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

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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 consumption. 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. Until now, 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
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[tia] OR cacao[tib] OR cocoa[tia] OR cocoa[tib] OR cocoa[tiab] OR cacao[tiab] OR cacao[tiab] OR chocolate[tiab] OR chocolate[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 text 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, flavanol, 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 carryover 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 11 (Statacorp), using the Dersimonian and Laird random-effects model (11). Results were considered statistically significant when P ≤ 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. P was used to assess the magnitude of heterogeneity. F was considered potentially significant at a threshold of P = 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 (15,17,19,22–28) 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>
<thead>
<tr>
<th>Study name</th>
<th>Design</th>
<th>Population</th>
<th>Age, y, (int/cont)</th>
<th>Control type</th>
<th>Flavonoid type</th>
<th>Route of administration</th>
<th>Control dose, mg/d</th>
<th>Total flavonoid dose, mg/d</th>
<th>Study size, n</th>
<th>Duration, d</th>
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<td>Bai (31)</td>
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<td>Diabetic</td>
<td>63.1/64.4 LFC</td>
<td>E</td>
<td>Drink</td>
<td>75</td>
<td>963</td>
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<td>Crews (32)</td>
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<td>Healthy volunteers</td>
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<td>Bar + drink</td>
<td>7.4</td>
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<td>Overweight volunteers</td>
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<td>Drink</td>
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<td>Grassi (29)</td>
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<td>Healthy men</td>
<td>29.3/30.3 White chocolate</td>
<td>N/A</td>
<td>Bar</td>
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<td>Mnuyayapa (35)</td>
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<td>51/51 LFC</td>
<td>Pro, E, C, Phen</td>
<td>Drink</td>
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<td>781</td>
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<td>Murphy (20)</td>
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<td>Drink</td>
<td>9</td>
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<td>68/78.9 LFC</td>
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<td>Polyphenols</td>
<td>Bar</td>
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<td>550</td>
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<td>Taubert (21)</td>
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<td>Bar</td>
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<td>63.4/63.7 White chocolate</td>
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<td>36/36 Average American diet</td>
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<td>Wang Polagruoto (28)</td>
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<td>HL, post-menopause</td>
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<td>SBP, DBP, P, TC, HDL, LDL, TG, BMI, FMD</td>
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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-flavanoid 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
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 non-linearity = 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...
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.

Acknowledgments

E.L.D., S.R.B., and M.G.S. designed the research; M.G.S., S.R.B., A.C.M., N.H.C., and C.E.M.C. performed the research; S.R.B. and E.L.D. performed the statistical analysis; M.G.S. wrote the manuscript; and M.G.S. and S.R.B. had primary responsibility for the integrity of the data and the accuracy of the analysis. All authors had full access to the data in the study. All authors read and approved the final manuscript.

Literature Cited


Cocoa and cardiovascular risk factors

1987


