Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials[1–3]

Lee Hooper, Colin Kay, Asmaa Abdelhamid, Paul A Kroon, Jeffrey S Cohn, Eric B Rimm, and Aedín Cassidy

ABSTRACT

Background: There is substantial interest in chocolate and flavan-3-ols for the prevention of cardiovascular disease (CVD).

Objective: The objective was to systematically review the effects of chocolate, cocoa, and flavan-3-ols on major CVD risk factors.

Design: We searched Medline, EMBASE, and Cochrane databases for randomized controlled trials (RCTs) of chocolate, cocoa, or flavan-3-ols. We contacted authors for additional data and conducted duplicate assessment of study inclusion, data extraction, validity, and random-effects meta-analyses.

Results: We included 42 acute or short-term chronic (≤18 wk) RCTs that comprised 1297 participants. Insulin resistance (HOMA-IR: −0.67; 95% CI: −0.98, −0.36) was improved by chocolate or cocoa due to significant reductions in serum insulin. Flow-mediated dilatation (FMD) improved after chronic (1.34%; 95% CI: 1.00%, 1.68%) and acute (3.19%; 95% CI: 2.04%, 4.33%) intakes. Effects on HOMA-IR and FMD remained stable to sensitivity analyses. We observed reductions in diastolic blood pressure (BP; −1.60 mm Hg; 95% CI: −2.77, −0.43 mm Hg) and mean arterial pressure (−1.64 mm Hg; 95% CI: −3.27, −0.01 mm Hg) and marginally significant effects on LDL (−0.07 mmol/L; 95% CI: −0.13, 0.00 mmol/L) and HDL (0.03 mmol/L; 95% CI: 0.00, 0.06 mmol/L) cholesterol. Chocolate or cocoa improved FMD regardless of the dose consumed, whereas doses >50 mg epicatechin/d resulted in greater effects on systolic and diastolic BP. GRADE (Grading of Recommendations, Assessment, Development and Evaluation, a tool to assess quality of evidence and strength of recommendations) suggested low- to moderate-quality evidence of beneficial effects, with no suggestion of negative effects. The strength of evidence was lowered due to unclear reporting for allocation concealment, dropouts, missing data on outcomes, and heterogeneity in biomarker results in some studies.

Conclusions: We found consistent acute and chronic benefits of chocolate or cocoa on FMD and previously unreported promising effects on insulin and HOMA-IR. Larger, longer-duration, and independently funded trials are required to confirm the potential cardiovascular benefits of cocoa flavan-3-ols. *Am J Clin Nutr* 2012;95:740–51.

INTRODUCTION

There is substantial interest in the potential role of chocolate and one of its primary bioactive components, flavan-3-ols, in prevention and management of CVD[4] (1–3). Several observational studies supported the association between high cocoa intake and reduced CVD risk and mortality (4, 5). Recent prospective data suggest that greater average intake (7.5 compared with 1.7 g/d) of total chocolate (24% of intake from dark chocolate) is associated with lower systolic (1.0 mm Hg) and diastolic (0.9 mm Hg) BP and a 10% lower 8-y risk of stroke (6). In patients with a previous myocardial infarction, eating chocolate twice a week compared with never eating chocolate was also associated with a 66% reduction in 8-y cardiac mortality (5). A growing body of in vitro evidence also supports potential beneficial effects of flavan-3-ols on cardiovascular risk through effects on endothelial function, inflammation, platelet function, angiotensin-converting enzyme activity and glucose transport (3, 7–11). However the relative impact of these mechanisms in vivo remains unclear.

Numerous short-term RCTs have examined mechanisms by which chocolate or cocoa and flavan-3-ols potentially reduce CVD risk, and some have been systematically reviewed (12–16). However, the predominant focus of these reviews has been on BP effects. An early systematic review examined effects of chocolate on CVD risk factors and suggested benefits on BP, lipid oxidation, HDL cholesterol, and inflammation, but included intervention and observational studies only up to early 2005 and without assessment of study validity (12). More recent reviews have addressed effects of chocolate or cocoa flavonoids on BP and reported statistically significant reductions in BP in trials of 2–18-wk duration (13, 15–17). Another systematic review observed a reduction in LDL cholesterol; however, this finding was not stable to sensitivity analysis in which lower-quality studies were removed or in which data from different study durations were used (18). In our own review of the literature through 2007 of all major flavonoid subclasses, we observed improvements by

[1] From the Norwich Medical School, University of East Anglia, Norwich, United Kingdom (LH, CK, AA, and AC); the Institute of Food Research, Norwich Research Park, Colney, Norwich, United Kingdom (PAK); the Heart Research Institute, Sydney, Australia (JSC); and the Departments of Epidemiology and Nutrition, Harvard School of Public Health, Channing Laboratory, and the Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (EBR).

[2] This study was unfunded and completed with internal support from the Department of Nutrition, Norwich Medical School, University of East Anglia, United Kingdom.

[3] Address correspondence to L Hooper, Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, United Kingdom. E-mail: l.hooper@uea.ac.uk.

[4] Abbreviations used: BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; FMD, flow-mediated dilatation; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; Hb A_{1c}, glycated hemoglobin; RCT, randomized controlled trial.

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chocolate in FMD and systolic and diastolic BP but without effects on HDL or LDL cholesterol. It also raised issues about the validity of included studies and the effects of chocolate supplementation on body weight (14). Because many further chocolate trials have been published over the past several years, there is a need for a review to assess the overall effects and validity of the data from RCTs of chocolate or cocoa on the major established risk factors for CVD, including insulin resistance and inflammation.

In the present study we systematically reviewed RCTs to assess the effects of chocolate, cocoa, and/or cocoa flavan-3-ols on CVD biomarkers. Specifically, we examined effects on the classic modifiable Framingham risk measures: systolic and diastolic BP and total, LDL, and HDL cholesterol (19, 20); in addition, given the reciprocal relation between insulin resistance and endothelial dysfunction (21), we also examined other independent predictors of CVD risk including fasting glucose, insulin, triglycerides, Hb A_{1c}, CRP, and FMD (22–25).

METHODS

The systematic review was conducted in accordance with Cochrane methodology and is presented in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (26, 27). No protocol for this review has been published.

We included parallel or crossover RCTs in adult participants (at any risk of CVD, but not critically ill) where the intervention was rich in chocolate, cocoa, cocoa extracts, or refined cocoa flavan-3-ols compared with a control group. Studies could be of any duration, but multifactorial interventions were excluded. Outcomes included FMD, lipoprotein concentrations, BP, fasting insulin, glucose, Hb A_{1c}, insulin resistance [by using HOMA-IR and QUICKI (quantitative insulin sensitivity check index)], insulin sensitivity index, mean arterial pressure, and CRP. Hard clinical cardiovascular events were extremely unlikely given the short duration of existing trials, so that although we searched for them, none were found.

Medline and EMBASE [both on Ovid (http://www.ovid.com/)] and the Cochrane Library (CENTRAL; http://www.thecochranelibrary.com/) databases were searched to May 2011 by using complex structured searches (*see* Supplemental Figure 1 under "Supplemental data" in the online issue). Bibliographies of included studies and relevant reviews were checked, and searches were not limited by language.

Titles, abstracts, and then potential full-text articles were assessed for inclusion independently by 2 reviewers, and disagreements discussed within the whole review group. Where inclusion of full-text articles was unclear (eg, where it was not clear in the published article whether intervention allocation was randomized) we attempted to contact study authors. Data were extracted independently by 2 separate reviewers, and differences were subsequently adjudicated. Data were taken from graphs where authors could not be contacted, and outcomes converted into common units. Changes in continuous variables from baseline to end of study were extracted, requested from authors, or calculated; relevant SDs were imputed (26) for all outcomes apart from FMD, for which insufficient data were available (because no single study reported both end data and change data with all relevant variances).

For parallel studies with more than one relevant intervention arm, intervention groups were combined and compared with the single control group. For crossover studies with more than one intervention arm, data were used from the intervention arm that was most suitably controlled. Parallel studies providing 2 intervention groups and 2 suitable control groups were reported as 2 substudies. Crossover studies were treated as parallel studies, with the total number of participants recorded in both intervention and control arms.

Trial quality characteristics were extracted in duplicate onto data extraction forms and included the following: allocation concealment (coded as adequate, unclear, or inadequate), participant blinding, provider blinding, outcome assessor blinding (each coded as yes, unclear, or no), and reporting of withdrawals (numbers of withdrawals in each group were clear, and reasons reported coded as done, partial, or not done) (28, 29). In addition, we assessed funding (coded as free of industry funding or involvement, not free of such funding, or unclear) and the similarity of saturated fat intake between the intervention and control arms ($\leq$2% of energy intake from saturated fat) as a marker of general dietary similarity between arms. We classified industry funding as a commercial source providing some or all monetary funding for a trial, a company carrying out a study "in house," or in cases in which any study author was employed by a relevant industry. We did not include companies providing intervention or control ingredients freely to studies as being industry funded. A trial was considered to be at low risk of bias in cases in which allocation concealment was adequate; participant, provider, and outcome assessor blinding were all coded "yes"; industry funding was absent; and study arms were similar in respect to saturated fat intake. All other trials were considered at moderate or high risk of bias.

Variability between studies was assessed by using I^2 (an estimate of the proportion of total observed variability due to genuine variation rather than to random error within studies; considered substantial when >50%) (26, 30). Intervention/control differences in outcomes were combined across studies by using mean differences in random-effects meta-analysis with Review Manager 5.0 software (31). For all outcomes, where there were $\geq$10 studies (26), we planned to explore effects of dose, study duration, sex, and intervention type and explored effects of baseline CVD risk at the suggestion of a referee.

We planned sensitivity analyses to assess the robustness of results to trial quality (eg, removing trials that were at medium to high risk of bias); however, all studies were at medium to high risk of bias due to poor reporting of allocation concealment, blinding, dropouts, and use of commercial funding. Instead, we ran 2 sensitivity analyses, removing studies without clear allocation concealment and removing trials funded by commercial interests. Funnel plots were used to assess for evidence of small study (or publication) bias (32). GRADEpro software (GRADE Working Group 2004–2007, version 3.6) was used to GRADE the level of evidence for cardiovascular health (33, 34).

RESULTS

A total of 1637 potentially relevant titles and abstracts were identified from searches on Medline, EMBASE, and the Cochrane Library, together with other reference sources and information provided by relevant experts. Ninety-eight articles were collected as full text and assessed for inclusion, and 56 studies were identified as being RCTs of chocolate, cocoa, or cocoa flavan-3-ols. We attempted to contact the authors of these 56 trials by e-mail

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(or by letter in cases in which e-mail addresses could not be located) to ask whether there were any further outcomes not reported in their published articles and for full data on outcomes mentioned but not presented in enough detail to include in meta-analysis. We established contact with 20 authors (*see* the Acknowledgments paragraph) and discussed 19 studies, and additional data were included in the review for 12 studies. Forty-two trials (8, 15, 35–75) reported outcomes relevant to this review and were included (**Figure 1**). The characteristics of the included 42 RCTs and their validity are described in Supplemental Tables 1 and 2 available under "Supplemental data" in the online issue, and details of chocolate or cocoa RCTs that did not report outcomes for this review are described in Supplemental Table 3 (under "Supplemental data" in the online issue).

The 42 trials included 1297 participants from Japan (3 trials; 36, 37, 66), the United States (11 trials; 40, 43, 44, 54, 56, 59, 61, 67, 74, 75), Australia (7 trials; 39, 41, 42, 45, 60, 62), Europe (18 trials; 35, 46, 48–52, 55, 57, 58, 63, 64, 68–73), and South America (1 trial; 47) and 2 trials (38) that were conducted in either the United States or Germany. Many participants were described as healthy volunteers (15 trials) or as overweight (6 trials), whereas others included subjects with elevated BP (7 trials) or serum total cholesterol (3 trials), type 2 diabetes (3 trials), raised CVD risk (2 trials), stable coronary artery disease (2 trials), congestive heart failure (1 trial), smoking-related endothelial dysfunction (1 trial), chronic fatigue (1 trial), or combined hypertension and impaired glucose tolerance (1 trial). Fifteen trials were of parallel design, and 26 were crossover studies (in one abstract, the study design was unclear).

Interventions were cocoa drinks in 21 trials, dark or milk chocolate in 15, cocoa supplements in 3, solid chocolate plus cocoa drinks in 2, and a whole diet (all foods provided) including cocoa powder and chocolate in one trial. These were compared with low flavan-3-ol versions of the same foods, drinks, or supplements and were fairly well controlled in 23 studies. In 7 trials, comparisons were not well matched (including cocoa and sugar drinks compared with sugar alone; chocolate compared with sham eating), whereas in 4 studies the placebo was unclear.

Study validity

The validity of the studies varied, but no studies were at low risk of bias (*see* Supplemental Table 2 available under "Supplemental data" in the online issue). Allocation concealment was adequate in 10 trials, not adequate in 1 trial, and unclear in the remainder of trials. Participant blinding was adequate in 24 trials, unclear in 5 trials, and inadequate in the remaining 13 trials, whereas provider/researcher blinding and outcome assessor blinding were adequate in approximately half of the studies and were unclear in the remainder. Eight studies reported no funding from commercial companies, 28 were commercially funded, and 6 were unclear with regard to funding. The assessed percentage of energy from saturated fat in the intervention group was $\leq$2% of energy of that in the control group in 8 studies, was $>$2% in 6 trials (suggesting significant dissimilarity of diet between intervention and control groups), and was not reported in 28 trials.

Effect of cocoa, chocolate, or both on FMD

Meta-analyses suggested acute improvement in FMD 2 h after ingestion of chocolate/cocoa (3.19%; 95% CI: 2.04%, 4.33%; 11 studies, 373 participants, I^2 = 84%) and after chronic intake (1.34%; 95% CI: 1.00%, 1.68%; 11 studies, 382 participants, I^2 = 0%) (**Figure 2**).

Effect of cocoa, chocolate, or both on measures of glucose and insulin metabolism and homeostasis

Results from the meta-analyses suggested significant reductions in fasting serum insulin concentrations (−2.65 μU/mL, without important heterogeneity; **Figure 3**), serum insulin after glucose challenge [−17 μU/mL; 95% CI: −20.7, −13.4 μU/mL, at 30 min (data not shown)], HOMA-IR (−0.67; Figure 3), and ISI (5.38; 95% CI: 1.81, 8.95; 2 trials, 70 participants, I^2 = 60%; data not shown) after chocolate or cocoa interventions. The interventions had no effect on fasting glucose (−0.02 mmol/L; Figure 3), Hb A_{1c} (mean difference: 0.02%; 95% CI: −0.09%, 0.14%; 3 studies, 104

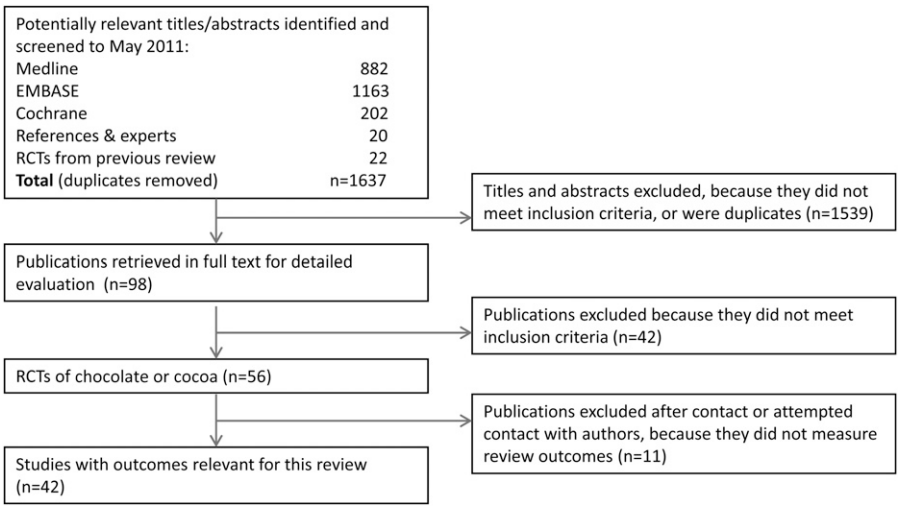

FIGURE 1. Flow diagram for the systematic review [PRISMA statement (27)]. Medline and EMBASE [both on Ovid (http://www.ovid.com/)] and the Cochrane Library (CENTRAL; http://www.thecochranelibrary.com/). PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCTs, randomized controlled trials.

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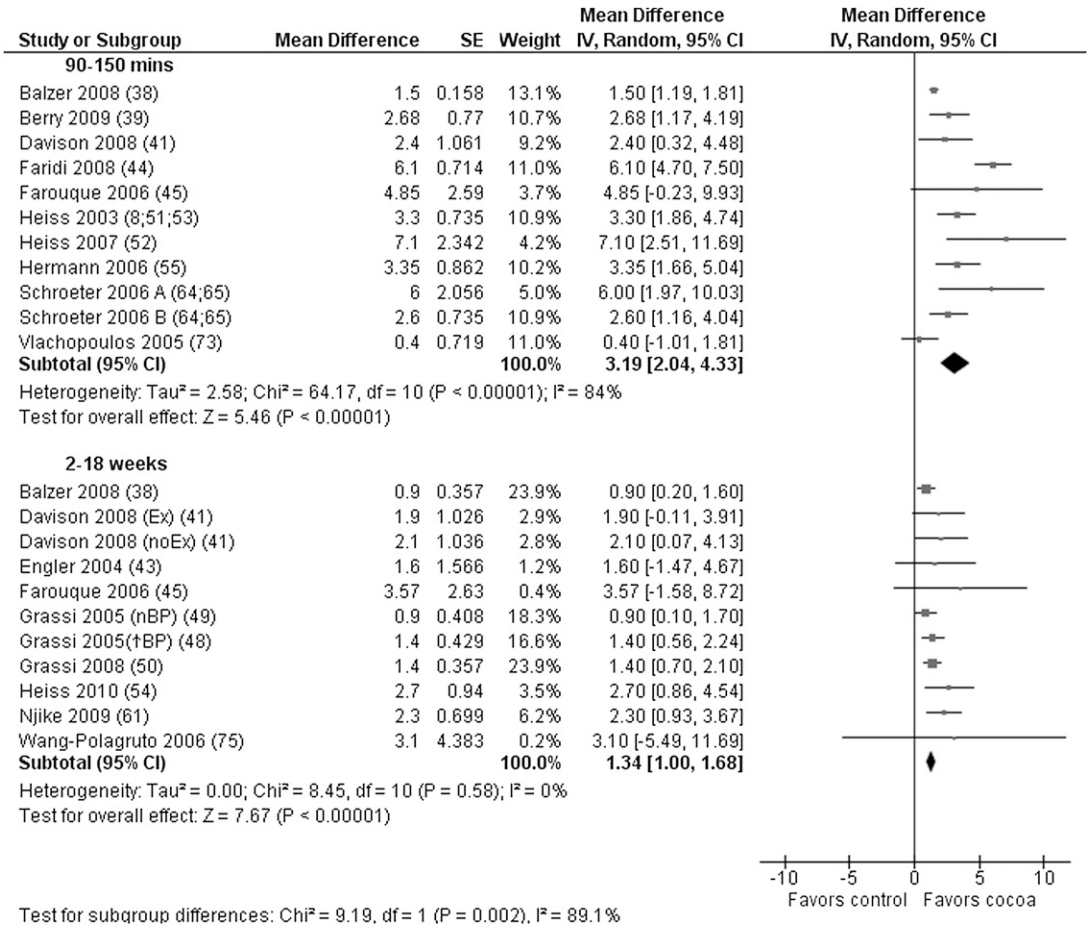

FIGURE 2. Effect of chocolate/cocoa flavan-3-ols on flow-mediated dilatation. Random-effects meta-analysis conducted by using IV methods. The Davison study consisted of 2 substudies: one gave intervention and control participants additional exercise [Davison 2008 (Ex) (41)], and one did not provide exercise [Davison 2008 (noEx) (41)]. Similarly, Grassi published 2 trials, one in subjects with raised BP at baseline [Grassi 2005 (↑BP) (48)] and one in subjects without raised BP [Grassi 2005 (nBP) (49)]. BP, blood pressure; IV, inverse variance.

participants; data not shown), or QUICKI (0.02; 95% CI: −0.00, 0.04; 4 trials, 148 participants, I^2 = 85%; data not shown). Fasting triglyceride concentrations were significantly reduced after intervention (−0.05 mmol/L; **Figure 4**); however, >40% of the effect came from 2 small substudies (one with 23 and one with 24 participants) of the Kurlandsky trial (56). Because the SDs as reported were surprisingly small, we re-ran the meta-analysis with the assumption that they should have been reported as SEs. With these updated data, the effect of intervention on triglycerides was no longer significant (−0.03 mmol/L; 95% CI: −0.08, 0.02 mmol/L; I^2 = 0%).

Effect of cocoa, chocolate, or both on other CVD risk biomarkers

Significant reductions in diastolic BP (−1.60 mm Hg; 95% CI: −2.77, −0.43 mm Hg; 22 trials, 918 participants, I^2 = 52%) and mean arterial pressure (−1.64 mm Hg; 95% CI: −3.27, −0.01; 4 trials, 163 participants, I^2 = 0%) (Figure 4) after chronic intake were observed. Marginally significant effects on LDL (−0.07 mmol/L; 95% CI: −0.14, −0.00 mmol/L; 21 studies, 986 participants, I^2 = 58%) and HDL (0.03 mmol/L; 95% CI: 0.00, 0.06 mmol/L; 21 studies, 986 participants, I^2 = 67%) cholesterol were found, but there were no significant effects on diastolic BP after acute intake, nor on CRP, total cholesterol, or systolic BP after acute or chronic intake. As a secondary analysis, we assessed the effects on body weight, BMI, and waist circumference, but few trials reported these outcomes.

Effect of dose, duration, treatment and placebo composition, CVD risk, and sex

Although we wanted to explore effects of epicatechin dose, study duration, treatment and placebo composition, baseline CVD risk, and sex on CVD risk factors, our ability to do this effectively was hindered by the small numbers of studies for each outcome, the limited number of studies that reported epicatechin dose, and the few studies that differentiated outcomes by sex. Meta-regression requires ≥10 studies per factor examined (26), so sufficient data would have been available to examine only study duration. Because there were sufficient data for subgroup analyses for CRP (10 studies), fasting glucose, acute and chronic FMD (11 studies each), total and LDL and HDL cholesterol (21 studies each), triglycerides (20 studies), systolic BP (23 studies), and diastolic BP (22 studies), we used subgrouping to sequentially examine effects of dose, duration, treatment and placebo composition, and baseline CVD risk, but not sex.

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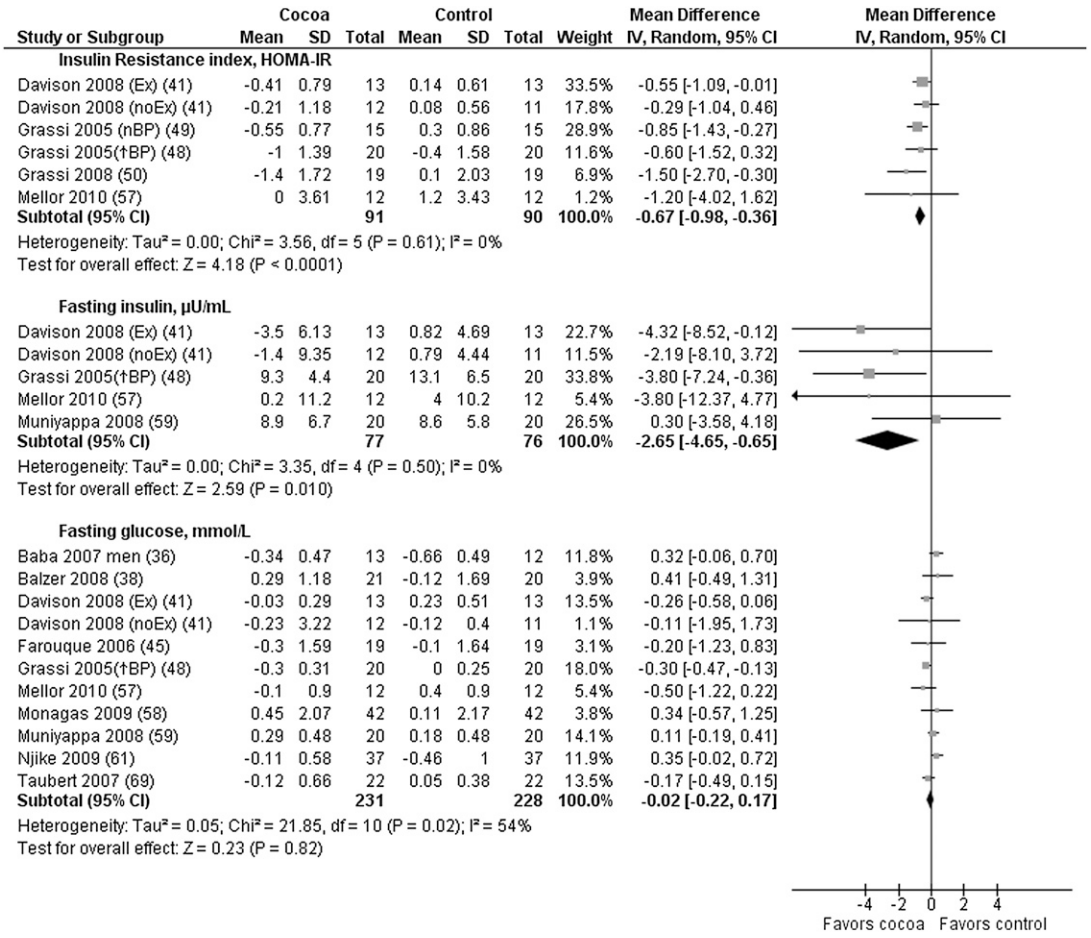

FIGURE 3. Effect of chocolate/cocoa flavan-3-ols on measures of glucose and insulin metabolism or homeostasis. Random-effects meta-analysis conducted by using IV methods. The Davison study consisted of 2 substudies: one gave intervention and control participants additional exercise [Davison 2008 (Ex) (41)], and one did not provide exercise [Davison 2008 (noEx) (41)]. Similarly, Grassi published 2 trials, one in subjects with raised blood pressure at baseline [Grassi 2005 (↑BP) (48)] and one in subjects without raised BP [Grassi 2005 (nBP) (49)]. IV, inverse variance.

There were significant improvements in FMD (both acutely and chronically) for all doses of epicatechin (**Table 1**). Such subgrouping appeared to reduce overall heterogeneity in the acute FMD data, and these data were consistent with greater effects at higher doses. There was no suggestion of heterogeneity in the chronic FMD data, and there were no clear differences in effect at different doses. Subgrouping by epicatechin dose suggested greater effects for systolic and diastolic BP at doses >50 mg/d. For fasting glucose and triglycerides, the data suggested improvement at moderate doses (50–100 mg epicatechin/d) but no effect at lower (<50 mg/d) or higher (>100 mg/d) doses. We did not assess the effect of epicatechin dose on HOMA-IR and fasting

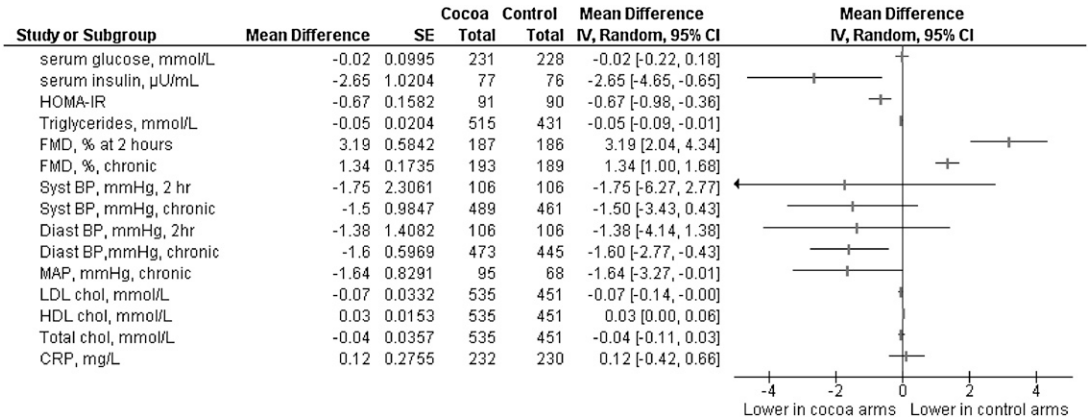

FIGURE 4. Effect of chocolate or cocoa flavan-3-ols on a range of cardiovascular disease risk biomarkers. Random-effects meta-analysis conducted by using IV methods. Data are presented for fasting serum glucose, insulin, and triglycerides. Minor differences between confidence intervals shown here and in other analyses within the article are due to rounding. chol, cholesterol; CRP, C-reactive protein; Diast BP, diastolic blood pressure; FMD, flow-mediated dilatation; IV, inverse variance; MAP, mean arterial pressure; Syst BP, systolic blood pressure.

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TABLE 1
The effect of epicatechin dose on cardiovascular risk biomarkers[1]

Outcome	Epicatechin dose	Mean effect (95% CI)[2]	No. of studies[3] (no. of participants)	I^2	*P* value for difference between subgroups
	mg/d			%	
Fasting glucose (mmol/L)	≤50	−0.01 (−0.24, 0.22)	3 (142)	68	0.22
	>50–100	−0.17 (−0.33, −0.02)	3 (106)	80	
	>100	0.09 (−0.20, 0.37)	2 (78)	0	
Triglycerides (mmol/L)	≤50	0.03 (−0.05, 0.11)	5 (217)	0	0.06
	>50–100	−0.13 (−0.23, −0.02)	6 (282)	0	
	>100	0.04 (−0.18, 0.25)	3 (138)	0	
FMD, 2 h (%)	≤50	No data	0 (0)		0.005
	>50–100	1.62 (1.33, 1.92)	3 (89)	74	
	>100	3.53 (2.22, 4.83)	4 (110)	43	
FMD, chronic (%)	≤50	2.18 (0.93, 3.43)	2 (95)	0	0.13
	>50–100	1.04 (0.59, 1.49)	3 (111)	0	
	>100	1.60 (0.95, 2.24)	3 (108)	0	
SBP, chronic (mm Hg)	≤50	0.10 (−2.20, 2.41)	6 (299)	33	0.002
	>50–100	−4.48 (−6.32, −2.63)	5 (161)	90	
	>100	−4.58 (−5.95, −3.21)	3 (110)	0	
DBP, chronic (mm Hg)	≤50	−0.38 (−1.97, 1.20)	6 (299)	7	0.001
	>50–100	−4.25 (−5.66, −2.85)	5 (161)	38	
	>100	−3.62 (−5.50, −1.74)	2 (78)	66	
LDL cholesterol (mmol/L)	≤50	−0.00 (−0.09, 0.08)	5 (217)	67	0.27
	>50–100	−0.02 (−0.08, 0.04)	6 (296)	71	
	>100	−0.14 (−0.28, 0.01)	4 (204)	63	
HDL cholesterol (mmol/L)	≤50	0.03 (−0.00, 0.07)	5 (217)	78	0.94
	>50–100	0.02 (0.00, 0.04)	6 (336)	81	
	>100	0.03 (−0.03, 0.09)	3 (124)	48	
Total cholesterol (mmol/L)	≤50	0.03 (−0.07, 0.13)	5 (217)	77	0.32
	>50–100	−0.06 (−0.13, 0.01)	6 (336)	51	
	>100	−0.05 (−0.29, 0.19)	3 (124)	55	
CRP (mg/L)	≤50	−0.50 (−1.32, 0.31)	2 (98)	0	0.60
	>50–100	−0.02 (−0.52, 0.47)	3 (111)	0	
	>100	−0.30 (−1.69, 1.09)	1 (38)	—	

[1] CRP, C-reactive protein; DBP, diastolic blood pressure; FMD, flow-mediated dilatation; SBP, systolic blood pressure.
[2] These meta-analyses all used fixed-effects mean differences to allow the assessment of *P* values for differences between subgroups.
[3] Because some studies did not state epicatechin dose, the total number of studies in this table does not add up to the total number of those included in the review.

insulin because there were too few studies for subgrouping or meta-regression.

When we stratified by duration, studies of acute and chronic intake improved FMD, whereas only studies of <3 wk reduced fasting glucose, LDL, and total cholesterol and only those studies of >3-wk duration increased HDL cholesterol (**Table 2**). There were no clear effects of duration on BP or triglycerides.

When we subgrouped studies comparing similar treatments and control comparisons (**Table 3** and Supplemental Table 4 under "Supplemental data" in the online issue), there were no significant differences in efficacy between the different treatment groups except for CRP. This was also true when we subgrouped studies by baseline CVD risk (Supplemental Table 5 under "Supplemental data" in the online issue); the only outcome for which there were differences in efficacy between subgroups was LDL cholesterol, but here there was no clear pattern in effect as CVD risk increased, and the difference in subgroups relied on only one trial.

In sensitivity analyses, removing studies funded by industry or where funding was unclear, the beneficial effects of chocolate or cocoa on HOMA-IR and FMD were retained; however, effects on BP and HDL and LDL cholesterol were no longer significant (Supplemental Table 6 under "Supplemental data" in the online issue). Similarly, removing studies with unclear allocation concealment retained the statistical significance of effects on FMD (both acutely and in short-term chronic trials) and HOMA-IR, and the short-term chronic effects on diastolic BP and the effects on systolic BP became statistically significant. No effects on triglycerides, fasting serum insulin, glucose, CRP, and LDL, HDL, and total cholesterol were observed (data not shown).

We identified several studies that had clearly assessed pertinent outcomes but that did not present the data in a usable format for meta-analysis. These included serum glucose (3 studies), postprandial triglyceride concentrations (1 study), BP (4 studies), lipids (3 studies), triglycerides (1 study), and CRP (3 studies). We are also aware of data on FMD awaiting full publication (1 study) and lipids. It is unclear what effect the addition of such missing data to the meta-analyses would have or the amount of additional missing data (from studies in which it was not clear that the outcome had been measured or in cases in which the entire study was not published). The funnel plots for systolic BP (the outcome with most included participants) did not show any evidence of small study bias, but because all included studies were small there was limited power, so the funnel plot does not exclude the possibility of missing studies or outcomes (26, 76). The funnel plot for FMD did suggest minor imbalance (Supplemental Figure 2 under "Supplemental data" in the online issue).

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TABLE 2
The effect of duration of intervention on cardiovascular risk biomarkers[1]

Outcome	Duration	Mean effect (95% CI)[2]	No. of studies (no. of participants)	I^2	P value for difference between subgroups
				%	
Fasting glucose (mmol/L)	<3 wk	−0.20 (−0.35, −0.04)	2 (80)	82	0.01
	3–6 wk	0.11 (−0.04, 0.25)	5 (281)	0	
	>6–26 wk	−0.10 (−0.29, 0.08)	5 (142)	44	
Triglycerides (mmol/L)	<3 wk	−0.02 (−0.12, 0.07)	6 (224)	52	0.63
	3–6 wk	−0.06 (−0.11, −0.02)	11 (624)	0	
	>6–26 wk	−0.01 (−0.15, 0.13)	5 (142)	0	
FMD (%)	Acute, 90–150 min	1.89 (1.61, 2.16)	11 (369)	84	0.07
	<3 wk	1.20 (0.68, 1.71)	3 (89)	0	
	3–6 wk	1.40 (0.93, 1.87)	7 (291)	6	
	>6–26 wk	2.00 (0.57, 3.43)	2 (49)	0	
SBP (mm Hg)	Acute, 90–150 min	−2.01 (−3.58, −0.45)	3 (212)	85	0.004
	<3 wk	−6.12 (−8.12, −4.12)	6 (220)	76	
	3–6 wk	−2.01 (−3.31, −0.72)	11 (592)	60	
	>6–26 wk	−2.35 (−4.89, 0.18)	6 (163)	8	
DBP (mm Hg)	Acute, 90–150 min	−1.14 (−2.30, 0.03)	3 (212)	79	0.02
	<3 wk	−3.27 (−4.80, −1.73)	6 (220)	62	
	3–6 wk	−0.41 (−1.50, 0.69)	10 (560)	0	
	>6–26 wk	−2.05 (−3.99, −0.12)	6 (163)	29	
LDL cholesterol (mmol/L)	<3 wk	−0.22 (−0.33, −0.11)	6 (224)	35	0.0004
	3–6 wk	0.02 (−0.02, 0.06)	11 (664)	55	
	>6–26 wk	−0.04 (−0.15, 0.07)	5 (142)	0	
HDL cholesterol (mmol/L)	<3 wk	−0.02 (−0.06, 0.02)	6 (224)	61	<0.0001
	3–6 wk	0.02 (0.01, 0.04)	10 (620)	58	
	>6–26 wk	0.15 (0.08, 0.21)	5 (142)	49	
Total cholesterol (mmol/L)	<3 wk	−0.17 (−0.30, −0.04)	6 (224)	67	0.06
	3–6 wk	−0.02 (−0.06, 0.03)	10 (620)	46	
	>6–26 wk	0.05 (−0.12, 0.22)	5 (142)	0	
CRP (mg/L)	<3 wk	−0.07 (−0.54, 0.40)	3 (108)	0	0.57
	3–6 wk	0.14 (−0.27, 0.54)	6 (330)	76	
	>6–26 wk	−0.80 (−2.76, 1.16)	1 (24)	—	

[1] CRP, C-reactive protein; DBP, diastolic blood pressure; FMD, flow-mediated dilatation; SBP, systolic blood pressure.
[2] These meta-analyses all used fixed-effects mean differences to allow the assessment of P values for differences between subgroups.

DISCUSSION

This systematic review and meta-analysis identified 42 RCTs (including 1297 participants) that randomly assigned participants to chocolate, cocoa, or cocoa flavan-3-ols in comparison with control groups in acute or short-term chronic interventions. To our knowledge, this is the first systematic review to show that chocolate or cocoa intervention reduces insulin resistance as a result of a decrease in insulin secretion. We also found strong beneficial effects on FMD from 11 chronic and 11 acute studies, which were stable to sensitivity analysis. Reductions in diastolic BP, triglycerides, and mean arterial pressure were also observed, but results were less stable in sensitivity analyses, as were the marginally significant effects on LDL and HDL cholesterol. No effects on systolic BP or CRP concentrations were observed. Interestingly, in line with other dietary interventions, beneficial effects on HDL cholesterol were greater in longer-term trials.

Our results support the reciprocal relation between insulin resistance and endothelial function and suggest that the effect of cocoa/chocolate interventions on fasting insulin concentrations and HOMA-IR may be associated with endothelial function (21, 22, 77). These data are further supported by in vitro studies showing effects of flavan-3-ols and their metabolites on glucose transport, bioavailability and bioactivity of nitric oxide, inflammation, platelet function, and angiotensin-converting enzyme activity (3, 7–11).

Our results suggest that epicatechin dose may be a key contributor to the effects observed. Increasing the epicatechin dose resulted in significant improvements in FMD after acute intake (Table 1). Doses of >50 mg epicatechin/d reduced systolic and diastolic BP, whereas doses <50 mg/d did not. These findings support oral administration of pure (−)-epicatechin mimicking acute vascular effects of flavan-3-ol–rich cocoa (64). This suggests that lower-dose studies may dilute the "true" response of chocolate or cocoa, thus reducing the apparent effectiveness within meta-analyses. Interestingly, for fasting glucose and triglycerides, beneficial effects were observed at only the 50–100-mg/d epicatechin dose and not at higher intakes. This may be due to chance because there are limited dose-response studies for analysis. Further long-term epicatechin dose-response studies are required, and epicatechin content should be reported in future studies.

No long-term trials have examined effects of chocolate, cocoa, or flavan-3-ols on the range of major CVD risk biomarkers. The longest trial was 18 wk, and only 7 trials were longer than 6 wk (Supplemental Table 1 under "Supplemental data" in the online issue). In analyses examining the impact of duration, effects on most outcomes were greatest in acute and in the shortest chronic (<3 wk) studies (Table 2). One exception was the observed benefit of chocolate or cocoa on HDL in longer-duration studies, which in line with observed effects of other dietary constituents

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TABLE 3
The effect of treatment and placebo composition on the endpoint measures[1]

Outcome	Type of comparison	Mean effect (95% CI)[2]	No. of studies (no. of participants)	I^2	P value for difference between subgroups
				%	
Fasting glucose (mmol/L)	Flavonoids vs no flavonoids[3]	0.41 (−0.49, 1.31)	1 (41)	—	0.49
	Cocoa vs no cocoa OR dark vs white chocolate	−0.14 (−0.26, −0.02)	8 (340)	62	
	Chocolate vs no chocolate[4]	−0.20 (−1.23, 0.83)	1 (38)	—	
Triglycerides (mmol/L)	Flavonoids vs no flavonoids	0.03 (−0.11, 0.17)	3 (222)	65	0.27
	Cocoa vs no cocoa OR dark vs white chocolate	−0.03 (−0.10, 0.03)	11 (463)	0	
	Chocolate vs no chocolate	−0.08 (−0.13, −0.02)	4 (131)	4	
FMD, chronic intake (%)	Flavonoids vs no flavonoids	1.16 (0.52, 1.80)	4 (111)	14	0.57
	Cocoa vs no cocoa OR dark vs white chocolate	1.40 (0.99, 1.81)	6 (231)	0	
	Chocolate vs no chocolate	3.57 (−1.58, 8.72)	1 (38)	—	
SBP, chronic intake (mm Hg)	Flavonoids vs no flavonoids	−4.14 (−5.95, −2.32)	4 (110)	66	0.35
	Cocoa vs no cocoa OR dark vs white chocolate	−3.28 (−4.68, −1.88)	14 (621)	73	
	Chocolate vs no chocolate	2.90 (−7.20, 13.00)	1 (21)	—	
DBP, chronic intake (mm Hg)	Flavonoids vs no flavonoids	0.71 (−2.08, 3.50)	3 (78)	11	0.12
	Cocoa vs no cocoa OR dark vs white chocolate	−2.10 (−3.10, −1.10)	14 (621)	53	
	Chocolate vs no chocolate	1.40 (−5.65, 8.45)	1 (21)	—	
LDL cholesterol (mmol/L)	Flavonoids vs no flavonoids	−0.00 (−0.07, 0.06)	4 (262)	67	0.23
	Cocoa vs no cocoa OR dark vs white chocolate	−0.05 (−0.11, 0.02)	11 (463)	57	
	Chocolate vs no chocolate	0.04 (−0.04, 0.12)	4 (131)	75	
HDL cholesterol (mmol/L)	Flavonoids vs no flavonoids	0.02 (−0.01, 0.04)	4 (262)	73	0.30
	Cocoa vs no cocoa OR dark vs white chocolate	0.04 (0.01, 0.06)	11 (463)	73	
	Chocolate vs no chocolate	0.04 (0.00, 0.08)	4 (131)	60	
Total cholesterol (mmol/L)	Flavonoids vs no flavonoids	−0.05 (−0.13, 0.02)	4 (262)	5	0.55
	Cocoa vs no cocoa OR dark vs white chocolate	0.01 (−0.07, 0.09)	11 (463)	63	
	Chocolate vs no chocolate	−0.02 (−0.10, 0.05)	4 (131)	54	
CRP (mg/L)	Flavonoids vs no flavonoids	1.10 (−3.02, 5.22)	1 (41)	—	0.0002
	Cocoa vs no cocoa OR dark vs white chocolate	−0.21 (−0.55, 0.12)	6 (290)	0	
	Chocolate vs no chocolate	1.78 (0.89, 2.67)	2 (41)	72	

[1] CRP, C-reactive protein; DBP, diastolic blood pressure; FMD, flow-mediated dilatation; SBP, systolic blood pressure.
[2] These meta-analyses were conducted by using fixed-effects mean differences to allow assessment of P values for differences between subgroups.
[3] Comparison included purified flavan-3-ol vs placebo or high flavan-3-ol cocoa vs low flavan-3-ol cocoa, where the main difference between intervention and control groups relates to flavan-3-ol content.
[4] Studies controlled only for differences in energy and sugar content between the intervention and control products.

on HDL. Chocolate or cocoa contains other potentially bioactive constituents in addition to flavan-3-ols, including stearic acid, potassium, and methylxanthines (78), which may be present in intervention but not in control products of many included studies (Table 3 and Supplemental Table 4 under "Supplemental data" in the online issue). In addition, 6 included trials showed large differences between intervention and control groups in saturated fat intake, which may influence CVD risk (Supplemental Table 2 under "Supplemental data" in the online issue). Subgrouping suggested that effects on FMD were likely due to flavan-3-ols, whereas other cocoa components may contribute to changes in glucose (79) and LDL (Table 3). Because the number of trials available for subgrouping was limited, such analyses should be interpreted with caution. Larger and longer-duration trials with optimally designed treatments and controls are required.

Compliance is typically better in short-duration studies in which the dietary intervention is modest and does not substantially interfere with the regular diet. Two of seven included studies that provided plasma metabolite data were unable to quantify differences in plasma epicatechin or flavan-3-ol/metabolites between the intervention and control group (data not shown). This suggests that the intervention doses were low, the analytic methods used were insufficient, or that compliance was poor in these studies. For the other 5 studies in which plasma concentrations were measured, the effectiveness of interventions in increasing blood flavan-3-ol/metabolite concentrations was clear.

No included trials were at low risk of bias, and the GRADE assessment of strength of evidence varied from very low to moderate (**Figure 5**) because of unclear reporting of allocation concealment and dropouts in many studies, missing outcome data, and heterogeneity in study results for some biomarkers. This low validity may lead to exaggerated suggestions of effectiveness. Other limitations were as follows: most studies were underpowered to assess effects appropriately, few trials were independently funded, most studies did not report measures of adherence or metabolism, many studies had unclear doses of flavonoids and potentially contained other bioactive constituents, and many studies had missing outcomes or were poorly reported. There were clearly missing data for several outcomes, and to reduce potential bias we contacted authors of studies in which any data were not usable to gain sufficient information to include them; this resulted in the inclusion of additional data from 12 studies. Twelve other trials had clearly measured or partially reported outcomes of interest that were not usable in meta-analysis. Another limitation was that all RCTs were small: the largest included 160 participants (of whom 40 were in the single control group), and the mean number of participants was

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Quality assessment							No of participants		Effect size (Absolute)	Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Cocoa, chocolate or cocoa flavan-3-ols	Control			
Cardiovascular events - not reported											
0	-	-	-	-	-	none	-	-	-		CRITICAL
Fasting serum glucose, mmol/L (follow-up 2-18 weeks; measured with: mmol/L; Better indicated by lower values)											
11	randomized trials	serious[1]	serious[2]	no serious indirectness	no serious imprecision	reporting bias[3]	231	228[4]	MD 0.02 lower (0.22 lower to 0.17 higher)	⊕OOO VERY LOW	IMPORTANT
Fasting serum insulin, μU/mL (follow-up 2-12 weeks; measured with: μU/mL; Better indicated by lower values)											
5	randomized trials	serious[1]	no serious inconsistency[5]	no serious indirectness	no serious imprecision	reporting bias[3]	77	76[4]	MD 2.65 lower (4.65 to 0.65 lower)	⊕⊕OO LOW	IMPORTANT
HOMA-IR (Insulin resistance index) (follow-up 2-12 weeks; Better indicated by lower values)											
6	randomized trials	serious[1]	no serious inconsistency[5]	no serious indirectness	no serious imprecision	reporting bias[3]	91	90[4]	MD 0.67 lower (0.98 to 0.36 lower)	⊕⊕OO LOW	IMPORTANT
Triglycerides (fasting), mmol/L (follow-up 2-18 weeks; measured with: mmol/L; Better indicated by lower values)											
20	randomized trials	serious[1]	no serious inconsistency[5]	no serious indirectness	no serious imprecision	reporting bias[3]	515	431[4]	MD 0.05 lower (0.09 to 0.01 lower)	⊕⊕OO LOW	IMPORTANT
FMD (flow-mediated dilatation) at 2 hours (acute), % (follow-up 90-150 mins; measured with: %; Better indicated by higher values)											
11	randomized trials	serious[1]	no serious inconsistency[6]	no serious indirectness	no serious imprecision	reporting bias[3] dose response gradient[7]	187	186[4]	MD 3.19 higher (2.04 to 4.33 higher)	⊕⊕⊕O MODERATE	IMPORTANT
FMD (flow-mediated dilatation) after chronic intake, % (follow-up 2-12 weeks; measured with: %; Better indicated by higher values)											
11	randomized trials	serious[1]	no serious inconsistency[5,8]	no serious indirectness	no serious imprecision	reporting bias[3]	176	172[4]	MD 1.34 higher (1 to 1.68 higher)	⊕⊕OO LOW	IMPORTANT
Systolic blood pressure, mmHg (follow-up 2-18 weeks; measured with: mmHg; Better indicated by lower values)											
23	randomized trials	serious[1]	serious[2]	no serious indirectness	no serious imprecision	reporting bias[3]	489	461[4]	MD 1.50 lower (3.43 lower to 0.3 higher)	⊕OOO VERY LOW	IMPORTANT
Diastolic blood pressure, mmHg (follow-up 2-18 weeks; measured with: mmHg; Better indicated by lower values)											
22	randomized trials	serious[1]	serious[2]	no serious indirectness	no serious imprecision	reporting bias[3]	473	445[4]	MD 1.60 lower (2.77 to 0.43 lower)	⊕OOO VERY LOW	IMPORTANT
Mean arterial pressure (MAP), mmHg (follow-up 1-12 weeks; measured with: mmHg; Better indicated by lower values)											
4	randomized trials	serious[1]	no serious inconsistency[5]	no serious indirectness	no serious imprecision	reporting bias[3]	95	68[4]	MD 1.64 lower (3.27 to 0.02 lower)	⊕⊕OO LOW	IMPORTANT
Low-density lipoprotein (LDL cholesterol), mmol/L (follow-up 2-18 weeks; measured with: mmol/L; Better indicated by lower values)											
21	randomized trials	serious[1]	serious[2]	no serious indirectness	no serious imprecision	reporting bias[3]	535	451[4]	MD 0.07 lower (0.13 lower to 0 higher)	⊕OOO VERY LOW	IMPORTANT
High-density lipoprotein (HDL cholesterol), mmol/L (follow-up 2-18 weeks; measured with: mmol/L; Better indicated by higher values)											
21	randomized trials	serious[1]	serious[2]	no serious indirectness	no serious imprecision	reporting bias[3]	535	451[4]	MD 0.03 higher (0 to 0.06 higher)	⊕OOO VERY LOW	IMPORTANT
Total cholesterol, mmol/L (follow-up 2-18 weeks; measured with: mmol/L; Better indicated by lower values)											
21	randomized trials	serious[1]	no serious inconsistency	no serious indirectness	no serious imprecision	reporting bias[3]	535	451[4]	MD 0.04 lower (0.11 lower to 0.03 higher)	⊕⊕OO LOW	IMPORTANT
C-reactive protein (CRP), mg/L (follow-up 2-8 weeks; measured with: mg/L; Better indicated by lower values)											
10	randomized trials	serious[1]	serious[2]	no serious indirectness	no serious imprecision	reporting bias[3]	232	230[4]	MD 0.12 higher (0.42 lower to 0.66 higher)	⊕OOO VERY LOW	IMPORTANT

FIGURE 5. GRADE summary of evidence for the effects of chocolate, cocoa and cocoa flavonoids on cardiovascular health biomarkers. [1]For these randomized controlled trials, allocation concealment was generally unclear, blinding was clearly adequate for participants and researchers in about half of trials, and incomplete accounting for participant losses was common. [2]I^2 was 50%, and the P value for heterogeneity was <0.10. [3]Publication bias cannot be ruled out on the basis of a funnel plot because all of the studies were small (so the funnel plot lacks power to identify any bias). Because studies were small, publication bias was more likely, and many of the studies located were funded by interested industry sources, or funding was not stated, making publication bias feasible. There was some evidence that outcomes were not reported in some studies in which results were not statistically significant. [4]For crossover studies, participants were counted twice (once for intervention and once for control arms). [5]I^2 was <50%, and the P value for heterogeneity was >0.10. [6]Despite some apparent heterogeneity in effect size (I^2 was 50% and the P value for heterogeneity was <0.10), the positive effect of chocolate or cocoa on acute flow-mediated dilatation was consistent and stable to sensitivity analyses. [7]Dose response was suggested by subgrouping, but not definitive (with only 7 randomized controlled trials that provided dose information). [8]As well as low levels of heterogeneity, effects on flow-mediated dilatation were highly stable to sensitivity analyses. GRADE, Grading of Recommendations, Assessment, Development, and Evaluation; MD, mean difference.

27. A study size <20 (11 of 42 studies recruited <20 participants and a further 10 included 20–21 participants) is associated with increased publication bias risk (80), and groups of small trials tend to report larger effect sizes than do large trials. This "small study effect" suggests that interpretation of pooled estimates from small studies should be cautious, with careful assessment of between-study differences (81, 82).

Many included studies were funded by industry, and in all areas of research evidence suggests that industry funding is associated with pro-industry conclusions, restrictions on publication of

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negative results, and delayed publication (81, 83). Our sensitivity analyses, which removed studies funded by industry or where funding was unclear, did not alter the observed effects on HOMA-IR and FMD, but other outcomes were no longer statistically significant (Supplemental Table 6 under "Supplemental data" in the online issue) possibly because of reduced statistical power. Similarly, removing studies with unclear allocation concealment retained the statistical significance of effects on FMD, HOMA-IR, and diastolic BP, supporting the stability of the observed effects on HOMA-IR and FMD.

The limited available observational data on the relation between chocolate intake and cardiovascular health suggest that consumption of more chocolate or cocoa is associated with lower BP and with lower risks of stroke and cardiovascular mortality, with stronger effects in men than in women (4–6, 84, 85). Intakes of chocolate and cocoa in higher-intake groups [eg, 7.5 g chocolate/d in the upper quintile of the EPIC (European Prospective Investigation into Cancer and Nutrition) study (6) and 4.2 g cocoa/d in the Zutphen Elderly Study (4)] are very low compared with the observed effective chocolate/cocoa flavan-3-ol doses in the included RCTs. In this meta-analysis, improvements in HOMA-IR or FMD were observed after twice-daily consumption of cocoa drinks containing 19, 22, or 54 g cocoa/d; 46 or 100 g dark chocolate/d; or 48 g chocolate plus 18 g cocoa/d. Fifty grams of chocolate/d provides ~230 kcal or ~10% of daily energy intake (86); there is therefore potential for this consumption to adversely affect weight if the diet is not isocalorically balanced.

Although findings that chocolate/cocoa improve HOMA-IR and FMD should be treated with caution due to the risk of bias inherent in the included trials, it is important to discuss the potential clinical importance of these effects. Growing evidence supports the role of insulin resistance and endothelial function as independent predictors of CVD risk (21, 22). In the San Antonio Heart Study, the OR of a CVD event comparing the extreme quintiles (top quintile of HOMA-IR: 4.80–41.7; bottom quintile: 0–1.03) was 1.94 (95% CI: 1.05, 3.59) (87), suggesting that the reduction in HOMA-IR of 0.7 that we observed may be clinically important. A recent meta-analysis found that each 1% increase in FMD was associated with a relative risk of cardiovascular events of 0.87 (95% CI: 0.83, 0.91) (25). The 1.3% increase associated with chronic chocolate intake in this review would have important consequences for cardiovascular risk, and in combination with improved HOMA-IR cardiovascular effects may be substantial.

To our knowledge, this is the first systematic review to comprehensively assess the overall effects and validity of the available RCT data on chocolate or cocoa on a range of important CVD risk factors, including insulin resistance. Our data highlight limitations of available short-duration trials, but several promising effects on biomarkers of CVD risk have emerged, including previously unreported beneficial effects on insulin and HOMA-IR, as well as benefits on FMD.

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