Katz A, Quon MJ. QUICKI is a useful index of insulin sensitivity in subjects with hypertension. Am J Physiol Endocrinol Metab. 2003;284:E804–12.

9. Carnevale Schianca GP, Sainaghi PP, Castello L, Rapetti R, Limoncini AM, Bartoli E. Comparison between HOMA-IR and ISI-gly in detecting subjects with the metabolic syndrome. Diabetes Metab Res Rev. 2006;22:111–7.

10. Elbourne DR, Altman DG, Higgins JP, Curtin F, Worthington HV, Vail A. Meta-analysis involving cross-over trials: methodological issues. Int J Epidemiol. 2002;31:140–9.

11. Higgins JP, Green S, editors. Analysing and reporting results. In: Cochrane handbook for systematic reviews of interventions 8.7.2 (updated May 2005). Cichester (UK): Wiley & Sons, Ltd; 2007.

12. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–34.

THE JOURNAL OF NUTRITION

13. Begg CB. Bias in meta-analysis detected by a simple, graphical test. Stat Med. 1985;4:1–9.

14. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009;339:b2700.

15. Allen RR, Carson L, Kwik-Uribe C, Evans EM, Erdman JW. Daily consumption of a dark chocolate containing flavanols and added sterol esters affects cardiovascular risk factors in a normotensive population with elevated cholesterol. J Nutr. 2008;138:725–31.

16. Heiss C, Jahn S, Taylor M, Real WM, Angeli FS, Wong ML, Amanile N, Prasad M, Rassaf T, Ottaviani JI, et al. Improvement of endothelial function with dietary flavanols is associated with mobilization of circulating angiogenic cells in patients with coronary artery disease. J Am Coll Cardiol. 2010;56:218–24.

17. Mellor DD, Sathyapalan T, Kilpatrick ES, Beckett S, Atkin SL. High-cocoa polyphenol-rich chocolate improves HDL cholesterol in Type 2 diabetes patients. Diabet Med. 2010;27:1318–21.

18. van den Bogaard B, Draijer R, Westerhof BE, van den Meiracker AH, van Montfrans GA, van den Born B-JH. Effects on peripheral and central blood pressure of cocoa with natural or high-dose theobromine: a randomized, double-blind crossover trial. Hypertension. 2010;56:839–46.

19. Wan Y, Vinson JA, Etherton TD, Proch J, Lazarus SA, Kris-Etherton PM. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. Am J Clin Nutr. 2001;74:596–602.

20. Murphy KJ, Chronopoulos AK, Singh I, Francis MA, Moriarty H, Pike MJ, Turner AH, Mann NJ, Sinclair AJ. Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. Am J Clin Nutr. 2003;77:1466–73.

21. Taubert D, Berkels R, Roesen R, Klaus W. Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. JAMA. 2003;290:1029–30.

22. Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, Kwak HK, Milbury P, Paul SM, Blumberg J, et al. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr. 2004;23:197–204.

23. Fraga CG, Actis-Goretta L, Ottaviani JI, Carrasquedo F, Lotito SB, Lazarus S, Schmitz HH, Keen CL. Regular consumption of flavonol-rich chocolate can improve oxidant stress in young soccer player. Clin Dev Immunol. 2005;12:11–7.

24. Grassi D, Lippi C, Necozione S, Desideri G, Ferri C. Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. Am J Clin Nutr. 2005;81:611–4.

25. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, Desideri G, Blumberg JB, Ferri C. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension. 2005;46:398–405.

26. Farouque HM, Leung M, Hope SA, Baldi M, Schechter C, Cameron JD, Meredith IT. Acute and chronic effects of flavanol-rich cocoa on vascular function in subjects with coronary artery disease: a randomized double-blind placebo-controlled study. Clin Sci (Lond). 2006;111:71–80.

27. Polagruto JA, Wang-Polagruto JF, Braun MM, Lee L, Kwik-Uribe C, Keen CL. Cocoa flavanol-enriched snack bars containing phytosterols effectively lower total and low-density lipoprotein cholesterol levels. J Am Diet Assoc. 2006;106:1804–13.

28. Wang-Polagruto JF, Villablanca AC, Polagruto JA, Lee L, Holt RR, Schrader HR, Ensunsa JL, Steinberg FM, Schmitz HH, Keen CL. Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholes-terolemic postmenopausal women. J Cardiovasc Pharmacol. 2006;47 Suppl 2:S177–86; discussion S206–9.

29. Shiina Y, Funabashi N, Lee K, Murayama T, Nakamura K, Wakatsuki Y, Daimon M, Komuro I. Acute effect of oral flavonoid-rich dark chocolate intake on coronary circulation, as compared with non-flavonoid white chocolate, by transthoracic Doppler echocardiography in healthy adults. Int J Cardiol. 2009;131:424–9.

30. Taubert D, Roesen R, Lehmann C, Jung N, Schomig E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. JAMA. 2007;298:49–60.

31. Balzer J, Rassaf T, Heiss C, Kleinbongard P, Lauer T, Merx M, Heussen N, Gross HB, Keen CL, Schroeter H, et al. Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-masked, randomized, controlled trial. J Am Coll Cardiol. 2008;51:2141–9.

32. Crews WD Jr, Harrison DW, Wright JW. A double-blind, placebo-controlled, randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health: clinical findings from a sample of healthy, cognitively intact older adults. Am J Clin Nutr. 2008;87:872–80.

33. Davison K, Coates AM, Buckley JD, Howe PRC. Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects. Int J Obes (Lond). 2008;32:1289–96.

34. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, Blumberg JB, Ferri C. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. J Nutr. 2008;138:1671–6.

35. Muniyappa R, Hall G, Kolodziej TL, Karne RJ, Crandon SK, Quon MJ. Cocoa consumption for 2 wk enhances insulin-mediated vasodilatation without improving blood pressure or insulin resistance in essential hypertension. Am J Clin Nutr. 2008;88:1685–96.

36. Monagas M, Khan N, Andres-Lacueva C, Casas R, Urpi-Sarda M, Llorach R, Lamuela-Raventos RM, Estruch R. Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. Am J Clin Nutr. 2009;90:1144–50.

37. Njike VY, Faridi Z, Shuval K, Dutta S, Kay CD, West SG, Kris-Etherton PM, Katz DL. Effects of sugar-sweetened and sugar-free cocoa on endothelial function in overweight adults. Int J Cardiol. 2011;149:83–8.

38. Ried K, Frank OR, Stocks NP. Dark chocolate or tomato extract for prehypertension: a randomised controlled trial. BMC Complement Altern Med. 2009;9:22.

39. Mathur S, Devaraj S, Grundy SM, Jialal I. Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. J Nutr. 2002;132:3663–7.

40. Moens AL, Goovaerts I, Claeys M, Vrints C. Flow-mediated vasodilation: a diagnostic instrument or an experimental tool? Chest. 2005;127:2254–63.

41. Bauer S, Ding EL, Smit LA. Cocoa consumption, cocoa flavonoids, and effects on cardiovascular risk factors: an evidence-based review. Curr Cardiovasc Risk Rep. 2011;5:120–7.