UNIVERSIDADE FEDERAL DE JUIZ DE FORA
CAMPUS GOVERNADOR VALADARES
GRADUAÇÃO EM FARMÁCIA

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Os efeitos dos produtos do cacau em indivíduos com síndrome metabólica e doenças relacionadas: uma revisão sistemática e meta-análise

Governador Valadares
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Trabalho de Conclusão de Curso (TCC) apresentado ao curso de Farmácia da Universidade Federal de Juiz de Fora, *campus* Governador Valadares, como requisito parcial para a conclusão do curso de Bacharel em Farmácia.

Orientador: Maisa Silva

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Aprovada em 07 de novembro de 2025

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The effects of cocoa products in individuals with metabolic syndrome and related diseases: a systematic review and meta-analysis

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Abstract

Cocoa supplementation has been shown to improve parameters related to metabolic syndrome, although results have been contradictory. We conducted a systematic review and meta-analysis to examine the effect of cocoa products on lipid and glycemic profiles, blood pressure levels, and anthropometric measurements in individuals with metabolic syndrome and related diseases. A search of PubMed, Web of Science, Embase, and Scopus was performed to identify randomized clinical trials (RCTs). To compare the effects of the cocoa product with placebo, mean differences with 95% confidence intervals (CIs) were pooled using a random-effects model. Subgroup analyses and meta-regression were performed to identify the source of heterogeneity, and study quality assessment was conducted using the GRADE approach. A total of 13 RCTs, including 16 arms, that investigated the effects of cocoa on parameters related to metabolic syndrome were included. The meta-analysis showed a significant effect of cocoa supplementation on triacylglycerol levels, and subgroup analysis revealed that cholesterol levels were reduced with cocoa supplementation in patients with dyslipidemia and diabetes. Furthermore, the meta-regression analysis revealed a decrease in glycemic and lipid profiles, as well as anthropometric parameters, in studies with longer durations of cocoa supplementation. Our study suggests that cocoa intake has beneficial effects and that the duration, type, and clinical status of patients were important determinants for favorable effects on biomarkers related to metabolic syndrome.

Keywords: Randomized clinical trials, cocoa, metabolic syndrome, metaanalysis

Resumo

A suplementação de cacau demonstrou melhorar os parâmetros relacionados à síndrome metabólica, embora os resultados sejam contraditórios. Realizamos uma revisão sistemática e meta-análise para examinar o efeito dos produtos do cacau nos perfis lipídicos e glicêmicos, níveis de pressão arterial e medidas antropométricas em indivíduos com síndrome metabólica e doenças relacionadas. Uma pesquisa no PubMed, Web of Science, Embase e Scopus foi realizada para identificar ensaios clínicos randomizados (ECRs). Para comparar os efeitos do produto do cacau com placebo, as diferenças médias com intervalos de confiança (ICs) de 95% foram agrupadas usando um modelo de efeitos aleatórios. Análises de subgrupos e meta-regressão foram realizadas para identificar a fonte de heterogeneidade, e a avaliação da qualidade do estudo foi conduzida usando a abordagem GRADE. Um total de 13 ECRs, incluindo 16 braços, que investigaram os efeitos do cacau em parâmetros relacionados à síndrome metabólica foram incluídos. A metanálise mostrou um efeito significativo da suplementação de cacau nos níveis de triacilglicerol, e a análise de subgrupos revelou que os níveis de colesterol foram reduzidos com a suplementação de cacau em pacientes com dislipidemia e diabetes. Além disso, a análise de meta-regressão revelou uma diminuição nos perfis glicêmico e lipídico, bem como nos parâmetros antropométricos, em estudos com maior duração de suplementação de cacau. Nosso estudo sugere que a ingestão de cacau tem efeitos benéficos e que a duração, o tipo e o estado clínico dos pacientes foram determinantes importantes para efeitos favoráveis sobre os biomarcadores relacionados à síndrome metabólica.

Palavras-chave: Ensaios clínicos randomizados, cacau, síndrome metabólica, meta-análise

1- Introduction

Metabolic syndrome (MetS) is characterized by the coexistence of cardiovascular risk factors and metabolic disorders, such as hypertension, insulin resistance, dyslipidemias – elevated triglyceride levels and reduced HDL levels – and increased waist circumference [1, 2]. The presence of MetS is a risk factor for other conditions, particularly increasing the risk of developing type 2 diabetes (DM2) by five times and the risk of cardiovascular diseases by three times [3]. Data from the International Diabetes Federation indicate that the prevalence of MetS in the Americas (33.4%) is the second highest in the world, surpassed only by the Eastern Mediterranean region (34.6%) [4]. It may be the result from a combination of genetic factors, dietary profile, and physical inactivity [5].

Strategies that can be implemented to reduce the risk of developing metabolic syndrome and associated diseases are important. Cocoa is a food rich in flavonoids and the consumption of these compounds can reduce the risk of developing DM2 and Non-Alcoholic Fatty Liver Disease (NAFLD) [6, 7]. Studies show that individuals who consume cocoa and products derived have smaller waist circumference, fasting blood glucose [6] and reduced the risk of incidence of hypertension [8]. However, the consumption of dark chocolate in individuals with mild hypertension promote a reduction in diastolic blood pressure, but no significant effects on vascular function, markers of glucose/lipid metabolism, renin-angiotensin-aldosterone system, and oxidative stress [9]. Results found in meta-analyses on the effect of cocoa on metabolic parameters also present discrepancies, as in the study by Arisi et al. in 2024 [10], cocoa showed positive effects on total cholesterol, LDL, fasting glucose, SBP and DBP, but did not produce a reduction effect on triglycerides, body weight, BMI, waist circumference, HDL cholesterol and glycated hemoglobin (HbA1c) in adults, with and without comorbidities.

On the other hand, the meta-analysis produced by Chen et al. in 2022[11], which found that consumption of cocoa products significantly reduced LDL cholesterol, triglycerides and glycemia in patients with type 2 diabetes.

Despite some publications on the effects of cocoa products on MetS-related parameters, we know of no meta-analysis in individuals with MetS and related diseases. Furthermore, previous studies have shown contradictory results, thus, this study aims to conduct a systematic review and meta-analysis of randomized controlled trials to evaluate the hypothesis that cocoa and its products have an ameliorating effect on parameters such as blood pressure, lipid and glycemic profile, and anthropometric parameters in individuals with MetS and related disorders.

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 [12], and PRISMA 2020 check list [13]. The review has been registered at PROSPERO international prospective register of systematic reviews CRD42024586659.

2.1- Search strategy and selection criteria

We used the PICOS model to determine the inclusion criteria standing for population (aged > 18 years old with metabolic syndrome or related disorders), intervention (cocoa supplementation), comparison (control group), outcome (MetS risk factors) and study (randomized controlled trials). 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

"blood pressure OR hypertension" OR "lipid" OR "cholesterol" OR "triglyceride" OR "blood glucose" AND "randomized controlled trial". The search was limited to studies published in the last ten years prior to the search date. A manual review of the reference lists of 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 (AGC, BGC and GNG). 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) human 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 cocoa supplementation on blood pressure, lipid profiles, blood glucose, weight, body mass index (BMI), waist circumference (WC), glycated hemoglobin (HbA1c), insulin, and homeostasis model assessment insulin resistance index (HOMA-IR) at baseline and follow-up; (IV) performed in adult subjects: (V) patients with metabolic syndrome or 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 cocoa and other substances were also excluded. Studies with cocoa supplementation and physical exercise or lifestyle intervention were not included in this review. Trials

evaluating multiple treatment arms (low- or high-dose cocoa) 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, the dose and type of cocoa supplementation, the duration of the study, health condition, and MetS risk factors (main outcomes).

Four investigators independently used the Cochrane Collaboration tool to assess risk of bias for each included trial RCTs [14]. 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. 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 [15]. 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: $SD = \sqrt{(SD_{pre-treatment})^2 + (SD_{post-treatment})^2 - 2R \times SD_{pre-treatment} \times SD_{post-treatment}}$, assuming a correlation coefficient (R) = 0.5 [16, 17]. 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 Cochrane's test [18]. 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. Based on the heterogeneity between included studies, a random effect or a fixed model was applied in the meta-analysis. The pooled effect size estimated using the DerSimonian-Laird Based random-effects model [19]. To investigate the potential sources of between-study heterogeneity, we carried out a subgroup analysis based on design of studies, dosage and type of cocoa supplementation, the duration of treatment, age and gender of participants of studies, presence or absence of dyslipidemia and diabetes. 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 age mean with pooled effect size.

3- Results

3.1- Search results

An overall of 2289 studies were retrieved through initial online database search and 7 additional records identified in other sources. After removing that did not meet the inclusion criteria, 24 references remained. These potentially relevant articles were examined for full text evaluation. The other 11 articles were excluded for the following reasons: data presentation inappropriate for quantitative synthesis in three trials, two articles with cocoa supplementation and other substances were discarded, three studies without placebo, two articles were supplementation and lifestyle modification and one non-randomized study. Thus, 13 studies were included in the meta-analysis, including 16 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, five studies addressed overweight patients [21-23], being one of them also with borderline criteria of metabolic syndrome [24] and one with pre-menopausal females with overweight/obesity [25]. Three studies included adults with diabetes, of those, one with type 2 diabetes [26], one with type 2 diabetes with hypertension [27] and one with diabetes and hypertension [28]. Besides those, prediabetic patients were selected by one study [29]. Nonalcoholic fatty liver disease was addressed by one study [30], as well as obesity [23], stage 1 hypertension [31], metabolic syndrome [32] and smokers with cardiovascular comorbidities [33].

The shortest clinical trial was 4 weeks, while the longest trial was 12 weeks, with sample sizes ranging from 15 to 100 participants. The mean age of participants ranged from 21 to 65 years. Two studies recruited only women [23, 25] and seven studies did not report the proportion of men and women who were recruited and analyzed in the control

and placebo groups [22, 29, 31-33]. Regarding the intervention method, one study used chocolate [33], four used dark chocolate [21, 28, 30, 32], two used cocoa with a high flavonol content [25, 27], one used cocoa flavonoids [24], two used cocoa powder [26, 31], one used cocoa products [23], one used cookies with flavonoids extracted from cocoa [22] and only one used a cocoa procyanidin supplement [29]. Cocoa dosages ranged from 2,5 to 28 g/day of cocoa.

Six RCTs, with eight arms, reported on SBP and DBP [24, 27-29, 31, 32], and twelve RCTs reported on blood lipid profiles, including nine RCTs, with ten arms, on triacylglycerol (TG) and total cholesterol (TC) [21-24, 26-28, 30-32], nine RCTs, with ten arms, on LDL [21, 22, 24, 26-32], and nine RCTs, with ten arms, on HDL [21-24, 26-28, 30-32]. Thirteen studies provided data on glycemic status markers, including seven RCTs, with eight arms reporting on fasting plasma glucose (FPG) [21-24, 27, 28, 31, 32], six RCTs, with seven arms on fasting insulin [21, 25, 27, 28, 31, 33], five RCT, with seven arms on HOMA-IR [21, 27, 29, 31, 32], and three on HbA1c [27, 28, 32]. Furthermore, nine studies, with ten arms, reported anthropometric measurements, such as BMI (seven RCTs, with eight arms) [21, 22, 24, 27, 30-33], WC (seven RCTs, with eight arms) [21, 22, 24, 27, 30-32], and weight (seven RCTs, with eight arms) [21, 22, 24, 25, 27, 31, 33].

3.2. Risk of bias assessment

The Cochrane bias evaluation was performed to evaluate study and reporting quality are shown in Figure 2. Five studies provided comprehensive explanations from the randomization process [22, 25, 27, 28, 31]. Two trials were considered high risk of bias due to deviations from intended intervention and due to missing outcome data [26, 33]. Two studies were showed high risk of bias due measurement of the outcomes [22,

33]. In four trials high risk of bias was found in selection of the reported result [21, 22, 27, 32].

3.3. Quantitative analysis

3.3.1. Effect of cocoa supplementation on blood lipid profiles

The effects of cocoa supplementation in ten studies were analyzed, and meta-analysis did not verify a significant change in TC levels (-0.14 mmol/L, 95% CI: -0.45, 0.16; p = 0.3606), with between-study heterogeneity (p = < .0001, I2 = 94.31%). The meta-analysis of ten studies did not verify a significant effect of cocoa supplementation on HDL levels (0.07 mmol/L, 95% CI: -0.01, 0.14; p = 0.0876), with between-study heterogeneity (p = <0.0001, I2 = 74.46%). Meta-analysis of ten studies revealed no significant effect on serum LDL levels when cocoa was administered (-0.21 mmol/L, 95% CI: -0.48, 0.05; p = 0.1170), with significant between-study heterogeneity (p = < .0001, I2 = 82.83%). Ten RCTs were used to investigate TG, and a significant effect of cocoa supplementation was found by meta-analysis (-0.21mmol/L, 95% CI: -0.40, -0.02; p = 0.0333), with between-study heterogeneity (p = < .0001, I2 = 96.23%) (Figure 3).

Subgroup analysis showed that cocoa supplementation decreases cholesterol levels in patients with dyslipidemia and diabetes and in individuals who were supplemented with cocoa compared to studies with chocolate (table 2). However, the duration and design of the study, the dosage used, the mean age and gender of the patients did not influence the levels of cholesterol (data not shown). We also performed subgroup analysis for HDL cholesterol levels. The subgroup analysis showed increased HDL levels in studies with a crossover design, and in patients who did not have dyslipidemia or diabetes. Surprisingly, HDL levels decreased in parallel-design studies, individuals over 50 years of age, and diabetics (table 2). However, the duration of the study, the dosage

and type of cocoa used and gender of the patients did not influence the levels of HDL cholesterol (data not shown).

Subgroup analyses of LDL cholesterol levels demonstrated that these levels were reduced in studies with parallel and crossover designs and in diabetic patients (table 2). On the other hand, the duration of the study, the dosage and type of cocoa used, gender and presence of dyslipidemia did not influence the levels of LDL cholesterol (data not shown). TG levels were decreased in a parallel-design study in individuals under 50 years of age, with diabetes and with and without dyslipidemia (table 2). The duration of the study, the dosage and type of cocoa used and gender did not influence the levels of TG (data not show).

3.3.2. Effect of cocoa supplementation on markers of glycemic status

Eight trials were used to evaluate the effect of cocoa supplementation on FPG, and no significant changes were found (-0.20mmol/L; 95% CI: -0.68, 0.28, p = 0.4106), with between-study heterogeneity (p =< .0001, I2 = 88,58%). Seven RCTs were used to measure insulin, and no significant change was observed (-0.97mU/L; 95% CI: -3.20, 1.27, p = 0.3969). The between-study heterogeneity was not significant (p = 0.1471, I2 = 36.87%). In addition, seven RCTs used in this review with no heterogeneity among them (p = 0.8306, I2 = 0%) demonstrated that HOMA-IR was not significantly influenced by cocoa supplementation (-0.21; 95% CI: −0.47, 0.04, p = 0.0962). The meta-analysis of HbA1c parameters was performed with three studies; however, there was no significant effect of cocoa supplementation (-0,48%; 95% CI: −1,28, 0.33, p = 0.2434), with between-study heterogeneity (p = 0.0178, I2 = 75.19%) (Figure 4).

Subgroup analysis showed that chocolate supplementation decreased glucose levels compared to studies that supplemented with cocoa (table 2). On the other hand, the

duration and design of the study, the dosage, gender or mean age and presence of dyslipidemia or diabetes did not influence the levels of glucose (data not shown).

3.4.3. Effect of cocoa supplementation on blood pressure

The overall results of the meta-analysis of eight studies investigating the effect of cocoa supplementation on SBP did not show a significant change (−2.16mmHg, 95% CI: -5.34, 1.02, p = 0.1839) and no between-study heterogeneity (p = 0.077, I2 = 46.15%). In addition, DBP was also not significantly changed (−1.46 mmHg, 95% CI: -3.70, 0.78, p = 0.2015). The between-study heterogeneity was significant (p = 0.04, I2 = 50.90%) (Figure 5). Subgroup analysis showed that chocolate supplementation decreased DBP compared to studies that supplemented with cocoa (table 2). However, the duration and design of the study, the dosage, gender or mean age and presence of dyslipidemia or diabetes did not influence the DBP (data not shown).

3.4.4. Effect of cocoa supplementation on anthropometric measures

Eight studies assessed the effect of cocoa supplementation on body weight, and no effects were observed (0.91 kg, 95% CI: -0.01, 1.83, p = 0.0532). The between-study heterogeneity was not significant (p = 0.5621, I2 = 0%). The meta-analysis of eight studies did not show a significant effect of cocoa administration on WC (-1.58cm, 95% CI: -4.14, 1.01, p = 0.2312), with between-study heterogeneity (p = 0.0002, I2 = 75.23%). Eight studies investigated the effect of cocoa supplementation on BMI. The pooled effect size did not show any significant effect on BMI (-0.28 kg m^{-2}, 95% CI: −0.82, 0.27, p = 0.3195), with between-study heterogeneity (p = 0.0056, I2 = 64.95%) (Figure 6).

Subgroup analysis showed that chocolate supplementation decreased BMI compared to studies that supplemented with cocoa (table 2). However, the duration and design of the study, the dosage, gender or mean age and presence of dyslipidemia or

diabetes did not influence the BMI (data not shown). Waist circumference was reduced in studies lasting more than 8 weeks and in individuals under 50 years of age and with dyslipidemia (Table 2). The design of the study, the dosage and type, gender and presence of diabetes did not influence the WC (data not shown).

3.5- Meta-regression

The meta-regression analysis was conducted to evaluate whether the changes in outcomes in response to cocoa supplementation could be associated with duration of intervention and age mean. The effect of cocoa supplementation on outcomes was independent of duration of intervention and age mean in SBP ($p = 0.095$ and $p= 0.1572$, respectively), DBP ($p = 0.7524$ and $p= 0.7548$, respectively), insulin ($p = 0.3869$ and $p= 0.6552$, respectively), HOMA ($p = 0.6205$ and $p= 0.7394$, respectively), total cholesterol ($p = 0.6465$ and $p= 0.9795$, respectively), HDL cholesterol ($p = 0.1820$ and $p= 0.0509$, respectively), LDL cholesterol ($p = 0.6291$ and $p= 0.8763$, respectively), and weight ($p = 0.5791$ and $p= 0.0845$, respectively).

On the other hand, meta-regression analysis revealed that cocoa supplementation decreased glucose ($p=0.0042$), HBA1c ($p=0.0046$), TG ($p=<0.0001$) levels and BMI ($p=<0.0001$) and WC ($p=<0.0001$) as the study duration increased. Furthermore, the mean age of study participants influenced HbA1c level ($p=0.047$), BMI ($p=0.0009$) and WC ($p=<0.0001$) (Figure 7).

3.6- Publication bias

Publication bias assessment was conducted using funnel plots and Egger's linear regression test. A visual inspection of the funnel plot revealed no publication bias in the

majority of included studies (data not shown). Egger's linear regression test confirmed the absence of publication bias (TC: p = 0.4508, LDL: p = 0.1974, TG: p= 0.9352, glucose: p = 0.5855, insulin: p = 0.0693, HbA1c: p = 0.7794, SBP: p = 0.9313, DBP: p = 0.5307, BMI: p = 0.5923, weight: p = 0.1270, WC: p= 0.3645). However, for HDL levels (p = 0.0214) and HOMA (p = 0.0374) Egger's linear regression test confirmed a publication bias.

3.7 - Quality of the evidence for the outcome using GRADE

GRADE categorized the quality of evidence into four levels: High, Moderate, Low and Very Low quality, ranging from confidence that the true effect approximates the estimate of the effect to that the true effect is likely to be substantially different from the estimate of the effect. All outcomes were judged as moderate or low quality of evidence, based on the GRADE approach, due to methodological limitations (risk of bias), the imprecision of pooled effects related to a small number of participants and publication bias since small studies with negative results were missing (Table 3).

4- Discussion

The results of this meta-analysis of thirteen studies, including sixteen arms, indicated that administration of cocoa significantly had no significant effects on arterial pressure, glycemic status markers and anthropometric parameters. However, cacao supplementation reduced triacylglycerol levels, without altering the other parameters of the lipid profile. In addition, cocoa supplementation decreases cholesterol levels in patients with dyslipidemia and diabetes. Cocoa supplementation compared to chocolate supplementation revealed a decrease in total cholesterol. On the other hand, subgroup analysis showed that chocolate supplementation decreased serum glucose levels, DBP and BMI when compared to studies that supplemented with cocoa. The meta-regression

analysis revealed cocoa supplementation decreased glucose, HbA1c, TG levels and BMI and WC as the study duration increased.

The significant reduction in triacylglycerol levels promoted by cocoa supplementation was found in other studies corroborating ours. A meta-analysis published in 2021, using studies with healthy people, found that chocolate supplementation reduced triacylglycerol levels [34]. Similar to that, two meta-analyses focusing on cardiovascular health [35, 36] found a significant reduction compared to placebo. However, a meta-analysis conducted in 2024 [10] with studies in adults with and without established comorbidities and a meta-analysis in 2021 [37] in patients with type 2 diabetes found no evidence of the effect of cocoa consumption on triacylglycerol levels. In addition, our meta-regression analysis, we found that studies with longer duration showed a greater reduction in triacylglycerol levels than studies with shorter duration. The flavanols, particularly the procyanidins, found in cacao, are potent lipase inhibitors in vitro [38, 39]; reduce acute postprandial [39] and fasting plasma triglycerides [40] and increase fecal lipid excretion [41] in animals and humans. It has been demonstrated that cocoa reduces blood triglycerides and lipid accumulation visceral and hepatic in animal models [41-44].

Corroborating our findings, other studies have not found significant changes in HDL and LDL cholesterol levels promoted by supplementation with cocoa products, such a systematic review conducted by Tan et al. 2021 [34] in healthy human subjects. On the other hand, the meta-analysis published in 2024 by Arisi et al. [10] found a reduction in LDL in unhealthy individuals, with no such effect in healthy individuals promoted by cocoa supplementation. The same reduction effect was verified for patients with diabetes in a meta-analysis developed by Darand et al using cocoa/dark chocolate in observational studies in 2021 [37]. Furthermore, meta-analyses conducted by Hooper et al. in 2012,

with chocolate, cocoa, and flavan-3-ols [35] and Lin et al. using cocoa flavanol in 2016 [36] revealed an increase in HDL cholesterol concentration.

We suggest that the discrepancies found in these studies for cholesterol metabolism may be due to the different health conditions of the individuals participating in the studies and also the type of cocoa product that was supplemented. Corroborating this hypothesis, in our subgroup analysis we found that cocoa supplementation was able to reduce cholesterol levels in patients with dyslipidemia and diabetes. Additionally, cocoa supplementation revealed a decrease in total cholesterol compared to chocolate supplementation.

Our results found that cocoa supplementation in individuals with metabolic syndrome and associated diseases was not able to promote a significant reduction in the glycemic profile. However, the results found in the literature are discrepant. A meta-analysis conducted in healthy adults or those with comorbidities found a reduction in blood glucose levels promoted by supplementation with cocoa products [10]. However, in the meta-analysis conducted by Hooper et al. [45] in adult participants with some risk of cardiovascular disease, did not observe a reduction in blood glucose with supplementation with cocoa products.

The metaregression analysis conducted in this study found that cocoa products were more efficient in improving the glycemic profile in a longer study. The meta-analysis conducted by Chen et al. (2022) [11], in adults with type II diabetes mellitus, also found that in long-term studies, cocoa products reduced serum glucose, and that the same effect was not found in short-term studies. Other studies in cells and healthy individuals have also indicated that the intake of cocoa products promotes long-term glucose homeostasis [46, 47].

Several mechanisms are suggested for improving glucose homeostasis, such as cocoa flavanols, through the slowing of digestion and absorption of carbohydrates in the intestinal tract [48], by inhibiting the digestive enzymes α-amylase and α-glucosidase, protect pancreatic b cells, the inhibition of the glucose transporter, glucose transporter 2 (GLUT2), increase insulin secretion, modulate intracellular signaling pathways and genes involved in gluconeogenesis and glycogenesis, and the promotion of the secretion of glucagon-like peptide 1 (GLP-1) [38, 49, 50].

The results of the current study showed that cocoa supplementation did not alter blood pressure. In agreement with these findings, the systematic review conducted by Tan et al [51], which evaluated the effects of cocoa and its derivatives in healthy individuals also reported no significant effect of chocolate and cocoa on BP. Similarly, the meta-analysis by Darand et al [52], conducted in patients with type 2 diabetes mellitus, found that cocoa supplementation had no effect on BP. However, contrasting results were observed in the meta-analyis by Amoah et al [53], which included individuals with normal or elevated BP and in the study by Jafarnejad et al [54], conducted in middle-aged and elderly individuals with or without metabolic syndrome and related conditions; both studies reported a BP-lowering effect of cocoa. The presence of potential sources of between-study heterogeneity in some of these meta-analyses could explain these different results.

Subgroup analysis revealed a reduction in DBP in the chocolate group, corroborating the meta-analysis developed by Tanghe et al. 2021 [55]. One of the most well-established mechanisms by which chocolate may reduce DBP involves the action of flavonoids, which promote endothelium-dependent vasodilation [56], thereby impacting peripheral vascular resistance. The observed decrease in DBP may be explained by its close association with peripheral vascular resistance, in contrast to systolic blood pressure

(SBP), which is more strongly influenced by cardiac output and the capacity of proximal arteries [57].

Our study corroborates the meta-analysis developed by Kord-Varkaneh et al. in 2019 [58] that showed no significant effect on anthropometric measurements after the treatment with cocoa. However, the duration of the studies was an important determinant in verifying the favorable effects on anthropometric measures. Studies suggest that consumption of dietary flavanol-containing substances could reduce BMI and WC [59, 60]. The mechanisms of the anti-obesity potential of flavonols are well investigated, although the exact mechanisms have not yet been clarified. Flavanols have shown a thermogenic effect [61]. They are able to downregulate expression of enzymes involved in biosynthesis of fatty acids, cholesterol and lipogenesis [62, 63]. Additionally, they act on the pancreatic enzymes lipase and amylase [41] and can suppress appetite through increasing GLP-1 and decreasing ghrelin concentration [48, 64].

In our study, we observed significant differences in some metabolic parameters when changing the type of cocoa product—chocolate or cocoa. This can be explained by the increased bioactivity recorded for cocoa delivered primarily in the form of chocolate [53]. This is attributed to the presence of nutrients such as fats that can increase polyphenol bioavailability and slow digestion. Furthermore, chocolate products are very heterogenous, containing variable amounts of cocoa solids, sugar, and fat [65], reflecting the high between-study heterogeneity.

The strengths of this meta-analysis include the synthesis of evidence from RCTs examining MetS-related parameters, detailed subgroup analyses, and a comprehensive assessment of potential biases. Furthermore, our subgroup analysis found that cocoa interventions may have effects on biomarkers of lipid and glucose metabolism, depending

on existing comorbidities, intervention duration, and types of cocoa supplementation. However, there are several potential limitations to our study. The heterogeneity of the studies included in this meta-analysis is the most important limitation. The included trials were conducted in individuals with different health conditions (obesity, type 2 diabetes, hyperlipidemia and hypertension, NAFLD, and metabolic syndrome). Another limitation is the difference in duration and type of cocoa supplementation. Different forms and amounts of cocoa, including flavanol-rich cocoa, procyanidin, cocoa powder, dark chocolate, and cookies, were included. In addition, our meta-analysis was limited by the small number of publications in the subgroup analyses, which resulted in considerable clinical and methodological heterogeneity between studies.

5. Conclusion

Our systematic review and meta-analysis showed that the consumption of cocoa significantly reduced triacylglycerol levels. Furthermore, our findings suggested a beneficial long-term effect of cocoa product intake for metabolic syndrome and related diseases, especially in terms of blood glucose, lipid metabolism, and anthropometric parameters. Further investigations are needed to elucidate the exact mechanisms of action of cacao, as well as the appropriate dose, type of supplementation, duration, and clinical conditions of the individuals.

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

AGC, BGC, GNG and MS contributed to the conception of the research. AGC, BGC, GNG and MS searched databases, screened articles and extracted data. LRM and MS performed statistical analysis; and AGC, BGC, GNG, 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 blood lipid profiles.

Figure 4 - Forest plot of the effect of cocoa supplementation on markers of glycemic status

Figure 5 - Forest plot of the effect of cocoa supplementation on blood pressure

Figure 6 - Forest plot of the effect of cocoa supplementation on anthropometric measures

Figure 7- Meta-regression plots of the association of mean changes in glucose concentrations with duration of treatment (A), association of mean changes in TG concentrations with duration of treatment (B), association of mean changes in plasma HbAc1 concentrations duration of treatment (C) and mean age (D), association of mean changes in BMI duration of treatment (E) and mean age (F) and association of WC duration of treatment (G) and mean age (H).

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, no blind	Placebo=17 Cocoa = 15	No reported	40 g of chocolate containing 70% cocoa/day	4 weeks	smokers with cardiovascular comorbidities	Weight, BMI, Insulin.
Simpson *et al.* 2023	double-blind, parallel	Placebo=16 Cocoa = 16	Placebo= 34.8 ± 9.13 Cocoa = 31.9± 11.20	high-flavanol cocoa (609 mg cocoa flavanols, 95 mg (-)-epicatechin)	4 weeks	Pre-menopausal females with overweight/ obesity	Weight, Insulin.
Leon-Flores *et al.* 2020	Blind, parallel	Placebo=11 Cocoa = 13	Placebo= 42±2.2 Cocoa = 48.3±1.9	cookies with flavonoids extracted from the cocoa containing 12.5 mg of EPI equivalents, twice a day.	8 weeks	overweight	Weight, BMI, WC, glucose, TC, TG, LDL, HDL,
Shiina *et al.* 2019 (1)	double-blind, crossover	11	59.3 ± 7.1	cacao procyanidin supplement (83.3 ± 2.7 mg/day)	4 weeks	Males prediabetic	LDL, HOMA, SBP, DBP
Shiina *et al.* 2019 (2)	double-blind, crossover	11	59.3 ± 7.1	cacao procyanidin supplement (83.3 ± 2.7 mg/day)	4 weeks	Females prediabetic	LDL, HOMA, SBP, DBP
Dicks *et al.* 2018	double-blind, parallel	Placebo=18 Cocoa = 17	Placebo= 62.8 ± 1.6 Cocoa = 65.6 ± 2.6	2.5 g/day of a flavanol-rich cocoa	12 weeks	Type 2 Diabetes and Hypertension	glucose, HbA_{1c}, insulin, HOMA, weight, WC, SBP, DBP, TC, TG, LDL, HDL

Leyva-Soto et al. 2018	double-blind, parallel	Placebo=42 Cocoa = 42	Placebo= 23.6 ± 3.5 Cocoa = 23.8 ±± 3.4	2 g of dark chocolate containing 70% cocoa	6 months	Metabolic syndrome	glucose, HbA_{1c}, HOMA, BMI, WC, SBP, DBP, TC, TG, LDL, HDL
Njike *et al.* 2016 (3)	double-blind, parallel	Placebo=26 Cocoa = 25	Placebo = 54.2 ± 10.1 Cocoa = 54.2 ± 10.1	10 g cocoa powder every day	8 weeks	stage 1 hypertension	glucose, insulin, HOMA, weight, BMI, WC, SBP, DBP
Njike *et al.* 2016 (4)	double-blind, parallel	Placebo=26 Cocoa = 24	Placebo = 53.0 ± 10.6 Cocoa = 53.0 ± 10.6	5 g cocoa powder every day	8 weeks	stage 1 hypertension	glucose, insulin, HOMA, weight, BMI, WC, SBP, DBP, TC, TG, LDL, HDL
McFarlin *et al.* 2015 (5)	double-blind, crossover	Overweight =7	22± 3	cocoa-containing product (12.7 g natural cocoa)	4 weeks	overweight	TC, TG, HDL, glucose
McFarlin *et al.* 2015 (6)	double-blind, crossover	Obese =7	21±3	cocoa-containing product (12.7 g natural cocoa)	4 weeks	obese	TC, TG, HDL, glucose
Munguía *et al.* 2015	double-blind, parallel	Placebo=5 (2 males) Cocoa = 10 (2 males)	No reported	cacao flavonoids (80 mg)	4 weeks	overweight subjects with borderline criteria of metabolic syndrome	weight, BMI, WC, SBP, DBP, glucose, TC, TG, LDL, HDL
Alavinejad et al. 2015	double-blind, parallel	Placebo=21 (18 males) Cocoa = 21 (15 males)	Placebo= 37.95 ± 10.34 Cocoa = 38.18 ± 11.04	30 gr dark chocolate (83%)	12 weeks	Nonalcoholic fatty liver disease	BMI, WC, glucose, TC, TG, LDL, HDL

Rostami *et al.* 2015	double-blind, parallel	Placebo=28 (12 males) Cocoa = 32 (12 males)	Placebo= 57.17 ± 7.86 Cocoa = 58.71 ± 9.07	25g dark chocolate	8 weeks	diabetes and hypertension	glucose, insulin, HbAc1, SBP, DBP, TC, TG, LDL, HDL
Parsaeyan *et al.* 2014	Parallel	Placebo=50 Cocoa = 50	54 ± 5	20g cocoa poder	6 weeks	type 2 diabetes	TC, TG, LDL, HDL
West *et al.* 2014	double-blind, crossover	30	51.7 ± 1.2	37 g/d of dark chocolate	4weeks	overweight	insulin, HOMA, TC, TG, LDL, HDL,weight, BMI, WC

Abbreviations: TG: triacylglycerol; TC: total-cholesterol; LDL: Low-density lipoprotein cholesterol; HDL: High-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance, BMI: Body mass index; WC: Waist circumstance; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Table 2 - The results of subgroup analyses

Subgrouped	No. of trials	Effect size (95% CI)	P Value	I^2 (%)	P heterogeneity	P for between subgroup heterogeneity
Cholesterol Type						<0.001
Cocoa	6	-0.20(-0.39, -0.01)	0.0311	81,9%	<0.001	
Chocolate	4	-0.05 (-0.47, 0.37)	0.8075	67.3%	0.0098	
Dyslipidemia						<0.001
Yes	2	-5.19 (-0.93, -0.26)	0.0005	0%	0.9337	
No	8	0.05 (-0.04, 0.16)	0.3267	84.4%	<0.001	
Diabetes						< 0.001
Yes	5	-0.35 (-0.64, -0.06)	0.0164	87.2%	<0.001	
No	5	-0.12 (- 0.27, 0.02)	0.2746	0%	0.1152	
HDL						
Study design						0.0563
Crossover	3	0.18 (0.03, 0.34)	0.0190	57.7%	0.1569	
Parallel	7	-0.03 (-0.05, -0.01)	0.0066	0%	0.0756	
Mean age						0.0472
< 50 years	5	0.11 (-0.01, 0.23)	0.0713	65.7%	0.0123	
> 50 years	4	-0.04 (-0.06, -0.02)	0.0006	0%	0.6968	
Dyslipidemia						0.0065
Yes	4	0.01 (-0.06, 0.08)	0.8396	56.9%	0.0173	

No	6	0.09 (0.03, 0.16)	0.0038	0%	0.0493	
Diabetes						0.0753
Yes	3	-0.04 (-0.06, -0.02)	0.0005	0%	0.5881	
No	7	0.09 (0.01, 0.18)	0.0240	55.3%	0.040	
LDL						0.1013
Study design						
Crossover	3	-1.05 (3.47, 1.36)	0.3910	98.1%	0.0015	
Parallel	7	-0.26(-0.50, -0.03)	0.0291	69.7%	0.0003	
Dyslipidemia						0.0073
Yes	4	-0.51(-0.70, -0.33)	<0.001	44.6%	0.2600	
No	6	-0.41 (-1.63, 0.81)	0.5107	97.4%	0.0046	
TG						
Study design						<0.001
Crossover	3	-0.03 (-0.18 0.12)	0.6859	87.92%	<0.0001	
Parallel	7	-0.35 (-0.56, -0.14)	0.0010	85.21%	<0.0001	
Mean age						<0.001
< 50 years	5	-0.23 (-0.66, 0.19)	0.2785	98.80%	<0.0001	
> 50 years	4	-0.22 (-0.27, -0.18)	<0.0001	0%	0.0825	
Dyslipidemia						<0.001
Yes	2	-0.73 (-1.10, -0.36)	<0.0001	55.34%	0.1345	
No	8	-0.15(-0.18, -0.13)	<0.001	0%	<0.001	
Diabetes						<0.001
Yes	5	-0.32(-0.35, -0.28)	<0.001	95.84%	<0.001	
No	5	-0.01 (-0.30, 0.27)	0.9241	97.4%	0.0046	
Glucose						
Type						<0.001
Cocoa	4	0.16 (-0.18, 0.51)	0.3559	54.4%	0.0150	
Chocolate	3	-0.64 (-1.32, -0.05)	0.0350	80.37%	0.0002	
BMI						
Type						0.0399
Cocoa	4	-0.06 (-0.30, 0.17)	0.6045	0%	0.6594	
Chocolate	4	-1.18 (-2.32, -0.05)	0.0408	28.6%	0.0089	
DBP						0.2858
Cocoa	6	-0.30(-3.29, 2.68)	0.8412	61.18%	0.2570	
Chocolate	2	-4.71 (-7.73, -1.68)	0.0023	0%	0.3555	
WC						
Duration						0.005
<8 weeks	3	0,323 (-2.69 3.32)	0.8343	0%	0.5205	
>8weeks	6	-3.00 (-4.09, -1.90)	<.0001	0%	0.0002	
Mean age						0.3370
< 50 years	3	-5.77 (-7.43, -4.10)	<0.0001	0%	0.8356	
> 50 years	4	-0.60 (-1.94, 0.73)	0.3757	0%	0.0825	
Dyslipidemia						0.7246
Yes	3	-5.71 (-7.57, -4.24)	<0.0001	0%	0.2740	
No	5	-0.54(-1.86, 0.76)	0.4138	0%	0.9013	

Table 3 - Summary of main results

Outcome	Studies	Participants	Effect Estimate	P value	Quality of Evidence (GRADE)
TC	10	404	-0.14 [-0.45, 0.16]	0.3606	⊕⊕⊕⊖ Moderate[1]
LDL	10	412	-0.21 [-0.48, 0.05]	0.1170	⊕⊕⊕⊖ Moderate[1]
HDL	10	404	0.07 [-0.01, 0.14]	0.0876	⊕⊕⊖⊖ Low[1,3]
TG	10	404	-0.21[-0.40, -0.02]	0.0333	⊕⊕⊕⊖ Moderate[1]
FPG	8	274	-0.20 [-0.68, 0.28]	0.4106	⊕⊕⊕⊖ Moderate[2]
Insulin	7	290	-0.97 [-3.20, 1.27]	0.3969	⊕⊕⊕⊖ Moderate[2]
HOMA	7	272	-0.21 [−0.47, 0.04]	0.0962	⊕⊕⊖⊖ Low[2,3]
HbA1c	3	179	-0,48 [−1,28, 0.33]	0.2434	⊕⊕⊖⊖ Low[1,2]
SPB	8	317	−2.16 [-5.34, 1.02]	0.1839	⊕⊕⊕⊖ Moderate[2]
DPB	8	317	−1.46 [-3.70, 0.78]	0.2015	⊕⊕⊕⊖ Moderate[2]
Weight	9	311	0.91 [-0.01, 1.83]	0.0532	⊕⊕⊕⊖ Moderate[2]
WC	8	331	-1.58 [-4.14, 1.01]	0.2312	⊕⊕⊖⊖ Low[1,2]
BMI	8	328	-0.28 [−0.82, 0.27]	0.3195	⊕⊕⊖⊖ 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.

[3]Publication bias: Literature were searched exhaustively. Funnel plots were conducted and showed asymmetry (small studies with negative results were missing).