UNIVERSIDADE FEDERAL DE JUIZ DE FORA

INSTITUTO DE CIÊNCIAS DA VIDA

DEPARTAMENTO DE NUTRIÇÃO

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Efeitos anti-inflamatórios da ingestão de cacau em pacientes com síndrome metabólica e distúrbios relacionados: uma revisão sistemática e meta-análise

Governador Valadares

2026

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Trabalho de Conclusão de Curso apresentado ao curso de Nutrição da Universidade Federal de Juiz de Fora como requisito parcial à obtenção do título de graduação em Nutrição.

Orientadora: Prof. Drª. Maísa Silva

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Ficha catalográfica elaborada através do programa de geração
automática da Biblioteca Universitária da UFJF,
com os dados fornecidos pelo(a) autor(a)

Santos Damião, Sabrina.
Efeitos anti-inflamatórios da ingestão de cacau em pacientes com síndrome metabólica e distúrbios relacionados: uma revisão sistemática e meta-análise / Sabrina Santos Damião. -- 2026.
37 p.

Orientador: Maísa Silva
Trabalho de Conclusão de Curso (graduação) - Universidade Federal de Juiz de Fora, Campus Avançado de Governador Valadares, Instituto de Ciências da Vida - ICV, 2026.

1. ensaios clínicos randomizados; . 2. cacau;. 3. síndrome metabólica;. 4. marcadores inflamatórios;. 5. meta-análise. I. Silva, Maísa, orient. II. Título.

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Aprovada em (dia) de (mês) de (ano)

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DEDICATÓRIA

Dedico este trabalho a Deus, aos meus pais, ao meu irmão e à minha orientadora, pelo apoio, incentivo e suporte ao longo de toda a minha trajetória até aqui.

RESUMO

Ensaios clínicos randomizados controlados investigaram o papel potencial do cacau na modulação de processos inflamatórios associados à síndrome metabólica, mas o efeito permanece controverso. Esta meta-análise teve como objetivo avaliar o impacto da suplementação de cacau e seus efeitos sobre biomarcadores inflamatórios em pacientes com síndrome metabólica e distúrbios relacionados. Foram realizadas buscas sistemáticas nas bases de dados PubMed, Scopus, Web of Science e Embase, até setembro de 2024. No total, 13 ensaios clínicos randomizados (ECR) foram incluídos na meta-análise, abrangendo 14 braços. Nossos resultados revelaram que a suplementação com cacau reduziu significativamente a proteína C-reativa (PCR) (-0,08 mg/ dL, IC 95%: -0,16, -0,001; p = 0,0451), enquanto nenhum efeito significativo foi observado sobre o fator de necrose tumoral alfa (TNF-α) (10,96 pg /mL, IC 95%: -0,16, 0,38; p = 0,4210) ou a interleucina-6 (IL-6) (-4,44 pg /mL, IC 95%: -0,90, 0,01; p = 0,0559). Nenhuma dessas análises demonstrou heterogeneidade entre os estudos. No geral, esta meta-análise sugere que a ingestão de cacau pode diminuir as concentrações de biomarcadores inflamatórios.

Palavras-chave: ensaios clínicos randomizados; cacau; síndrome metabólica; marcadores inflamatórios; meta-análise

ABSTRACT

Randomized controlled trials have investigated the potential role of cocoa in modulating inflammatory processes associated with metabolic syndrome, but the effect remains controversial. This meta-analysis aimed to evaluate the impact of cocoa supplementation on inflammatory biomarkers in patients with metabolic syndrome and related disorders. PubMed, Scopus, Web of Science, and Embase were systematically searched until September, 2024. In total, 13 RCTs were included for meta-analysis, including 14 arms. Our findings revealed that cocoa supplementation significantly reduced CRP (-0.08 mg/dL, 95 % CI: -0.16, -0.001 p= 0.0451), while no significant effects were observed on TNF-α (10.96 pg/mL, 95 % CI: -0.16, 0.38; p = 0.4210), or IL-6 (-4.44 pg/mL, 95 % CI: -0.90, 0.01; p= 0.0559). None of these analyses showed heterogeneity between the studies. Overall, this meta-analysis suggests that cocoa intake may lower inflammatory biomarkers concentrations.

Keywords: randomized clinical trials; cocoa; metabolic syndrome, inflammatory markers, meta-analysis

SUMÁRIO

FOLHA DE APRESENTAÇÃO

O Trabalho de Conclusão de Curso intitulado “Efeitos anti-inflamatórios da ingestão de cacau em pacientes com síndrome metabólica e distúrbios relacionados: uma revisão sistemática e meta-análise” resultou na elaboração de um artigo científico redigido em língua inglesa, com vistas à submissão à *International Journal of Food Sciences and Nutrition* (Taylor & Francis). Assim, o manuscrito encontra-se estruturado e formatado de acordo com as normas e diretrizes exigidas pelo periódico para submissão.

Effects anti-inflammatory of cocoa intake in patients with metabolic syndrome and related disorders: a systematic review and meta-analysis

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Abstract

Randomized controlled trials (RCTs) have investigated the potential role of cocoa in modulating inflammatory processes associated with metabolic syndrome. Nevertheless, the evidence to date remains conflicting and inconclusive. Therefore, the present study aimed to perform a systematic review and meta-analysis to evaluate the impact of cocoa supplementation on inflammatory biomarkers, including tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and interleukin-6 (IL-6), in patients with metabolic syndrome and related disorders. PubMed, Scopus, Web of Science, and Embase were systematically searched for relevant publications until September, 2024. The review has been registered at PROSPERO (CRD42024597034). To evaluate the effects of cocoa compared with placebo, pooled mean differences with 95% confidence intervals (CI) were calculated using a random-effects model. In addition, meta-regression analyses were applied, and the overall certainty of evidence was evaluated using the GRADE methodology. Publication bias was evaluated using funnel plots and Egger's tests. In total, 13 RCTs were included for meta-analysis, including 14 arms. Our findings revealed that cocoa supplementation significantly reduced CRP (-0.08 mg/dL, 95 % CI: -0.16, -0.001 p= 0.0451), while no significant effects were observed on TNF-α (10.96 pg/mL, 95 % CI: -0.16, 0.38; p = 0.4210), or IL-6 (-4.44 pg/mL, 95 % CI: -0.90, 0.01; p= 0.0559). None of these analyses showed heterogeneity between the studies. Overall, this meta-analysis suggests that cocoa intake may lower inflammatory biomarkers concentrations.

Keywords: randomized clinical trials; cocoa; metabolic syndrome, inflammatory markers, meta-analysis

1. Introduction

Cocoa, a product derived from the beans of the *Theobroma cacao* plant, is a rich source of a variety of bioactive flavonoid components, such as procyanidins, epicatechin, catechin, methylxanthines and primarily theobromine. It also contains significant amounts of dietary fiber, proteins, lipids, vitamins, and minerals, which contribute to its increasing relevance in health-related research (1). Numerous studies have demonstrated that cocoa flavonoid possess a range of beneficial biological activities, including potent anti-inflammatory (2), and antioxidant effects (3), enhancement of HDL-cholesterol levels (4), reduction of blood pressure, and improvement of endothelium-dependent vasodilation (5). These effects have been observed in the context of chronic conditions such as diabetes, hypertension, dyslipidemia, and metabolic syndrome (6).

Metabolic syndrome (MetS) is defined as a cluster of interconnected cardiometabolic risk factors, including abdominal obesity, dyslipidemia, hypertension, and insulin resistance, which collectively elevate the risk of type 2 diabetes and cardiovascular diseases (7). This condition is intrinsically associated with chronic low-grade inflammation, driven by adipose tissue dysfunction and abnormal cytokine production. Specifically, visceral adiposity promotes the release of pro-inflammatory mediators such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), which exacerbate insulin resistance, endothelial dysfunction, and metabolic dysregulation (8, 9). These biomarkers not only reflect systemic inflammation but also actively contribute to the progression of MetS and its comorbidities.

The effects of cocoa on inflammatory parameters in patients with metabolic disorders show controversial results. In an RCT carried out with smokers with severe cardiovascular

comorbidities, subjected to the consumption of chocolate with 70% cocoa, no significant differences were observed in serum CRP values (6). On the other hand, a study carried out with diabetic patients supplemented with dark chocolate found that the levels of CRP, TNF-α and IL-6 decreased (10). Previous meta-analyses observed that the consumption of cocoa-derived products in patients with type 2 diabetes decreased CRP levels in the long term (11). However, the meta-analysis developed by Behzadi et al., in 2024 (12), found that cocoa supplementation in adults did not promote significant changes in the inflammatory markers CRP, IL-6 and TNF-α.

Due conflicting results reported in the literature and the apparent absence of recent meta-analyses addressing the effects of cocoa on inflammatory mediators in patients with disorders related to metabolic syndrome, we conducted the present meta-analysis to evaluate the impact of cocoa consumption on circulating levels of CRP, IL-6 and TNF-α in this population.

2. Materials and Methods

The systematic review was conducted and reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (13), and PRISMA 2020 check list (14). The review has been registered at PROSPERO international prospective register of systematic reviews (no. CRD42024597034).

2.1.Search strategy and selection criteria

Medline, Scopus, Web of Science and Embase databases were searched using the following search terms in titles and abstracts (also in combination with MESH terms): “cocoa” OR “cacao” OR “chocolate AND “Metabolic syndrome” “OR “diabetes” OR “obese” OR “hypertension” OR “coronary heart disease” OR “non-alcoholic fatty liver disease” OR

"hypercholesterolemia" OR "polycystic ovary syndrome" AND "inflammation" AND "randomized controlled trial." A manual review of the reference lists in each identified study was also conducted. Literature searches were conducted from database inception until September, 2024. When applicable, attempts were also made to contact investigators for clarification or additional unpublished data. No language restrictions were imposed.

The search was performed independently by three authors (SSD, AM, GAAA). In case of disagreement, a fourth investigator was consulted (MS). Any discrepancies among the reviewers were resolved through consensus.

2.2.Inclusion/exclusion criteria

All clinical trials were then entered for final meta-analysis if they had the following criteria: (I) Random trials with either crossover design or parallel,; (II) the subjects in the trial were exposed to the intervention for a minimum of 1 weeks; (III) reported the impact of cacao or polyphenol-rich chocolate supplementation on CRP, TNF-α, IL-6 before and after the trial in the intervention and placebo groups; IV) performed in adult subjects: V) patients with metabolic syndrome and related disorders. In this meta-analysis, letters, short communications, reviews, animal studies and *in vitro* were excluded from the analysis. Duplicate studies, trials without sufficient data and the intervention used a mixture of cacao and other substances were also excluded. Trials evaluating multiple treatment arms (low- or high-dose cacao or different comorbidities) were included in the meta-analysis as a separate trial.

2.3.Data extraction and quality assessment

Eligible studies were reviewed and the following data were abstracted: study characteristics (authors and publication year), study design, population information (number

and gender of participants), the dose and type of cacao supplementation, the duration of the study, health condition, and inflammatory markers (main outcomes).

Five investigators independently used the Cochrane Collaboration tool to assess risk of bias for each included trial RCTs (15). This tool presents a slight difference in the evaluation of studies with parallel design and crossover studies. Disagreements between investigators were resolved by consultation with the senior investigator. Quality was assessed according to the following criteria: randomization process, deviations from intended interventions, missing outcomes data, measurement of outcomes, and selection of the reported results. And for crossover studies an additional criterion on timing of identification and recruitment of individuals. Each domain was graded (low, high, or some concerns) based on the available information in the study. All disagreements were resolved by discussion.

We assessed the quality of evidence for each category using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (16). We rated the quality of evidence of the outcomes across trials using GRADE-provided criteria, including study risk-of-bias, inconsistency, indirectness, imprecision, and publication bias. GRADE categorized the quality of evidence into four levels: High, Moderate, Low and Very Low quality.

2.4. Statistical analysis

For each factor, we extracted the mean at baseline and post-intervention, from both the intervention and control groups. Standard deviations (SDs) of the mean differences were calculated using the following formula: SD = square root [(SDpre-treatment)2 + (SDpost-treatment)2 − (2R × SDpre-treatment × SDpost-treatment)], assuming a correlation coefficient (R) = 0.5 (17, 18). The meta-analysis was performed using R software (R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org, 2020).

Statistic heterogeneity of treatment effects between studies was formally tested with Cochran's test (19). The I^2 statistic was also examined, and we considered an I^2 value >50% and >75% to indicate substantial and considerable heterogeneity, respectively, between the trials. Effect sizes were presented as mean differences with 95% confidence intervals, and p-values < 0.05 were considered as statistically significant. Publication bias was assessed by the Egger's test and represented graphically by funnel plots (20). Sensitivity analyses were also performed by removing 1 study at a time, to assess any impact of study quality on the effect estimates. Meta-regression analysis was carried out to investigate the association between the duration of the intervention and the mean age with pooled effect size.

3. Results

3.1. Search results

An overall of 262 studies were retrieved through initial online database search. And 12 additional records identified in other sources. After removing that did not meet the inclusion criteria, 23 references remained. These potentially relevant articles were examined for full text evaluation. The other 10 articles were excluded for the following reasons: outcomes were not measured in one trial, five studies did not have a placebo group, one non-randomized study and one study was short-term supplementation. In addition, in two articles the data presentation was inappropriate for quantitative synthesis. Thus, 13 studies were included in the meta-analysis, including 14 arms (Figure 1).

The main characteristics of trials included in this meta-analysis are summarized in Table 1. The selected studies enrolled subjects with different health conditions, three studies included adults with type 2 diabetes (10, 21, 22). Three studies included overweight/obese patients (23-25). One study included patients with non-alcoholic fatty liver disease (26). Four studies included hypertension (5, 21, 27, 28). Coronary and cardiovascular diseases were

included on four studies, one of them with congestive heart failure (29), one with high risk of cardiovascular disease (30), and one with smokers with cardiovascular comorbidities (6).

Treatment duration ranged from 2 weeks to 12 weeks and sample size ranged from 20 to 100 participants. These studies reported the average age of participants, which ranged between 37.95 and 69.7 years. The type of cocoa product used was cocoa powder cocoa in 3 studies (28-30), dark chocolate or chocolate in 6 studies (6, 10, 21, 24-26), and 3 studies with flavanol-rich cocoa (5, 22, 27). Dosages of chocolate or chocolate used were from 5 g/day to 100 g/day. Eleven RCTs, with twelve arms, reported on C-reactive protein (CRP) (5, 6, 10, 21-23, 25, 26, 28-31), four on interleukin-6 (IL-6) (24, 25, 27, 30) and four studies measured on tumor necrosis factor-alpha (TNF-α) (10, 24, 25, 27).

3.2.Risk of bias assessment

The Cochrane bias evaluation was performed to evaluate study and reporting quality are shown in figure 2. Eight studies provided comprehensive explanations of random sequence generation (10, 21-23, 25, 27-29). Six studies had unclear or high risk due of deviations from intended interventions (5, 6, 10, 21, 23, 30). Only one article had high risk due to missing outcomes data (6). And two studies had high risk in measurement of outcomes (5, 6). On the other hand, only four studies did not present a risk of selection of the reported results (10, 23, 25, 29).

3.3.Quantitative analysis

3.3.1. Effect of cocoa on C-reactive protein (CRP)

The effects of cocoa supplementation in twelve studies were analyzed, meta-analysis verify a significant effect on CRP levels (-0.08 mg/dL, 95 % CI: -0.16, -0.001 p= 0.0451), without between-study heterogeneity (p = 0.8240, I2 = 0%) (Figure 3-A).

3.3.2. Effect of cocoa on interleukin-6 (IL-6)

Sensitivity analysis revealed that the heterogeneity of the included studies was highly affected by the study of Jafarirad et al. (10) (-1.33 pg/mL, 95 % CI: -2.75, 0.08). When this study was removed from the analysis, the heterogeneity changed from 95.32% to 0%. Therefore, we decided to remove this study from the analysis. The meta-analysis of four studies no revealed significant change in serum IL-6 levels in which cocoa was administered (10.96 pg/mL, 95 % CI: -0.16, 0.38; p = 0.4210), without between-study heterogeneity (p = 0.9890, I2 = 0%) (Figure 3-B).

3.3.3. Effect of cocoa on tumor necrosis factor-alpha (TNF-α)

Four RCTs were used to investigate TNF-α and no significant effect of the cocoa supplementation was found by meta-analysis (-4.44 pg/mL, 95 % CI: -0.90, 0.01; p= 0.0559), without between-study heterogeneity (p = 0.0815, I2 = 55.34%) (Figure 3-C).

3.4.Meta-Regression Analysis

We performed meta-regression analysis to evaluate the association between the duration of the intervention with cocoa supplementation and mean age of participants with anti-inflammatory profile. Meta-regression analysis did suggest any significant association between changes in CRP, IL-6 e TNF-α levels with mean age. Furthermore, this analysis revealed that IL-6. CRP and TNF-α levels were not associated with study duration (Figure 4).

3.5- Publication bias

Publication bias assessment through funnel plot and Egger's linear regression test. Egger's linear regression test confirmed the absence of publication of bias in CRP: p =

0.2338, IL-6: p = 0.2272, and TNFα: p = 0.5140. On a visual inspection of funnel plot depicted no publication bias in included studies (data not presented).

3.6. Quality of the evidence for the outcome using GRADE

The outcomes were rated as high or low quality of evidence using the GRADE approach. Due risk of bias (methodological limitations) and publication bias since small studies with negative results were missing, some studies had reduced scores (Table 2).

4. Discussion

According to our meta-analysis of thirteen trials and fourteen arms, intervention with cocoa was associated with a significant reduction in serum CRP. However, it could not significantly affect IL-6 and TNF-α levels in patients with metabolic syndrome and related disorders.

Chronic inflammation is associated with an increased risk of cardiovascular events (32), and vascular impairment associated with diabetes (33), furthermore, obesity has been reported to be a major determinant of low-grade chronic inflammation (34). Increased adiposity promotes pro-inflammatory polarization of macrophages, with subsequent increased production of pro-inflammatory cytokines and adipokines, thus inducing low-grade general systemic inflammation that contributes to insulin resistance, DM2 and MetS. In addition, excess lipids that cannot be stored in adipose tissue are deposited in other organs (such as the liver, skeletal muscle, and blood vessels), leading to the expression of pro-inflammatory mediators, resulting in low-grade systemic chronic inflammation (35). During this process, adipose tissue produces and releases a variety of pro-inflammatory and anti-inflammatory factors, including adipokines and cytokines such as TNF-α and IL-6 (36).

Studies suggest that higher flavonoid intake can inhibit inflammatory response by reducing the levels of important pro-inflammatory mediators (37, 38). The flavonoids acting as anti-inflammatory agents through strengthening antioxidant activities or displaying regulatory properties in various pathways of inflammation (39). In particular, oxidative imbalance causes the upregulation of pro-inflammatory cytokines (such as TNF-alpha and IL-6) and inflammatory molecules (such as VCAM-1, ICAM-1, and NF-κB) (40). Cocoa is a rich dietary source of various bioactive flavonoid compounds compared to other foods (41) and it plays a crucial role in multiple chronic inflammatory conditions (42). The mechanisms by which cocoa may attenuate inflammatory responses include its antioxidant capacity, mediating pro-inflammatory pathways through NF-κB and modulating signaling cascades. Furthermore, cocoa polyphenol inhibits cyclooxygenase-2 (COX-2), lipoxygenase (LOX), Activator protein-1 (AP-1), and Mitogen-activated protein kinases (MAPKs). A combination of these signals suppresses the production of pro-inflammatory mediators such as TNF-α and IL-6 (43).

An increase in a pro-inflammatory marker, such as IL-6, stimulates the liver to synthesize C-reactive protein (CRP), which is a marker of chronic low-grade inflammation, and elevated levels are associated with increased risk of insulin resistance, cardiovascular events, and disease progression (44, 45). Our study found that CRP serum levels decreased with cocoa supplementation in MetS patients and related disorders. One meta-analysis developed in 2022 by Chen et al, in individuals with type 2 diabetes patients, also corroborate our result (11). However, Behzadi et. al in 2024, carried out a meta-analysis containing 18 RCTs in healthy or unhealthy individuals, did not significantly reduce CRP levels (12). The discrepancies found in these studies may be due to several factors, including differences in flavonoid dosage, duration of intervention, participants baseline health status, type of CRP

measurement, and geographical location of the studies. These elements likely contributed to the observed heterogeneity in outcomes.

IL-6 is produced by numerous different cell types, including adipocytes, endothelial cells, pancreatic β-cells, macrophages and monocytes (46). It is pro-inflammatory cytokine that is intrinsically linked to metabolic syndrome parameters, such as hypertension, elevated BMI, and insulin resistance (47, 48). Studies describes elevated IL-6 as a feature of chronic low-grade inflammation observed in patients with obesity, type 2 diabetes, and related metabolic disorders (47, 49). Our meta-analysis found no significant reduction in serum levels promoved for cocoa intervention, possibly due to different degrees of inflammation among the individuals included in the study.

TNF-α is a proinflammatory cytokine produced predominantly by activated macrophages. TNF-α expression increases in obesity and IR in humans (34), while TNF-α treatment induces IR in adipose tissue (50). Serum TNF-α levels decrease during weight loss (51). Elevated levels are associated with chronic inflammation in diseases such as obesity, hypertension, type 2 diabetes mellitus, and metabolic syndrome (52, 53). TNF-α causes systemic acute-phase response via the release of other pro-inflammatory cytokines, such as IL-6 and the reduction of anti-inflammatory adiponectins (50). The meta-analysis by Chen et al. (11) demonstrated that long-term consumption of cocoa polyphenols can improve cardiometabolic biomarkers in individuals with T2DM by inhibiting the expression of pro-inflammatory mediators, including TNF-α. Another meta-analysis (12) evaluated the dose-response effect of dark chocolate/cocoa consumption on inflammatory biomarkers and found a significant decrease in TNF-α levels only in unhealthy adult participants. Thus, we can suggest that, in addition to variations in the duration of interventions, the types of products used, and the preparation methods of cocoa supplementation, the individual's clinical condition influences the action of cocoa on anti-inflammatory markers. This hypothesis was

also investigated by Sitarek *et. al,* in 2024 (54), who examined the effect of cocoa supplementation on different markers of inflammation in healthy and unhealthy individuals. For healthy subjects, out of a total of 14 biomarkers, 6 showed positive results regarding the effect of cocoa-derived products or chocolate on inflammation (42.86%). However, for subjects with a history of health problems, 22 of the 33 biomarkers indicated that cocoa-derived products or chocolate had a positive effect on inflammation (66.67%).

Discrepancies findings regarding cocoa's impact on inflammation markers may also be influenced by the human body's response to inflammation. Every human being has a different body response that is influenced by genetic polymorphisms and epigenomic events. Another topic that may result in inconsistent findings is the intercorrelation between the food matrix and anti-inflammatory activity. Muhammad et al., 2019 (55) found that flavonoids in coconut drinks are easier for the body to absorb, than in complex structured foods such as chocolate bars. As variations in the flavanol dose and chemical profile of the cocoa products used, as industrial processing, particularly alkalization, can destroy 60–90% of the bioactive flavanols (56).

In our study, we observed a decrease in CRP promoted by cocoa supplementation; however, this effect was not observed in the levels of cytokines IL-6 and TNF, this can be explained by the half-life of these markers in the plasm. CRP has a plasma half-life of 19 hours (57) and may take several days to return to baseline levels (58). However, the plasma half-life of IL-6 is relatively short, less than six hours (59), and TNF-α has a relatively short half-life once released into the extracellular environment, typically ranging from 20 min to a few hours (60). This short half-life means that IL-6 and TNF-α levels can change rapidly, leading to greater complexity in their measurement. Furthermore, the number of studies included in this meta-analysis that measured these cytokines was very small, which may also have contributed to the effects of cocoa not being observed.

This meta-analysis demonstrates several key strengths. The systematic review and meta-analysis were rigorously conducted in accordance with PRISMA guidelines. Egger's regression test revealed no significant asymmetry in the funnel plot, supporting the robustness of the overall effect estimate. Notably, no heterogeneity was observed among the included studies, and a meta-regression was performed to investigate potential additional associations. Furthermore, the review is registered with PROSPERO and incorporates a comprehensive GRADE evaluation to assess the quality of evidence.

However, the present meta-analysis has some limitations. Most significantly is the low number of trials that were available for the meta-analysis, and this low number of studies was reflected in few individuals, mainly in the analysis of IL-6 and TNFα. In addition, we identified large variations in designs and duration studies, dosage and type cocoa, age and comorbidities of participants. Furthermore, different inflammation levels presented by the individuals included in this review may mask the anti-inflammatory action promoted by cocoa.

5. Conclusion

This meta-analysis of RCTs demonstrated the beneficial effects of cocoa supplementation on inflammatory markers among patients with metabolic syndrome and related disorders. This study demonstrated a significant decrease in the circulating levels of CRP; however, we did not verify a significant effect of supplementation with cocoa on plasma TNFα and IL-6 concentrations. Thus, cocoa may act as an effective adjunct in modulating inflammation in patients with MetS and related disorders. However, these results highlight the importance of standardizing cocoa interventions, extending study durations and participants' health status in future research to accurately determine their anti-inflammatory potential in metabolic syndrome and related disorders.

6. Conflict of interest

The authors declare no conflicts of interest.

7. Funding source

This research did receive sources of funding through VIC/UFJF scholarships.

8. Author contribution

SSD, AM, GAA and MS contributed to the conception of the research. SSD, AM, GAA and MS searched databases, screened articles and extracted data. LRM and MS performed statistical analysis; and SSD, AM, GAA, LRM and MS contributed to writing the manuscript.

9. Acknowledgment

None.

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Figure legends:

Figure 1 - Literature search and review flow chart for selection of studies.

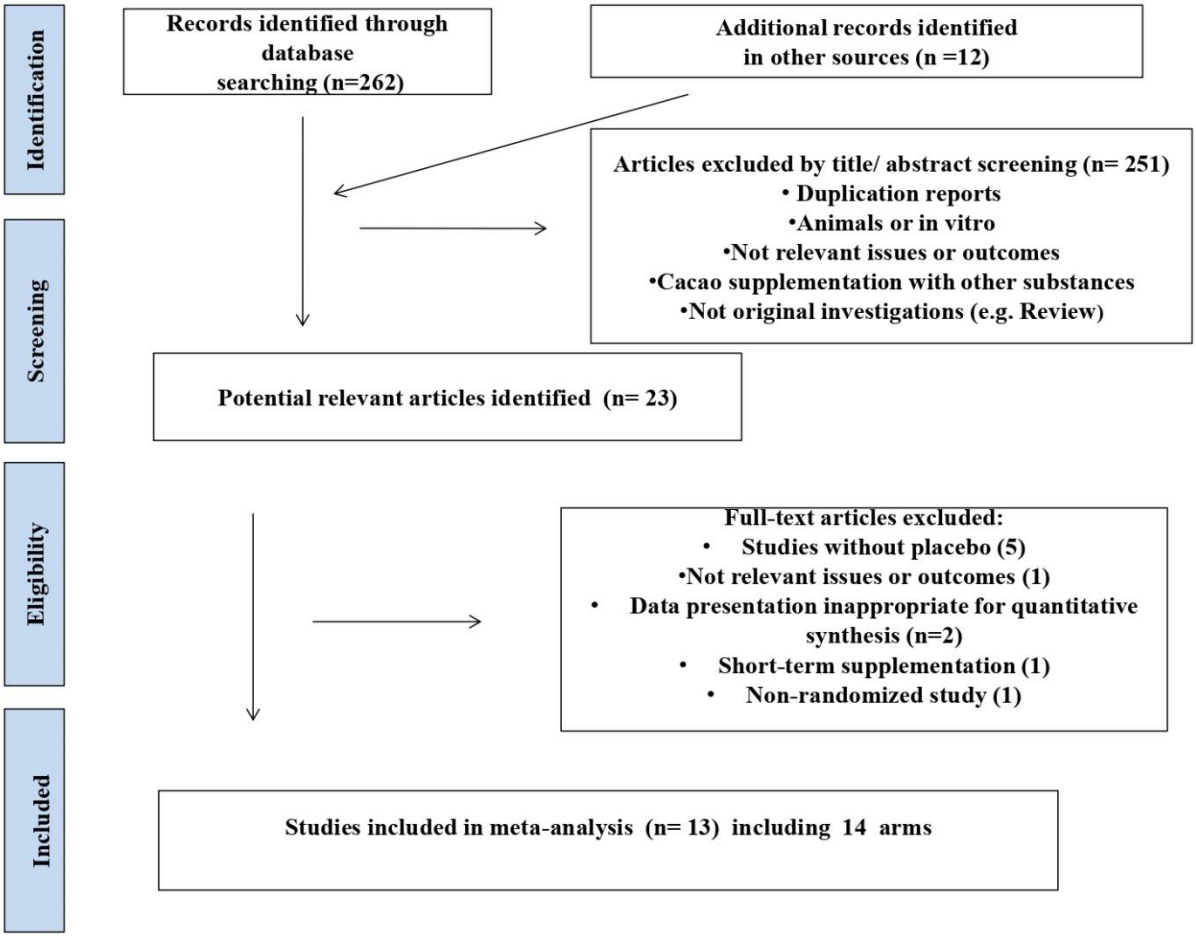

Figure 2 - The summary of review authors' judgments about each risk of bias item for included studies.

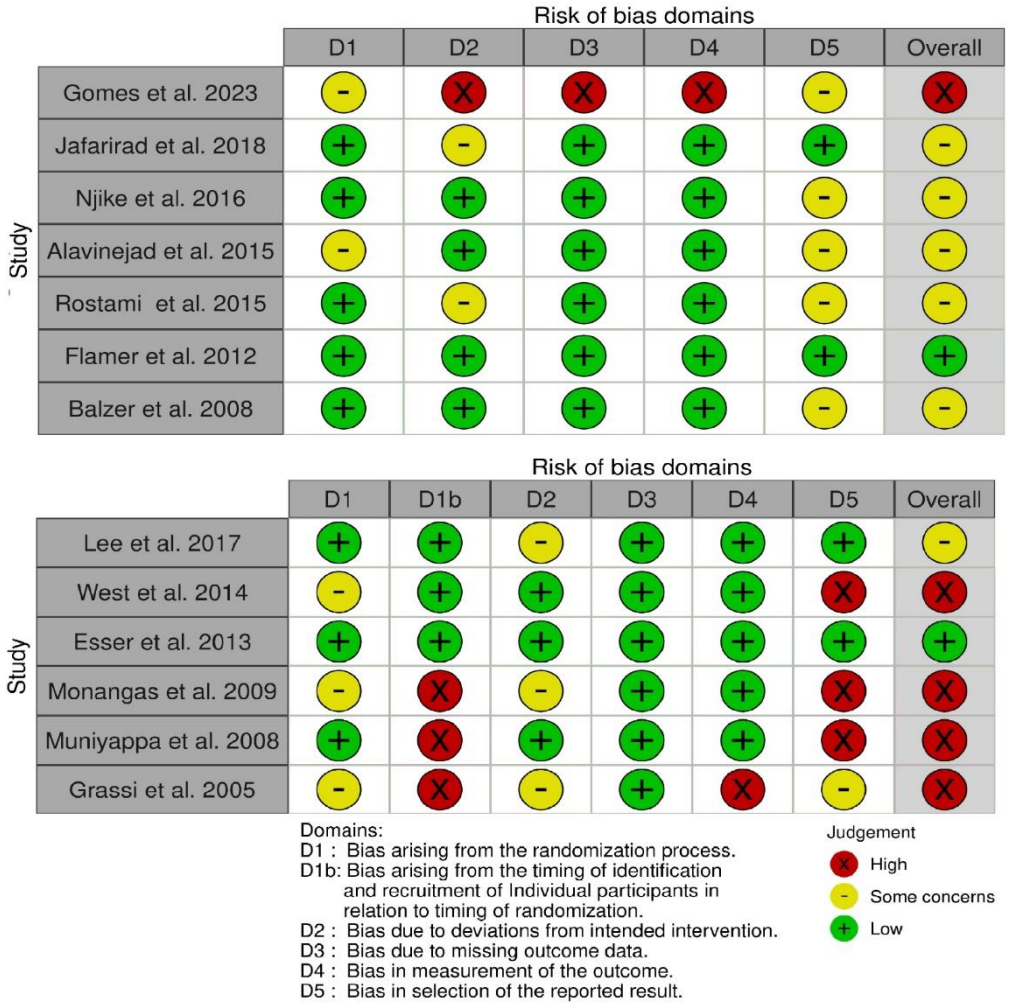

Figure 3 - Forest plot of the effect of cocoa supplementation on plasma C-reactive protein concentrations (A) and plasma IL-6 concentrations (B) and association plasma TNFα concentrations (C).

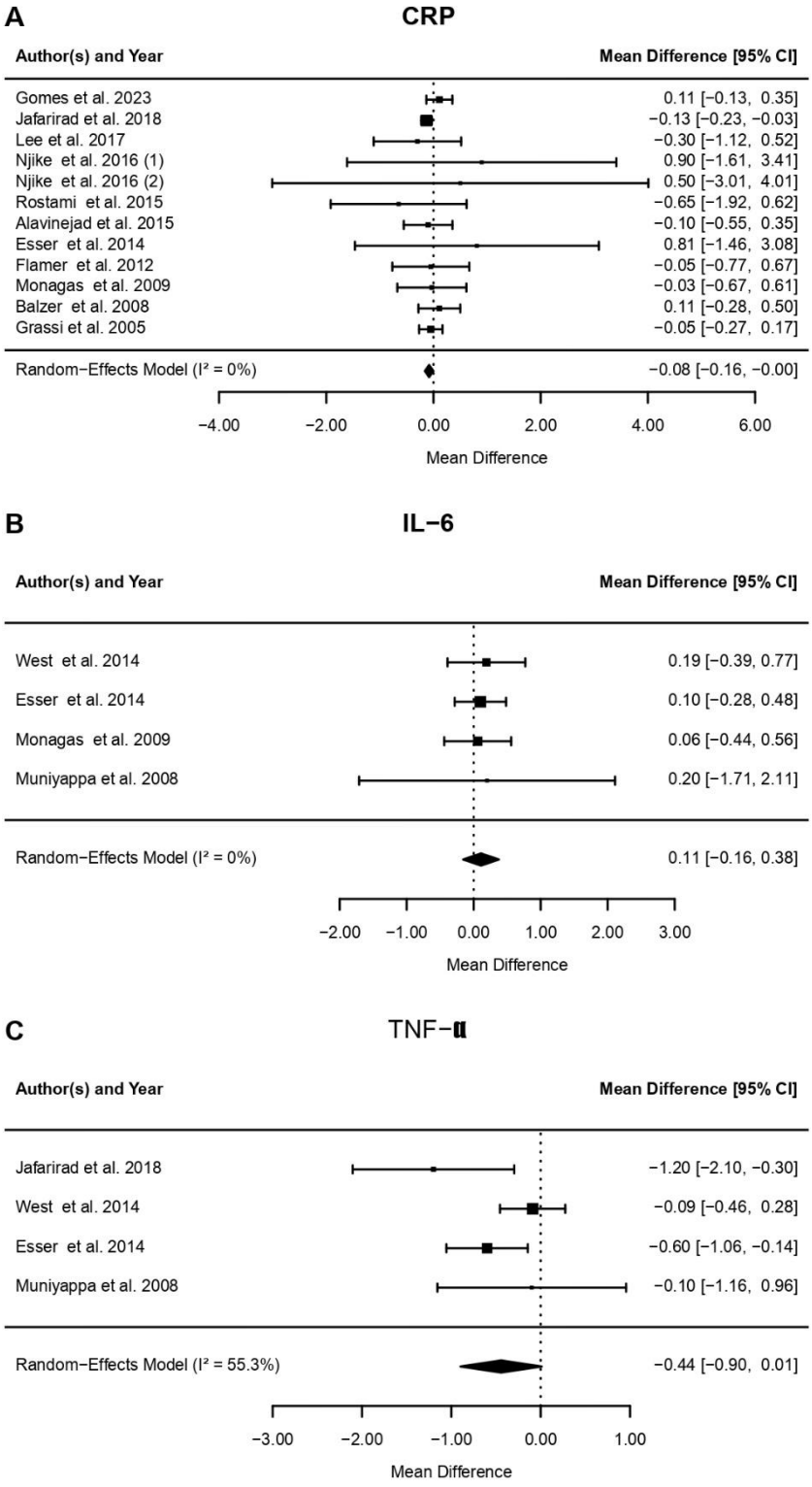

Figure 4 - Meta-regression plots of the association of mean changes in plasma C-reactive protein concentrations with duration of treatment (A) and mean age (B), association of mean changes in plasma IL-6 concentrations with duration of treatment (C) and mean age (D), an association of mean changes in plasma TNFα concentrations duration of treatment (E) and mean age (F).

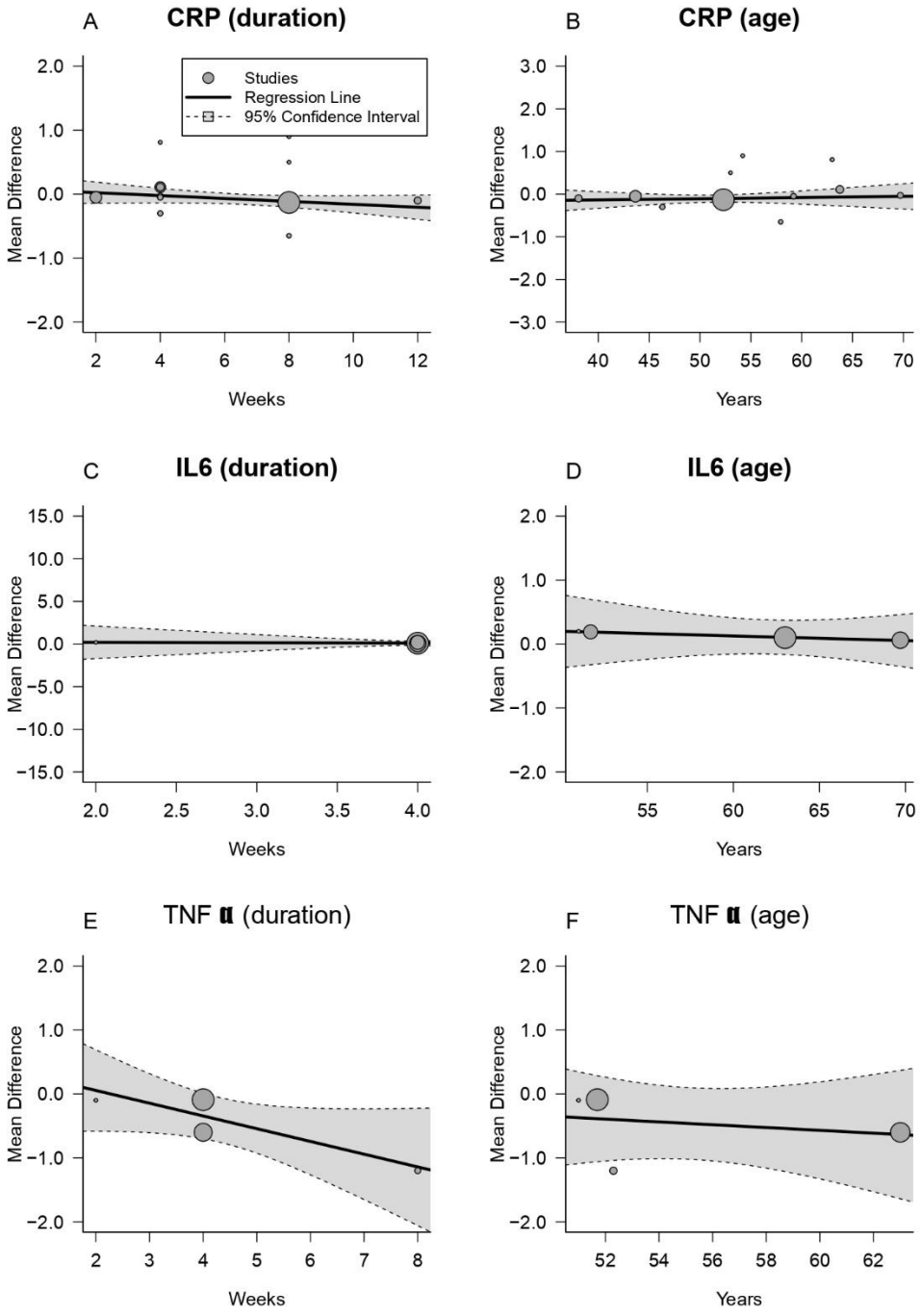

Table 1- Characteristics of included studies.

First author (publication year)	Design	Number of participants/ Gender	Mean age (years)	Type and amount of cocoa intake	Duration	Notes about participants	Main outcomes
Gomes *et al.* 2023	Parallel	Placebo=17 Cocoa = 15	No reported	40 g/d of chocolate containing 70% cocoa/day	4 weeks	smokers with cardiovascular comorbidities	CRP
Jafarirad *et al.* 2018	Parallel	Placebo=23 Cocoa = 21	52.3 ± 6.4	30 g/d dark chocolate	8 weeks	type 2 diabetes	CRP, TNFα, IL-6
Lee *et al.* 2017	Crossover	31	46.3 ± 1.8	18 g/d of cocoa powder and 43 g/d of dark chocolate	4 weeks	overweight and obese	CRP
Njike *et al.* 2016 (1)	double-blind, parallel	Placebo=26 Cocoa = 25	Placebo = 54.2 ± 10.1 Cocoa = 54.2 ± 10.1	10 g/d cocoa powder .	8 weeks	stage 1 hypertension	CRP
Njike *et al.* 2016 (2)	double-blind, parallel	Placebo=26 Cocoa = 24	Placebo = 53.0 ± 10.6 Cocoa = 53.0 ± 10.6	5 g/d cocoa powder	8 weeks	stage 1 hypertension	CRP
Rostami *et al.* 2015	double-blind, parallel	Placebo=28 Cocoa = 32	Placebo= 57.2 ± 7.9 Cocoa = 58.7 ± 9.1	25g/d dark chocolate	8 weeks	diabetes and hypertension	CRP

Alavinejad et al. 2015	double-blind, parallel	Placebo=21 Cocoa = 21	Placebo= 37.9 ± 10.3 Cocoa = 38.2 ± 11.1	30 g/d dark chocolate	12 weeks	Nonalcoholic fatty liver disease	CRP
West *et al.* 2014	double-blind, crossover	30	51.7 ± 1.2	37 g/d dark chocolate	4 weeks	Overweight	TNFα, IL-6
Esser *et al.* 2014	Double-blind crossover	41	63 ± 5	70 g/d chocolate	4 weeks	Overweight	CRP, TNFα, IL-6
Flamer *et al.* 2012	double-blind, parallel	Placebo=10 Cocoa = 10	Placebo = 58.1± 11.9 Cocoa = 60.3± 10.1	80 g/d serving with 70% cocoa powder	4 weeks	Congestive heart failure	CRP
Monagas *et al.* 2009	Crossover	42	69.7 ± 11.5	40 g/d cocoa powder	4 weeks	high risk of cardiovascular disease	CRP, IL-6
Balzer *et al.* 2008	double-blind, parallel	Placebo=20 Cocoa = 21	Placebo= 64.4± 8.6 Cocoa = 63.1± 8.3	flavanol-rich cocoa (321 mg flavanols/d)	4 weeks	type 2 diabetes	CRP
Muniyappa *et al.* 2008	Double-blind crossover	20	51± 1.5	flavanol-rich cocoa drink (900 mg flavanols/d)	2 weeks	Hypertension	TNFα, IL-6
Grassi et al. 2005	blind crossover	20	43.65±7.8	100 g/d flavanol-rich dark chocolate	2 weeks	Hypertension	CRP

Abbreviations: C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α).

Table 2 - Summary of main results

Outcome	Studies	Participants	Effect Estimate	P value	Quality of evidence (GRADE)
CRP	12	474	-0,08 [0.16, -0.001]	0.0451	⊕⊕⊕⊕ High
IL-6	4	133	10.96 [-0.16, 0.38]	0.4210	⊕⊕⊖⊖ Low1,2
TNF-α	4	135	-4.44 [-0.90, 0.01]	0.0559	⊕⊕⊖⊖ Low[1,2]

[1]Methodological limitations (risk of bias).

[2]Downgraded due to imprecision (95% confidence interval of the pooled effect includes no effect and negative effect), lack of precision < 400 participants.