

## **Cocoa effects on blood pressure, endothelial function, and inflammation: a systematic review**

### **Efeitos do cacau sobre a pressão arterial, a função endotelial e a inflamação: uma revisão sistemática**

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**ABSTRACT**

Hypertension has affected 1,13 billion people worldwide and is one of the main risk factors for cardiovascular disease, which has a high mortality rate. The aim of the study was to review the effect of cocoa on systolic and diastolic blood pressure in hypertensive patients, and its alterations in endothelial function and inflammation. We searched PubMed, Scopus, Science Direct, Lilacs and MedLine databases in August 2018. Inclusion criteria were limited to hypertensive individuals, cardiovascular diseases, intervention and randomized clinical studies; published since 2000 and in English, Spanish and Portuguese languages. A total of 433 articles were found in the search, which 13 met the inclusion criteria. The trials had a sample of 19 to 122 participants. The cocoa was offered as chocolate bars and cocoa beverage. Chocolate bars doses ranged from 6 g to 100 g/day and cocoa powder doses ranged from 5 g to 31 g/day. Intervention ranged from 2 to 18 weeks. The results observed revealed a significant blood pressure-reducing effect of cocoa, an increase in flow-mediated dilation in the evaluation of endothelial function, and no changes in antioxidant and anti-inflammatory capacity were observed after intervention. The conclusion suggests that cocoa has a blood pressure lowering effect, but new studies are still required with hypertensive individuals so that their mechanisms are better evaluated and their efficacy proven.

**Keywords:** cocoa, chocolate, polyphenols, hypertension, inflammation.

**RESUMO**

A hipertensão afeta 1,13 bilhão de pessoas em todo o mundo e é um dos principais fatores de risco para doenças cardiovasculares, com alta taxa de mortalidade. O objetivo do estudo foi analisar o efeito do cacau na pressão arterial sistólica e diastólica em pacientes hipertensos e suas alterações na função endotelial e na inflamação. Fizemos buscas nos bancos de dados PubMed, Scopus, Science Direct, Lilacs e MedLine em agosto de 2018. Os critérios de inclusão foram limitados a indivíduos hipertensos, doenças cardiovasculares, intervenção e estudos clínicos randomizados; publicados desde 2000 e nos idiomas inglês, espanhol e português. Um total de 433 artigos foi encontrado na pesquisa, dos quais 13 atenderam aos critérios de inclusão. Os estudos tinham uma amostra de 19 a 122 participantes. O cacau foi oferecido como barras de chocolate e bebida de cacau. As doses de barras de chocolate variaram de 6 g a 100 g/dia e as doses de cacau em pó variaram de 5 g a 31 g/dia. A intervenção variou de 2 a 18 semanas. Os resultados observados revelaram um efeito significativo do cacau na redução da pressão arterial, um aumento na dilatação mediada pelo fluxo na avaliação da função endotelial e nenhuma alteração na capacidade antioxidante e anti-inflamatória foi observada após a intervenção. A conclusão sugere que o cacau tem um efeito redutor da pressão arterial, mas ainda são necessários novos estudos com indivíduos hipertensos para que seus mecanismos sejam melhor avaliados e sua eficácia comprovada.

**Palavras-chave:** cacau, chocolate, polifenóis, hipertensão, inflamação.

## 1 INTRODUCTION

Hypertension (HTN) has affected 1,13 billion people around the world and its complications causes 9,4 million deaths each year<sup>(1, 2)</sup>. Besides, it is one of the risk factors for Cardiovascular Disease (CVD), which was the disease responsible for more deaths in the world in 2016<sup>(3)</sup>.

Experimental studies have suggested that polyphenols, found in large quantities in cocoa, have protective effects against CVD and its risk factors. Mainly the class of tannins and flavonoids are being studied due to their capacity to reduce blood pressure, antioxidant, anticoagulant and anti-inflammatory effects, improvement of glucose metabolism and lipid profile and effects on endothelial function by improvement in flow-mediated dilatation (FMD)<sup>(4, 5, 6, 42)</sup>. These effects can be explained by improving the oxidative status, promoted by flavonoids, through the neutralization of free radicals<sup>(43)</sup>.

The explanation of the protective effect against CVD is on the fact that cocoa is rich in monomeric flavonoids (epicatechin and catechin) and oligomeric flavonoids (procyanidins) that have antioxidant action and capacity of activate the enzyme endothelial nitric oxide synthase (eNOS), increasing the bioavailability of Nitric Oxide (NO), resulting in vessel relaxation. Studies demonstrate that this capacity of vasodilatation from the increased production of NO interferes in the reduction of peripheral vascular resistance and improves the vascular and metabolic actions of insulin, in addition, reduces blood pressure<sup>(7, 8, 9)</sup>.

Cocoa also has effects on another CVD risk factor, inflammation. Its high polyphenols content has been associated with the improvement in inflammatory process. The explanation is in the fact that polyphenols have an action in the modulation of the signaling cascade, in the inhibition of the expression of genes involved in inflammation and in the suppression of chronic inflammation, in addition to being antioxidants<sup>(6, 10, 11)</sup>. In detail, cocoa flavonoids have an action on the suppression of Cyclooxygenase-2 (COX-2) expression, inhibit the production and expression of TNF- $\alpha$ , from the modulation of NF-kB, and the production of lipopolysaccharide induced cytokines (LPS), as IL-8. However, cocoa's anti-inflammatory capacity it is not totally elucidated on the literature<sup>(12, 13, 14, 15)</sup>.

Despite the innumerous studies about cocoa, the answers about the effects on HTN and your metabolic consequences, as well as the clinical relevance of its action are still unclear. The different methodological approaches used, the use of different forms and quantities of cocoa in studies and insufficient studies focusing on hypertensive patients, and their effects on endothelial function and inflammation may explain the lack of consensus on the use of cocoa in clinical practice<sup>(16, 17)</sup>.

Thus, the objective of this study is to systematically review the literature and assesses the effect of cocoa intake on blood pressure, the doses administered, and their efficacy in hypertensive individuals.

## 2 METHODS

### 2.1 DEFINITION OF THE OBJECT OF STUDY/INTERVENTION

The intervention was based on the electronic search for studies that evaluated, daily doses or at a specific moment, placebo or food containing cocoa in its composition and was performed with elevated blood pressure individuals. The HTN is defined when the arterial pressure levels are equal to or higher than 140 mmHg of systolic blood pressure and/or 90 mmHg of diastolic blood pressure<sup>(2)</sup>. Furthermore, there is currently a new category of prehypertensive individuals who presents the blood pressure levels between 120-129 mmHg of systolic blood pressure and/or 80-89 mmHg of diastolic blood pressure and have a risk of progression to hypertension<sup>(18)</sup>.

The consumption of cocoa was considered in bars of chocolate, cocoa powder and cocoa powder-based beverages, without minimal exposure.

### 2.2 SEARCH METHODS AND CRITERIA FOR ELIGIBILITY

The search was defined based on the PICOS strategy: population, intervention, comparative, outcome and study design. Three reviewers selected the following keywords: *blood pressure, high blood pressure, hypertension, theobroma cacao, cocoa, cacao* and *endothelial* that were combined with the AND and OR connectors, applying the criteria: keywords most commonly used in the literature and being a health descriptor (MeSH and DeCS).

It was conducted in August 2018 in the following databases: SCIENCE DIRECT, SCOPUS, PUBMED, LILACS and MEDLINE and an example of search strategy used was "CACAO" or "COCOA" and "HYPERTENSION" and "ENDOTHELIAL". Inclusion criteria were limited to hypertensive individuals with cardiovascular diseases, clinical intervention studies and randomized studies; published since 2000 in English, Spanish and Portuguese.

Were excluded experimental studies that did not use humans, observational studies, case-control, meta-analysis articles, systematic review, letter to the editor, studies with healthy individuals and studies with other diseases besides hypertension and cardiovascular diseases (chronic kidney disease, neurological diseases, metabolic syndrome, inflammatory diseases,

autoimmune diseases, hepatopathy, thyroid-related diseases, cancer, pulmonary diseases, gastrointestinal diseases or any other severe systematic disease).

## 2.3 PRIMARY AND SECONDARY OUTCOMES

The primary outcomes investigated in this study were Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP); the secondary outcomes were flow-mediated dilatation (FMD), oxidative stress, antioxidant capacity and inflammatory biomarkers IL-6, IL-8, IL-10, TNF- $\alpha$  and PCR.

## 2.4 DATA EXTRACTION

The data extraction of the included articles was realized by two authors review using a Microsoft Excel spreadsheet, based on the tool prepared by Pereira and Galvão<sup>(19)</sup> on how to prepare a systematic review, in which data were extracted from 13 articles included in this study. After that, the information of the spreadsheet was cross-checked.

## 2.5 ASSESSMENT OF RISK OF BIAS

Two review authors assessed the risk of bias separately. The differences in the assessment of the studies were resolved by discussion and agreement with a third reviewer. The articles were assessed according to the risk of bias based on the Cochrane Collaboration (RoB - Risk of Bias) tool. The aspects were assessed individually, being classified as Low risk of bias, Uncertain risk of bias and High risk of bias. The overall assessment of the study is based on: if one or more aspects are classified as uncertain bias risk, the study is classified as uncertain risk, the same occurs in the classification for High Risk of bias. The study is only classified as low risk of bias if it obtains this classification in all aspects.

# 3 RESULTS

## 3.1 SEARCH AND DATA EXTRACTION

The research found 433 articles through search strategies. The duplicates were excluded, and, after that, the articles underwent two eligibility assessment criteria based on the defined criteria; first, they were assessment by title and abstract, later, they were assessment by the full reading of the articles. At the end, 13 articles were included in this systematic review. The selection steps are shown in figure 1.

## 3.2 DESCRIPTION OF STUDIES

The included studies in this review were published from 2005 to 2016 and involved adults and the elderly. Due to the low number of studies found in the literature search, were considered studies that used men and women, postmenopausal women, glucose intolerant participants and those with type 1 and type 2 diabetes in the intervention. In relation to blood pressure (BP) levels, the studies evaluated not only hypertensive individuals, but also individuals with normotension, prehypertension, mild hypertension and stage 1 hypertension.

The forms of cocoa used were in chocolate bars and cocoa powder-based beverages. The bar doses ranged from 6g to 100g/day and the powder doses ranged from 5g to 31g/day. Intervention time ranged from 2 to 18 weeks. The studies used a sample of 19 to 122 participants, which means that there was no regularity in relation to sample size, and some studies showed significant loss of participants until their conclusion. Only a few studies used placebo in the intervention, and they had difficulty to find something similar to cocoa products. Table 1 shows all the information related to the 13 studies included in this review.

## 3.3 RISK OF BIAS RESULTS

### 3.3.1 Random Sequence Generation

Six trials described the method used for randomization <sup>(20, 22, 23, 24, 25, 27)</sup>. Another six trials reported that there was randomization, but without detailing the method used <sup>(8, 9, 15, 18, 19, 20)</sup>. One trial reported that the randomization was performed by an employee who did not participate in the study, so it was classified as High risk of bias <sup>(12)</sup>.

### 3.3.2 Allocation

Eight trials described adequate allocation concealment <sup>(8,9, 20, 23, 24, 25, 26, 27)</sup>. Three trials did not provide sufficient information on allocation concealment <sup>(16, 28, 29)</sup>. Two trials were classified as High Risk of Bias due to lack of placebo for cocoa in the control groups <sup>(15, 16)</sup>.

### 3.3.3 Blinding

Four trials compared the non-blind cocoa group with the white chocolate control group, being classified as Uncertain Risk <sup>(8, 24, 26, 29)</sup>. In addition, three trials did not report if the participants were blinded <sup>(27, 28, 29)</sup>. Two other trials did not blind the participants <sup>(21, 22)</sup> and only one trial was not blinded because the study was an interventional clinic <sup>(20)</sup>.

### 3.3.4 Incomplete Outcome

In two trials, there was a loss of about 30% of the sample <sup>(9, 29)</sup>. Two trials reported insufficient information on the reason for the losses <sup>(27, 28)</sup>.

### 3.3.5 Selective Outcome

All trials provided insufficient information to judge.

### 3.3.6 Other Sources of Bias

We consider a source of funding as an influence for the possibility of bias. One article was sponsored by industry <sup>(25)</sup>.

In table 2, it is possible to find the classification by domain of all the studies included in this review for the Risk of Bias, according to the author's assessment that was based on the *Cochrane's* tool.

## 3.4 EFFECTS OF COCOA INTERVENTION ON HYPERTENSION, ENDOTHELIAL FUNCTION AND INFLAMMATION

The analysis of the 13 articles revealed, mostly, a significant result in the reduction of SBP and DBP in the intervention group compared to the initial characteristics of the study and the placebo group. The values of the results extracted from the selected articles in this review are presented in table 3.

However, Muniyappa *et al.* <sup>(9)</sup> and Riedel *et al.* <sup>(22)</sup> showed no significant changes in blood pressure in the groups evaluated. In addition, one study showed a significant increase in ambulatory 24-hour systolic blood pressure compared to placebo, although, central SBP showed a significant reduction <sup>(25)</sup>.

The doses that were most effective in reducing SBP and DBP were 25g and 100g daily <sup>(26, 29)</sup>. The lowest dose had 450mg of flavonoids in its composition. Both studies used chocolate bars. The intervention duration were 8 weeks and 15 days, respectively.

In the evaluation of endothelial function, the FMD method was used in Grassi *et al.* <sup>(8)</sup>, Njikeet *et al.* <sup>(23)</sup> and Grassi *et al.* <sup>(29)</sup>. The expected result is an increase in FMD after consumption of cocoa products, and only Njikeet *et al.* <sup>(23)</sup> found no significantly positive result ( $p = 0.6920$ ). In this study, two intervention groups were used, one with 5g and the other with 10g of cocoa powder, and a placebo group. The study reports that the highest dose of cocoa showed better results <sup>(23)</sup>. The only one who evaluated a plasma marker of vasodilation was Taubert *et al.* <sup>(24)</sup>,



who used S-nitrosoglutathione, which showed significantly positive results ( $p < 0.001$ ) at the end of the study.

Muniyappa *et al.*<sup>(9)</sup>, Nogueira *et al.*<sup>(20)</sup>, Njikeet *et al.*<sup>(23)</sup>, Rostami *et al.*<sup>(26)</sup>, Deschet *et al.*<sup>(27)</sup> and Grassi *et al.*<sup>(29)</sup> assessed the anti-inflammatory capacity of cocoa through biochemical parameters PCR, ICAM-1, adipocins, TNF- $\alpha$  and IL-6. Of these studies, only Rostami *et al.*<sup>(26)</sup> presented significant results ( $p = 0.043$ ) for the reduction of inflammation assessed by reducing PCR.

Regarding the antioxidant capacity of cocoa, only Taubert *et al.*<sup>(24)</sup> evaluated alterations through the 8-isoprostane biomarker, which showed no alterations at the end of the intervention.

#### 4 DISCUSSION

According to the most studies, the cocoa intervention has a significantly reducing effect on blood pressure; the studies that obtained the best results were Grassi *et al.*<sup>(29)</sup> and Rostami *et al.*<sup>(26)</sup>. They used in periods of 15 days and 8 weeks, respectively, chocolate bars in the amount of 100g and 25g daily, and Rostami *et al.*<sup>(26)</sup> used 83% cocoa chocolate with 450 mg of flavonoids.

A study that can be highlighted is Davison *et al.*<sup>(16)</sup>, carried out for 6 weeks, in which the daily dose of 1052 mg of flavonoids, offered in cocoa beverage, showed a significant reduction in SBP and DBP. There is a suggestion in the literature that about 900 mg of flavonoids have a positive effect on blood pressure<sup>(31)</sup>. These studies suggest that it is important to dimension the quantity of flavonoids of the product used in the intervention, since it is pointed as the main agent of the reducing effect of blood pressure present in cocoa.

The mechanism by which the cocoa has effect in the BP is explained through the capacity of the flavonoids to activate the enzyme eNOS, providing vasodilation stimulated by the production of NO. This relaxation of the vascular smooth muscle leads to a reduction in blood pressure<sup>(8,9,32)</sup>. In addition, the consumption of cocoa increases the sensitivity to insulin, which has action in the production of NO through its cellular signaling pathway, modulating the regulation of NO synthase. Another factor that supports in this reducing mechanism is the antioxidant capacity of the flavonoids and the inhibition of the angiotensin-converting enzyme (ACE), thus, improving the oxidative stress and, consequently, favoring the production and availability of NO<sup>(14,33)</sup>.

From this, we can observe a limitation of this review which was the inclusion of studies that used in the intervention individuals with impaired glucose tolerance<sup>(8)</sup>, type 1 and type 2 diabetes mellitus<sup>(26,27)</sup>. Therefore, it is important to observe the results found in these studies



since they may not have been as effective because of the deficiency and insulin resistance that exists in these cases <sup>(34)</sup>.

It is also important to highlight the variability of cocoa products used, most of the studies assessed used chocolate bars rich in cocoa <sup>(8,20, 21, 22, 24, 26, 27, 29)</sup>, but cocoa powder was also used in the preparation of beverages <sup>(9, 16, 23, 25, 28)</sup>. This is important because the processing of cocoa can negatively interfere in the final concentration of polyphenols, which is the class indicated as responsible for the effects on oxidation, inflammation and endothelial function. And, thus, affect the outcomes related to these aspects <sup>(10, 35)</sup>.

In endothelial dysfunction, there is a decrease in endothelium-dependent relaxation due to low bioavailability of NO, increased vasoconstrictor effects leading to increased inflammation and vascular lesions <sup>(36)</sup>. Currently, the gold standard for evaluation of noninvasive endothelial function is FMD, which indicates endothelial vasodilation mediated by NO <sup>(37)</sup>. Epicatechin, a flavonoid compound, is the main responsible for the improvement in endothelial function by stimulating NO synthesis <sup>(37)</sup>. In this review, two of the three studies that assessed FMD presented significant results in the improvement of endothelial function, they used 65.97 mg ( $p < 0.0001$ ) and 110.9 mg ( $p < 0.05$ ) of epicatechin <sup>(8, 29)</sup>. The concentration of epicatechin in both studies was higher than that used by Njike *et al.* <sup>(23)</sup> who used chocolate with 26 mg of epicatechin and cocoa beverage with 19.8 mg and did not present significant results ( $p = 0.6920$ ). These findings are similar to the suggested in Vlachoianis *et al.* <sup>(31)</sup> that a daily dose of epicatechin around 100 mg has effects on FMD.

Inflammation is recognized as a triggering factor for endothelial dysfunction, CVD and HTN. Its relationship was established through studies, which evaluated normotensive individuals, and observed that altered biomarkers of inflammation in plasma were a risk factor for the development of HTN. Thus, they suggested that substances that help reduce blood pressure, in this case polyphenols, also affect the inflammatory process <sup>(9, 36)</sup>.

Polyphenols are indicated as promoters of anti-inflammatory mediators and stimulate the NO production, alter the recruitment of inflammatory cells of the circulation and make it difficult for them to migrate to the subendothelial space, reducing the formation of atherosclerotic plaque <sup>(38)</sup>. CRP is one of the most evaluated pro-inflammatory proteins in the studies; its transcription is commanded by IL-6 <sup>(36)</sup>. It was evaluated by 6 studies in this review, and only the study by Rostami *et al.* <sup>(26)</sup> found a significant reduction ( $p=0.043$ ). However, this result was only observed when compared to the beginning of the intervention and, when compared to the placebo group, there were no changes.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine, responsible for NF- $\kappa$ B inhibition, resulting in the reduction of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$  <sup>(36)</sup>. Some of these cytokines were evaluated in the studies of this review (TNF- $\alpha$ , IL-6, MCP-1 and 8-isoprostane) and showed no significant differences at the end. This supports what has already been placed by Grassi *et al.* <sup>(29)</sup> that perhaps the evaluation method of these concentrations, the dose used and the duration of intervention were not sufficient to obtain results in relation to the anti-inflammatory capacity of cocoa, or that perhaps the proposed mechanism of action was not fully elucidated.

Cocoa is also known for its antioxidant capacity, according to Lee *et al.* <sup>(39)</sup>, this capacity is higher than the wine, green tea and black tea, also known for this characteristic <sup>(39)</sup>. This explanation is in its high content of flavonoids which, due to its chemical structure, has the capacity to eliminate free radicals <sup>(40)</sup>. A study with male smokers that used chocolate in the intervention found that the antioxidant capacity increased 2 hours after consumption <sup>(41)</sup>. A different result from that found in a study evaluated in this review, using non-smoking men and women, in which there were no changes after intervention with cocoa <sup>(24)</sup>.

The heterogeneity of the HTN classification of the populations used showed a difficulty in compiling and extracting a statement regarding to the outcome of the studies. In addition, this influences the outcomes in the reduction of blood pressure, since, as has already been pointed out, the higher the blood pressure level at the beginning, the better the results will be at the end of the study <sup>(24)</sup>. What was also confirmed by the review articles that used hypertensive individuals, they presented better outcomes in relation to the reduction in BP compared to the beginning of the study and compared to studies with prehypertensive individuals <sup>(8, 24, 29)</sup>.

Another important limitation of studies involving cocoa is the difficulty of finding a placebo that has similar sensory characteristics, especially when using cocoa powder and chocolate bar. Four studies in this review used white chocolate (flavonoids-free) as the control group and all studies presented significant results in the reduction of blood pressure <sup>(8, 24, 26, 29)</sup>. According to meta-analysis, the use of white chocolate may cause overestimation of the results of the intervention group, since this is one of the factors that alters the blinding of the participants, leading to a possible risk of bias in the results <sup>(30)</sup>.

The acceptability of chocolate and the possibility of maintaining the cocoa intervention for a long time was assessed through reports and acceptability scales filled in by the participants of one of the selected studies. 20% of the participants reported difficulty in accepting the intervention and only 73% would be willing to continue using the daily dose, even after the intervention <sup>(22)</sup>. One option used in this study as a placebo and suggested by the participants as

a form of intervention was the use of capsules. Thus, it is possible to facilitate the blinding of the participants, increase their attendance and control the capsules component in future studies, since commercial chocolates differ in content according to the processing used <sup>(17)</sup>.

Despite facilitating the study method, the use of capsules is contradictory, in addition to making the product more expensive for later use by the population, there is a loss of inclusion of cocoa in the usual diet in its integral form, as food, taking advantage of its functionality for health, as well as its sensory characteristics.

Some of the articles reported the quantity of polyphenols supplied by the intervention per day; two of them reported the quantity of polyphenols <sup>(20, 24)</sup> and four the polyphenolic subclass <sup>(9, 16, 25, 27)</sup>, while other studies only the form of intervention. The lack of standardization in the content of the intervention negatively implies in evidencing any discovery in relation to the dose used for effective results of cocoa use.

Currently, there are several studies that evaluate the effect of cocoa in healthy individuals, while those with hypertensive individuals are insufficient, so for there to be a consensus on the use of cocoa in clinical practice, it is important to have more studies that use this population, that are well controlled, with a larger number of participants and longer intervention time. In addition, recognized characteristics of cocoa such as antioxidant and anti-inflammatory capacity are not yet well elucidated and do not present results in the literature in hypertensive individuals. New randomized clinical trials and analysis of biomarkers that were not seen in any of the studies cited in this review, such as IL-1 $\beta$ , would therefore be necessary to increase the evidences on the anti-inflammatory activity of cocoa.

## 5 CONCLUSION

Our systematic review concluded that cocoa has the ability to reduce blood pressure with a minimum daily intake of 1 week. However, randomized clinical trials in hypertensive individuals are still insufficient in the literature. Thus, new studies are needed to reach a consensus regarding the effect dose and the intervention time.

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Table 1 – Characteristics of the articles included in this review

<i>Reference/Year</i>	<i>Study design</i>	<i>Blinding</i>	<i>N</i>	<i>Population characteristics</i>	<i>Age</i>	<i>Sex ratio</i>	<i>Intervention</i>	<i>Outcome</i>	<i>Duration</i>	<i>Sample losses</i>
Grassi, D. <i>et al.</i> , 2008 <sup>(8)</sup>	Randomized	Single-blind	19	Stage 1 hypertension and glucose intolerance	44	M:11 /W:8	100g Black chocolate / 100g White chocolate	Flavanol Rich Dark Chocolate group: ↑ FMD, and after 15 days ↓ SBP, DBP e MAPA	15 days	NI
Muniyappa, R. <i>et al.</i> , 2008 <sup>(9)</sup>	Randomized, CR, P CTR	Double-blind	29	Stage 1 hypertension	21-65	NI	31g of cocoa powder (≈450mg of flavonoids) /31g of Placebo (≈14mg of flavonoids) in 150 ml of warm water 2x per day	There were no significant changes in blood pressure in both groups	2 weeks	9
Nogueira, L. P. <i>et al.</i> , 2012 <sup>(20)</sup>	Clinical Intervention	Not Blind	28	Stage 1 hypertension	44	M:10/W:10	50g of chocolate 70% cocoa (2135mg of polyphenols)	Mild ↓ in SBP and DBP in 24h day and night	4 weeks	8
Koli, R. <i>et al.</i> , 2015 <sup>(21)</sup>	Randomized, CR, P CTR	Not Blind	30	Mild hypertension	45,8	M:19/W:11	49g of dark chocolate	There were no significant changes in 24h blood pressure in the intervention group. Blood pressure ↓ significantly throughout the study.	8 weeks	8
Ried, K. <i>et al.</i> , 2009 <sup>(22)</sup>	Randomized, CTR	-	39	Prehypertension	22-73	CG – M:5 / W:5 IG – M:7 / W:4	50g of dark chocolate with 70% of cocoa per day/ tomato extract (15 mg of lycopene) / placebo (soy oil – 1 capsule per day)	There were no significant changes in blood pressure in both groups	8 weeks	14
REFEREN CE/ YEAR	Study design	BLINDIN G	N	Population characteristics	AGE	Sex ratio	Intervention	Outcome	Duration	SAMP LE LOSSE S
NJIKE, V. Y. ET AL., 2016 (23)	Randomized, CTR	DOUBLE- BLIND	122	Stage 1 hypertension	53,6	M: 59 /W:63	5g of cocoa powder / 10g of cocoa powder/ placebo	10g Group: ↓ SBP e DBP in patients using ACE inhibitors.	8 weeks	21



DAVISON, K. ET AL., 2010 (16)	Randomized	DOUBLE-BLIND	59	Prehypertension, Mild hypertension and postmenopausal women	NI	NI	Cocoa beverage with 33, 372, 712 or 1052 mg of flavonoids	Participants who used $\beta$ -blockers showed improvement in endothelial function. Overall effect: $\downarrow$ SBP, DBP and MAP. 1052 mg Group: $\downarrow$ SBP and DBP	6 weeks	7
TAUBERT, D. ET AL., 2007 (24)	Randomized, CTR	BLIND INVESTIGATOR	44	Prehypertension and Stage 1 hypertension	55-75	M:20/W:24	6,3g of dark chocolate (30mg of polyphenols) /day / 5,6 g of white chocolate/day	Dark chocolate group: $\downarrow$ SBP and DBP; $\uparrow$ S-nitrosoglutathione after 12 weeks; 4 individuals changed from stage 1 hypertension to prehypertension	18 weeks	0
BOGAARD, B. ET AL., 2010 (25)	Randomized, P CTR, CR	DOUBLE-BLIND	42	Prehypertension, Stage 1 hypertension and postmenopausal women	62	M:32 /W:10	Placebo / cocoa powder rich in flavonoids with a natural dose of theobromine (106mg) / cocoa powder rich in flavonoids with a high dose of theobromine (979mg)	Central SBP $\downarrow$ and cocoa beverage $\uparrow$ significant the SBP of 24h ambulatory compared to placebo	3 weeks	3
ROSTAMI, A. ET AL. 2015 (26)	Randomized, P CTR	DOUBLE-BLIND	68	Hypertension and diabetes mellitus type 2	35-70	DCG – M :12/ W:20 WCG – M:12/ W:16	25g of dark chocolate (450mg of flavonoids) /day / 25g of white chocolate/day	In relation to initial characteristics: DCG $\downarrow$ significant SBP, DBP, hbA1c and PCR. In relation to WCG: the DCG showed a $\downarrow$ in SBP and DBP	8 weeks	8
REFEREN CE/ YEAR	Study design	BLINDIN G	N	Population characteristics	AGE	Sex ratio	Intervention	Outcome	Duration	SAMP LE LOSSE S

DESCH, S. ET AL., 2010(27)	Randomized	NI	102	Prehypertension, Stage 1 hypertension and diabetes mellitus type 1 and 2	NI	NI	6 g of dark chocolate (5mg of epicatechin) / 25 g of dark chocolate (21mg of epicatechin)	Both groups: ↓ significant the mean and SBP of 24h In the 6g group: there were significant ↓ in DBP	3 months	11
ALLEYN E, T. ET AL., 2014(28)	CR	NI	45	Mild hypertension and normotensive	35-60	NI	5 g of cocoa in 125 mL of water / Placebo 1 / Placebo 2	Mild hypertension: ↓ SBP and DBP Hypertension: ↓ SBP and DBP Normotensive: there were no changes	1 week	9
GRASSI, D. ET AL., 2005 (29)	Randomized, CR, P, CTR	NI	35	Essential Hypertension and Normotensive	EH: 43,65 NT: 33,9	EH - M:10/W:10 NT - M:7/W:8	100g of dark chocolate bar / 90g of White chocolate bar (flavonoids free)	Compared to the beginning of the study: dark chocolate in hypertensive: ↓ SBP, DBP, MAPA ↑FMD. For normotensive: ↑FMD after dark chocolate	15 days	NI

Source: Own authorship. N= Trial sample; M= Man; W= Woman; CTR= Controlled; CR= Crossed; CTR = Placebo Controlled; NI=Not Informed; CG=Control Group; IG= Intervention Group; DCG=Dark Chocolate Group; WCG=White Chocolate Group; EH=Essential Hypertension; NT=Normotensive

Table 2. Risk of Bias

Selected Studies	Random sequence generation	Allocation concealment	Blinding of participants and professionals	Blinding of outcome assessment	Incomplete outcome data	Selective reporting
Grassi, D. et al 2008 <sup>(8)</sup>	Unclear	Low	Unclear	Unclear	Low	Unclear
Muniyappa, R et al, 2008 <sup>(9)</sup>	Unclear	Low	Low	Low	Unclear	Unclear
Grassi, D. et al, 2005 <sup>(29)</sup>	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Nogueira, L. P. et al, 2012 <sup>(20)</sup>	Low	Low	High	High	Low	Unclear
Koli, R. et al 2015 <sup>(21)</sup>	Unclear	High	High	Unclear	Low	Unclear
Ried, k. et al, 2009 <sup>(22)</sup>	Low	High	High	Low	Low	Unclear
Njike, V. Y. et al, 2016 <sup>(23)</sup>	Low	Baixo risco	Low	Low	Low	Unclear
Davison, k. et al, 2010 <sup>(16)</sup>	High	Unclear	Low	Low	Low	Unclear
Taubert, D. et al 2007 <sup>(24)</sup>	Low	Low	Unclear	Low	Low	Unclear
Bogaard, B. et al, 2010 <sup>(25)</sup>	Low	Low	Unclear	Unclear	Low	Unclear
Rostami, A. et al 2015 <sup>(26)</sup>	Unclear	Low	Unclear	Unclear	Low	Unclear
Desch, S. et al, 2010 <sup>(27)</sup>	Low	Low	Unclear	Low	Unclear	Unclear
Alleyne, T. et al, 2014 <sup>(28)</sup>	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear

Source: Own authorship.

Table 3. Results of interventions extracted from selected articles

Autor	$\Delta$ beginning/end OR Intervention/control group SBP (mmHg)	$\Delta$ beginning/end OR Intervention/control group DBP (mmHg)	p Value
Grassi <i>et al.</i> , 2005 <sup>(29)</sup>	-11.0 ± 6.3	-6.2 ± 4.2	SBP: p < 0.0001 / DBP: p < 0.0001
Grassi <i>et al.</i> , 2008 <sup>(8)</sup>	NI	NI	P < 0.05
Taubert <i>et al.</i> , 2007 <sup>(24)</sup>	-2,9 ± 1,6	-1,9 ± 1,0	SBP: p < 0,001 / DBP: p < 0,001
Muniyappa <i>et al.</i> , 2008 <sup>(9)</sup>	NI	NI	SBP: p = 0,74 / DBP: p = 0,48

Ried <i>et al.</i> , 2009 <sup>(22)</sup>	NI	NI	SBP – week 0-12: p = 0,23 / week 0-24: p = 0,79 DBP – week 0-12: p = 0,49 / week 0-24: p = 1,0
Davison <i>et al.</i> , 2010 <sup>(16)</sup>	<b>1052mg of flavonoids</b> Ambulatory: -4.1 ± -12.1 24h: -3.8 ± -5.5	<b>1052mg of flavonoids</b> Ambulatory: -2.0 ± -3.2 24h: -2.1 ± -3.2	<b>1052mg of flavonoids</b> 24h MAP: p < 0,001 SBP: p < 0,02 / DBP: p < 0,04
Bogaard <i>et al.</i> , 2010 <sup>(25)</sup>	24h: + 3,2 ± 1,1	24h: + 1,3 ± 0,6	24h SBP: p < 0,01 / DBP: p = 0,04
Nogueira <i>et al.</i> , 2012 <sup>(20)</sup>	NI	NI	24h SBP: p = 0,41 / DBP: p = 0,37
Rostami <i>et al.</i> , 2014 <sup>(26)</sup>	-6.4 ± 6.25	-5.93 ± 6.25	SBP: p = 0,001 / DBP: p = 0,001
Koli <i>et al.</i> , 2015 <sup>(21)</sup>	-7	-3	p = 0,016
Desch <i>et al.</i> , 2010 <sup>(27)</sup>	NI	NI	<b>6g of chocolate</b> 24h SBP: p = 0,009 / DBP: p = 0,01 <b>25g of chocolate</b> 24h SBP: p = 0,02 / DBP: p = 0,20
Alleyne <i>et al.</i> , 2014 <sup>(28)</sup>	-18	-14	SBP: p = 0,0001 / DBP: p = 0,0001
Njike <i>et al.</i> , 2016 <sup>(23)</sup>	<b>10g of cocoa</b> Ambulatory: 0.6 ± 8.5 24h: -1.3 ± 10.0 <b>5g of cocoa</b> ambulatory: -0.4 ± 11.4 24h: -1.1 ± 6.5	<b>10g of cocoa</b> Ambulatory: 0.1 ± 5.8 24h: -0.2 ± 5.7 <b>5g of cocoa</b> Ambulatory: -0.8 ± 7.0 24h: -0.8 ± 4.2	<b>10g of cocoa</b> Ambulatory SBP p = 0,1371 / DBP p = 0,1285 24h SBP p = 0,2981 / DBP p = 0,5898 <b>5g of cocoa</b> Ambulatory SBP p = 0,6457 / DBP p = 0,8359 24h SBP p = 0,7779 / DBP p = 0,837

Source: Own authorship. NI = Not Informed.