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Evaluation of blood pressure lowering effects of cocoa flavanols in diabetes mellitus: A systematic review and meta-analysis

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ABSTRACT

In healthy people, cocoa-derived flavanols (CF) improve blood pressure (BP). This meta-analysis investigates whether CF also affect BP in diabetic patients. PubMed, Web of Science, and Embase were consulted to retrieve eligible randomized controlled trials. A random-effects model and the Grades of Recommendation, Assessment, Development and Evaluation (GRADE)-approach were used for analyses and quality of evidence respectively. Of 267 citations, 11 trials were identified, studying either type 2 diabetic populations only (subgroup A) or type 2 diabetic patients plus non-diabetic subjects with increased cardiovascular risk (subgroup B1) or type 1 plus type 2 diabetic patients (subgroup B2). Mid/long-term CF consumption decreased BP slightly, however, only reaching statistical significance for diastolic BP in subgroup B1 (-1.89 mmHg, 95% CI: −3.24, −0.54, I^2 = 55%). Considerable heterogeneity between studies and low quality of evidence caused poor quality evidence of minimal effects of CF ingestion on BP in diabetic patients.

1. Introduction

According to the European Food Safety Authority (EFSA), flavanols derived from the seeds of *Theobroma cacao*, the cocoa bean, help to preserve endothelium-dependent vasodilation in healthy populations, if ingested in quantities exceeding 200 mg cocoa-derived flavanols (CF)/day. This equals 10 g high-flavanol dark chocolate or 2.5 g high-flavanol cocoa powder (EFSA Panel on Dietetic Products et al., 2012). However, it is unclear to what extent CF also enhance vasodilation and other vascular functions in people with increased cardiovascular risk, such as hypertension and diabetes mellitus (DM).

Flavanols are natural substances from the flavonoid family, a class of polyphenols (Manach et al., 2004), which can be found in cocoa products, but also in several fruits, beans, teas, and red wines (Arts et al., 2000; Manach et al., 2004).

In vitro- and animal studies, as well as reports from healthy volunteers have, indeed, suggested that CF improve cardiovascular health by enhancing endothelial function (Engler et al., 2004; Schroeter et al., 2006), inhibiting angiotensin converting enzymes (Actis-Goretta et al., 2006; Persson et al., 2011), lowering blood pressure (BP) (Ried et al., 2017; Taubert et al., 2007), influencing various inflammatory processes (Goya et al., 2016), and preventing platelet aggregation (Bordeaux et al., 2007; Hermann et al., 2006). Epicatechin, a highly active monomeric form of CF, is believed to be mainly responsible for these vascular effects, although this is still debated (Aprotosoaie, Miron, et al., 2016; Rodriguez-Mateos et al., 2018; Schroeter et al., 2010).

Abbreviations: BP (SBP – DBP), Blood Pressure (Systolic – Diastolic); BMI, Body Mass Index; CF, Cocoa-derived flavanols; CI, Confidence Interval; DM (T1DM – T2DM), Diabetes Mellitus (type 1 Diabetes Mellitus - type 2 Diabetes Mellitus); GRADE, Grades of Recommendation, Assessment, Development and Evaluation; I^2, Heterogeneity; NO, Nitric Oxide; RCT, Randomized-controlled trial.

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In addition, CF are suggested to act as antioxidants (Keen et al., 2005). In vivo, CF increase plasma antioxidant capacity (Rein et al., 2000) and reduce lipid peroxidation in humans (Wiswedel et al., 2004). CF also increase bioavailability of nitric oxide (NO) (Heiss et al., 2003; Heiss et al., 2005), by inhibiting endothelial Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase and by enhancing endothelial Nitric Oxide Synthase (eNOS) production and activity (Schewe et al., 2008).

The latter is of particular interest. In type 1 and type 2 DM (T1DM and T2DM respectively), NO-depletion is considered crucial in the development of DM-associated hypertension and vascular complications (Giacco et al., 2010; Honing et al., 1998). Therefore, CF could potentially influence the development and/or progress of DM-associated vascular complications in particular; given the high world-wide prevalence of DM and its associated vascular complications, this could have a serious preventive and/or therapeutic impact.

Yet, little research has been performed specifically on CF-induced vascular benefits in DM; the available reports study relatively small samples, have divergent study designs, and yield inconclusive results (Ayoobi et al., 2017; Dicks et al., 2018; Mellor et al., 2010; Rynarzewski et al., 2019). Considering the theoretical background, the potential impact, and the promising results in healthy and hypertensive subjects (Cooper et al., 2008; Hooper et al., 2008; Ried et al., 2017), we therefore performed a *meta*-analysis to evaluate the evidence for an effect of CF on BP reduction and/or improvement of vascular function in patients with T1DM and/or T2DM. We only focused on CF and only randomized-controlled trials were considered for inclusion.

2. Materials and methods

This *meta*-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2015) and was registered in PROSPERO, a database of systematic review protocols (registration number: CRD42018112229, https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=112229).

2.1. Literature sourcing

Keywords, Medical Subject Headings (MeSH) terms, and synonyms were inserted in 3 electronic databases (PubMed, Web of Science, and Embase) to identify potentially relevant studies published up to August 13th, 2020. The search terms included: diabetes mellitus, chocolate, cocoa flavanols, epicatechin, catechin, and vascular functioning (e.g. vascular stiffness, vascular resistance, blood pressure, blood circulation, and endothelial function) (Table S1). A manual search in reference lists of the included studies was also conducted. No limitations on language or date of publication were set. All search strategies are presented in Table S2.

2.2. Study selection

As described in PROSPERO and our literature search (Tables S1 and S2), eligible studies included randomized controlled trials (RCT) investigating vascular effects of CF administration, regardless of duration of intake, in patients with all types of DM. Citations were excluded if no full report of original research was published (e.g. protocols, letters, and guidelines) or if the full text was unavailable. After removal of duplicates, 2 researchers (KVW, AT) screened titles and abstracts, and subsequently full texts independently (κ-coefficient 0.94). In case of disagreement the authors deliberated until consensus was reached.

As is customary, we considered a *meta*-analysis of 4 or less publications to be unreliable. Because parameters of vascular function yielded less than 4 publications, we only focused on BP as a primary outcome. Thus, we could analyze 11 papers with comparable study populations (patients with DM) and intervention (mid/long-term administration of flavanols extracted from the cocoa bean only).

2.3. Data extraction

The following data were extracted from the included papers: (1) author, year of publication, and study design, (2) study population (intervention versus control group), (3) relevant information concerning the flavanol intervention (form and content/ day), (4) relevant information concerning the control treatment, (5) frequency of ingestion (single dose versus split-doses), (6) time (duration of intervention), (7) method of BP measurement, (8) miscellaneous information, and (9) effect on BP. These were outlined in an evidence table by 2 researchers (KVW, AT), independently. A third researcher (PC) adjudicated in case of disagreement.

A subdivision was made based on the populations studied in each paper, i.e. either T2DM patients only (subgroup A, 5 papers (Ayoobi et al., 2017; Curtis et al., 2013; Dicks et al., 2018; Mellor et al., 2010; Rostami et al., 2015)), non-diabetics plus T2DM combined (subgroup B1, 4 papers (Desideri et al., 2012; Gutiérrez-Salmeán et al., 2016; Mastroiacovo et al., 2015; Sorond et al., 2013)), or non-diabetics, T1DM, and T2DM combined (subgroup B2, 2 papers (Desch et al., 2010; Monagas et al., 2009)). If provided, the percentage of each type included in each paper is outlined in the evidence table (Table 1).

2.4. Risk of bias and quality of evidence

To assess the risk of bias within studies, the revised Cochrane risk-of-bias tool for randomized trials (RoB 2) was used. Five different domains on potential biases were evaluated independently by 2 researchers (PC, AT) and, in case of disagreement, by a third researcher (AVG): bias arising from (1) the randomization process, (2) deviations from intended interventions, (3) missing outcome data, (4) measurement of the outcome, and (5) selection of the reported result. Each domain was rated as 'low risk', 'some concerns' or 'high risk', based on signaling questions and on the associated Cochrane guidelines (Higgins et al., 2019). Afterwards, an overall risk of bias could be adjudicated for each publication by summation of the ratings on each domain. In case of a crossover (CO)-study, the signaling questions of each domain were adapted according to the Cochrane guidelines (Higgins et al., 2019).

No citation was excluded for the analyses based on risk of bias.

Finally, the strength of the body of evidence was assessed using the Grades of Recommendation, Assessment, Development and Evaluation (GRADE)- approach (Higgins et al., 2019). Publication bias was evaluated in total sample of included papers using the Egger's test (Egger et al., 1997) in R (version.string R version 3.6.1, 2019–07-05), however, also visually in subgroups through funnel plots.

2.5. Data synthesis and analysis

Data synthesis was performed by one researcher (AT), supervised by another researcher (AVG). Review Manager (RevMan) (Computer program, Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used for data analyses and creation of Figs. 2, 3, 4, and 5 and Supplementary Figures S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, and S15. A random-effects model was chosen a priori since not all papers had a study population of solely patients with DM and we anticipated that not all interventional or placebo supplementations would be similar in dose, substance, and form.

Standardization of the mean difference or the mean standard deviation was not necessary, since all studies reported SBP and DBP values in mmHg. Treatment effects of CF on outcomes were calculated from differences in mean changes (calculator in RevMan and Cochrane guidelines and formulae (Higgins et al., 2019)) within treatment groups and implementing hedges g to account for smaller study samples. Values pre- and post-intervention were analyzed separately except for 1 study (Monagas et al., 2009), who did not report baseline values for each

Table 1
Table of evidence and characteristics of included studies.

Paper /Study Design	Study Population		Flavanol Intervention		Control treatment	Frequency	time	BP assessment[1]	Miscellaneous	Effect on BP[2]
	Intervention group	Control group	Form	Flavanol content/ day						
Subgroup A: T2DM only										
Ayoobi et al., 2017* (Ayoobi et al., 2017) RCT - SB	• T2DM for 4.1 ± 0.3 y • 14 F, 7 M • Age: 50.6 ± 1.6 y	• T2DM for 3.8 ± 0.3 y • 13 F, 10 M • Age: 50.7 ± 1.6 y	30 g 84% dark chocolate	no information	no intervention	1x /d	8 w	sitting; after 10 min rest; frequency not specified	• oral anti-DM drugs only • no info on anti-HT drugs • HbA1c not reported	• SBP ↓ (-7.2 ± 1.6 mmHg) • DBP ↓ (-6.3 ± 1.7 mmHg)
Curtis et al., 2013 * (Curtis et al., 2013)RCT- DB	• T2DM for 5.0 y (median, 95% CI: 4.9; 9.2) • 47 F • Age: 62.1 ± 0.7 y • HbA1c: 7.1 ± 0.1%	• T2DM for 5.0 y (median, 95% CI: 4.4; 7.2) • 46 F • Age: 63.0 ± 0.8 y • HbA1c: 7.3 ± 0.2%	27 g flavonoid enriched chocolate	• flavan-3-ols: 850 mg • EC: 90 mg • Isoflavones: 100 mg	placebo chocolate (no info on flavanol content)	2x /d (lunch + evening)	52 w	• Aortic central BP, Ambulatory BP • 2 h, 10 min intervals	• postmenopausal women only • insulin allowed • anti-HT drugs allowed	No change
Dicks et al., 2018 (Dicks et al., 2018)RCT- DB	• T2DM for 6.7 ± 1,4 y • 10 F, 7 M • Age: 65.6 ± 2.6 y • HbA1c: 6.4%	• T2DM for 7.2 ± 1.0 y • 7 F, 11 M • Age: 62.8 ± 1.6 y • HbA1c: 6.5%	5 × 0.5 g cocoa powder capsules	• FL: 207.5 mg, • EC: 40.4 mg • C: 13.6 mg	5 × 0.5 g pure microcrystalline cellulose	3 in morning, 2 in evening	12 w	2 (3) measurements, 1–2 min intervals; fasting state	• all subjects had HT • oral drugs only	No change
Mellor et al., 2010 * (Mellor et al., 2010)CO– DB	• T2DM for 18.0 ± 1.4 *m* • 5 F, 7 M • Age: 68 y (median, range 42–71) • HbA1c: 6.4 ± 0.2%	cross-over	45 g high polyphenol chocolate, 85% cocoa solids	• EC: 16.6 mg	low polyphenol chocolate (<2 mg EC)	3x /d	8 w	sitting; after 10 min rest; frequency not specified; fasting state	• oral drugs only	No change
Rostami et al., 2015* (Rostami et al., 2015) RCT- DB	• T2DM for 7.5 ± 0.8 *m* • 20 F, 12 M • Age: 58.7 ± 1.6 y • HbA1c: 7.2 ± 0.2%	• T2DM for 7.9 ± 0.7 *m* • 16 F, 12 M • Age: 57.2 ± 1.5 y • HbA1c 7.6 ± 0.2%	25 g dark chocolate, 83% cocoa solids	• flavonoids: 450 mg	white chocolate (no flavonoids)	1x /d	8 w	sitting; after 10 min rest; 2 measurements	• all subjects had HT • oral drugs only	• SBP ↓ (-6.6 ± 1.9 mmHg) • DBP ↓ (-4.9 ± 1.9 mmHg)
Subgroup B1: mixed population with T2DM										
Desideri et al., 2012 (Desideri et al., 2012) RCT- DB	• Elderly, mild cognitive impairment, 2 groups a) high FL dose: 16 F, 14 M; Age: 71.2 ± 0.9 y; 16% T2DM; b) intermediate FL dose: 17 F, 13 M; Age: 71.3 ± 0.8 y; 20% T2DM	• Elderly, mild cognitive impairment, 1 group c) low FL dose:14 F, 16 M; Age: 71.0 ± 0.8 y; 23% T2DM	Cocoa drink with intermediate OR high FL content	• High: • FL: 993 mg • EC: 185 mg • C: 62 mg • Other: 41 g caffeine, 458 mg theobromine • Intermediate: • FL: 520 mg • EC: 95 mg • C: 35 mg • Other: 44 g caffeine, 429 mg theobromine	Cocoa drink with low FL content • FL: 48 mg • EC: 5 mg • C: 8 mg • Other: 46 g caffeine, 400 mg theobromine	1x /d (morning)	8 w	sitting; after 15 min rest, 4 measurements, non-fasting state	• 70% of high, 73% of intermediate, and 77% of low FL had HT • oral drugs only • no info on duration of DM or HbA1c	• High FL: SBP ↓ (-10.0 ± 0.6 mmHg) ; DBP ↓ (-4.8 ± 0.3 mmHg) • Intermediate FL SBP ↓ (-8.2 ± 0.6 mmHg) ; DBP ↓ (-3.4 ± 0.4 mmHg) • Low FL: SBP ↓ (-1.4 ± 1.0 mmHg) ; DBP ↓ (-0.9 ± 0.6 mmHg) • Effect of High > intermediate > low (p = 0.0018 for SBP and 0.007 for DBP)
Gutiérez-Salmeán et al., 2016 (Gutiérrez-Salmeán et al., 2016)RCT- DB	• Hyper-triglyceridemia • 20 F/M • Age: 18–55 y	• Hyper-triglyceridemia • 10 F/M • Age: 18–55 y	Hard gelatin capsules	• EC: 100 mg	Hard gelatin capsules, inactive placebo	2x /d 2 capsules lunch-dinner (30 min before meal)	4 w	frequency not specified	• insulin use, HT, and use of betablockers were exclusion criteria • % DM not specified	No change

(*continued on next page*)

Table 1 (*continued*)

Paper /Study Design	Study Population		Flavanol Intervention		Control treatment	Frequency	time	BP assessment[1]	Miscellaneous	Effect on BP[2]
	Intervention group	Control group	Form	Flavanol content/ day						
									• no info on duration of DM or HbA1c	
Mastroiacovo et al., 2015 (Mastroiacovo et al., 2015) RCT- DB	• Elderly, cognitive intact, 2 groups: a) high FL dose:18 F, 12 M; Age: 70.0 ± 0.9 y; 16% T2DM; b) intermediate FL dose: 19 F, 11 M; Age: 68,7 ± 0.7 y; 16% T2DM	• Elderly, cognitive intact, 1 group: c) low FL dose:16 F, 14 M; Age: 70.0 ± 0.8 y; 23% T2DM	Cocoa drink with intermediate OR high FL content	• High: • FL: 993 mg • EC: 185 mg • C: 62 mg • Other: 41 g caffeine, 458 mg theobromine • Intermediate: • FL: 520 mg • EC: 95 mg • C: 35 mg • Other: 44 g caffeine, 429 mg theobromine	Cocoa drink with low FL content • FL: 48 mg • EC: 5 mg • C: 8 mg • Other: 46 g caffeine, 400 mg theobromine	1x /d (morning)	8 w	sitting; after 15 min rest, 4 measurements, non-fasting state	• 53% of high, 63% of intermediate and 50% of low FL had HT • oral drugs only • no info on duration of DM or HbA1c	• High FL: SBP ↓ (-7.8 ± 0.6 mmHg) ; DBP ↓ (-4.8 ± 0.4 mmHg) • Intermediate FL SBP ↓ (-6.8 ± 0.6 mmHg) ; DBP ↓ (-3.2 ± 0.4 mmHg) • Low FL: SBP ↓ (-1.6 ± 1.1 mmHg) ; DBP ↓ (-1.6 ± 0.7 mmHg) • Effect of High > intermediate > low ($p < 0.0001$ for SBP and DBP)
Sorond et al., 2013 (Sorond et al., 2013) RCT-DB	• Elderly • 31 F, 29 M • Age: 72.9 ± 0.7 y • 53.3% T2DM	Equals intervention	FL-rich cocoa powder + water	• FL: 1218 mg	FL-poor cocoa powder + water; • FL: 26 mg /d	2x /d	4 w	median of 3 values	• 90% had HT • no info on anti-DM or anti-HT drugs • no info on duration of DM or HbA1c	No change
Subgroup B2: mixed population with T1DM and T2DM										
Desch et al., 2010 (Desch et al., 2010) RCT-SB	• CV-risk • 3 F, 40 M • Age: 65.2 ± 1.2 y • 30% DM T1 or T2	• CV-risk • 17 F, 31 M • Age: 66.8 ± 1.1 y • 46% DM T1 or T2	25 g dark Chocolate	• EC: 21 mg • C: 7 mg • Other: 12 mg caffeine, 105 mg theobromine	6 g dark chocolate • EC: 5 mg • C: 1.7 mg • Other: 3.1 mg caffeine, 26.4 mg theobromine	1x /d, 2 h post-dinner	12 w	• 24-h BP, 30-min intervals	• 99% use anti- HT drugs • insulin allowed • no info on duration of DM or HbA1c	• SBP ↓ in both groups (25 g/d: −2.8 ± 1.1 mmHg; 6 g/d: −3.4 ± 0.5 mmHg), NS difference between 2 groups • DBP ↓ only in control group (6 g/d: −1.8 ± 0.4 mmHg), NS difference between 2 groups
Monagas et al., 2009 (Monagas et al., 2009) CO-SB	• DM or CV-risk factors • 23 F, 19 M • Age: 69.7 ± 1.8 y	cross-over	2 × 20 g cocoa powder + 250 ml skim milk	• EC: 46.08 mg • C: 10.41 mg • Other: 0.44 g theobromine	Skim milk, 500 ml/d	2x /d	4 w	3 measurements	• no info on anti-DM or anti-HT drugs • % DM not specified • no info on duration of DM or HbA1c	No change

Data are expressed in mean ± SEM unless described differently. Subgroup A = papers investigating diabetic populations only; subgroup B1 = papers investigating mixed populations, i.e. type 2 diabetic plus non-diabetic; subgroup B2 = papers investigating mixed populations, i.e. type 1 and/or 2 diabetic plus non-diabetic.

* papers performed in a geographical area in which dietary average flavanol intake is high (>400 mg/day); [1]all blood pressures were measured using automated sphygmomanometers; [2]only significant changes for blood pressure are reported; BP = blood pressure (systolic SBP, diastolic DBP); C = catechins; CI = confidence interval; CO = crossover trial; CV = Cardiovascular; d = day; DB = double blinded trial; DM = Diabetes Mellitus (T1 = type 1, T2 = type2); EC = epicatechins; FL = flavanols; h = hours; HT = hypertension; min = minutes; m = months; NS = non-significance; RCT = Randomized Controlled Trial; rep = repetitions; S = significance; SB = single blinded trial (only investigators are blinded); w = weeks; y = year

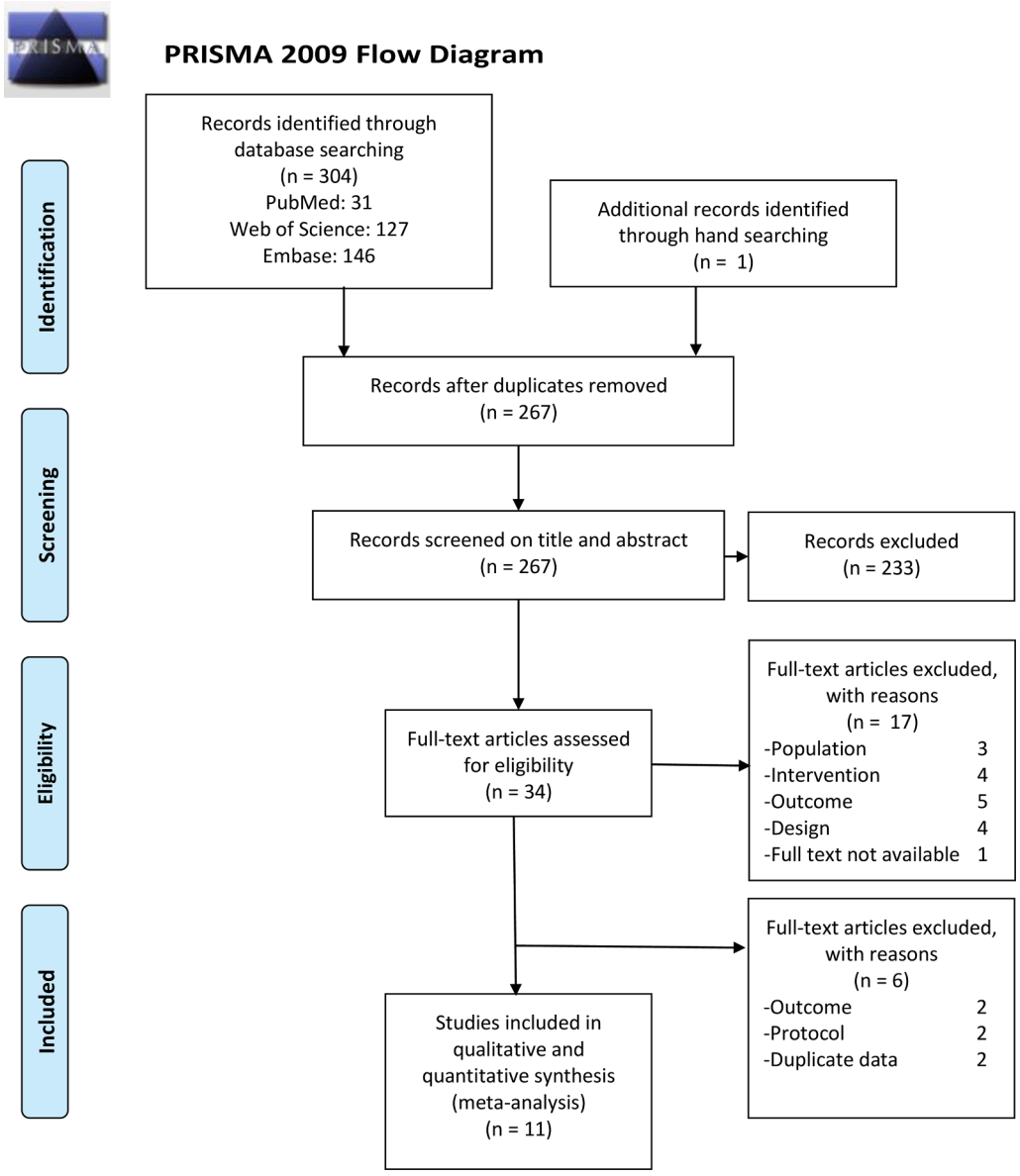

Fig. 1. Flow diagram showing the study selection process.

group. If certain information was not provided in an included paper, we followed Cochrane guidelines (Higgins et al., 2019) and used the calculator in RevMan to calculate the required data. When studies did not describe mean treatment effect scores and related standard deviations/ standard errors (Desch et al., 2010; Dicks et al., 2018; Gutiérrez-Salmeán et al., 2016; Mellor et al., 2010; Monagas et al., 2009; Sorond et al., 2013), effect scores were obtained by subtracting the final mean from the baseline mean and related standard deviations were computed using formulae provided by the Cochrane guidelines (Higgins et al., 2019). Since all correlation coefficients were above 0.5, we had to impute standard deviations for changes from baseline when not reported. Spearman correlation coefficients were used because these are the most conservative. Furthermore, 2 studies reported on 3 different intervention groups (Desideri et al., 2012; Mastroiacovo et al., 2015). Data of these interventions were combined as defined in the Cochrane handbook (Higgins et al., 2019).

The included publications were parallel RCT, apart from 2 CO-studies (Mellor et al., 2010; Monagas et al., 2009). Hence, 'generic inverse variance' was applied in RevMan as a method of analysis (Curtin et al., 2002; Elbourne et al., 2002).

If SBP and DBP were not reported separately (Balzer et al., 2008; Ramirez-Sanchez et al., 2013), or if relevant data could not be calculated (Ayoobi et al., 2017), corresponding authors were contacted by e-mail. In case of no answer, papers were excluded. In addition, if papers described a mixed population of whom only a certain percentage had T1DM or T2DM (Desch et al., 2010; Desideri et al., 2012; Gutiérrez-Salmeán et al., 2016; Mastroiacovo et al., 2015; Monagas et al., 2009; Sorond et al., 2013), corresponding authors were contacted by e-mail to obtain separate results for non-diabetic and diabetic subjects, with subdivision depending on type of DM. In case of no answer, the publication was included but data of the population (non-diabetic and diabetic subjects) was used in its entirety to evaluate the effect of CF.

Treatment effects of mid/long-term intake of CF on DBP and SBP were analyzed in the total group and then separately in each subgroup: subgroup A (T2DM only), B1 (non-diabetic plus T2DM), and B2 (non-diabetic, T1DM, and T2DM). To further examine which factors might explain possible heterogeneity in these results, the following additional subgroup analyses were performed on the total of included papers: split-dose versus single dose administration, dose of epicatechins administered/ day, composition of the placebo formula, BP at baseline, sex, age,

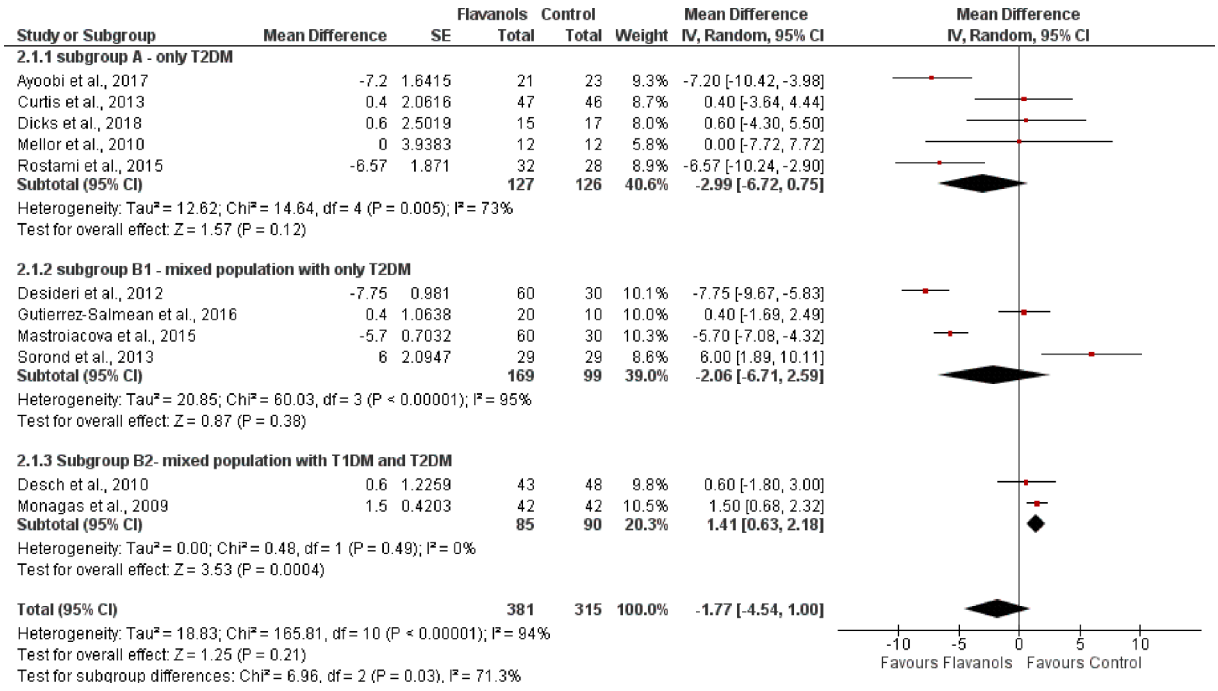

Fig. 2. Forest plot Systolic Blood Pressure. Solid diamonds represent the pooled estimates for each subgroup (A, B1 and B2) and in total; horizontal lines represent 95% confidence interval; red dots represent point estimate of each study; I^2 and p values for heterogeneity and subgroup differences are shown. Subgroup A = papers investigating diabetic populations only; subgroup B1 = papers investigating mixed populations, i.e. type 2 diabetic plus non-diabetic; subgroup B2 = papers investigating mixed populations, i.e. type 1 and/or 2 diabetic plus non-diabetic.

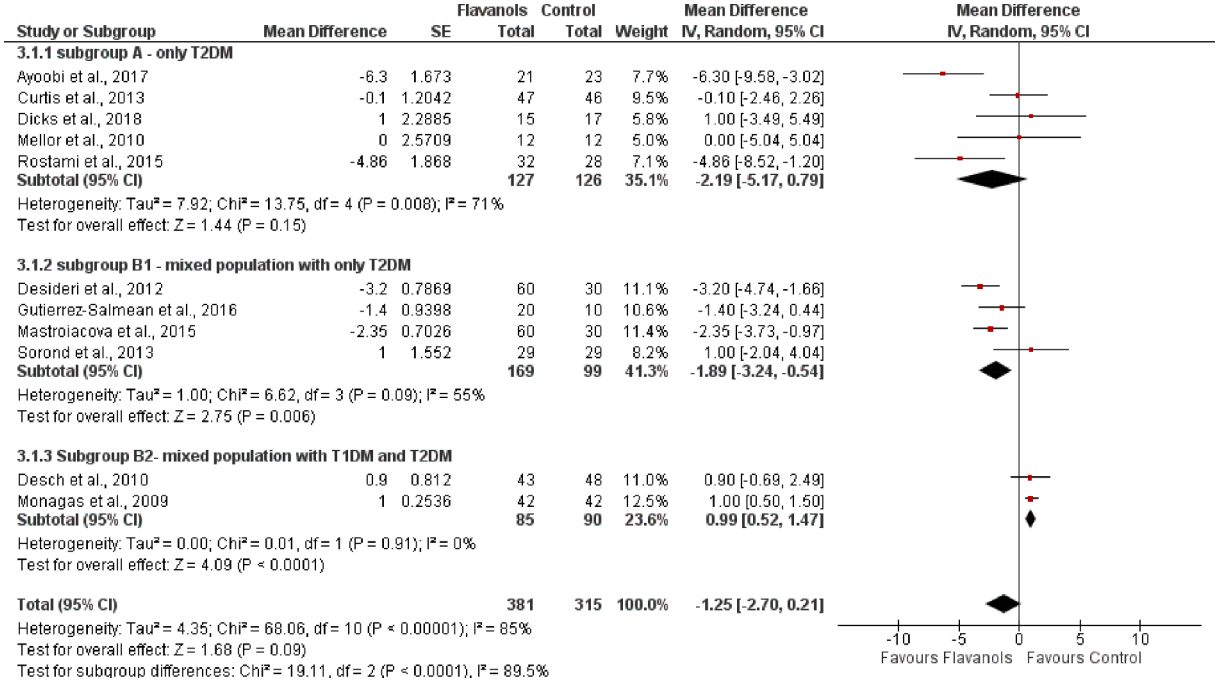

Fig. 3. Forest plot Diastolic Blood Pressure. Solid diamonds represent the pooled estimates for each subgroup (A, B1 and B2) and in total; horizontal lines represent 95% confidence interval; red dots represent point estimate of each study; I^2 and p values for heterogeneity and subgroup differences are shown. Subgroup A = papers investigating diabetic populations only; subgroup B1 = papers investigating mixed populations, i.e. type 2 diabetic plus non-diabetic; subgroup B2 = papers investigating mixed populations, i.e. type 1 and/or 2 diabetic plus non-diabetic.

body mass index (BMI), and geographical differences in average daily flavanol intake. The cut-off point for age was based on the conventional definition of 'elderly' (Orimo et al., 2006) and the cut-off point for geographical differences in average daily flavanol intake was based on a report identifying regions with high (>400 mg) versus low (<400 mg) dietary flavanol ingestion (Escobar-Cévoli et al., 2017). Subgroup analyses based on percentage of cocoa, total daily dose of CF or medication use were not possible because data were either lacking or too heterogenous to analyze. For each analysis, the level of statistical heterogeneity (I^2, < 40%: might not be important and ≥ 75%: considerable

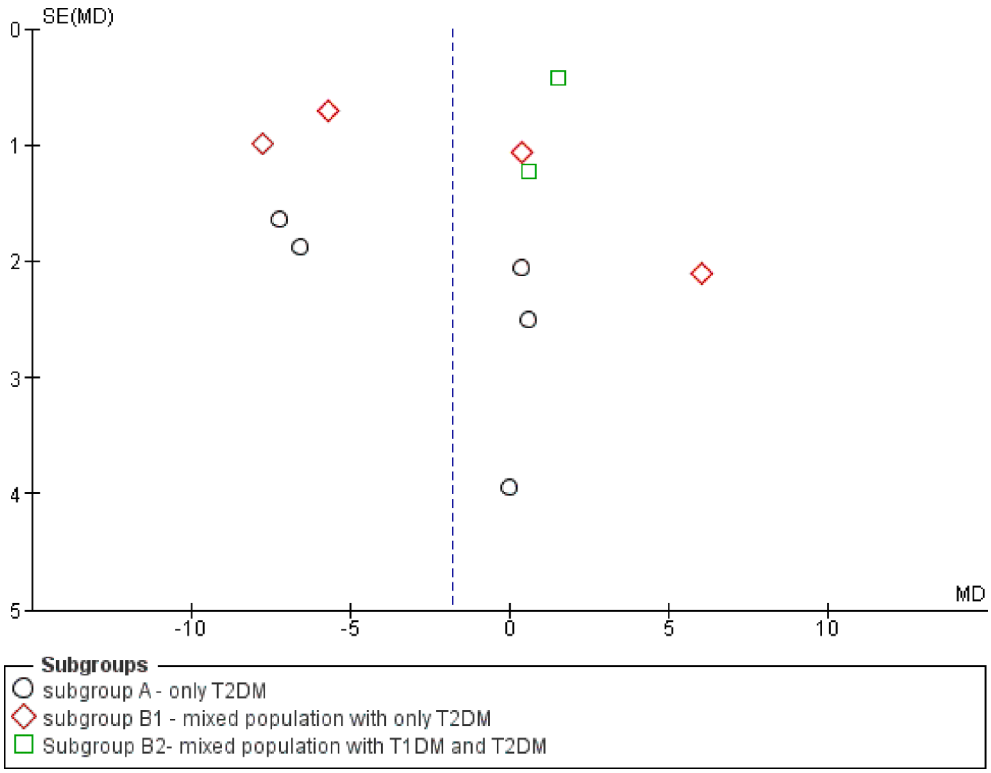

Fig. 4. Funnel plot Systolic Blood Pressure . The x-axis represents the mean difference; the y-axis represents the standard error of the mean difference; black open circles = subgroup A = papers investigating diabetic populations only; red open diamonds = subgroup B1 = papers investigating mixed populations, i.e. type 2 diabetic plus non-diabetic; green open squares = subgroup B2 = papers investigating mixed populations, i.e. type 1 and/or 2 diabetic plus non-diabetic.

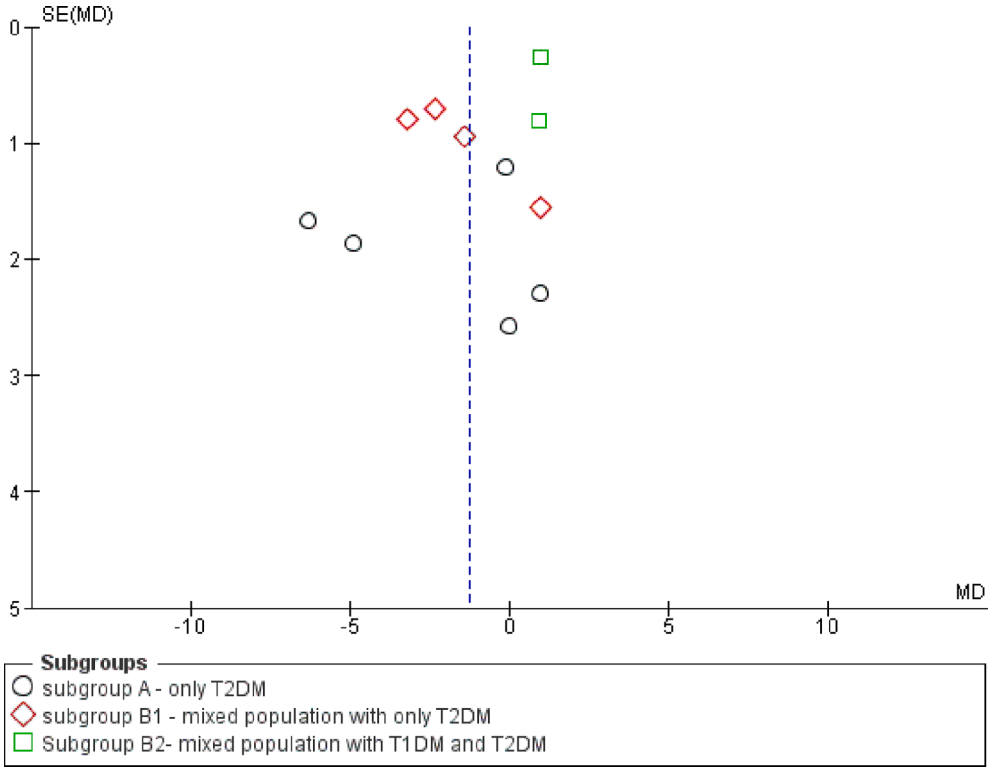

Fig. 5. Funnel plot Diastolic Blood Pressure. The x-axis represents the mean difference; the y-axis represents the standard error of the mean difference; black open circles = subgroup A = papers investigating diabetic populations only; red open diamonds = subgroup B1 = papers investigating mixed populations, i.e. type 2 diabetic plus non-diabetic; green open squares = subgroup B2 = papers investigating mixed populations, i.e. type 1 and/or 2 diabetic plus non-diabetic.

heterogeneity (Higgins et al., 2019)) was described. The level of statistical significance was set at an alpha-level below 0.05. Results are reported and depicted in Forest plots (mean difference and 95% CI).

3. Results

266 papers were identified for screening after literature search and 1 was additionally included after a manual search (Mastroiacovo et al., 2015) (Fig. 1). 233 citations were excluded after screening on title and abstract, and another 17 after subsequent screening on full text. 6 additional reports were excluded: 2 for not providing separate data on SBP and DBP (Balzer et al., 2008; Ramirez-Sanchez et al., 2013), 2 for presenting BP data that were also published elsewhere (Curtis et al., 2012; Haghighat et al., 2013), and 2 for investigating one-time (versus mid/long-term) CF administration (Basu et al., 2015; Rynarzewski et al., 2019).

3.1. Characteristics of eligible studies

All papers were published between 2009 and 2018 (Table 1). Sample sizes ranged from 12 − 60 in the intervention groups and from 10 − 48 in the control groups. All but one study (Curtis et al., 2013) included both sexes. Except in one publication (Gutiérrez-Salmeán et al., 2016), the mean age of each study population was > 50 years old and the greater part of subjects used antihypertensive (Curtis et al., 2013; Desch et al., 2010; Desideri et al., 2012; Dicks et al., 2018; Mastroiacovo et al., 2015; Mellor et al., 2010; Rostami et al., 2015) and antidiabetic drugs (Ayoobi et al., 2017; Curtis et al., 2013; Desch et al., 2010; Desideri et al., 2012; Dicks et al., 2018; Mastroiacovo et al., 2015; Mellor et al., 2010). Only in subgroup A, papers reported mean disease duration (7 months − 7 years) and mean baseline HbA1c values (6.4% − 7.6%) of the patients with T2DM. In other subgroups, these data were absent.

The intervention and placebo formulae varied considerably between the different studies as to the durations of CF intake (4 weeks − 1 year), as well as the CF-containing products used, and the administered amounts and frequencies. CF was administered in the form of dark chocolate (24–45 g), cocoa powder (2.5–58 g) or capsules, containing 207.5 mg – 993 mg flavanols and/or 16.6 mg – 185 mg epicatechins per day; they were given in one batch (Ayoobi et al., 2017; Desch et al., 2010; Desideri et al., 2012; Mastroiacovo et al., 2015; Rostami et al., 2015) or distributed over the day (Curtis et al., 2013; Dicks et al., 2018; Gutiérrez-Salmeán et al., 2016; Mellor et al., 2010; Monagas et al., 2009; Sorond et al., 2013). Placebo formulae were either not defined (Gutiérrez-Salmeán et al., 2016), or consisted of either no intervention (Ayoobi et al., 2017), only milk (Monagas et al., 2009), capsules with microcrystalline cellulose (Dicks et al., 2018), white (Rostami et al., 2015) or placebo (Curtis et al., 2013) chocolate, flavanol-poor chocolate (<2 mg epicatechin) (Mellor et al., 2010), flavanol-poor cocoa powder (26 mg flavanols (Sorond et al., 2013) or 48 mg flavanols and 5 mg epicatechin (Desideri et al., 2012; Mastroiacovo et al., 2015)) or a minute quantity of dark chocolate (6 g containing 5 mg epicatechin) (Desch et al., 2010).

Finally, very little information was provided on time of BP measurement and time between ingestion of antihypertensive medication and BP assessment. In addition, the methods of BP measurements varied between the included papers: 2 averaged two measurements (Dicks et al., 2018; Rostami et al., 2015), 4 assessed the mean of 3–4 times (Desideri et al., 2012; Mastroiacovo et al., 2015; Monagas et al., 2009; Sorond et al., 2013), 1 measured for 2 h with 10 min intervals (Curtis et al., 2013), 1 measured for 24 h with 30 min intervals (Desch et al., 2010), and 3 did not specify the frequency (Ayoobi et al., 2017; Gutiérrez-Salmeán et al., 2016; Mellor et al., 2010). Timing of the measurements was only reported by 4 citations, of which 2 measured in fasting state (Dicks et al., 2018; Mellor et al., 2010) and 2 in non-fasting state (Desideri et al., 2012; Mastroiacovo et al., 2015).

3.2. Risk of bias of eligible studies

The major reason for scoring papers as 'high risk' (Ayoobi et al., 2017; Curtis et al., 2013; Gutiérrez-Salmeán et al., 2016; Monagas et al., 2009; Sorond et al., 2013) or 'some concerns' (Desch et al., 2010; Dicks et al., 2018; Mellor et al., 2010; Rostami et al., 2015) was based on a negative score on domain 2 (bias due to deviations from intended interventions) and/or domain 5 (bias due to selection of the reported results) (Table S3). If the placebo differed visibly from the CF source (e.g. white chocolate versus dark chocolate (Rostami et al., 2015)), the impossibility of blinding participants and researchers led to a negative score on domain 2. Moreover, most trials were not analyzed in accordance with a pre-specified plan, which was finalized before unblinded outcome data were available for analysis (domain 5).

Based on the overall GRADE assessment (Table S4), the quality of the body of evidence appeared to be low (Schünemann et al., 2013). This appraisal can partly be explained by inconsistencies in results between studies (Table S4, Figs. 2 and 3). In addition, the funnel plot for DBP is rather symmetrical, but the funnel plot for SBP seems relatively asymmetrical, which could indicate publication bias (Figs. 4 and 5). However, based on the Egger's test, no publication bias is present in total sample of included studies, nor for SBP ($p = 0.50$), nor for DBP ($p = 0.06$).

3.3. SBP

No statistically significant effect on SBP was seen in subgroup A, subgroup B1 or in group A + B together (Fig. 2): mean treatment effect was –2.99 mmHg in subgroup A (95% CI : −6.72, 0.75, $I^2 = 73\%$, 127 participants in the intervention group and 126 participants in the control group), −2.06 mmHg in subgroup B1 (95% CI : −6.71, 2.59, $I^2 = 95\%$, 169 participants in the intervention group and 99 participants in the control group), and −1.77 mmHg in group A + B together (95% CI: −4.54, 1.00, $I^2 = 94\%$, 381 participants in the intervention group and 315 participants in the control group).

In subgroup B2 a statistically significant increase in SBP was observed (mean treatment effect + 1.41 mmHg, 95% CI : 0.63, 2.18, $I^2 = 0\%$, 85 participants in the intervention group and 90 participants in the control group).

3.4. DBP

CF induced a statistically significant decrease in DBP (Fig. 3) in subgroup B1 (mean treatment effect −1.89 mmHg; 95% CI : −3.24, −0.54, $I^2 = 55\%$, 169 participants in the intervention group and 99 participants in the control group).

There was no statistically significant effect of CF on DBP in either subgroup A (mean treatment effect −2.19 mmHg, 95% CI : −5.17, 0.79, $I^2 = 71\%$, 127 participants in the intervention group and 126 participants in the control group) or in group A + B together (mean treatment effect −1.25 mmHg, 95% CI: −2.70, 0.21, $I^2 = 85\%$, 381 participants in the intervention group and 315 participants in the control group).

Similarly to SBP, an increase in DBP in subgroup B2 was indicated (mean treatment effect + 0.99 mmHg, 95% CI : 0.52, 1.47, $I^2 = 0\%$, 85 participants in the intervention group and 90 participants in the control group).

3.5. Additional subgroup analyses

3.5.1. SBP

We performed additional subgroup analyses to assess the influence of various variables on our outcomes. These indicated that the effect of CF on SBP could have been influenced by single versus split-dose CF ingestion, the amount of epicatechins administered/ day, BP at baseline, sex, and usual dietary CF content. In studies administrating CF as a single dose, SBP decreased by 5.28 mmHg (95% CI : −8.15, −2.41, $I^2 = 87\%$), whereas a split-dose administration even increased SBP by 1.42

mmHg (95% CI : 0.26, 2.58), $I^2 = 20\%$) (Figure S1). Epicatechin content of 16 – 46 mg/ day, induced an increase of SBP by 1.37 mmHg (95% CI : 0.61, 2.14, $I^2 = 0\%$), while daily doses of epicatechins between 90 and 185 mg did not affect SBP (mean treatment effect: −3.36, 95% CI : −7.20, 0.47, $I^2 = 93\%$) (Figure S2). When at least 50% of all subjects in each paper had systolic hypertension (≥140 mmHg) at baseline, CF lowered SBP by 5.14 mmHg (95% CI: −7.51, −2.78, $I^2 = 69\%$), whereas no effect was observed when <50% had elevated SBP's at baseline (mean treatment effect + 0.23 mmHg 95% CI : −2.20, 2.65, $I^2 = 85\%$) (Figure S3). Furthermore, papers with a study population of >60% female participants showed a decrease of 5.04 mmHg (95% CI : −7.65, −2.42, $I^2 = 69\%$), whereas in trials with equal sex distribution (mean treatment effect −0.06 mmHg, 95% CI : −5.33, 5.21, $I^2 = 95\%$) no impact of CF on SBP was identified (Figure S4).

Subgroup analysis based on the geographical area in which the studies were performed indicated that in areas in which average daily flavanol ingestion normally exceeds 400 mg (e.g. Iran, UK, and Poland), CF tended to lower blood pressure by 3.86 mmHg (95% CI : −7.92, 0.20, $I^2 = 72\%$, $p = 0.06$). Contrastingly, in areas in which no more than 400 mg of flavanols are generally ingested daily (e.g. Germany, Mediterranean countries, and US), CF administration did not influence SBP (mean treatment effect: −0.99, 95% CI: −5.00, 3.01, $I^2 = 96\%$) (Figure S5).

The equilibration of intervention and placebo formula with caffeine and theobromine, age or BMI, did not seem to influence the effect of CF on SBP (Figures S6, S7, and S8).

3.5.2. DBP

Similarly, subgroup analyses showed that CF effect on DBP could have been influenced by single dose versus split-dose CF administration, the amount of epicatechins administered/ day, and sex, but in addition also by age, but not by dietary flavanol use. In studies with single CF doses, DBP dropped by 2.82 mmHg (95% CI : −5.00, −0.65, $I^2 = 83\%$), whereas split-dose studies showed no effect on DBP (mean treatment effect + 0.38 mmHg, 95% CI : −0.57, 1.33, $I^2 = 26\%$) (Figure S9). Epicatechin content ranging from 90 to 185 mg/ day decreased DBP by 1.99 mmHg (95% CI : −3.15, −0.83, $I^2 = 44\%$), while daily doses of epicatechins ranging from 16 to 46 mg increased DBP by 0.98 mmHg (95% CI : 0.51, 1.45, $I^2 = 0\%$) (Figure S10). Sex, again, appeared relevant: CF lowered DBP in papers with a study population of >60% female participants, −3.08 mmHg (95% CI: −5.45, −0.71, $I^2 = 72\%$), but not with equal sex distribution (mean treatment effect −0.20 mmHg, 95% CI : −2.58, 2.18, $I^2 = 85\%$) (Figure S11). Papers studying ages below 65 years found decreases in DBP by 2.87 mmHg (95% CI : −5.52, −0.23, $I^2 = 75\%$), as opposed to publications on mean ages above 65 years, which observed no effect on DBP (mean treatment effect: −0.41 mmHg, 95% CI: −2.11, 1.29, $I^2 = 86\%$) (Figure S12).

The equilibration of intervention and placebo formula with caffeine and theobromine, BMI or geographical differences in dietary flavanol use, did not seem to influence the effect of CF on DBP (Figures S13, S14, and S15). Since all participants in each study had normal ranges for DBP at baseline, the influence of baseline elevated DBP could not be assessed in a subgroup analysis.

4. Discussion

The aim of this systematic review and *meta*-analysis was to identify whether CF affect BP and/or vascular function in patients with DM. The paucity of reports (<4 publications on comparable vascular functions), however, confined us to the effects on BP only. Our analysis shows low quality of evidence due to risk of bias, inconsistency and heterogeneity among the publications, and imprecision of the available reports (GRADE). At best, there are weak indications of slight improvement in SBP and DBP after mid/long-term CF ingestion. Possibly, CF effects are greater when they contain at least 90 mg of epicatechin, when given in a single dose, and when subjects are female, younger and hypertensive. However, as mentioned, no definite conclusions can be drawn, neither positive nor negative.

4.1. Effects of cocoa flavanols (CF) on blood pressure (BP)

Our *meta*-analysis suggests that CF reduces DBP, but not SBP, by ~ 1–2 mmHg. This is compatible with the postulated mechanism of action: if, indeed, CF effects are largely achieved through increased NO bioavailability (Aprotosoaie, Miron, et al., 2016; Schewe et al., 2008) (see above), this may primarily affect peripheral vascular resistance (Cooke & Dzau, 1997) and hence DBP. SBP is predominantly associated with cardiac output rather than peripheral vascular resistance (Bouman et al., 2008). Nonetheless, SBP also showed a slight decrease of 1–3 mmHg, but this did not reach statistical significance.

Although small, these changes may not be irrelevant (Cook et al., 1995; Stamler, 1991; Whelton et al., 2002). In general populations aged 35 to 64 years, reductions of 2 mmHg in DBP and 2–5 mmHg in SBP decreased mortality by 3–7% (Stamler, 1991) and the risk of developing diastolic hypertension, coronary heart diseases and stroke/transient ischemic attack by 17%, 6%, and 15% respectively (Cook et al., 1995). Of note, in our *meta*-analysis, 4 out of 11 studies found statistically significant reductions in SBP of ≥ 5.70 mmHg and in DBP of ≥ 2.35 mmHg (Ayoobi et al., 2017; Desideri et al., 2012; Mastroiacovo et al., 2015; Rostami et al., 2015).

4.2. Heterogeneity

As mentioned, however, there was marked clinical and methodological heterogeneity between the citations, both in the actual intervention (administered dose, daily frequency, and the nature of both intervention and placebo formulae), and in the population characteristics (sex, BMI, age, the stage of disease (concerning DM, hypertension or other cardiovascular conditions), use of antihypertensive/ antidiabetic medication, type of DM, and geographical location). All of these aspects may have influenced the outcomes.

4.2.1. Heterogeneity in intervention

In order to address this heterogeneity, we performed various subgroup analyses where possible. These suggested that SBP and DBP decreased with one-time daily, but not split- dose administration. This is not surprising considering the absorption time and half-life of CF: epicatechin concentrations reach their maximum 2 to 3 h after ingestion (Baba et al., 2000; Richelle et al., 1999). However, conclusions must be drawn with caution: possibly, the timing of the split-doses relative to the timing and technique of the BP measurement (single measurement, multiple measurements, 24-hour blood pressure monitoring) could have affected the effective (peak) plasma dose at the time of BP measurement and therefore the actual outcomes as well as their comparability.

This comparability is further challenged by the composition of intervention and placebo formulae. Some, but not all studies equilibrated their placebo formula to the intervention: in order to isolate CF effects from other vaso-active compounds of cocoa, they added theobromine and/or caffeine to the placebo formula (Aprotosoaie, Luca, et al., 2016; Echeverri et al., 2010). Although our subgroup analysis did not identify a statistical effect of equilibration, given the paucity of studies analyzed, we cannot exclude that equilibration clouded (i.e. underestimated) overall effects.

In addition, CF composition and dose varied; since it was not even reported in all studies, we directed our subgroup analysis on epicatechin content, which, interestingly, was reported much more precisely. Only 90 mg of epicatechins/ day or more lowered BP. Although, again, this conclusion must be drawn with caution given the limitations of our *meta*-analysis in general and the subgroup analyses in particular (see below 4.3), it underlines the relevance of the (sub)content of the administered CF. A significant fraction of flavanols, including epicatechin and its oligomeric forms (procyanidins), are metabolized by colonic microbiota before absorption (Del Rio et al., 2013) into

biologically active metabolites. Ex vivo, but not in vivo human studies, suggest that these metabolites could exert vascular effects in their own right, among others by reducing endothelial oxidative stress (Álvarez-Cilleros et al., 2018; Álvarez-Cilleros et al., 2018; Fernandez-Millan et al., 2014). However, the clinical relevance of the latter is unclear (Rodriguez-Mateos et al., 2018).

Furthermore, the studies did not correct for baseline dietary flavanol content, which was only assessed in 2 citations (Desideri et al., 2012; Mastroiacovo et al., 2015). We therefore performed a subgroup analysis based on geographical location of the studies as a next best option. Somewhat to our surprise, CF seemed to lower BP ($p = 0.06$) in high-flavanol areas (average daily ingestion of the general population $>$ 400 mg, e.g. Iran, UK, and Poland (Escobar-Cévoli et al., 2017)), but not in low flavanol areas (average daily ingestion $<$ 400 mg, e.g. Germany, Mediterranean countries, and US). Again, the meaning of this outcome is unclear, since geographical area was not the only difference between the trials, and, as mentioned, most results were not corrected for individual daily flavanol intake.

4.2.2. Heterogeneity in study population

Not surprisingly, the heterogeneity of the baseline characteristics of the investigated populations further challenges the comparability of the results. Our subgroup analyses did not identify effects of BMI, in contrast to sex and age: CF seemed more effective in lowering SBP and DBP in women than in men. This is compatible with previous reports on sex differences in the regulation of vascular tone (De Angelis et al., 2004; Thompson & Khalil, 2003), and might be a concept worth considering in future research and perhaps clinical practice. Similarly, DBP, but not SBP, decreased in people under, but not over 65 years of age. Ried et al. (2017) report similar findings, using a cut-off point of 50 years; the authors postulate that the structural arterial changes in the elderly subdue vascular reactivity to physiological stimuli (Ried et al., 2017).

Nevertheless, CF effects in general seem more pronounced in the presence of vascular dysfunction (Aprotosoaie, Miron, et al., 2016; Kerimi & Williamson, 2015). For instance, a *meta*-analysis in non-diabetic subjects showed better CF-induced BP reduction in hypertensive than pre-hypertensive people, whereas there was no effect in normotensive people (Ried et al., 2017). We therefore investigated the effect of baseline hypertension in the study populations of our *meta*-analysis. Although mean BP was described as normal in all publications (120–140/ 70–85 mmHg), almost all studies reported the use of antihypertensive medication in several subjects, and standard deviations of BP were relatively high (up to 17 for baseline-SBP and 10 for baseline-DBP (Mellor et al., 2010)). Hence, we may conclude that a considerable fraction of the participants had essential hypertension (SBP $\geq$ 140 and DBP $\geq$ 90 (Hypertension. (13 september, 2019)) at baseline. And indeed, our subgroup analyses suggested stronger BP lowering effects in presence of baseline systolic hypertension; analysis for DBP was, unfortunately, not statistically feasible.

We could not correct for the use of antihypertensive drugs per se, again due to data heterogeneity. Nonetheless, this could be relevant considering their intrinsic vasoactive effects which might have amplified or annihilated CF effects. The same is true for antidiabetic drugs and insulin, which may stimulate NO synthase by themselves (Ferrannini & Cushman, 2012) and therefore affect the outcome, and for factors on which little or no information was given, such as the duration of DM and the presence of diabetic vascular complications.

4.3. Limitations

As mentioned, our *meta*-analysis was limited by the small amount of publications, the relatively small sample size of each and the considerable clinical and methodological heterogeneity amongst the papers, even on important clinical aspects and confounders. We describe these different aspects and their possible influence on the outcomes in our discussion. The subgroup analyses, which we performed in order to gain more insight into these factors, were based on rather arbitrary cut-off points and were binary by design. Other cut-offs and/or non-binary correction for confounding factors could perhaps have yielded different results. Combined with the rather high risk of bias and inconsistency, this results in low quality of evidence.

Therefore, we feel that all results, particularly those of the subgroup analyses should be interpreted with caution; at best, they indicate directions on which future research could be based.

5. Conclusion

Although CF seem to be promising nutraceuticals with potential beneficial effects on vascular health in the general population, the clinical evidence corroborating this theoretical notion is very weak in DM.

Our *meta*-analysis, restricted to BP effects, suggests that, at best, there is weak evidence for a reduction of DBP, but not SBP, by 1–2 mmHg, after mid/long-term CF administration; these effects seem stronger in female, younger and hypertensive people, when CF is ingested in 1 daily batch, and when epicatechin content is high enough. However, the marked heterogeneity among the available small studies challenges the drawing of unequivocal conclusions. Nevertheless, the world-wide prevalence of DM and the associated cardiovascular morbidity warrant further exploration of the possible role CF might play in its therapy or prevention. Especially given the proven, consistent CF effects in healthy and hypertensive people as well as the theoretical background CFs influence on NO bioavailability, we highly recommend further research using larger sample sizes and correction for the above-mentioned confounders.

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CRediT authorship contribution statement

Anouk Tanghe: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Elsa Heyman:** Conceptuialization, Writing - review & editing. **Karsten Vanden Wyngaert:** Investigation, Validation, Writing - review & editing. **Ans Van Ginckel:** Formal analysis, Investigation, Methodology, Validation, Writing - review & editing. **Bert Celie:** Conceptualization, Writing - original draft. **Ernst Rietzschel:** Conceptualization, Writing - review & editing. **Patrick Calders:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Visualization, Writing - original draft. **Samyah Shadid:** Conceptualization, Project administration, Supervision, Visualization, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2021.104399.

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