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REVIEW

The action of phytochemicals present in cocoa in the prevention of vascular dysfunction and atherosclerosis

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Abstract

Background: Chronic non-communicable diseases (NCDs), including cardiovascular diseases (CVDs), have caused many deaths worldwide. Atherosclerotic plaque formation is common in individuals with CVDs. Thus, antioxidant and anti-inflammatory nutritional strategies can be used to prevent or inhibit this process. Due to its higher concentrations of cocoa, dark chocolate is considered a functional food owing to the presence and action of phytochemical compounds, with anti-inflammatory and antioxidant actions. However, the recommended amounts of these compounds to prevent atherosclerosis have not yet been fully elucidated.
Aim: To review the effects of cocoa and dark chocolate intake on the prevention of cardiovascular dysfunction and atherosclerosis.
Methods: This narrative review was based on a search of PubMed and Lilacs. The search was conducted from September 2021 to February 2022 using the following keywords: flavonoids, cocoa, atherosclerosis, oxidative stress, and inflammation. The inclusion criteria were original articles, meta-analyses, and experimental and clinical studies published between 2002 and 2022 in English, focusing on the subject addressed. The exclusion criteria were the title and abstract reading and duplication of articles in the databases.
Results: The antioxidant and anti-inflammatory functions of phytochemicals in cocoa and dark chocolate are related to the modulation of nitric oxide via activation/phosphorylation and acting as a vasodilator. Furthermore, these phytochemicals reduce the formation of reactive oxygen species and activate antioxidant enzymes. The anti-inflammatory activities are related to the modulation of nuclear factor kappa B in the reduction of inflammatory markers, such as tumor necrosis factor-alpha, C-reactive protein, and pro-inflammatory cytokines, as well as in the reduction of adhesion molecules in the wall of the vases.
Conclusion: The main phytochemicals present in cocoa and dark chocolates are catechins and their epicatechin isomers, which are responsible for improving inflammatory, metabolic, and antioxidant profiles. Its consumption can be encouraged, but with caution, owing to the caloric supply and forms of chocolate production, as these factors can reduce the presence of flavonoids in its composition.
Relevance for patients: The antioxidant and anti-inflammatory functions of the phytochemicals in cocoa and dark chocolate are responsible for modulating nitric oxide via activation/phosphorylation and acting as a vasodilator. Reducing the formation of reactive oxygen species, as well as activating antioxidant enzymes. As for the anti-inflammatory activities, they modulate the nuclear factor kappa B, reducing inflammatory markers, thus improving the antioxidant and inflammatory profile of these patients.

Keywords: flavonoids, cocoa, atherosclerosis, oxidative stress, inflammation

1. Introduction

Chronic non-communicable diseases (NCDs) have a multifactorial etiology, which is determined by genetic factors and modifiable factors, such as eating habits, smoking, obesity, and sedentary lifestyle[1,2]. NCDs, including cardiovascular diseases (CVDs), have caused a larger number of deaths worldwide. According to data from the World Health Organization, for which global statistics were calculated, approximately 44% of all deaths are related to chronic non-communicable diseases, accounting for seven of the ten main causes of death worldwide [3].

In the pathophysiological process of CVDs, the formation of atherosclerotic plaques is common in all cases [4]. Cardiovascular episodes are mainly associated with unstable plaques and their ruptures, which have intense inflammatory activity and highly thrombogenic lipid material, presenting themselves as a determining factor in the clinical manifestations of atherosclerosis [4]. Excessive production of reactive oxygen species (ROS) results in oxidative stress, which is related to atherosclerosis [5].

Thus, antioxidant and anti-inflammatory nutritional strategies can be used as allies for the prevention or reduction of atherogenesis [6]. Some foods, such as fruits, vegetables, teas, red wine, and dark chocolate, have characteristics that offer health benefits due to the action of their bioactive compounds [7].

Cocoa present in chocolates, particularly in dark chocolates, is rich in flavonoids associated with benefits for cardiovascular health [8]. These benefits are related to antioxidant action, increased insulin sensitivity, decreased platelet aggregation, lower expression of adhesion molecules, and activation of nitric oxide, a potent vasodilator that helps reduce blood pressure [9].

Despite several studies addressing the benefits of the antioxidant and anti-inflammatory action of dark chocolate or cocoa, there are still inconsistencies regarding the phytochemicals present in this food, the benefits of its consumption, and the adequate amount to be ingested. Thus, this study aimed to review the literature how the phytochemicals present in cocoa and dark chocolate modulate the atherogenesis process, and consequently, prevent atherosclerosis.

2. Methodology

This is a narrative review based on a search of PubMed, Scopus, Web of Science, SciElo, and Lilacs. The search was performed from September 2021 to February 2022, using the following keywords: flavonoids, cocoa, atherosclerosis, oxidative stress, and inflammation. The inclusion criteria were original articles, meta-analyses, and experimental and clinical studies published between 2002 and 2022 in English, focusing on the subject addressed. The exclusion

criteria were the title and abstract reading and duplication of articles in the databases. The selection was initially made by one author and later by a second author.

3. Phytochemicals in cocoa

At present, cocoa is one of the most consumed products worldwide and is widely used by the food industry [10.] There are at least three groups of substances in cocoa beans with beneficial health effects: (i) flavonoids (catechin and proanthocyanidins), (ii) theobromine/caffeine, and (iii) minerals (magnesium, iron, and zinc) [11.] It is known that the term flavonoids is considered the generic name for all compounds included in the subfamilies, such as flavonols, flavones, flavanones, and flavanols (ex: catechin), anthocyanins, and isoflavones [12.] Because they are contained in cocoa, in this study, the class of greatest interest was flavanols. The basic chemical structure of flavonoids consists of two aromatic rings, A and B, connected by an oxygenated heterocycle C, with flavanols being the predominant forms (-) - epicatechin, (+) - epicatechin, (-) - catechin, and (+) - catechins (monomeric), procyanidins (oligomeric), and proanthocyanidins [13]. They are known to be composed of catechins, proanthocyanidins, and procyanidins or condensed tannins that are formed from the bond between (+) monomers, catechin, and (–) - epicatechin, allowing the formation of catechin dimers, oligomers, and polymers [14].

According to Rothwell (2013), the flavanols with the highest concentrations in cocoa are epicatechins, followed by catechins. How much the dimeric procyanidins, Ottaviani et al. (2012) suggested that their role as a mediator of beneficial effects to the organism contributes little to the systemic pool of flavonoids, and their bioactivity is related to the generation of phenolic metabolites after biotransformation by the intestinal microbiota [15].

In addition to polyphenols, cocoa contains theobromine (approximately 2% to 3% of the bean weight), a plant alkaloid of the methylxanthine family, with antioxidant potential, which acts on the central nervous system [16]. According to Sansone et al. (2016), theobromine positively affects the absorption of epicatechins, showing the synergism between the bioactive compounds present in cocoa [17].

Several factors can influence the type and amount of flavonoids present in foods containing cocoa and chocolate, including the geographic origin of cocoa production, type of cultivation, harvesting, post-harvest, and processing practices [18]. Studies performed with the Kuna Indians, natives of islands off the coast of Panama, and consumers of large amounts of cocoa per day showed that the Kuna Indians have lower blood pressure values compared to other Pan-American civilizations [19]. The factors involved are environmental and not genetic, as this cardiovascular protection was lost by the Kuna Indians who migrated to the urban part of Panama City, where cocoa consumption was replaced by other foods low in flavonoids [20].

In addition, Schroeter et al. (2006) found that urinary levels of flavanol metabolites expressed as epicatechin equivalents are more than six times higher in island dwellers off the coast of Panama, who consumed approximately 600 to 900 mg of flavonoids per day compared to inhabitants of the western continent [21].

A study by Ottaviani et al. (2015) evaluated the safety and efficacy of consuming cocoa flavonoids in healthy adults and concluded that ingestion in amounts of up to 2,000 mg/day in 12 weeks had no adverse effects on the health of men and women [22]. In Europe, the estimated average intake of flavonoids from cocoa is 105 mg/day [23]. Thus, it is possible to observe that flavonoid intake varies widely according to geographic location.

4. Properties of the phytochemical compounds of cocoa

4.1 Anti-inflammatory activity

The anti-inflammatory mechanisms of phytochemical compounds in cocoa have not yet been fully elucidated. Several benefits of flavanol (-) -epicatechin (EC) from cocoa have been attributed to its antioxidant and anti-inflammatory properties. Ruijters et al. (2014) investigated whether EC can prevent the deterioration of the anti-inflammatory effect of glucocorticoid cortisol (GC) in the presence of oxidative stress. Cortisol reduces inflammation in differentiated monocytes. Oxidative stress quenches the anti-inflammatory effects of cortisol, leading to cortisol resistance. EC reduces intracellular oxidative stress and the development of cortisol resistance. Thus, it was possible to prove the mechanism by which EC exerts its anti-inflammatory and antioxidant action [24].

In addition, cocoa consumption leads to a reduction in the levels of inflammatory markers and proinflammatory molecules. Jafarirad et al. (2018) observed that 84% cocoa dark chocolate supplementation reduced inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL- 6) in type 2 diabetic patients [25].

Sarriá et al. (2014) concluded that regular consumption of a fiber-rich cocoa product containing 416.4 mg of polyphenols for 4 weeks decreased plasma concentrations of the cytokines IL-1b and IL-10 while slightly reducing other concentrations of molecules pro-inflammatory [26]. Eskandari et al. (2020) observed similar results, as well as a reduction in leptin, resistin, and monocyte chemoattractant protein 1 (MCP-1), and an increase in the concentration of adipokines with anti-inflammatory properties such as irisin and adiponectin [27].

Another aspect in which the anti-inflammatory activity of cocoa is related to the modulation of the synthesis of eicosanoids is through the action of procyanidins, which inhibit the action of 5-lipoxygenase (LOX-5) via MAPK kinase, an enzyme involved in the synthesis of

leukotrienes, with arachidonic acid as its precursor [28.] Inhibition of LOX-5 confers anti-inflammatory, vasoprotective, and anti-bronchoconstrictor effects. Furthermore, Alvarez-Cilleros et al. (2020) believed that the anti-inflammatory properties are mainly related to modulation via intracellular phosphorylation of nuclear factor kappa B (NF-κB), and found that cocoa ingestion prevents its phosphorylation, as well as increased regulation of nitric oxide synthase (iNOS) in the rat aorta, thus supporting the anti-inflammatory effect of cocoa [29].

4.2 Effects of cocoa phytochemicals on atherosclerosis

The accumulation of fat in the arterial walls is associated with low-density lipoproteins (LDLs), which, owing to the increase in intimal permeability, favor their retention in the subendothelial space, causing an inflammatory process through adhesion molecules such as VCAM-1 (molecule cell adhesion-1), ICAM-1 (intercellular adhesion molecule-1), E-selectin, and MCP-1 [30]. Such molecules promote migration, adhesion, and accumulation of lymphocytes and monocytes in the arterial wall, which a posteriori differentiate into macrophages and capture oxidized LDLs [29,31.]

According to Khan et al. (2012), the consumption of 40 g of dark chocolate can modulate the lipid profile, increase high-density lipoprotein (HDL) levels, and reduce oxidized low-density lipoprotein (LDL) levels in patients with high cardiovascular risk [32.] This is an important role of cocoa polyphenols in lipid metabolism, as they bind to LDL particles, inhibit oxidation [32, 33], and reduce platelet aggregation. This is supported by Innes et al. (2003), who observed that ingestion of 100 g of dark chocolate reduced platelet aggregation and adhesion [34].

Endothelial nitric oxide synthase (eNOS) is one of the most prominent pathways in the atherosclerotic process and is responsible for the production of nitric oxide, which is important for the preservation of vascular health, with an action proportional to cell function. Therefore, the lower the availability of NO, the lower its function [35], In addition, it is known that foods with a high flavonoid content can stimulate the production of NO [36].

In addition, some studies have analyzed the bioactivity of metabolites such as acid 3,4-dihydroxyphenylacetic acid (DHPAA), 2,3-dihydroxybenzoic acid (DHBA), and 3-hydroxyphenylpropionic acid (HPPA) generated by the intestinal microbiota from flavonols, which were able to improve endothelial function and prevent oxidative stress in human endothelial cells [37].

Vázquez-Agel et al. (2013) evaluated the activation of NF-kB and adhesion molecules (ICAM, VCAM, E-selectin) in 18 healthy volunteers, measured 6 h before and after consumption of 40 g of cocoa powder. They observed a significant reduction in NF-kB activation and ICAM-

1 concentration when consumed with water, and no change when consumed with milk. In addition, there was a significant increase after the milk-only intervention; E-selectin decreased only after the intervention with cocoa and water. No significant change was observed in VCAM-1 concentration, confirming the action of cocoa in reducing NF-kB [38].

Table 1 - Studies on cocoa or chocolate consumption on metabolic outcomes between 2011-2020.

Author	Intervention	% cocoa	Phytochemicals	Results
Vázquez-Angell, M. et al. 2011[38]	18 healthy volunteers; 40g of cocoa powder with milk or with water or and only milk; Initial and 6h after each intervention.	100%	Epicatechin 28.2 mg Catechin 8.2 mg Procyanidins B2 25.5 mg Quercetin 0.23 mg	Reduction of NF-kB activation; reduction of ICAM-1 and E-selectin adhesion molecules
Nanetti, L. et al. 2011[36]	In vitro; 25 women; 25 men; 50 g dark chocolate; 3 weeks consumption.	NI	Epicatechin 151.5 mg Catechin 25.3 mg Total Procyanidin 108 mg	Increase in HDL and NO; LDL and peroxynitrite reduction
Grassi, D. et al. 2012 [39]	12 healthy volunteers; 100 g/d dark chocolate or flavanol-free white chocolate for 3 days.	NI	Epicatechin 447 mg Catechin 59 mg Quercetin 14 mg	Increase in DFM; prevented the increase in isoprostane and endothelin
Khan, N. et al. 2012 [32]	19 men; 23 women risk volunteers; 40g of cocoa powder with 500 ml of skimmed milk/day or only 500 mL/day of skimmed milk for 4 weeks.	100%	Epicatechin 46.8 mg Catechin 10.41 mg Procyanidin B2 36.54 mg Proanthocyanidins (t)425.7 mg	HDL increase; LDLox reduction; increased excretion of flavanol metabolites

Curtis, P. J. et al. 2013 [40]	118 postmenopausal diabetic women; 27 g flavonoid-enriched chocolate/d + 100 mg isoflavones or matched placebo	NI	Epicatechin 90 mg	Significant improvement in BP
Loffredo, L. et al. 2014 [41]	14 men and 6 women with peripheral artery disease (PAD); 40 g of dark chocolate (>85% cocoa) or 40 g of milk chocolate (≤35% cocoa).	85%	Epicatechin 0.59 mg/ml Catechin 0.32 mg	Increased serum NOX; decreased isoprostanes and NOX2; increase in ON and decrease in E-selectin and VCAM1
Sarriá, B. et al. 2014 [26]	24 moderately hypercholesterolaemic volunteers; two servings (15g each) of a cocoa product rich in fiber in milk or only milk (control); 4 weeks.	NI	Polyphenols 417 mg/d	HDL increase and IL10; reduced glucose and IL-1;
McFarlin, B. K. et al. 2015 [31]	24 women young; natural cocoa-containing product (12.7 g cocoa) or isocaloric cocoa-free placebo daily; 4 weeks.	100%	Epicatechin 48 mg Catechin 13.6 mg	HDL increase
Hammer, A. et al. 2015 [42]	21 volunteers with symptomatic PAD; each patient on 2 days, with an interval of 7 days, at baseline and 2 hours after ingestion of 50 g dark chocolate or 50 g white respectively.	70%	Catechin 0.27 mg/g Epicatechin 0.9 mg/g	There were no changes in endothelial function

Jafarirad, S. et al. 2018[25]	44 volunteers with T2D; 30g of 84% dark chocolate (n=21); control group received only TLC guidelines (n=23); during 8 weeks.	84%	NI	Reduced fasting glucose, Hb A1C, LDL, triglycerides; TNF-a, IL-6 and hs-CRP
Cavarretta, E. et al. 2018[45]	24 elite football players; 20g every 12h dark chocolate (>85% cocoa) intake or a control group for 30 days.	85%	NI	Positively modulated redox status and reduced exercise-induced muscle injury biomarkers in elite soccer athletes
Munguia, L. et al. 2019 [45]	74 volunteers; no-flavonoid (NF) or flavonoid-rich natural cocoa (F). Once/day for up to 12-weeks with 22 g of a dry powder that was reconstituted with water just before consumption.	100%	Epicatechin 25 mg Proanthocyanidin 154mg Theobromine 103 mg	Decreased blood glucose, LDL, and triglycerides; HDL increase
Eskandari, M. et al. 2020 [27]	48 obese adolescent boys; 4 groups: jump rope exercise (JRE) + white chocolate (JW; n = 13), JRE + dark chocolate (JD; n = 13), dark chocolate (DS; n = 12) or control (C; n = 12). JW and JD groups performed JRE 3 times per week for 6 weeks. Participants in the DS and JD groups consumed 30 g of dark chocolate 83% of cocoa for 6 weeks.	83%	Epicatechin 160 mg Theobromine 960 mg	C/Q reduction, MG, PCR, TNF-a, IL-6, leptin, resistin, RBP-4, and MCP-1 and increased irisin and adiponectin
Regecova, V. et al. 2020 [44]	47 volunteers; two tests of mental arithmetic one before chocolate administration and the second one 2 hours after chocolate (1 mg/g of body weight) ingestion	85%	NI	Buffer cardiovascular reactivity to stress in healthy young women

Caption: NF-kB - nuclear factor kappa B; ICAM-1 and E-selectin - adhesion molecules; HDL-high density lipoprotein; ON-nitric oxide; LDL - low density lipoprotein; DFM- flow-mediated dilation; BP- blood pressure; NOX - NADPH oxidase enzyme complex; IL-1, IL10, IL6 - interleukins 1, 10 and 6; Hb A1C-glycated hemoglobin A1C; TNF-a- tumor necrosis factor-alpha; hs-CRP - high sensitivity C-reactive protein; W/Q - hip and waist ratio; MG- fat mass; RBP-4-retinol-binding protein; MCP1- monocyte chemoattractant protein 1. ;JRE- jump rope exercise; JW- white chocolate; JD: dark chocolate supplementation; C-Control; NF-no-flavonoid; F- flavonoid; PAD-peripheral artery disease.NI: Not identified. Source: Prepared by the author (2022).

Thus, according to studies carried out with cocoa, it was possible to observe an improvement in the lipid profile and inflammatory mediators, reducing NF-kB and adhesion molecules. In studies performed with chocolate, a reduction in adhesion molecules and an increase in nitric oxide were observed. The amount of chocolate and the percentage of cocoa were very heterogeneous, making it difficult to establish a precise recommendation. Finally, regarding the levels of phytochemicals present in both samples, there was no pattern of analysis, and it is still undetermined which associations and quantities would be recommended to obtain these effects. It is important to note that chocolate should be consumed with caution.

According to Counet. et al., (2004), among the factors that can influence the type and amount of flavonoids present in cocoa and chocolate, processing must be considered since the main interest during chocolate processing is to preserve the nutrients that occur naturally in cocoa beans. Thus, food processing methods, such as fermentation and baking, can decrease the final flavonoid content [18].

Among the studies of this review, it is important to emphasize that some evaluated the flavonoids present in cocoa but did not establish the total content of this polyphenol. Others only evaluated the percentages of cocoa concentration, which is one of the limitations of this review that most of the studies found that the concentration of cocoa in chocolate was considered. Therefore, few studies have considered flavonoids in milligrams, which is considered a bias in studies to better understand the number of milligrams of this compound available in the cocoa consumed by individuals. Thus, further studies are needed to carry out this conversion and quantify the flavonoids in cocoa.

Conclusion

Studies have shown that the main phytochemicals present in cocoa and dark chocolates are catechins and their epicatechin isomer, which are responsible for attenuating the atherosclerotic process, reducing the activation of NF-kB, adhesion molecules, and pro-inflammatory cytokines, and increasing the levels of anti-inflammatory cytokines such as IL-10. They also help reduce fasting blood glucose and glycated hemoglobin and improve the lipid profile with a reduction in LDL and triglycerides and an increase in HDL. They also modulate some hormones of the reward system and exert antioxidant actions. Therefore, its consumption should be encouraged, but with caution due to the caloric supply and forms of chocolate production, as these factors can reduce the presence of flavonoids in their composition. Quantification of these bioactive compounds should be encouraged to complete the recommendation of the percentage of cocoa in chocolate.

Declaration of Interest

The authors declare no conflict of interest.

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References

1. Arking DE & Chakravarti A. Understanding cardiovascular disease through the lens of genome-wide association studies. Trends Genet 2009;25:387-94.

2. James SL, Abate D, Abate KH *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet 2018;392:1789-858.

3. World Health Organization. The top 10 causes of death. [Electronic resource]. URL: https://www. WHO. int/news-room/fact-sheets/detail/the-top-10-causes-of-death, 2020.

4. Frostegård J. Immunity, atherosclerosis, and cardiovascular disease. BMC Med 2013;11:117.

5. Yilmaz MI, Romano M, Basarali MK *et al.* The Effect of Corrected Inflammation, Oxidative Stress and Endothelial Dysfunction on Fmd Levels in Patients with Selected Chronic Diseases: A Quasi-Experimental Study. Sci Rep 2020; 10:9018.

6. Fernández-Murga L, Tarín JJ, García-Perez MA *et al.* The impact of chocolate on cardiovascular health. Maturitas 2011; 69: 312-21.

7. Chaves DF, Solis MY, Gandin P, *et al.* Acute effects of isocaloric meals with different fiber and antioxidant contents on inflammatory markers in healthy individuals. Ann Nutr Metab 2013; 62:164-168.

8. Martin FP, Rezzi S, Peré-Trepat E, *et al.* Metabolic effects of dark chocolate consumption on energy, gut microbiota, and stress-related metabolism in free-living subjects. J Proteome Res 2009; 8:5568-79.

9. Garcia JP, Santana A, Baruqui DL *et al.* The Cardiovascular effects of chocolate. Rev Cardiovasc Med 2018; 19:123-127.

10. International Cocoa Organization. Growing Cocoa: Origins of Cocoa and Its Spread Around the World. https://www.icco.org/about-cocoa/growing-cocoa.html (accessed Jan 2022); 2013.

11. García-Blanco T, Dávalos A, Visioli F. Tea, cocoa, coffee, and affective disorders: vicious or virtuous cycle?. J Affect Disord 2017; 224: 61-68.

12. Manach C, Scalbert A, Morand C, *et al.* Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004; 79; 727-47.

13. Desch S, Schmidt J, Kobler D *et al.* Effect of cocoa products on blood pressure: systematic review and meta-analysis. Am J Hypertens 2010;23: 97-103.

14. Cifuentes-Gomez T, Rodriguez-Mateos A, Gonzalez-Salvador I *et al.* Factors Affecting the Absorption, Metabolism, and Excretion of Cocoa Flavanols in Humans. J Agric Food Chem 2015; 63; 7615-23.

15. Ottaviani JI, Kwik-Uribe C, Keen CL *et al.* Intake of dietary procyanidins does not contribute to the pool of circulating flavanols in humans. Am J Clin Nutr 2012; 95: 851-8.

16. Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. Antioxid Redox Signal 2011; 15:2779-811.

17. Sansone R, Ottaviani JI, Rodriguez-Mateos A, *et al.* Methylxanthines enhance the effects of cocoa flavanols on cardiovascular function: randomized, double-masked controlled studies. Am J Clin Nutr 2017; 105: 352-360.

18. Counet C, Ouwerx, C, Rosoux, D, Collin, S. (2004). Relationship between procyanidin and flavor contents of cocoa liquors from different origins. Journal of agricultural and food chemistry 2004;52:20, 6243-6249.

19. Bayard V, Chamorro F, Motta J, Hollenberg NK. Does flavanol intake influence mortality from nitric oxide-dependent processes? Ischemic heart disease, stroke, diabetes mellitus, and cancer in Panama. International journal of medical sciences 2007; 4:1-53.

20. Hollenberg N K, Martinez G, McCullough M, Meinking T, Passan D, Preston M, Vicaria-Clement, M. Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. Hypertension 1997;29:1,171-176.

21. Schroeter H, Heiss C, Balzer, J, Kleinbongard, P, Keen CL, Hollenberg N K, Kelm M. Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proceedings of the National Academy of Sciences 2006;103:4,1024-1029.

22. Ottaviani JI, Balz M, Kimball J *et al.* Safety and efficacy of cocoa flavanol intake in healthy adults: a randomized, controlled, double-masked trial. Am J Clin Nutr 2015; 102:1425-35.

23.Vogiatzoglou A, Mulligan AA, Luben RN, *et al.* Assessment of the dietary intake of total flavan-3-ols, monomeric flavan-3-ols, proanthocyanidins, and theaflavins in the European Union. Br J Nutr 2014; 111:1463-73.

24. Ruijters EJ, Haenen GR, Weseler AR *et al.* The cocoa flavanol (-)-epicatechin protects the cortisol response. Pharmacol Res 2014;79: 28-33.

25. Jafarirad S, Ayoobi N, Karandish M, *et al.* Dark Chocolate Effect on Serum Adiponectin, Biochemical and Inflammatory Parameters in Diabetic Patients: A Randomized Clinical Trial. Int J Prev Med 2018; 9: 86.

26. Sarriá B, Martínez-López S, Sierra-Cinos JL, *et al.* Regular consumption of a cocoa product improves the cardiometabolic profile in healthy ad moderately hypercholesterolaemic adults. Br J Nutr 2014;111:122-34.

27. Eskandari M, Hooshmand Moghadam B, Bagheri R, *et al.* Effects of Interval Jump Rope Exercise Combined with Dark Chocolate Supplementation on Inflammatory Adipokine, Cytokine Concentrations, and Body Composition in Obese Adolescent Boys. Nutrients 2020; 12: 3011.

28. Schramm DD, Wang JF, Holt RR, *et al.* Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. Am J Clin Nutr 2001;73:36-40.

29.Álvarez-Cilleros D, López-Oliva ME, Ramos S *et al.* Preventive effect of cocoa flavanols against glucotoxicity-induced vascular inflammation in the arteria of diabetic rats and on the inflammatory process in TNF-α-stimulated endothelial cells. Food Chem Toxicol 2020; 146: 1118-24.

30. Csányi G, Taylor WR, Pagano PJ (2009) NOX and inflammation in the vascular adventitia. Free Radic Biol Med, 2009; 47:1254-66.

31.McFarlin BK, Venable AS, Henning AL *et al.* Natural cocoa consumption: Potential to reduce atherogenic factors?. J Nutr Biochem 2015; 26: 626-32.

32. Khan N, Monagas M, Andres-Lacueva C, *et al.* Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in subjects at high risk of cardiovascular disease. Nutr Metab Cardiovasc Dis 2012; 22: 1046-53.

33. Rios LY, Gonthier MP, Rémésy C, *et al.* Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. Am J Clin Nutr 2003;77:912-8.

34. Innes AJ, Kennedy G, McLaren M, *et al.* Dark chocolate inhibits platelet aggregation in healthy volunteers. Platelets 2003; 14:325-7.

35. Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. Clin Sci (Lond) 2011;120, 357-75.

36. Nanetti L, Raffaelli F, Tranquilli AL, *et al.* Effect of consumption of dark chocolate on oxidative stress in lipoproteins and platelets in women and men. Appetite 2012; 58:400-5.

37.Álvarez-Cilleros D, Ramos S, Goya L *et al.* Colonic metabolites from flavanols stimulate nitric oxide production in human endothelial cells and protect against oxidative stress-induced toxicity and endothelial dysfunction. Food Chem Toxicol 2018;115:88-97.

38. Vázquez-Agell M, Urpi-Sarda M, Sacanella E, *et al.* Cocoa consumption reduces NF-κB activation in peripheral blood mononuclear cells in humans. Nutr Metab Cardiovasc Dis 2013;23:257-63.

39. Grassi D, Desideri G, Necozione S, *et al.* Protective effects of flavanol-rich dark chocolate on endothelial function and wave reflection during acute hyperglycemia. Hypertension 2012;60: 827-832.

40. Curtis PJ, Potter J, Kroon PA, *et al.* Vascular function and atherosclerosis progression after 1 y of flavonoid intake in statin-treated postmenopausal women with type 2 diabetes: a double-blind randomized controlled trial. Am J Clin Nutr 2013; 97: 936-942.

41. Loffredo L, Perri L, Catasca E *et al.* Dark chocolate acutely improves walking autonomy in patients with peripheral artery disease. J Am Heart Assoc 2014;3:1072.

42. Hammer A, Koppensteiner R, Steiner S *et al.* Dark chocolate and vascular function in patients with peripheral artery disease: a randomized, controlled cross-over trial. Clin Hemorheol Microcirc 2015;59:145-153.

43. Munguia L, Rubio-Gayosso I, Ramirez-Sanchez I *et al.* High Flavonoid Cocoa Supplement Ameliorates Plasma Oxidative Stress and Inflammation Levels While Improving Mobility and Quality of Life in Older Subjects: A Double-Blind Randomized Clinical Trial. J Gerontol A Biol Sci Med Sci 2019; 74:1620-1627.

44. Regecova, V., Jurkovicova, J., Babjakova, J., & Bernatova, I. The effect of a single dose of dark chocolate on cardiovascular parameters and their reactivity to mental stress. Journal of the American College of Nutrition, 2020;*39*:414-421.

45. Cavarretta E, Peruzzi M, Del Vescovo, R, Di Pilla F, Gobbi G, Serdoz A, Carnevale, R. Dark chocolate intake positively modulates redox status and markers of muscular damage in elite football athletes: a randomized controlled study. Oxidative medicine and cellular longevity, 2018;1-10.