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Cocoa Flavanol Intake and Biomarkers for Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials[1–4]

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Abstract

Background: Cocoa flavanols may improve cardiometabolic health. Evidence from small short-term randomized clinical trials (RCTs) remains inconsistent, and large long-term RCTs testing the efficacy of cocoa flavanols are still lacking.

Objective: We performed a systematic review and meta-analysis of RCTs to quantify the effect of cocoa flavanol intake on cardiometabolic biomarkers.

Methods: We searched PubMed, Web of Science, and the Cochrane Library for RCTs that evaluated the effects of cocoa flavanols on biomarkers relevant to vascular disease pathways among adults. Data were extracted following a standardized protocol. We used DerSimonian and Laird random-effect models to compute the weighted mean differences (WMDs) and 95% CIs. We also examined potential modification by intervention duration, design, age, sex, comorbidities, and the form and amount of cocoa flavanol intake.

Results: We included 19 RCTs that comprised 1131 participants, and the number of studies for a specific biomarker varied. The amount of cocoa flavanols ranged from 166 to 2110 mg/d, and intervention duration ranged from 2 to 52 wk. Cocoa flavanol intake significantly improved insulin sensitivity and lipid profile. The WMDs between treatment and placebo were −0.10 mmol/L (95% CI: −0.16, −0.04 mmol/L) for total triglycerides, 0.06 mmol/L (95% CI: 0.02, 0.09 mmol/L) for HDL cholesterol, −2.33 μIU/mL (95% CI: −3.47, −1.19 μIU/mL) for fasting insulin, −0.93 (95% CI: −1.31, −0.55) for the homeostatic model assessment of insulin resistance, 0.03 (95% CI: 0.01, 0.05) for the quantitative insulin sensitivity check index, 2.54 (95% CI: 0.63, 4.44) for the insulin sensitivity index, −0.83 mg/dL (95% CI: −0.88, −0.77 mg/dL) for C-reactive protein, and 85.6 ng/mL (95% CI: 16.0, 155 ng/mL) for vascular cell adhesion molecule 1. No significant associations were found for other biomarkers. None of the modifiers seemed to qualitatively modify the effects of cocoa flavanol intake.

Conclusions: Our study suggests that cocoa flavanol intake has favorable effects on select cardiometabolic biomarkers among adults. These findings support the need for large long-term RCTs to assess whether cocoa flavanol intake reduces the risk of diabetes and cardiovascular events. *J Nutr* 2016;146:2325–33.

Keywords: cocoa flavanols, cardiometabolic health, randomized controlled trials, meta-analysis, biomarkers

Introduction

Cardiometabolic diseases are among the leading causes of morbidity and mortality worldwide (1, 2). In observational studies, dietary intake of flavanol-rich cocoa products, such as dark chocolate, has been associated with a reduced risk of cardiometabolic diseases, including cardiovascular disease (3), hypertension (4), metabolic syndrome (5), and diabetes (6). Given these possible protective effects on cardiometabolic health, cocoa products may add to the armamentarium of bioactives.

Cocoa products are generally considered a source of dietary flavanols that may underlie their purported health benefits (5–8), although their flavanol profile and content vary by cultivars and fermentation procedures. Evidence from previous meta-analyses of randomized clinical trials (RCTs)[10] suggest that chocolate, cocoa, or cocoa flavanols may lower blood pressure (9) and improve cardiometabolic health (10, 11). Unfortunately, the evidence from RCTs for cocoa flavanols remains limited (10).

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Synthesized evidence from observational studies has also shown that chocolate consumption may reduce the risk of ischemic heart disease (12) and stroke (13). Hypothesized mechanisms that underlie potential associations between cocoa flavanols and a reduced risk of chronic diseases include improvements in the lipid profile (14), insulin sensitivity (15, 16), and endothelial function (17) and the alleviation of systemic inflammation (18), thrombosis (19, 20), and oxidation (14, 21).

We therefore conducted a meta-analysis of RCTs to assess the effects of cocoa flavanol intake on a variety of circulating cardiometabolic biomarkers. We also examined whether the effects of cocoa flavanols differ by study design, participant age, sex, intervention duration, existing comorbidities, and the form and amount of cocoa flavanol intake.

Methods

Data sources and searches. We followed a standardized protocol in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to conduct this meta-analysis (22). Two investigators independently conducted literature searches of PubMed, Web of Science, and the Cochrane Central Register of Controlled Trials published from January 1965 (index date) to December 2015 with the use of terms from Medical Subject Headings, including cacao, cocoa, chocolate, clinical trial, controlled clinical trial, and RCT. The search was limited to trials on human participants and articles in English. All relevant studies and review articles (including meta-analysis) and the reference lists of the identified articles were checked manually. Any disagreement between the 2 investigators was resolved by consensus. An institutional review board review was not applicable because we conducted a systematic review and meta-analysis, which do not directly involve human subjects.

Study selection. Articles were included if the study was *1*) an RCT that assigned ≥1 group of participants to cocoa products, chocolate, or cocoa flavanol supplements and 1 group to placebo and *2*) circulating cardiometabolic biomarkers in blood samples, including plasma, serum, and whole blood, were measured at baseline and at the end of each intervention. All abstracts that reported the effects of cocoa flavanols on cardiometabolic biomarkers were included for screening. Studies were excluded if *1*) the study design was not an RCT or there was no placebo group; *2*) the intervention was not cocoa products, chocolate, or cocoa flavanol supplements; *3*) the biomarker concentrations were monitored ≤1 wk after the acute intervention; *4*) the amount of cocoa flavanols in the active intervention was <100 mg/d; *5*) the participants were pregnant women, children, or adolescents; or *6*) values of outcome measures at the end of the trial or changes from baseline were not reported.

In total, 320 articles were retrieved from the literature search, and 6 additional articles were retrieved from cross-reference and expert sources (**Figure 1**). We excluded 239 articles after reviewing the titles and abstracts and 68 more after examining the full text. The final set of articles for our systematic review and meta-analysis included 1131 participants from 19 unique RCTs.

Data extraction and quality assessment. Data were extracted according to a pre-established protocol. The following information was extracted from the included RCTs: general information (first author's name, year of publication, title); study characteristics (study design, eligibility criteria, trial quality, intervention duration, and the form and amount of cocoa flavanol intake); participant characteristics (age, proportion of men, race/ethnicity, and comorbidities); and outcome measures (definition of outcomes, statistical methods, pre- and post-intervention means and SDs, sample size of each arm, and adverse events). Methodologic quality was assessed with the use of the Cochrane Collaboration's tool for assessing the risk of bias (23) and included random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. For each trial, the risk of bias was reported as low, unclear, or high. The criteria used for quality assessment have been described in detail elsewhere (23).

Data synthesis and analysis. Mean changes and SDs of cardiometabolic biomarkers from baseline in the treatment and placebo groups were used to calculate the weighted mean differences (WMDs) and 95% CIs with the use of the DerSimonian and Laird random-effects models (24). Between-study heterogeneity was examined with the use of I^2 statistics (25), with an I^2 of 25%, 50%, and 75% denoting low, medium, and high heterogeneity, respectively. Begg's and Egger's tests formally tested for publication bias (26, 27). If there were any evidence of publication bias, the trim and fill method evaluated its impact (28).

Meta-regressions evaluated the overall impact of the predetermined potential modifiers, including study design, the form of cocoa flavanol intake, the amount of cocoa flavanol intake, age, sex, intervention duration, and existing comorbidity. Cutoffs of 200 and 600 mg/d for the categorical cocoa flavanol amount tested were selected based on prior knowledge and what has been used in previous studies. The categorical

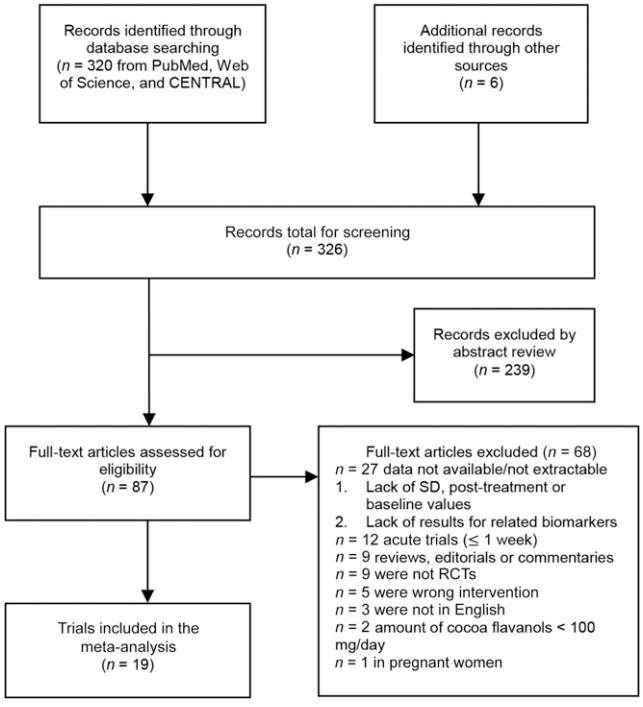

FIGURE 1 Flowchart of the study selection of 19 RCTs eligible for the meta-analysis. In total, 326 articles were identified that evaluated the effect of cocoa flavanols on cardiometabolic biomarkers. We excluded 239 articles after abstract review and 68 after full-text examination. After exclusion, 19 RCTs (n = 1131) were included in the meta-analysis. RCT, randomized control trial.

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[4] Supplemental Figures 1–4 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[10] Abbreviations: ISI, insulin sensitivity index; QUICKI, quantitative insulin sensitivity check index; RCT, randomized clinical trial; VCAM-1, vascular cell adhesion molecule 1; WMD, weighted mean difference.

TABLE 1 Characteristics of trials included in the meta-analysis[1]

Author (reference)	Year	Country	n_I/n_C	Age, y	Men, %	Comorbidity	Cocoa flavanols, mg/d	Cocoa flavanol form	Duration, wk	Source of blood measurements	Fasting status
Grassi et al. (30)	2005	Italy	15/15	34	47	None	500	Chocolate bars	2	Serum: lipids; plasma: insulin and glucose	Yes
Grassi et al. (31)	2005	Italy	20/20	44	50	Hypertensive	500	Chocolate bars	2	Serum: lipids; plasma: insulin and glucose	Yes
Farouque et al. (32)	2006	Australia	20/20	61	75	Coronary artery disease	444	Chocolate bars and beverage	6	Serum: lipids, lipoproteins, and glucose; plasma: insulin and biomarkers of endothelial function	Yes
Baba et al. (33)	2007	Japan	13/12	38	43	None	199	Beverage	12	Plasma	Yes
Shiina et al. (34)	2009	Japan	20/19	30	100	None	550	Chocolate bars	2	Serum	NR
Balzer et al. (35)	2008	Germany	21/20	64	29	Diabetic	963	Beverage	4	Plasma	Yes
Davison et al. (36)	2008	Australia	12/12	45	33	Overweight	902	Beverage	12	Plasma	Yes
			12/13	45	31	Overweight	902	Beverage	12	Plasma	Yes
Grassi et al. (4)	2008	Italy	19/19	45	58	Hypertensive	1080	Chocolate bars	2	Serum: lipids, CRP, and insulin; plasma: glucose	Yes
Muniyappa et al. (37)	2008	United States	20/20	51	40	Hypertensive	902	Beverage	2	Serum: lipids, biomarkers of endothelial function, and adipocytokines; plasma: glucose and insulin	Yes
Monagas et al. (38)	2009	Spain	42/42	70	45	High CV risk	495	Beverage	4	Serum: glucose, lipids, inflammatory biomarkers, biomarkers of endothelial function	Yes
Njike et al. (39)	2011	United States	38/39	52	15	Overweight	805	Beverage	6	Serum	Yes
Mellor et al. (40)	2010	United Kingdom	12/12	68	58	Diabetic	166	Chocolate bars	8	Serum: insulin and lipids; plasma: glucose	Yes
Almoosawi et al. (41)	2012	United Kingdom	21/21	NR	0	None	200	Beverage	4	NR	Yes
			21/21	NR	0	Overweight	200	Beverage	4	NR	Yes
Curtis et al. (42)	2012	United Kingdom	50/59	62	0	Diabetic	850	Chocolate bars	52	Plasma	Yes
Desideri et al. (43)	2012	Italy	30/30	71	48	Cognitively impaired	990	Beverage	8	Plasma	Yes
Neufingerl et al. (44)	2013	France	37/37	55	50	None	325	Beverage	4	Serum	Yes
			32/37	55	50	None	325	Beverage	4	Serum	Yes
Sarria et al. (45)	2014	Spain	24/24	27	46	None	400	Beverage	2	Serum: lipids, lipoprotein, glucose, urea, uric acid, and creatinine	Yes
			20/20	30	45	High cholesterol	400	Beverage	2	Plasma: inflammatory biomarkers	Yes
West et al. (46)	2014	United States	30/30	53	0	None	814	Chocolate bars and beverage	4	Serum: lipids, lipoproteins, CRP, and insulin; plasma: inflammatory biomarkers and glucose	Yes
D'Anna et al. (47)	2014	Italy	30/30	56	0	Metabolic syndrome	2110	Beverage	24	Serum	Yes

[1] CRP, C-reactive protein; n_I, sample size of the intervention group; n_C, sample size of the placebo group; NR, not reported.

variable for intervention duration was created based on the median duration of all included RCTs.

$P \leq 0.05$ was considered significant except for the tests of publication bias ($P = 0.10$) (29). All statistical analyses were performed with Stata version 13 (StataCorp LP).

Results

Figure 1 shows how the participants from the 19 RCTs were selected for the meta-analysis. The actual number of studies synthesized varied across biomarkers. Characteristics of eligible trials are summarized in **Table 1**. Among all participants, the proportion of men ranged from 0% to 100%, with a mean age of 30–71 y. The amount of cocoa flavanols tested for the RCTs ranged from 166 to 2110 mg/d, and intervention duration ranged from 2 to 52 wk. Among the included RCTs, 11 trials used a crossover design, 8 trials used a placebo-controlled parallel design, and 13 trials were conducted among participants with existing comorbidities, including hypertension, coronary artery disease, diabetes, overweight, hypercholesterolemia, metabolic syndrome, or mild cognitive impairment.

Description of study quality. The quality of studies included was heterogeneous (**Figure 2**). Random sequence generation and allocation concealment were reported in 6 trials. Allocation concealment was also reported in 6 trials. The risk of potential performance bias was low in 7 trials. Among 6 trials with available information on whether outcome assessment was blinded, the risk of detection bias was high in only 1 trial. The outcome data were incomplete in 2 trials. The risk of another bias was high in 2 trials because of poor compliance or other study-specific limitations.

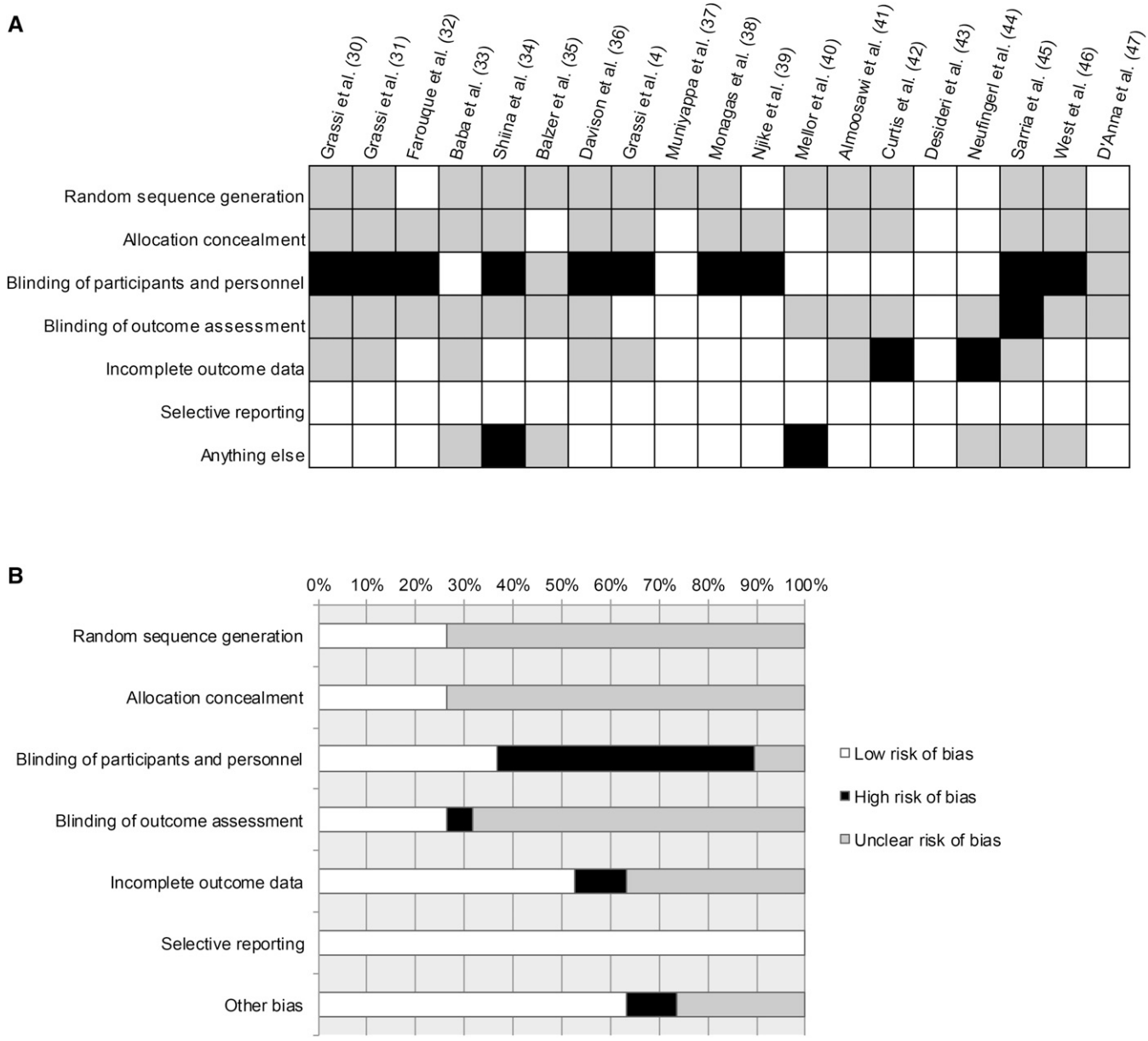

FIGURE 2 Assessment of the risk of bias for 19 selected randomized control trials: summary for items of bias. (A) Risk of bias for all trials included in the meta-analysis presented for individual trials (represented by author name and year) as low, high, or unclear risk of bias in each assessment item; (B) risk of bias for all trials included in the meta-analysis presented as the percentages of trials with low, high, or unclear risk of bias in each assessment item.

TABLE 2 WMDs in biomarkers in cocoa flavonol intervention groups compared with placebo groups[1]

Biomarkers	*n*	WMD (95% CI)	*P*	I^2, %
Glucose, mmol/L	18	−0.19 (−0.40, 0.03)	0.09	82
Insulin, μIU/mL	10	−2.33 (−3.47, −1.19)	<0.001	66
HOMA-IR	11	−0.93 (−1.31, −0.55)	<0.001	85
HbA1c, %	3	0.00 (−0.23, 0.24)	0.99	0
QUICKI	7	0.03 (0.01, 0.05)	0.01	97
ISI	4	2.54 (0.63, 4.44)	0.01	86
TC, mmol/L	19	−0.07 (−0.15, 0.01)	0.10	27
TG, mmol/L	20	−0.10 (−0.16, −0.04)	0.001	28
HDL cholesterol, mmol/L	20	0.06 (0.02, 0.09)	0.001	71
LDL cholesterol, mmol/L	19	−0.26 (−0.56, 0.04)	0.09	95
ApoAI, g/L	2	0.05 (−0.04, 0.14)	0.29	0
ApoB, g/L	2	−0.02 (−0.08, 0.05)	0.65	0
CRP, mg/dL	5	−0.83 (−0.88, −0.77)	<0.001	0
IL-6, pg/mL	3	0.04 (−0.34, 0.43)	0.82	0
TNF-α, pg/mL	2	−0.09 (−0.45, 0.27)	0.62	0
ICAM-1, ng/mL	4	−0.52 (−4.30, 3.27)	0.79	0
VCAM-1, ng/mL	3	85.6 (16.0, 155)	0.02	0
MCP-1, pg/mL	2	−8.49 (−67.1, 50.1)	0.78	0
Creatinine, mg/L	4	−0.22 (−0.82, 0.38)	0.47	77
Uric acid, mg/L	3	−0.64 (−2.79, 1.50)	0.56	0
Urea, mg/dL	3	−13.7 (−62.7, 35.2)	0.58	71
E-selectin, ng/mL	3	−2.48 (−10.5, 5.52)	0.54	0
P-selectin, ng/mL	2	5.96 (−28.6, 40.5)	0.74	29
Adiponectin, μg/mL	2	0.78 (−1.73, 3.28)	0.54	0
Oxidized LDL, unit/L	2	2.88 (−3.49, 9.24)	0.38	0

[1] CRP, C-reactive protein; HbA1c, glycated hemoglobin; ICAM-1, intercellular adhesion molecule 1; ISI, insulin sensitivity index; MCP-1, monocyte chemoattractant protein 1; QUICKI, quantitative insulin sensitivity check index; TC, total cholesterol; VCAM-1, vascular cell adhesion molecule 1; WMD, weighted mean difference.

Lipid and lipoprotein biomarkers. The number of trials included for each lipid and lipoprotein biomarker is shown in **Table 2**. Cocoa flavanol intake significantly lowered TGs ($P < 0.001$) and increased HDL cholesterol concentrations ($P < 0.001$) compared with placebo (Table 2). The WMDs were −0.10 mmol/L (95% CI: −0.16, −0.04 mmol/L; $I^2 = 28\%$) for TGs (Table 2, **Supplemental Figure 1**) and 0.06 mmol/L (95% CI: 0.02, 0.09 mmol/L; $I^2 = 71\%$) for HDL cholesterol (Table 2, **Supplemental Figure 2**). The *P* values of the Egger's or Begg's tests for all lipid biomarkers were >0.10, suggesting that the small study effects were not significant. After correcting for multiple comparisons, the associations observed for TGs and HDL cholesterol remained significant ($q = 0.002$ and 0.003).

Biomarkers of insulin resistance. Table 2 also shows the effects of cocoa flavanol intake on insulin resistance. Fasting insulin concentrations and HOMA-IR were each significantly lower in the cocoa flavanol groups than the placebo group (each $P < 0.001$), whereas the quantitative insulin sensitivity check index (QUICKI) and insulin sensitivity index (ISI) were significantly improved among the cocoa flavanol groups compared with placebo (each $P = 0.01$) (Table 2). The WMDs between the cocoa flavanol and placebo groups were −2.33 μIU/mL (95% CI: −3.47, −1.19 μIU/mL; $I^2 = 66\%$) for fasting insulin (Table 2, **Supplemental Figure 3**) and −0.93 (95% CI: −1.31, −0.55; $I^2 = 85\%$) for HOMA-IR (Table 2, **Supplemental Figure 4**). The WMD for QUICKI was 0.03 (95% CI: 0.01, 0.05; $I^2 = 97\%$), and the WMD for ISI based on postintervention values was 2.54 (95% CI: 0.63, 4.44; $I^2 = 86\%$) (Table 2). There was no evidence of publication bias from the Egger's or Begg's tests (all $P > 0.10$). After correcting for multiple comparisons, the associations observed for insulin ($q < 0.001$), HOMA-IR ($q < 0.001$), QUICKI ($q = 0.02$), and ISI ($q = 0.02$) remained significant.

Other biomarkers. The WMDs were −0.22 mg/L (95% CI: −0.82, 0.38 mg/L; $I^2 = 77\%$) for creatinine and −0.64 mg/L (95% CI: −2.79, 1.50 mg/L; $I^2 = 0\%$) for uric acid. Because of the limited information reported by individual trials, we were unable to compute WMDs of change scores for other biomarkers. Therefore, postintervention values and change scores reported in the original articles were used for those biomarkers. A significant difference between the cocoa flavanol intervention and placebo groups was found for C-reactive protein (WMD = −0.83 mg/dL; 95% CI: −0.88, −0.77 mg/dL; $P < 0.001$; $I^2 = 0\%$) and vascular cell adhesion molecule 1 (VCAM-1) (WMD = 85.6 mg/L; 95% CI: 16.0, 155 mg/L; $P = 0.02$; $I^2 = 0\%$) (Table 2). After correcting for multiple comparisons, the associations observed for C-reactive protein ($q < 0.001$) and VCAM-1 ($q = 0.02$) remained significant. With a relatively limited number of RCTs available, our analyses did not show any statistically significant overall effects of cocoa flavanols on biomarkers of oxidative stress.

Modifying effects of age, sex, duration, design, comorbidities, and form and amount of cocoa flavanols. Meta-regressions and subgroup analyses were conducted for biomarkers with ≥10 trials included in the primary analyses. Age and sex did not modify the effects of cocoa flavanol intake on lipid metabolism and insulin resistance (all $P_{\text{meta-regression}} \geq 0.05$). Cocoa flavanols improved the profiles of lipid metabolism and insulin resistance regardless of intervention duration, design, or

TABLE 3 WMDs in lipids and insulin resistance-related biomarkers in cocoa flavonol intervention groups compared with placebo groups by health status[1]

	With existing comorbidities			Without existing comorbidities			
Biomarker	*n*	WMD (95% CI)	*P*	*n*	WMD (95% CI)	*P*	*P*-meta-regression
Glucose, mmol/L	13	−0.22 (−0.48, 0.04)	0.10	2	0.16 (−0.25, 0.57)	0.44	0.31
Insulin, μIU/mL	7	−2.63 (−4.20, −1.01)	0.001	0	NA	NA	NA
HOMA-IR	7	−1.15 (−1.71, −0.60)	<0.001	1	−0.71 (−1.24, −0.18)	0.01	0.62
TC, mmol/L	12	−0.12 (−0.25, 0.02)	0.10	6	−0.03 (-0.14, 0.09)	0.67	0.40
TG, mmol/L	13	−0.09 (−0.14, −0.03)	0.002	6	−0.17 (−0.34, −0.00)	0.05	0.22
HDL cholesterol, mmol/L	13	0.05 (0.00, 0.09)	0.04	6	0.08 (0.02, 0.15)	0.02	0.38
LDL cholesterol, mmol/L	12	−0.35 (−0.77, 0.08)	0.11	6	−0.06 (−0.18, 0.07)	0.37	0.35

[1] TC, total cholesterol; WMD, weighted mean difference.

TABLE 4 WMDs in lipids and insulin resistance-related biomarkers in cocoa flavonol intervention groups compared with placebo groups by trial design[1]

	Crossover			Parallel			
Biomarker	*n*	WMD (95% CI)	*P*	*n*	WMD (95% CI)	*P*	*P*-meta-regression
Glucose, mmol/L	10	−0.09 (−0.27, 0.10)	0.36	8	−0.31 (−0.69, 0.06)	0.10	0.23
Insulin, μIU/mL	6	−2.02 (−3.49,-0.56)	0.01	4	−3.08 (−5.37, −0.79)	0.01	0.47
HOMA-IR	7	−1.00 (−1.51,-0.49)	<0.001	4	−0.82 (−1.40, −0.25)	0.01	0.68
TC, mmol/L	9	−0.03 (−0.15, 0.10)	0.69	10	−0.13 (−0.23, −0.02)	0.02	0.26
TG, mmol/L	9	−0.07 (−0.13, −0.01)	0.02	11	−0.11 (−0.21, −0.01)	0.03	0.58
HDL cholesterol, mmol/L	9	0.06 (0.02, 0.10)	0.003	11	0.05 (−0.00, 0.11)	0.06	0.88
LDL cholesterol, mmol/L	9	−0.04 −0.21, 0.13)	0.62	10	−0.39 (−0.86, 0.08)	0.10	0.21

[1] TC, total cholesterol; WMD, weighted mean difference.

form of cocoa flavanols (**Tables 3–7**). Cocoa flavanol intake <200 mg/d was significantly associated with elevated circulating HDL cholesterol concentrations (WMD = 0.19 mmol/L; 95% CI: 0.12, 0.26 mmol/L; $P < 0.001$); cocoa flavanol intake between ≥200 and <600 mg/d showed significant beneficial effects on fasting glucose (WMD = −0.26 mmol/L; 95% CI: −0.40, −0.13 mmol/L; $P < 0.001$), fasting insulin (WMD = −2.43 μIU/mL; 95% CI: −4.81, −0.05 μIU/mL; $P = 0.05$), HOMA-IR (WMD = −0.72; 95% CI: −1.15, −0.29; $P = 0.001$), and HDL cholesterol (WMD = 0.06 mmol/L; 95% CI: 0.02, 0.09 mmol/L; $P = 0.001$); and cocoa flavanol intake ≥600 mg/d significantly reduced fasting insulin (WMD = −2.19 μIU/mL; 95% CI: −3.69, −0.69 μIU/mL; $P = 0.004$), HOMA-IR (WMD = −1.05; 95% CI: −1.69, −0.41; $P = 0.001$), and TGs (WMD = −0.09 mmol/L; 95% CI: −0.16, −0.02 mmol/L; $P = 0.01$) (Table 6).

Discussion

In this systematic review and meta-analysis of 1131 participants from 19 RCTs, we found that cocoa flavanol intake from cocoa products, chocolate, or cocoa flavanol supplements significantly improved biomarkers of lipid metabolism and insulin resistance. Our meta-analysis of RCTs is among the first to our knowledge to characterize how cocoa flavanols affect cardiometabolic biomarkers. We found that cocoa flavanol intake may reduce dyslipidemia, insulin resistance, and systemic inflammation, which are all major subclinical risk factors for cardiometabolic diseases.

The favorable associations between cocoa flavanols and cardiometabolic health have been reported in 3 major US prospective cohort studies: Nurses' Health Study I, Nurses' Health Study II, and the Health Professionals Follow-Up Study. Wedick et al. (48) followed >130,000 women and men, identified 12,611 incident type 2 diabetes cases, and reported a modestly reduced risk in type 2 diabetes of similar magnitude across quintiles 2–5 of flavanol intake compared with the lowest quintile (pooled HRs: 0.92, 0.91, 0.94, and 0.91, respectively). Similar findings were also reported in the European Prospective Investigation into Cancer and Nutrition study (49, 50). However, these findings were not confirmed in a study of 35,816 postmenopausal women (51).

Flavanols have been shown to inhibit glucosidase and glucose absorption from the intestine, protect pancreatic β cells, increase insulin secretion, activate insulin receptors and glucose uptake in insulin-sensitive tissues, and modulate intracellular signaling pathways and genes involved in gluconeogenesis and glycogenesis (15, 52). Flavanols may also improve insulin sensitivity by increasing NO bioavailability and inhibiting production of reactive oxygen species and nitrogen species (5, 6). The increased bioavailability of NO may also mediate the beneficial effects of cocoa flavanols on endothelial function (53). Experimental studies have shown that cocoa supplementation slowed body weight gain, increased plasma concentrations of adiponectin, and attenuated insulin resistance, as indicated by improved HOMA-IR (54, 55). In addition, a growing body of evidence derived from both in vitro studies and animal studies also demonstrates the antidyslipidemia and anti-inflammation effects of cocoa and cocoa flavanols (14, 56, 57) (58–60). However, evidence from humans is restricted to observational studies and smaller short-term trials. Only a few studies to our knowledge have synthesized the evidence for the effects of cocoa flavanol intake on specific biological parameters beyond those characterized by excess oxidative stress.

Previous reviews and meta-analyses have supported the notion that chocolate, cocoa products, and cocoa flavanol

TABLE 5 WMDs in lipids and insulin resistance-related biomarkers in cocoa flavonol intervention groups compared with placebo groups by intervention duration[1]

	>4 wk			≤4 wk			
Biomarker	*n*	WMD (95% CI)	*P*	*n*	WMD (95% CI)	*P*	*P*-meta-regression
Glucose, mmol/L	9	−0.26 (−0.66, 0.15)	0.21	9	−0.14 (−0.29, 0.02)	0.09	0.08
Insulin, μIU/mL	5	−3.12 (−5.06, −1.19)	0.002	5	−1.88 (−3.43, −0.33)	0.02	0.70
HOMA-IR	5	−0.87 (−1.38, −0.35)	0.001	6	−0.98 (−1.52, −0.45)	<0.001	0.66
TC, mmol/L	8	−0.07 (−0.22, 0.09)	0.38	11	−0.08 (−0.18, 0.02)	0.11	0.88
TG, mmol/L	9	−0.08 (−0.15, −0.01)	0.03	11	−0.15 (−0.26, −0.04)	0.01	0.89
HDL cholesterol, mmol/L	9	0.09 (0.04, 0.15)	0.001	11	0.03 (−0.01, 0.07)	0.11	0.52
LDL cholesterol, mmol/L	8	−0.44 (−1.00, 0.13)	0.13	11	−0.10 (−0.21, 0.01)	0.06	0.72

[1] TC, total cholesterol; WMD, weighted mean difference.

TABLE 6 WMDs in lipids and insulin resistance-related biomarkers in cocoa flavonol intervention groups compared with placebo groups by amount of cocoa flavanols[1]

	<200 mg/d			≥200 to <600 mg/d			≥600 mg/d			
	n	WMD (95% CI)	*P*	*n*	WMD (95% CI)	*P*	*n*	WMD (95% CI)	*P*	*P*-meta-regression
Glucose, mmol/L	2	0.31 (−0.06, 0.68)	0.10	7	−0.26 (−0.40, −0.13)	<0.001	9	−0.20 (−0.56, 0.16)	0.29	0.02
Insulin, μIU/mL	1	−3.80 (−8.24, 0.64)	0.09	3	−2.43 (−4.81, −0.05)	0.05	6	−2.19 (−3.69, −0.69)	0.004	0.86
HOMA-IR	1	−1.20 (−2.57, 0.17)	0.09	4	−0.72 (−1.15, −0.29)	0.001	6	−1.05 (−1.69, −0.41)	0.001	0.28
TC, mmol/L	2	−0.07 (−0.39, 0.26)	0.68	9	−0.09 (−0.20, 0.02)	0.10	8	−0.06 (−0.22, 0.10)	0.46	0.90
TG, mmol/L	2	−0.11 (−0.36, 0.14)	0.40	9	−0.13 (−0.26, 0.01)	0.06	9	−0.09 (−0.16, −0.02)	0.01	0.44
HDL cholesterol, mmol/L	2	0.19 (0.12, 0.26)	<0.001	9	0.06 (0.02, 0.09)	0.001	9	0.02 (−0.04, 0.08)	0.54	0.48
LDL cholesterol, mmol/L	2	−0.25 (−0.58, −0.09)	0.15	9	−0.13 (−0.28, 0.01)	0.07	8	−0.40 (−1.02, 0.21)	0.20	0.31

[1] TC, total cholesterol; WMD, weighted mean difference.

supplements may improve cardiometabolic health (10–13, 61, 62). A recent systematic review suggests beneficial effects of food sources of flavan-3-ols (green tea and cocoa) on cardiovascular health (57). Ding et al. (12) reported in a systematic review of observational studies that cocoa consumption may reduce ischemic heart disease mortality. In a meta-analysis of prospective cohorts of men, Larsson et al. (62) found that chocolate consumption was associated with a lower risk of stroke (RR = 0.83 comparing the highest and the lowest quartile of chocolate consumption). For intermediate cardiometabolic biomarkers, a meta-analysis of 20 RCTs reported that cocoa product consumption had a small but statistically significant effect on lowering blood pressure (−2.8 mm Hg systolic and −2.2 mm Hg diastolic) (63). Another meta-analysis of RCTs conducted by Hooper et al. (10) showed that both flow-mediated dilation and HOMA-IR were also improved after chocolate consumption, and Shrime et al. (64) showed beneficial effects of cocoa products of lowering blood pressure and improving insulin sensitivity, lipid profiles, and flow-mediated dilation in a meta-analysis of short-term studies. Our updated meta-analysis of RCTs has not only updated previous findings (10) but also added cardiometabolic biomarkers involved in lipid metabolism, insulin resistance, systemic inflammation, renal function, and oxidative stress.

Strengths of this meta-analysis include the synthesis of evidence from RCTs that examined both conventional and novel cardiometabolic biomarkers, detailed subgroup analyses for potential effect modifiers, and a comprehensive evaluation of potential bias. Our meta-analysis is among the first to our knowledge to synthesize evidence for the novel, less-studied cardiometabolic biomarkers. In addition, our subgroup analysis found that cocoa flavanol interventions may have consistent effects on the biomarkers of lipid metabolism and insulin resistance regardless of age, sex, existing comorbidities, intervention duration, RCT design, and the form and amount of cocoa flavanols. Although the meta-regression analysis showed that the difference between people with and without comorbidities did not reach statistically significant cutoffs, possibly because of the limited number of trials available, the benefits for people with existing comorbidities seemed to be more substantial than those without comorbidities, which warrant further investigations.

There are several potential limitations to our study. First, although 19 trials were included in the meta-analysis, the number of studies for a specific biomarker varied. The number of available RCTs is especially limited for lipoprotein(a), oxidized LDL, VCAM-1, and leptin. In addition, the effect sizes were small to moderate for most biomarkers. Therefore, findings for those biomarkers need to be confirmed by further investigations and interpreted with caution. In addition, the precision of the estimates from individual RCTs was subject to small samples and variable intervention durations. In addition to providing comprehensive evidence for biomarkers of lipid metabolism and insulin resistance, our study highlights the urgent need for large long-term RCTs that improve our understanding of how the short-term benefits of cocoa flavanol intake on cardiometabolic biomarkers may be translated into clinical outcomes. Second, the subgroup analyses were restricted to biomarkers with ≥10 studies, and cutoffs used for categorizing modifiers were selected on an ad hoc basis. Third, because of the heterogeneity of cocoa flavanol interventions and limited evidence reported from RCTs, we were not able to distinguish the effects of different active compounds of cocoa flavanol-rich foods. However, we sought to maximize those data available

TABLE 7 WMDs in lipids and insulin resistance-related biomarkers in cocoa flavonol intervention groups compared with placebo groups by form of cocoa flavanols[1]

	Chocolate bars			Powder or powder-based beverage			Combined			
Biomarker	*n*	WMD (95% CI)	*P*	*n*	WMD (95% CI)	*P*	*n*	WMD (95% CI)	*P*	*P*-meta-regression
Glucose, mmol/L	5	−0.36 (−0.52, −0.19)	<0.001	11	−0.10 (−0.42, 0.21)	0.52	2	0.05 (−0.19, 0.29)	0.70	0.40
Insulin, μIU/mL	5	−2.21 (−3.70, −0.73)	0.003	4	−2.71 (−5.86, 0.44)	0.09	1	−2.30 (−3.99, −0.61)	0.01	0.98
HOMA-IR	7	−1.06 (−1.61, −0.51)	<0.001	3	−0.82 (−1.60, −0.04)	0.04	1	−0.47 (−0.85, −0.09)	0.01	0.72
TC, mmol/L	6	−0.08 (−0.24, 0.08)	0.31	12	−0.04 (−0.14, 0.06)	0.42	1	−0.30 (−0.64, 0.04)	0.08	0.49
TG, mmol/L	5	−0.21 (−0.37, −0.05)	0.01	13	−0.09 (−0.14, −0.05)	<0.001	2	0.14 (−0.20, 0.48)	0.42	0.12
HDL cholesterol, mmol/L	6	0.06 (0.004, 0.12)	0.04	13	0.06 (0.01, 0.10)	0.02	1	0.03 (−0.05, 0.11)	0.48	0.94
LDL cholesterol, mmol/L	5	−0.17 (−0.32, −0.02)	0.03	12	−0.28 (−0.76, 0.20)	0.25	2	−0.43 (−0.70, −0.16)	0.002	0.89

[1] TC, total cholesterol; WMD, weighted mean difference.

from RCTs and found that any favorable effects of cocoa flavanols on cardiometabolic risk factors did not seem to be modified by the form or amount of cocoa flavanols. In addition, because of the limited number of trials available, studies that used different sources of blood for biomarker measurements were synthesized together, which may have introduced additional heterogeneity. Fourth, as shown in Figure 2, our results may be prone to the inherent weaknesses of individual RCTs.

In summary, our meta-analysis of RCTs indicates that cocoa flavanol intake from cocoa products, chocolate, or cocoa flavanol supplements may have modest but significant benefits in lipid metabolism, insulin resistance, and systemic inflammation. Further investigations, particularly large long-term RCTs, are urgently needed to confirm or refute whether cocoa flavanols represent a promising bioactive in the prevention of cardiometabolic diseases.

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XL, LW, and SL designed the study; XL, IZ, and AL collected the data; XL performed the statistical analysis and drafted the manuscript; XL, JEM, HDS, LW, and SL critically revised the manuscript for important intellectual content; and SL supervised all the work and had primary responsibility for the final content. All authors read and approved the final manuscript.

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