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Review

Impact of cocoa flavanols on human health

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ABSTRACT

Cocoa is a source of flavanols, and these phenolic compounds exert beneficial effects on health and aging, and reduce the risk of suffering chronic diseases (cardiovascular diseases, metabolic disorders, cancer). An increasing body of evidence has emerged to suggest that cocoa flavanols potentially are important chemopreventive natural agents. This review summarizes human studies from the past two decades, providing data related to the effects derived from cocoa intake on health and disease. Most human studies have reported beneficial effects of cocoa consumption on health and chronic diseases; however, outcomes are not unequivocal. Review of human studies enable to identify different mechanisms of action for cocoa, although they are not fully understood at present. In addition, it remains unclear whether cocoa consumption should be recommended to healthy subjects or to patients and what is the appropriate dosage or duration of cocoa consumption. Elucidation of information regarding these crucial issues could lead to cocoa use as an approach for decreasing the risk of certain chronic diseases, as well as improving health and quality of life.

1. Introduction

Cocoa and its products are consumed worldwide due to their pleasant taste. Cocoa constitutes a rich source of polyphenols, mainly flavanols, and numerous health-promoting effects have been attributed to these natural compounds. Different studies have demonstrated an association between cocoa intake and decreased risk of diverse chronic diseases, such as cardiovascular diseases, metabolic disorders and cancer (Andujar et al., 2012; Martín, Goya and Ramos, 2013; Martín, Goya & Ramos, 2016). In addition, cocoa consumption positively affects the nervous system, visual function and skin, among other outcomes (Andujar et al., 2012; Martín et al., 2013, 2016). Several mechanisms underlying these biological actions have been associated with the beneficial effects exerted by cocoa on health and disease. These include its antioxidant, anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-obesity and anti-allergic activities, which in turn have been connected to the regulation of numerous signaling pathways (Ali et al., 2014; Andujar et al., 2012; Martín et al., 2013; 2016; Rodríguez-Pérez et al., 2017). More recently, attention has been directed towards examination of the bidirectional interaction between cocoa flavanols and gastrointestinal microbiota. Such research is driven by the fact that this interplay seems to have a crucial role in promoting or inhibiting microbial growth in order to render subsequent effects on health and disease (Sorrenti et al., 2020). In view of all of these positive preventive properties, cocoa has attracted great interest. Nonetheless, unequivocal evidence that this food exerts beneficial effects on health and acts against the aforementioned diseases in humans has not been completely established at present, whilst potential mechanisms also remain unproven. The purpose of the present review is to summarize the evidence produced over the past two decades from human studies of the link between cocoa intake and its beneficial or preventive effects on health and diseases including cardiovascular disease, diabetes, metabolic syndrome, obesity and cancer. In this way, a systematic, comprehensive search of the literature published between 2000 and October 2020 on cocoa or cocoa flavanols in the context of human studies and selected chronic diseases and health was performed. PubMed and ISI Web of Knowledge databases were used to search for relevant trials. The following search terms and Boolean operators were employed: cocoa AND human AND (clinical trial OR intervention OR epidemiological OR epidemiology) AND (health OR disease OR diabetes OR obesity OR cancer OR cardiovascular OR brain OR cognition OR cognitive OR allergy OR eye OR immunity OR immune OR microbiota OR bacteria OR virus OR sepsis).

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Abbreviations

AI	augmentation index
ARIC	Atherosclerosis Risk In Communities
BDNF	brain-derived neurotrophic factor
BMI	body mass index
BP	blood pressure
BW	body weight
Cho	cholesterol
CFS	chronic fatigue syndrome
CRP	C-reactive protein
CVD	cardiovascular disease
EC	(−)-epicatechin
FMD	flow mediated dilatation
HbA1c	glycated haemoglobin
HDL	high density lipoprotein
HC	high-consumer
HOMA-IR	homeostatic model assessment of insulin resistance
IL	interleukin
LC	low-consumer
LDL	low-density lipoprotein
MC	moderate-consumer
MDA	malondialdehyde
MED	minimal erythema dose
NHANES	National Health and Nutrition Examination Survey
PWV	pulse wave velocity
T2D	type 2 diabetes
TG	triglyceride
TMAO	trimethylamine *N*-oxide
TNFα	tumour necrosis factor α

2. Cocoa

Cocoa and its derived products are consumed worldwide, mainly as chocolate. In 2018/19, almost 5 million tons of cocoa were produced. The major cocoa producers are the Ivory Coast and Ghana as they cover 60% of all cocoa used for chocolate production around the world (Del Prete and Samoggi, 2020). However, the largest chocolate manufacturers are based in North America and Europe, with the Swiss being the main consumers (8.8 kg/year/per capita) and the Chinese being the lowest (100 g/year/per capita) (Del Prete and Samoggi, 2020).

Cocoa constitutes a rich source of fiber (40-26%), lipids (24-10%), proteins (20-15%), carbohydrates (15%), and micronutrients (<2%) including minerals (P, Ca, K, Na, Mg, Zn, Cu) and vitamins (A, B, E) (Kim et al., 2014). Cocoa also contains high amounts of methylxanthines (theobromine and caffeine) and phenolic compounds named flavanols (Table 1). Indeed, dried unfermented cocoa beans comprise around 13.5% phenols, which are present as monomeric (−)-epicatechin (EC) and (+)-catechin, dimeric procyanidins, (especially procyanidin B2 and B1) and oligomers and polymers (Table 1) (Martín and Ramos, 2016). Cocoa also contains other polyphenols in lower amounts, such as flavones (luteolin, apigenin), flavanones (naringenin), flavonols (quercetin, isoquercitrin, hyperoside, etc.), phenolic acids and anthocyanins (Table 1) (Martín et al., 2016; Martín and Ramos, 2016).

Following cocoa consumption, flavanols remain stable in the digestive system until they reach the small intestine where they are absorbed. Monomers rapidly reach the liver and are conjugated into sulphates, glucuronides and methylated metabolites by phase II enzymes (Sorrenti et al., 2020). Further, some enterohepatic recirculation can take place through the elimination of some flavanol via the bile (Sorrenti et al., 2020). With regards to oligomers, procyanidins are poorly absorbed in the gastrointestinal tract and are mainly metabolized together with monomeric flavanols in the large intestine by microbiota. Indeed, it has been estimated that only 5–10% of polyphenols are absorbed in the small intestine, with the remaining (90–95%) reaching the colon (Sorrenti et al., 2020). These microbial metabolites peak around 9–48 h after intake and correspond to phenyl-γ-valerolactones and different phenolic acids (phenylvaleric acids, m-hydroxyphenylpropionic acid, m-hydroxyphenylacetic acid, and m-hydroxybenzoic acid) (Mena et al., 2019). Importantly, gut metabolite concentration in blood has been estimated to be 10-100-fold higher than the parent compound (Urpi-Sarda et al., 2009).

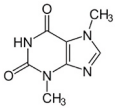
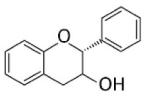
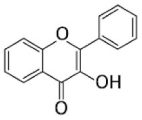
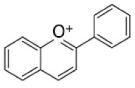
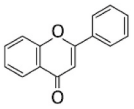
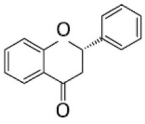
3. Effects of cocoa on cardiovascular disease

Over recent decades, the cardiovascular effects of cocoa and its derivative products have been extensively investigated in many human trials. Epidemiological studies generally support the existence of beneficial associations between cocoa products and reduced cardiovascular disease (CVD) risk. A meta-analysis of seven observational studies (114,009 participants) showed that greater chocolate consumption was linked with a 37% lower risk of CVD and a 29% lower risk of stroke (Buitrago-Lopez et al., 2011). Likewise, a meta-analysis on data from nine studies with 157,809 participants, demonstrated a significant association between chocolate consumption and reduced risk of coronary artery disease (Kwok et al., 2015). Two additional meta-analyses of prospective studies have also shown that chocolate intake reduced the risk of heart failure (Gong et al., 2017), as well as coronary heart disease and stroke (Yuan et al., 2017). Larsson et al. (2016) backed up these findings in another meta-analysis of six prospective cohort studies including 67,640 patients. Here it was found that high chocolate consumption was associated with a decreased risk of myocardial infarction and ischemic heart disease. Interestingly, the most recent meta-analysis including 23 prospective studies (405,304 participants) also evaluated dose-response associations between chocolate consumption and CVD incidence (Ren et al., 2019). Results indicated that the optimal dose of chocolate consumption for reducing CVD risk was 45 g/week, whilst higher levels of chocolate intake potentially induced adverse effects associated with high sugar consumption. In accordance with these aforementioned observational studies, a number of clinical trials have also demonstrated a beneficial impact of cocoa and cocoa derived products on CVD. The most noteworthy of these pertain to a number of meta-analyses of clinical trials which have uncovered potential effects of cocoa products on several cardiovascular risk factors, including endothelial function, blood pressure (BP), lipid metabolism and vascular stiffness (Table 2).

3.1. Endothelial function

The relationship between cocoa flavanols and endothelial function has been investigated in several interventional studies. Most of these have convincingly shown that consumption of flavanol-rich cocoa products produces vascular benefits. An early systematic review conducted by Hooper et al. (2012) identified 42 clinical trials (1297 participants) which examined the effects of cocoa products on several CVD risk factors, including endothelial function, insulin resistance, BP and lipid profile. A meta-analysis performed within this review of a subgroup of 11 clinical trials (382 participants) indicated that the consumption of cocoa products leads to beneficial effects on endothelial dysfunction, assessed via flow-mediated dilation (FMD). Overall, acute and chronic ingestion of cocoa products has been seen to increase FMD in both healthy subjects (Grassi et al., 2005a; Vlachopoulos et al., 2005) and individuals at higher risk of CVD, such as smokers (Heiss et al., 2005), diabetics (Balzer et al., 2008), individuals suffering hypertension (Grassi et al., 2005b) and those who are overweight or obese (Davison et al.,

Table 1
Basic chemical structure of phytochemicals found in cocoa.

Phytochemical groups	Representative phytochemicals
Methylxanthines	Theobromine Caffeine
	
Phenolic compounds Flavanols	(−)-Epicatechin> (+)-Catechin Epigallocatechin-3-gallate Epigallocatechin Procyanidin B1 (epicatechin-(4β→8)-catechin) Procyanidin B2 (epicatechin-(4β→8)-epicatechin) Procyanidin B2–*O*-gallate (epicatechin-3-*O*-gallate-(4β→8)-epicatechin) Procyanidin B2-3,3-di-*O*-gallate (epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate) Procyanidin B3 (catechin-(4α→8)-catechin) Procyanidin B4 (catechin-(4α→8)-epicatechin) Procyanidin B4–*O*-gallate (catechin-(4β→8)-epicatechin-3-*O*-gallate) Procyanidin C1 (epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin) Procyanidin D (epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin)
	
Flavonols	Quercetin Quercetin-3-*O*-arabinoside Hyperoside (quercetin-3-*O*-galactoside) Isoquercitrin (quercetin-3-*O*-glucoside)
	
Anthocyanins	Cyanidin 3-α-L-arabinosidyl cyanidin 3-β-D-arabinosidyl cyanidin
	
Flavones	Luteolin Luteolin-7-O-glucoside Orientin (luteolin-8-C-glucoside) Isoorientin (luteolin-6-C-glucoside) Apigenin Vitexin (apigenin-8-C-glucoside) Isovitexin (apgein-6-C-glucoside)
	
Flavanones	Naringenin Prunin (naringenin-7-O-glucoside) Hesperidin Eriodictyol
	
Phenolic acids	Chlorogenic acid Vanillic acid Coumaric acid Phlorectic acid Caffeic acid Ferulic acid Phenylacetic acid Syringic acid
	

2008; Faridi et al., 2008; Njike et al., 2011). Consequently, the European Food Safety Authority (EFSA) claimed for the first time in 2012 that "a cause-and-effect relationship may be established between the consumption of cocoa flavanols and maintenance of normal endothelium-dependent vasodilation". In order to obtain this effect, 2.5 g of high-flavanol cocoa powder or 10 g of high-flavanol dark chocolate containing 200 mg of cocoa flavanols should be consumed daily (EFSA, 2012). Following this, further trials were performed which have recently been reviewed by Ebaditabar and collaborators (2020). In this latest systematic review, a meta-analysis of 17 studies involving 615 participants confirmed the protective effects of both acute (1–2 h) and chronic (2–84 days) consumption of dark chocolate on FMD. Moreover, this beneficial effect has been confirmed in new trials carried out with healthy individuals (Dower et al., 2016; Loffredo et al., 2018; Monahan et al., 2011), smokers (Loffredo et al., 2011), overweight and obese (Esser et al., 2014; West et al., 2014) individuals, and participants with artery disease (Loffredo et al., 2014). In addition, greater FMD improvements have been detected in studies administering doses of dark chocolate that are higher than 20 mg/day or following chronic consumption (over a period of longer than a month).

Noteworthy findings were also reported by Sun et al. (2019) who recently evaluated the effects of the main bioactive flavanols contained in cocoa products. These are postulated to positively influence endothelial function and the study sought to uncover optimal doses. This updated systematic review and meta-analysis of 15 clinical trials (730 participants) provided further information regarding the improved endothelial function seen following cocoa flavanol intake in studies lasting longer than 2 weeks. More interestingly, treatments of the interventions included in this study showed optimal effects on FMD following total flavanol, (−)-epicatechin and (+)-catechin ingestion of 710, 95 or 25 mg, respectively. Since FMD has been inversely associated with future cardiovascular events, all of these studies provide further evidence to support a causal link between cocoa intake and reduced CVD risk.

3.2. Blood pressure

High blood pressure (BP) and hypertension are other critical risk factors for CVD. Interestingly, it has been indicated that a reduction of 5 mm Hg in systolic BP may decrease risk of cardiovascular events by about 20% over a period of 5 years (Martiniuk et al., 2007). Modest reductions in BP may, therefore, protect against cardiovascular risk factors. Over recent years, numerous randomized clinical trials have investigated the BP-lowering effects of cocoa consumption. These trials have been systematically reviewed through several meta-analyses (Desch et al., 2010; Jafarnejad et al., 2020; Ried et al., 2010, 2017; Taubert et al., 2007). The first review examining the effect of cocoa- and flavanol-rich dark chocolate was performed by Tauber et al., in 2007. In this review, five randomized controlled studies involving 173 individuals showed reductions of 4.7 mmHg and 2.8 mm in systolic and diastolic BP, respectively, following cocoa intake. Desch et al. (2010) reported similar results in a later meta-analysis of 10 randomized controlled trials (297 individuals). Likewise, the above cited meta-analysis performed by Hooper et al. (2012) of 22 trials involving 918 participants, demonstrated significant reductions in diastolic BP following cocoa or chocolate administration. In addition, in a pooled meta-analysis of 15 trials including 290 individuals, Ried et al. (2010) described significant BP-reducing effects following cocoa/chocolate ingestion. Interestingly, BP was most notably reduced in hypertensive or pre-hypertensive patients, with BP not being reduced in normotensive individuals. A further meta-analysis carried out by Ried et al. (2017) of 35 studies (1804 participants) reported a small but significant effect of flavanol-rich cocoa products (mean 670 mg flavanols), reducing BP (systolic and diastolic) by 1.76 mmHg. Once again, the most remarkable BP-lowering effect of cocoa was observed within patients who had high BP at study outset. This suggests that baseline BP may play a role in the extent to which cocoa reduces BP. Some authors have also concluded that the age of individuals could play a key role in the effects of cocoa on BP. Along these lines, younger individuals are seen to be the best responders, with these being expressed through greater BP reductions. In this sense, the aim of the most recent systematic review performed by Jafarnejad and collaborators (2020) was to investigate the effect of cocoa consumption on BP in middle-aged and elderly subjects. Meta-analysis of 13 studies (758 participants) demonstrated an inverse association between cocoa consumption and BP in these populations. However, no beneficial effects of cocoa consumption on systolic/diastolic BP in normotensive or middle-aged subjects was detected. Altogether, these data suggest that flavonoid-rich chocolate might promote overall BP-lowering effects in hypertensive individuals, although

Table 2
Summary of major meta-analysis evaluating cocoa and cocoa products intake and cardiovascular outcomes.

Reference	Type of study	Number of studies	Number of participants	Main outcomes
(Buitrago-Lopez et al., 2011)	Prospective	7	114,009	Chocolate consumption might be associated with a one third reduction in the risk of developing cardiovascular disease.
(Kwok et al., 2015)	Prospective	9	157,809	Higher chocolate intake is associated with a lower risk of future cardiovascular Events
(Larsson et al., 2016)	Prospective	6	67,640	Chocolate consumption was associated with lower risk of myocardial infarction and ischemic heart disease
(Gong et al., 2017)	Prospective	5	106,109	Chocolate consumption in moderation may be associated with a decreased risk of heart failure
(Yuan et al., 2017)	Prospective	14	508,705	Consuming chocolate in moderation, i.e. < 6 servings/week, is associated with decreased risks of coronary heart disease, stroke, and diabetes
(Ren et al., 2019)	Prospective	23	405,304	Chocolate consumption may be associated with reduced risk of CVD at <100 g/week consumption.
(Hooper et al., 2012)	RCT	42	1297	Improved FMD, insulin resistance, diastolic BP, triglycerides, and mean arterial pressure
(Sun et al., 2019)	RCT	15	730	Optimal effect on FMD with 710 mg of total flavanols, 95 mg of (−)-epicatechin or 25 mg of (+)-catechin
(Ebaditabar et al., 2020)	RCT	17	615	Beneficial effect of acute and chronic consumption of dark chocolate and flavonoids on FMD.
(Taubert et al., 2007)	RCT	5	173	Consumption of foods rich in cocoa cause a decrease in 4.7 mm Hg in systolic and 2.8 mmHg in diastolic BP
(Desch et al., 2010)	RCT	10	297	Dark chocolate and cocoa beverages intake produce a decrease of 4.5 mm Hg in systolic and 2.5 mm Hg in diastolic BP.
(Ried et al., 2010)	RCT	15	290	Dark chocolate can reduce hypertension. Flavanol-rich chocolate did not significantly reduce mean BP below 140 mmHg systolic or 80 mmHg diastolic
(Ried et al., 2017)	RCT	35	1804	Flavanol-rich cocoa products, containing 670 mg flavanols have a small but significant effect in reducing BP compared with control
(Jafarnejad et al., 2020)	RCT	13	758	Inverse association between cocoa consumption and SBP/DBP. Not effect of cocoa on BP in normotensive/elevated blood pressure subjects.
(Jia et al., 2010)	RCT	8	215	Short-term cocoa consumption significantly reduced Cho. The changes were dependent on the dose of cocoa and the healthy status of participants
(Tokede et al., 2011)	RCT	10	320	Short-term cocoa intake reduced Cho and LDL-Cho levels but no major effects on HDL-Cho and TG.
(Shrime et al., 2011)	RCT	24	1106	Cocoa consumption significantly improves BP, circulating lipid levels, insulin resistance, and FMD.
(Lin et al., 2016)	RCT	19	1131	Cocoa flavanol supplements may have modest but significant benefits in lipid metabolism, insulin resistance, and systemic inflammation.
(Jafarnejad et al., 2020)	RCT	14	1035	Cocoa products consumption markedly reduces PWV and AIx both in short- and long-term studies

AIx: augmentation index; **BP**: blood pressure; **Cho:** Cholesterol; **DBP:** diastolic blood pressure; **FMD:** flow mediated dilatation; **HDL**: high-density lipoprotein; **LDL:** low-density lipoprotein; **PWV:** pulse wave velocity; **RCT:** randomized controlled trial; **SBP:** systolic blood pressure; **TG:** triglycerides.

analysed studies are too heterogeneous to enable firm conclusions. Further long-term studies are, therefore, needed to verify the strength of relationship between cocoa intake and beneficial BP effects.

3.3. Lipid profile

The potential relationship between cocoa and reduced CVD risk could also be associated with improvements in the circulating lipid profile pertaining to levels of cholesterol (Cho), triglycerides (TG), high density lipoprotein (HDL) and low-density lipoprotein (LDL). Many studies have investigated the effect of supplementation with cocoa products on the lipid profile of healthy individuals (Almoosawi et al., 2012; Fraga et al., 2005; Grassi et al., 2005a; Neufingerl et al., 2013; Wan et al., 2001), as well as that of hypertensive (Grassi et al., 2005b; Muniyappa et al., 2008), hypercholesterolemic (Baba et al., 2007; Sarriá et al., 2014), overweight (Almoosawi et al., 2012; Davison et al., 2008; Khan et al., 2012; Njike et al., 2011) and diabetic (Balzer et al., 2008; Basu et al., 2015; Curtis et al., 2012; Mellor et al., 2010) individuals. Nevertheless, the effects of cocoa product intake on the lipid profile are still controversial. An earlier meta-analysis performed by Jia et al. (2010) of eight randomized trials (215 participants) indicated that short-term cocoa consumption significantly reduced Cho and LDL-Cho, even at low doses (daily polyphenol consumption of <260 mg), in individuals presenting cardiovascular risks factors. Tokede et al. (2011) described similar outcomes in a meta-analysis of 10 studies (320 individuals). Their study showed a reduction of Cho and LDL-Cho levels (6.2 and 5.9 mg/dL, respectively), although HDL-Cho and TG levels did not change. On the contrary, Hooper et al. (2012), in consideration of 21 studies and 986 participants, reported only marginally significant effects on LDL-Cho and Cho levels, although, in longer-term trials (lasting more than 3 weeks), HDL-Cho levels were increased by an average of 1.8 mg/dL. Similar results were reported in a later meta-analysis (24 randomized controlled trials, 1106 participants) which demonstrated an increase in HDL-Cho levels of 1.9 mg/dL following two weeks of cocoa product consumption (Shrime et al., 2011). In a more recent systematic review and meta-analysis of 1131 participants from 19 randomized clinical trials, cocoa flavanol intake significantly improved biomarkers of lipid metabolism (Lin et al., 2016). Further, whilst TG levels decreased and HDL-Cho values increased, no significant associations were found for LDL-Cho and total Cho. Overall, due to the considerable heterogeneity seen between different publications, effects of cocoa consumption on the lipid profile are controversial and further studies are needed to fully define potential benefits in this regard.

3.4. Vascular stiffness

Arterial stiffness is a consequence of biological aging and arteriosclerosis. It is also a designated marker of vascular dysfunction and a major predictor of cardiovascular outcomes (Vlachopoulos et al., 2010). Several clinical trials are reported in the literature to have examined the relationship between cocoa product intake and reliable measures of arterial stiffness. Such studies use measures of carotid-femoral pulse wave velocity (PWV), aortic pulse pressure or the augmentation index (AIx). In a recent prospective analysis, chocolate intake was significantly associated with PWV in a non-linear fashion. Specifically, the highest levels of PWV were found in individuals who rarely ate chocolate,

Table 3
Human epidemiologic studies and interventional trials of the effects of cocoa intake on obesity[a].

Reference	Type of study	Participants	Time	Dose (day)	Main outcomes
(Golomb et al., 2012)	Cross-sectional (observational)	1018 (BMI = 28, 20–85 y)	6 months	Times per week eating 28 g chocolate, 1 oz (FFQ)	↓BMI
(O'Neil et al., 2011)	NHANES Cross-sectional (observational)	15,023	6 years	Candy consumer/non-consumer	= BW, ↓CRP, ↓BP, ↓TG, ↓LDL-Cho, ↑HDL-Cho
(Greenberg and Buijsse, 2013)	ARIC cohort (observational)	28,562 (BMI = 28, 45–64 y)	6 years	Times per week eating 1 oz. chocolate (FFQ)	↑BW in dose-response manner at long term
(Greenberg et al., 2015)	WHI	107,243 post-menopausal women	3 years	Chocolate-candy consumer (FFQ)	↑BMI
(Munguía et al., 2015)	RCDB, parallel	15 adults (10 overweight +5 controls)	4 weeks	Cacao bean extract powder (80 mg flavonoids)	↓BW, ↓BMI, ↓WC, ↓TG/HDL-Cho ratio, ↓MDA, ↓carbonyls of proteins
(Almoosawi et al., 2012)	RCSB, crossover	42 adult women (13 overweight + 8 obese + 21 controls)	4 weeks	Dark chocolate (20 g, 500 mg polyphenols)	↓BW, =WC, ↓BP, =Insulin, =HOMA-IR, = salivary cortisol
(Njike et al., 2011)	RCDB, crossover	39 overweight (6 males + 33 females)	6 weeks	11 g natural cocoa powder	No effect on BW, BMI, WC, BP, FMD, total-Cho, TG, HDL-Cho, LDL-Cho, Glucose, LDLox
(Davison et al., 2008)	RCDB, parallel	49 adults (18 males + 31 females BMI˃25)	12 weeks	Cocoa powder (902 mg flavanol)	No effect on BW, BMI, WC, total-Cho, TG, HDL-Cho, LDL-Cho, Glucose, Insulin. ↑FMD, ↓insulin resistance, ↓BP
(Ibero-Baraibar et al. 2014, 2016)	RCDB, parallel	50 middle-aged obese (23 males + 27 females BMI = 28–32)	4 weeks	Cocoa extract (1.4 g cocoa, 654.3 mg of polyphenols)	No effect on BW, BMI, WC ↓LDLox, ↓MPO, ↓plasma homovanillic acid ↓sICAM-1, = sVCAM-1
(Nickols-Richardson et al., 2014)	RCSB, parallel	60 premenopausal women (BMI = 25–43)	18 weeks	Cocoa beverage (270 mg flavanols)	= BW, ↓BP, ↓Glucose, ↓Insulin, = total-Cho
Massolt et al. (2010)	RCDB, crossover	12 healthy women (BMI˃25)	Acute	Dark chocolate (30 g, 85% cocoa)	↓appetite, ↓ghrelin
(Sørensen and Astrup, 2011)	RCDB, crossover	16 young healthy men	Acute	Dark chocolate (100 g, 70% cocoa)	↑satiety, ↓desire to eat sweets, ↓energy intake
(Marsh et al., 2017)	RCDB, crossover	14 premenopausal women	Acute	Dark chocolate (84 g, 80% cocoa)	↓food intake. No effect on ghrelin, leptin, mood
(West et al., 2014)	RCDB, crossover	30 adult females (BMI = 25–37)	4 weeks	37 g dark chocolate+ 22 g cocoa (total flavanols 814 mg)	No effect on BW, BMI, WC, hip circumference, FMD, BP, Glucose, Insulin, TG, LDL-Cho, HDL-Cho, IL-1, IL-6, TNF-α
(Ribeiro-Vieira et al., 2017)	RCDB, parallel	60 females (30 controls + 30 cases, BMI = 25–35)	6 weeks	Cocoa beverage (39.1 g cocoa having 29.55 mg gallic acid equivalents)	No effect on BW, BMI, WC, ↑propionic acid
(Wiese et al., 2019)	RCDB, parallel	30 middle-aged (15 males + 15 females BMI = 30–35)	4 weeks	10 g dark chocolate (10 mg flavanols)	No effect on BW, TG, LDL, HDL. ↓MDA, ↓LDL-Px ↑*Lactobacillus*, ↓corneocyte exfoliation
(Angiletta et al., 2018)	RCDB, crossover	20 adults (10 males + 10 females BMI˃27)	5 days	28 g cocoa powder (30–900 mg flavanols/d)	No effect on BMI, body mass, fat mass, lean mass, TMAO

ARIC: Atherosclerosis Risk In Communities; **BMI**: body mass index; **BP**: blood pressure; **BW**: body weight; **Cho:** Cholesterol; **CRP**: C-reactive protein; **FFQ:** food frequency questionnaire; **FMD:** flow mediated dilatation; **HDL**: high-density lipoprotein; **HOMA-IR**: Homeostasis Model Assessment of Insulin Resistance; **ICAM-1**: intercellular adhesion molecule-1; **IL**: interleukin; **LDL:** low-density lipoprotein; **LDL-Px:** LDL peroxidase; **MDA:** malondialdehyde; **MPO:** myeloperoxidase; **NHANES**: National Health and Nutrition Examination Survey; **RCDB**: Randomized Controlled; **RCDB**: Randomized Controlled Doble-Blind; **RCSB**: Randomized Controlled Single-Blind; **TG:** triglycerides; **TMAO**, trimethylamine *N*-oxide; **TNF-α:** tumour necrosis factor-α; **WC:** waist circumference; **VCAM-1**: vascular cell adhesion molecule-1;**WHI**: Women's Health Initiative.

[a] The arrow indicates an increase (↑) or decrease (↓) in the levels of the different parameters analysed, " = " symbol designates unchanged parameters.

whereas the lowest levels were seen in those who consumed moderate amounts of chocolate (once per week) (Crichton et al., 2016). Although the available evidence suggests that cocoa and cocoa derived products might have protective effects on arterial stiffness, findings from clinical studies have reported mixed results. Some randomized trials have shown that arterial stiffness is reduced by regular high-cocoa intake (Grassi et al., 2015; Pereira et al., 2014; West et al., 2014). However, some other studies have reported that chocolate consumption has little or no effect on arterial stiffness (Recio-Rodríguez et al., 2012; Vlachopoulos et al., 2005). In addition, BP is considered to be a powerful confounding factor in relation to PWV. Thus, regular cocoa consumption could reduce BP, which, in turn, diminishes arterial stiffness. Indeed, many studies have demonstrated that cocoa product intake simultaneously decreased BP and arterial stiffness (Grassi et al., 2015; Pereira et al., 2014; Sansone et al., 2015). However, Nishiwaki et al. (2019) recently demonstrated in a group of healthy young individuals that regular high-cocoa chocolate ingestion can directly reduce arterial stiffness (PWV) without affecting BP. Additionally, in a very recent systematic review and meta-analysis of 14 randomized clinical trials including 1035 individuals, Jafari-Azad et al. (2020) evaluated the effect of cocoa intake on vascular stiffness. They reported that cocoa product consumption markedly reduced PWV and AIx in both short- and long-term studies. This was especially the case in males, chronic intakes of ≤4 weeks and acute intakes of ≤120 min. These findings suggest that regular chocolate consumption can improve arterial stiffness by promoting vascular health and, thus, reducing the risk of CVD.

In summary, all available data from prospective and clinical trials clearly indicate that moderate consumption of cocoa and cocoa derived products may have beneficial consequences for a variety of cardiovascular conditions. There is strong evidence that cocoa consumption increases FMD, whereas moderate evidence supports a relationship with improved blood pressure, lipid profile and arterial stiffness. Interestingly, beneficial effects seem to be emphasized in individuals presenting some level of dysfunction prior to study outset. The optimal cocoa flavanol dose for inducing these effects is suggested to be around 900 mg or greater and this could be obtained by ingesting flavanol-rich cocoas (Vlachojannis et al., 2016). However, more research is required to elucidate the optimal quantities of cocoa for consumption in order to produce short- or long-term beneficial effects on cardiovascular risk.

The molecular mechanisms through which cocoa products mediate

these cardiovascular benefits have been related to the ability of cocoa flavanols and their derived metabolites to improve endothelial nitric oxide (NO) formation (Ferri et al., 2015; Jiménez et al., 2012). This facilitates vasodilatation and modulates the renin-angiotensin-aldosterone system (Actis-Goretta et al., 2006; Álvarez-Cilleros et al., 2018). The anti-oxidative and anti-inflammatory properties of cocoa may also play a role given that oxidative stress and inflammation are associated with arterial damage (Martin and Ramos, 2017).

4. Effects of cocoa against obesity

Interventional and observational studies have associated cocoa/chocolate intake with the prevention of obesity in humans (Table 3). In this sense, a cross-sectional study conducted with 1018 participants (20–85 years) found that adults who consumed chocolate more frequently had a lower body mass index (BMI) than those who ate chocolate less often (Golomb et al., 2012). These findings were not explained by calorie intake (frequent chocolate intake was linked to more overall calories), physical activity, or any of the other examined potential confounders. On the other hand, no association was found between chocolate candy consumption and body weight in the National Health and Nutrition Examination Survey (NHANES) study. It is important to note that no differentiation was made between sugar and chocolate candy consumption in this work (O'Neil et al., 2011). Interestingly, in a prospective study involving the Atherosclerosis Risk in Communities (ARIC) cohort, chocolate consumption was connected, in a dose-response manner, with greater long-term weight gain. However, chocolate intake was related with lower body weight in participants with serious pre-existing obesity-related illnesses (Greenberg and Buijsse, 2013). Similarly, in a cohort of post-menopausal American women enrolled in the Women's Health Initiative (three-year follow up study, 1999–2004), greater chocolate-candy intake was observed to be linked witha greater likelihood of future weight gain (Greenberg et al., 2015).

In the same way as seen in epidemiological studies, inconsistent results have also been reported in randomized clinical trials (Table 3). Munguía et al. (2015) studied a group of 15 overweight individuals (20–60 years) with borderline metabolic syndrome in a double blind, placebo-controlled, clinical pilot trial. Volunteers received either a cacao bean extract powder (80 mg flavonoids) or a sugar-free placebo powder (similar characteristics to the cocoa powder but without flavonoids) once a day. After 4 weeks, significant reductions in body weight, BMI, waist circumference, triacylglycerols, TG/HDL ratio and oxidative stress-related parameters [malondialdehyde (MDA) and protein carbonyls] were observed in the cocoa supplementation group. This suggested that cacao flavonoids without added sugar efficiently modulated anthropometric and cardiometabolic risk factors in overweight individuals. Similarly, in a single-blind randomized placebo-controlled cross-over study, participants received either 20 g of polyphenol-rich dark chocolate (500 mg polyphenols) or 20 g of placebo dark chocolate for 4 weeks, separated by a 2-week washout period (Almoosawi et al., 2012). Significantly greater reductions in body weight were observed in the post-polyphenol-rich dark chocolate group compared with the placebo group, although no differences in waist circumference were detected. Certain cardiovascular risk factors were also improved, for instance, systolic and diastolic BP decreased after polyphenol-rich dark chocolate administration, although no significant changes were observed in the lipid profile (Almoosawi et al., 2012). In contrast, other human studies did not report any modifications to body weight and/or BMI in overweight/obese subjects receiving cocoa/chocolate. In this sense, in a randomized, controlled, crossover trial in which a sugar-free cocoa beverage and a sugar-sweetened cocoa beverage were administrated every day for 6 weeks, body weight, BMI, waist circumference and endothelial function (FMD) did not change when compared to a sugar-sweetened cocoa-free placebo group (Njike et al., 2011). In addition, Davison et al. (2008) investigated the effects of high- and low-flavanol cocoa (902 mg and 36 mg flavanol content, respectively) intake over 12 weeks, in combination with exercise, on fat and endothelial function in 49 obese volunteers (18–65 years). Despite the fact that body weight, BMI and waist circumference did not change because of dark chocolate consumption, improved endothelial function (FMD), insulin resistance, and diastolic and mean BP was reported regardless of exercise engagement. Likewise, a 4-week randomized, parallel and double-blind study compared 25 participants who received meals supplemented with 1.4 g of cocoa extract (645.3 mg of polyphenols) with 25 participants who received control meals characterized by a 15% energy restriction. Participant' body weight, waist circumference, total fat mass and truncal fat mass improved due to the intervention in both groups (Ibero-Baraibar et al., 2014). All of the observations made above indicate that cocoa supplementation did not have any additional effects on examined parameters, with beneficial outcomes instead being attributed to dietary calorie restriction (Ibero-Baraibar et al., 2014). Importantly, greater reductions in oxidized LDL-Cho and myeloperoxidase were observed only in the cocoa supplementation group. Similarly, another study evaluating the effects of 18 weeks of cocoa consumption (236 mL sugar-free natural cocoa beverage plus one 1.45 oz dark chocolate square twice a day every day, providing 270 mg of flavanols) in 60 overweight/obese premenopausal women showed improved BP, glucose and insulin levels in participants consuming a sugar-free cocoa beverage, in addition to similar body weight reductions (Nickols-Richardson et al., 2014). A randomized, placebo-controlled, 4-week, cross-over study conducted by West et al. (2014) with volunteers aged between 40 and 64 years who consumed 37 g/d of dark chocolate and a sugar-free cocoa beverage (22 g/d, 814 mg/d total flavanols), also reported no changes to body weight, BMI, WC or hip circumference. Further, no changes were found in FMD and fasting BP, whereas arterial stiffness was only reduced in women. Recently, a systematic review and meta-analysis of 30 randomized clinical trials involving 710 normal weight and 713 obese individuals explored the relationship between cocoa/dark chocolate consumption and body weight, BMI and waist circumference. In this meta-analysis, it was concluded that cocoa/dark chocolate supplementation did not modify anthropometric measures, with reduced bodyweight and BMI only being found in a subgroup consuming $\geq$30 g/day over 4–8 weeks (Kord-Varkaneh et al., 2019).

With regards to the effects of cocoa/chocolate on satiety, a clinical trial with 12 women (BMI = 18–25 kg/m^2) found that appetite was suppressed after smelling dark chocolate (85% cocoa). This satiety response was inversely correlated with ghrelin levels (Massolt et al., 2010). Similarly, in a randomized crossover study with 16 young, healthy, normal-weight men, dark chocolate consumption (70% cocoa) 2 h prior to consumption of an *ad libitum* meal promoted satiety. In comparison to milk chocolate (30% cocoa) intake under the same conditions, dark chocolate consumption diminished the desire to eat something sweet and decreased energy intake (17%) (Sørensen and Astrup, 2011). In accordance with these findings, 14 postmenopausal women (50–65 years) participated in three experimental trials, with a one-week gap between conditions. Participants were presented with the foodstuffs pertaining to each condition [cocoa (80%) 'dark' chocolate, cocoa (35%) 'milk' chocolate and cocoa butter 'white' chocolate (0% cocoa solids)], which were all energy matched and then instructed to eat until "comfortably full" (Marsh et al., 2017). Dark chocolate condition led to reduced subsequent food intake in comparison to the milk and white chocolate conditions. In contrast, active ghrelin and leptin levels did not differ between conditions, whilst mood was also unchanged (Marsh et al., 2017).

Obesity is a multifactorial disease that is associated with mental disorders (anxiety and depression) and dysbiosis. In consideration of this, Ibero-Baraibar et al. (2016) conducted a 4-week intervention trial in which 50 overweight/obese individuals were submitted to a 15% energy-restricted diet and assigned to receive 1.4 g a day of cocoa extract (645 mg polyphenol content). At the end of the study, cocoa

Table 4
Human interventional trials of the effects of cocoa intake in T2D patients[a].

Reference	Type of study	Participants	Time	Dose (day)	Main outcomes
(Balzer et al., 2008)	RCDB, parallel	41 T2D patients	30 days	Flavanol-rich cocoa (963 mg CF)	↑FMD. No effect on BP, LDL-Cho, HDL-Cho, Glucose, Insulin and HbA1c
(Mellor et al., 2010)	RCDB, crossover	12 hypertensive T2D patients	8 weeks	Dark chocolate (450 mg CF)	↑HDL-Cho, ↓BP. No effect on HOMA-IR, LDL-Cho, Glucose, Insulin and HbA1c
(Curtis et al., 2012)	RCDB, parallel	93 post-menopausal T2D women	1 year	850 mg flavanols and 100 mg isoflavones	↓HOMA-IR, ↓Insulin, ↓LDL-Cho. No effect on Glucose, HbA1c and HDL-Cho
(Curtis et al., 2013)	RCDB, parallel	93 post-menopausal T2D women	1 year	850 mg flavanols and 100 mg isoflavones	↓PWV, ↓PP. No effect on AIx and BP
Parsaeyan et al. (2014)	RCDB, parallel	100 T2D patients	6 weeks	Cocoa powder (20 g)	↓LDL-Cho, ↓HDL-Cho ↓TG, ↓CRP, ↓IL-6, ↓TNF-α,
(Rostami et al., 2015)	RCDB, parallel	60 hypertensive T2D patients	8 weeks	Dark chocolate (450 mg CF)>	↓BP. No effect on Glucose, HbA1c, HDL-Cho, LDL-Cho and CRP
(Basu et al., 2015)	RCDB, crossover	18 T2D patients	Acute	Cocoa beverage (960 mg polyphenols)	↑Insulin, ↑HDL-Cho. No effect on glucose, HOMA-IR, LDL-Cho and TG
(Ayoobi et al., 2017)	RCSB, parallel	44 T2D patients	8 weeks	Dark chocolate with 84% of cocoa (30 g)	↓BP, ↓waist circumference. No effect on nitric oxide levels
(Dicks et al., 2018)	RCDB, parallel	42 hypertensive T2D patients	12 weeks	Capsules (83.6 mg CF)	No effect on Glucose, HbA1c, Insulin, HOMA-IR, BP, LDL-Cho, HDL-Cho and TG
(Jafarirad et al., 2018)	RCDB, parallel	44 T2D patients	8 weeks	Dark chocolate (84% cocoa, 30 g)	↓Glucose, ↓HbA1c, ↓LDL-Cho, ↑HDL-Cho ↓TG, ↓CRP, ↓TNF-α, ↓IL-6
(Rynarzewski et al., 2019)	RCDB, crossover	42 hypertensive T2D patients	Acute	Capsules (83.6 mg CF)	No effect on Glucose, Insulin, HOMA-IR, BP, LDL-Cho, HDL-Cho and TG
(Davis et al., 2020)	RCDB, crossover	18 T2D patients	Acute	Cocoa beverage (960 mg polyphenols)	↓VLDL, ↓Chylomicron Particles, ↓IL-8

AIx: augmentation index; **BP**: blood pressure; **CF**: cocoa flavanol; **Cho:** Cholesterol; **CRP**: C-reactive protein; **FMD:** flow mediated dilatation; **GSH**: glutathione; **HbA1c:** haemoglobin glycosylated; **HDL**: high-density lipoprotein; **HOMA-IR**: Homeostasis Model Assessment of Insulin Resistance; **IL**: interleukin; **IR**: insulin resistance; **LDL:** low-density lipoprotein; **PP:** pulse pressure; **PWV:** pulse wave velocity; **RCDB**: Randomized Controlled Doble-Blind; **RCSB**: Randomized Controlled Single-Blind; **T2D**: type 2 diabetes; **TG:** triglycerides; **TNF-α:** tumour necrosis factor-α; **VLDL:** very low density lipoprotein.
[a] The arrow indicates an increase (↑) or decrease (↓) in the levels of the different parameters analysed.

consumption was associated with significant increases in plasma homovanillic acid levels, which, in turn, were related to a reduction in depressive symptoms.

As mentioned above, altered gut microbiota has been detected in obese individuals. The reduction or elimination of these alterations has been suggested as leading to improved health. In this sense, 60 overweight women (20–50 years) received a drink of cocoa (39.1 g) or cocoa and unripe banana flavor (54.1 g) dissolved in 150 mL of water in a double-blinded randomized 6-week clinical trial. Neither of these beverages modified body weight, BMI or waist circumference, however, they did improve dyspepsia and gastrointestinal symptoms (bowel habits, consistency of feces), whilst also increasing the production of propionic acid favoring the reshape of unbalanced gut microbiota. Anti-inflammatory effects were only seen in relation to cocoa consumption (Ribeiro-Vieira et al., 2017). Similarly, prebiotic effects were reported in a double-blinded 4-week trial in which 30 obese volunteers received lycopene alone (7 or 30 mg) or in combination with dark chocolate (10 g containing 10 mg of flavanols). Dark chocolate consumption did not influence body weight, TG, LDL-Cho or HDL-Cho and decreased markers of oxidative stress (MDA and LDL peroxidase) (Wiese et al., 2019). Interestingly, dark chocolate also led to greater *Lactobacillus* abundance. This was related to reduced corneocyte exfoliation, in addition to improved gut, blood and liver lipid metabolism (Wiese et al., 2019). In this regard, it could also be mentioned that certain metabolites generated by gut microbes from dietary substrates have been linked to different diseases. An example of this comes from trimethylamine *N*-oxide (TMAO), which has been associated with CVD (Ramos and Martín, 2021). In a crossover blinded design made up of five-day treatment periods followed by a 10-day washout, adult participants (25–55 years) received one of three cocoa treatments differentiated according to flavanol content (180, 400 and 900 mg flavanols/day). It was reported that this short-term intake of cocoa flavanols did not affect BMI, body mass, fat mass, lean mass or plasma TMAO levels in obese individuals at risk of T2D (Angiletta et al., 2018).

Altogether, more evidence is needed to clarify the impact of cocoa and its derived products on obesity as present studies have provided equivocal information regarding BMI and waist circumference. However, positive outcomes can be seen with regards to beneficial effects on cardiovascular markers (BP, FMD, etc.) in obese adults, with such effects also contributing towards the alleviation of disease. Moreover, cellular and pre-clinical works have demonstrated that cocoa could exert anti-obesogenic effects through different mechanisms, such as lipid metabolism modulation (decreased lipogenesis and enhanced lipolysis), reduced adipogenesis (inhibited adipocyte differentiation and growth), attenuated inflammatory response and oxidative stress (Ali et al., 2014; Rodríguez-Pérez et al., 2017), and microbiota reshaping (Gu et al., 2014).

5. Effects of cocoa against diabetes

In recent years, several large prospective cohort studies have suggested that moderate consumption of cocoa and cocoa-derived products may reduce risk of type 2 diabetes (T2D) (Crichton et al., 2017; Greenberg, 2015; Matsumoto et al., 2015; Oba et al., 2010). More recently, these results were confirmed by Maskarinec et al. (2019) in the Multi-ethnic Cohort (MEC) Study including 151,691 individuals of Native Hawaiian, Japanese American, Latino, African American and white ancestry. In this study, participants with a high intake of chocolate products and cocoa-derived flavanols experienced a reduced risk of developing T2D even after controlling for sugar intake, diet quality and other dietary aspects. Nonetheless, it should be considered that only a relatively small number of clinical interventional studies have evaluated the effects of cocoa products in diabetic patients (Table 4). It is noteworthy that most of these studies have investigated the potential beneficial effects of cocoa on, not only, glucose control but, also, vascular function. These studies emphasize the likely benefit of these aspects when it comes to reducing the risk of cardiovascular events within this population.

Balzer et al. (2008) were the first to demonstrate that 30-day supplementation with cocoa flavanols (963 mg/day) in drug-treated T2D

patients produced a significant increase in fasting FMD. In contrast, BP, heart rate and glycemic control were unaffected. In a smaller cohort (12 hypertensive patients with T2D), Mellor et al. (2010) showed that daily dark chocolate consumption (450 mg of flavanols) over a period of 8 weeks, improved HDL-Cho levels and decreased BP, without affecting insulin resistance or glycemic control. In a longer study with diabetic women, daily flavonoid-enriched chocolate intake (850 mg of flavanols and 100 mg of isoflavones) over a one-year period led to reduced insulin levels and insulin resistance (Homeostatic Model Assessment for Insulin Resistance, HOMA-IR) (Curtis et al., 2012). However, no effect on glycosylated hemoglobin (HbA1c) or glucose was detected. In addition, a combined flavonoid intervention also improved lipoprotein status and arterial stiffness, reducing the cardiovascular risk of this population (Curtis et al., 2013). Similarly, daily high-polyphenol dark chocolate intake (450 mg of polyphenols) over an 8-week period improved cardiovascular risk indices in diabetic and hypertensive patients by decreasing systolic and diastolic BP (Rostami et al., 2015). However, dark chocolate supplementation was not effective in improving fasting blood glucose, insulin and HbA1c levels in these patients. In the same way, Ayoobi and collaborators (2017) reported that the consumption of 30 g of dark chocolate (84% of cocoa solids) a day for 8 weeks reduced systolic and diastolic BP and waist circumference, and improved body composition in diabetic patients. It is interesting to note that cardiovascular diseases in diabetic patients have been widely associated with elevated oxidative stress and low-grade inflammation. In this regard, Parsaeyan et al. (2014) evaluated the effects of cocoa supplementation on the lipid profile, lipid peroxidation and inflammatory markers in T2D patients. After six weeks of intervention characterized by a daily intake of 10 g of cocoa powder, lipid peroxidation was inhibited and levels of Cho, TG, LDL-Cho and inflammation indicators (tumor-necrosis factor [TNF]-α and interleukin [IL]-6) in plasma were reduced in diabetic participants. However, no data on clinical markers associated with diabetes were reported in this study. Likewise, Jafarirad et al. (2018) showed that daily supplementation with 30 g of dark chocolate (84% cocoa) over an 8-week period decreased levels of inflammatory markers, such as hs- (C-reactive protein)-CRP, TNF-α and IL-6 in diabetic patients. Interestingly, fasting blood glucose, HbA1c, LDL-Cho and TG values also decreased in the dark chocolate group. In contrast, Dicks and collaborators (2018) did not find any changes in serum glucose, insulin, lipid levels or BP in stably treated patients with T2D and hypertension following regular intake of 2.5 g/day of a flavanol-rich cocoa powder for 12 days. Most participants received oral hypoglycemic drugs, as well as lipid- and BP-lowering drugs, in order to ensure tight metabolic and BP control. Thus, the lack of effect of cocoa on cardiometabolic parameters may be due to pharmaceutical polytherapy, which partly modulates the same molecular targets as cocoa flavanols.

Along with the potential effects of long-term cocoa consumption in diabetic patients, its postprandial effects on metabolic parameters have also been investigated in a small number of clinical trials. In patients with T2D, increased postprandial hyperglycemia and hypertriglyceridemia may damage vascular function and lead to a higher incidence of CVD. Indeed, the mitigation of metabolic stress during the postprandial state is a main objective of T2D management. Despite this, Basu and collaborators (2015) showed no-postprandial benefits in relation to glucose metabolism in T2D patients when a flavanol-rich cocoa drink (960 mg of polyphenols) was ingested together with a fast-food-style meal. Similarly, Rynarzewski et al. (2019) described that 2.5 g of flavanol-rich cocoa powder, ingested as part of a meal suitable for diabetics, did not affect postprandial glucose, lipid metabolism or BP in stably-treated diabetics. On the contrary, Davis et al. (2020) more recently reported that consumption of 20 g of a polyphenol-rich cocoa drink (960 mg of polyphenols) with a high-fat dietary content may alleviate postprandial dyslipidemia and inflammation in adults with T2D.

Overall, these results indicate that short-term consumption of cocoa and cocoa-derived products by diabetic patients could have some beneficial effects on glycemic control and, to a greater extent, on factors implicated in CVD risk. Likely mechanisms seem to be related with both the proved beneficial effects of cocoa flavanols on vascular function and their ability to modulate key proteins involved in the insulin signaling pathway, inflammation, oxidative stress and microbiota (Álvarez-Cilleros et al., 2020; Martin et al., 2017; Martín et al., 2016).

6. Effects of cocoa on cognitive functions

The etiology of age-associated cognitive decline is complex, however, increasing evidence supports the hypothesis that cardiovascular alterations, oxidative stress and neuroinflammation may play a crucial role in the pathophysiology of cognitive processes (Grassi et al., 2016). Consequently, a great amount of attention has been given to the potential beneficial effects of cocoa on cerebrovascular risk factors and cognitive function.

Cognitive function in humans is usually estimated through a variety of standardized tests which evaluate attention, memory, decision making, language comprehension, or a range of other mental processes (Socci et al., 2017). Outcomes from a number of human clinical studies suggest that cocoa and cocoa-derived products may induce positive effects on several of these cognitive processes, including attention, working memory and processing speed (Haskell-Ramsay et al., 2018; Socci et al., 2017) (Table 5). In a very recent systematic review, Barrera-Reyes et al. (2020) identified twelve randomized clinical trials conducted between 2006 and 2018, which evaluated the effect of cocoa polyphenols on cognitive function in healthy subjects (Table 5). Additionally, three new human studies have been carried out in the last two years on this topic. Of these trials, ten showed significant effects on cognitive function, whereas the remaining five did not find significant effects. Nonetheless, it is interesting to note that cocoa and its components may have exerted certain impacts on the brain (increase plasma levels of brain-derived neurotrophic factor or activate brain specific regions), although these effects were not reflected in cognitive tests (Camfield et al., 2012; Francis et al., 2006).

Scholey et al. (2010) provided the first piece of direct evidence of certain effects of cocoa consumption on cognitive functions. Acute administration of chocolate drinks containing 520 and 994 mg of cocoa flavanols improved working memory and attenuated mental fatigue induced by demanding tasks in healthy adults. Likewise, Massee et al. (2015) reported similar improvements in cognitive performance and mental fatigue in healthy young individuals after the ingestion of a cocoa tablet containing 250 mg of catechin. Acute beneficial effects of cocoa have also been demonstrated under normal cognitive conditions in young healthy adults. In this regard, Field et al. (2011) demonstrated that a single dose of dark chocolate (773 mg of flavonoids, 222 mg of theobromine and 38 mg of caffeine) improved visual contrast sensitivity and cognitive task performance. Karabay et al. (2018) have also demonstrated that the consumption of a cocoa drink containing 374 mg of cocoa flavanols improved visual search efficiency, although it did not facilitate temporal attention. This suggests that flavanols can affect specific cognitive functions. More recently, Lamport et al. (2020) demonstrated improvements in verbal episodic memory in healthy young individuals, 2 h following acute consumption of a standard 70% cocoa dark chocolate bar. Whilst the exact flavonoid content used in this study was unknown, it was estimated using validated databases to contain approximately 80–90 mg of flavonoids. Remarkably, acute beneficial effects were obtained with this cocoa flavanol content which was lower than that used in most other acute studies. Finally, one study failed to provide evidence of any acute effects on cognition performance in older adults following the administration of 500 mg of cocoa flavanols (Pase et al., 2013).

Interestingly, most randomized controlled trials have investigated the effects on human cognitive function following repeated consumption of cocoa polyphenols. Desideri et al. (2012) evaluated the effect of daily consumption of cocoa drinks with high (994 mg) and intermediate (520

Table 5
Human interventional trials of the effects of cocoa intake on cognition[a].

Reference	Type of study	Participants	Time	Dose (day)	Main outcomes
(Francis et al., 2006)	RCDB, crossover	16 (healthy young)	5 days	Dark chocolate (172 mg CF)	↑ blood oxygenation levels and activation in specific cortex areas. No effect on cognitive task
(Crews et al., 2008)	RCDB, parallel	101 (healthy older adults)	6 weeks	Dark chocolate and cocoa drink (754 mg CF)	No effects on cognitive and cardiovascular outcomes
(Scholey et al., 2010)	RCDB, crossover	30 (healthy adults)	Acute	Cocoa drink (520 and 994 mg)	↑ working memory and attenuate mental fatigue
(Field et al., 2011)	RCSB, crossover	30 (healthy young)	Acute	Chocolate bars (773 mg CF)	↑visual contrast sensitivity, ↑spatial memory and performance, ↓reaction time, = coherent motion
(Desideri et al., 2012)	RCDB, parallel	90 (elderly adults with MCI)	8 weeks	Cocoa drink (520 and 994 mg CF)	↑processing speed, ↑executive function ↑working memory
(Camfield et al., 2012)	RCDB, parallel	63 (healthy middle-aged)	30 days	Cocoa drink (250 and 500 mg CF)	↑neural efficiency. No effect on spatial working memory
(Pase et al., 2013)	RCDB, parallel	72 (healthy middle-aged)	30 days	Cocoa drink (250 and 500 mg CF)	No effect on cognition performance
(Brickman et al., 2014)	RCDB, parallel	37 (healthy older adults)	3 months	Cocoa supplement (900 mg CF)	↑cerebral blood volume in the dentate gyrus ↑ memory task
(Massee et al., 2015)	RCDB, parallel	40 (healthy young adults)	4 weeks	Cocoa bars (250 mg CF)	↑cognitive performance and attenuate mental fatigue
(Mastroiacovo et al., 2015)	RCDB, parallel	90 (healthy older adults)	8 weeks	Cocoa drink (520 and 993 mg CF)	↑cerebral blood volume, ↑neuronal functionality
(Neshatdoust et al., 2016)	RCDB, crossover	40 (healthy older adults)	4 weeks	Cocoa drink (494 mg CF)	↑levels of BNDF, ↑global cognition
Karabay et al. (2018)	RCDB, crossover	48 (healthy young)	Acute	Cocoa drink (374 and 747 mg CF)	↑visual search efficiency and spatial attention No effect on temporal attention
(Sumiyoshi et al., 2019)	RCSB, crossover	20 (healthy young)	4 weeks	Dark chocolate bar (540 mg of polyphenols)	↑processing speed, focused attention, and sustained attention. ↑levels NGF in plasma. No differences in PCBF and BDNF
(Lamport et al., 2020)	RCSB, parallel	98 (healthy young adults)	Acute	Dark chocolate bar (85 mg of CF)	↑verbal episodic memory
Suominen et al. (2020)	RCSB, parallel	100 (healthy young adults)	8 weeks	Dark chocolate (410 mg CF)	No effect in verbal fluency, mental flexibility, processing speed and executive functioning.

BDNF: brain-derived neurotrophic factor; **CF:** Cocoa flavanols: **MCI:** Mild Cognitive Impairment; **NGF:** nerve growth factor; **PCBF:** prefrontal cerebral blood flow; **RCDB**: Randomized Controlled Doble-Blind; **RCSB**: Randomized Controlled Single-Blind.

[a] The arrow indicates an increase (↑) or decrease (↓) in the levels or activity of the different parameters analysed, " = " symbol designates unchanged parameters.

mg) levels of flavonoids during 8 weeks in elderly people with mild cognitive impairment. They reported that cocoa supplementation improved some aspects of age-related cognitive dysfunction, including processing speed, executive function and working memory. Moreover, in a similar study, Mastroiacovo et al. (2015) observed identical results in elderly people with normal cognitive function. Notably, it has been suggested that long-term cocoa intake may be effective for improving cognition through neuronal functionality and enhancements to cerebral blood flow in memory-related brain areas. Healthy older adults who consumed a high-flavanol cocoa (900 mg) for 3 months had a greater cerebral blood volume in the dentate gyrus, a region in the hippocampal whose function declines with human aging (Brickman et al., 2014). This increase in cerebral blood volume was correlated with improved performance of a dentate gyrus-dependent memory task. Similarly, healthy elderly individuals who took a daily supplement of high-flavonoid cocoa (494 mg) for 28 days, presented cognitive improvements that were linked to changes in serum levels of a neurotrophic factor known to have neuroprotective effects, namely, brain-derived neurotrophic factor (BDNF) (Neshatdoust et al., 2016). Similar results have recently been found in healthy young individuals who consumed 70% cocoa dark chocolate enriched with flavonoids and methylxanthines every day for 30 days (Sumiyoshi et al., 2019). Dark chocolate intake increased levels of neurotrophic nerve growth factor in plasma and enhanced cognitive function and performance.

Despite this promising evidence, the heterogeneity of employed study designs (sample sizes, cocoa doses, intervention length and participant characteristics) means that mixed outcomes have been produced. For example, in healthy young females, a sub-chronic (5 days) daily intake of 172 mg cocoa flavanols was associated with increased blood oxygen levels and activation of specific cortex areas, previously associated with high-level cognition, without evoking any significant changes to cognitive variables (Francis et al., 2006). Similarly, chronic (30-day) intake of a cocoa drink with 250 and 500 mg of cocoa flavanols in middle-aged volunteers, effectively improved cognition by increasing neural efficiency (Camfield et al., 2012). Nevertheless, these changes were not accompanied by concomitant improvements in spatial working memory accuracy. It is of note that the first study of the cognitive effects of cocoa in healthy older adults, did not show significant effects on cognitive and cardiovascular outcomes, following daily cocoa flavanols intake (805 mg) over a 6-week period (Crews et al., 2008). In line with these findings, Suominen et al. (2020) recently found no effects on cognition in healthy older adults who consumed dark chocolate containing 410 mg of flavanols per day for eight weeks.

Collectively, the evidence obtained from human clinical studies seems to support that cocoa and cocoa-derived product consumption could improve general cognition and memory in both young and old individuals. Two main mechanisms of action have been proposed to explain the potential beneficial effects of cocoa intake on these processes. The first one pertains to the direct interaction of flavonoids with signaling pathways that promote neuronal function and brain connectivity (Carrillo et al., 2019). The second mechanism is related to the ability of cocoa to improve cerebral blood flow, inducing changes in memory processing (Haskell-Ramsay et al., 2018). Interestingly, a third mechanism of action is now emerging which involves the gut-brain axis and the ability of gut microbiota to improve the bioavailability of polyphenols (Ezra-Nevo et al., 2020).

Table 6
Human interventional trials of the effects of cocoa intake on aging[a].

Reference	Type of study	Participants	Time	Dose (day)	Main outcomes
(Mostofsky et al., 2010)	Prospective cohort study	31,823 women (48–83 y)	9 years	Times eating chocolate per month or week (FFQ)	↓heart failure hospitalization. ↓deaths in comparison to no regular chocolate consumers
(Moreira et al., 2016)	Longitudinal prospective study	531 (276 males+255 females, 67–79 y)	2 years	frequency of chocolate consumption and portion size (FFQ)	↓risk for cognitive decline
(Sorond et al., 2008)	RCDM, parallel	34 (healthy, 59–83 y)	2 weeks	Cocoa drink (18.2 and 451.1 mg flavanols)	↑cerebral blood flow
(Lamport et al., 2015)	RCDB, crossover	18 (healthy, 10 males+8 females, 55–65 y)	Acute (2 h)	Cocoa drink (23 and 494 mg flavanols)	↑cerebral blood flow
(Desideri et al., 2012)	RCDB, parallel	90 with MCI (61–85 y)	8 weeks	Cocoa drink (48 mg, 520 mg and 993 mg flavanols)	↓time for trail making test, ↑verbal fluency, ↓BP, ↓Glucose. No effect on insulin, TC, LDL-Cho, HDL-Cho, TG, isoprostanes
(Mastroiacovo et al., 2015)	RCDB, parallel	90 without MCI (61–85 y)	8 weeks	Cocoa drink (48 mg, 520 mg and 993 mg flavanols)	↓time for trail making test, ↑verbal fluency, ↓BP, ↓Glucose, ↓Insulin, ↓total Cho, ↓LDL-Cho, = HDL-Cho, ↓TG, = isoprostanes
(Brickman et al., 2014)	RCDB, parallel	37 (healthy, 50–69 y)	3 months	Cocoa supplement (45 and 900 mg flavanols)	↑dentate gyrus cerebral blood volume ↑memory task
(Sorond et al., 2013)	RCSB, parallel	60 (healthy,29 males+31 females, >65 y)	24 h and 30 days	Cocoa drink (26 and 1218 mg flavanols)	↑neurovascular coupling and cognition, ↑visual spatial attention, = BP, = task responses, = cerebral blood flow
(Heiss et al., 2015)	RCDB, parallel	22 young healthy males (<35 y) 20 elderly healthy males (50–80 y)	14 days	Cocoa drink (900 mg cocoa flavanols)	↓FMD, ↓systolic BP, ↓pulse wave velocity, ↓peripheral resistance, ↑arteriolar and microvascular vasodilatation capacity, ↑red cell deformability, ↑diastolic BP, = cardiac output
(Munguia et al., 2019)	RCDB, parallel	60 subjects (55–70 y)	12 weeks	Cocoa drink (179 mg cocoa flavanols+30 min walk)	↑mobility, ↑skeletal muscle index, ↓MDA, ↓carbonyl proteins
(McDermott et al., 2020)	RCDB, parallel	44 subjects with PAD (˃ 60 y)	6 months	Cocoa drink (15 g cocoa+75 mg epicatechin)	↑6-min walking distance

BP: blood pressure; **Cho**: cholesterol; **FFQ**: food-frequency questionnaire; **FMD:** flow mediated dilatation; **HDL**: high-density lipoprotein; **LDL:** low-density lipoprotein; **MCI:** Mild Cognitive Impairment; **MDA**: malondialdehyde; **PAD:** Peripheral Artery Disease; **RCDB**: Randomized Controlled Double-Blind; **RCSB**: Randomized Controlled Single-Blind; **TG:** triglycerides.

[a] The arrow indicates an increase (↑) or decrease (↓) in the levels or activity of the different parameters analysed, " = " symbol designates unchanged parameters.

7. Effects of cocoa on aging

In recent years, cocoa consumption has been studied in relation to various complications during aging, such as cognitive decline, cerebral blood circulation and cardiovascular disease (Table 6). In this sense, the Swedish Mammography Cohort followed 31,823 women (48–83 years) over 9 years, considering the outcomes of hospitalization due to heart failure and death in relation to chocolate intake (Mostofsky et al., 2010). It was observed that a regular moderate chocolate intake (1–3 servings per month and 1–2 servings per week) was associated with lower rates of hospitalization due to heart failure or death. This protective effect was not observed with intakes of ≥1 serving per day (Mostofsky et al., 2010). Similarly, a prospective study involving 531 participants aged ≥65 years (65–79 years) followed up over 2 years showed that chocolate intake was related with a 41% lower risk of cognitive decline (Moreira et al., 2016).

Several interventional studies have been carried out to clarify the effects of cocoa on different processes during aging and elucidate mechanisms of action (Table 6). In this regard, improvements in cerebral blood flow of the middle cerebral artery have been detected that were associated with better cognitive performance following daily intake of a flavanol-rich cocoa (451.1 mg) for 1 or 2 weeks in healthy elderly adults (Sorond et al., 2008). Similarly, Lamport et al. (2015) demonstrated that healthy older adults (55–65 years) who drank a high flavanol beverage (494 mg flavanols) experienced higher cerebral blood flow 2 h following intake. This was expressed as enhanced regional perfusion throughout the brain, particularly in the anterior cingulate cortex and the central opercular cortex of the parietal lobe. Additionally, in mildly cognitive impaired and healthy elderly participants (61–85 years), cognitive function was assessed after 8 weeks of consuming different drinks containing various amounts of cocoa flavanols (993 [high], 520 [moderate] and 43 mg [low]) (Desideri et al., 2012; Mastroiacovo et al., 2015). At the end of the study, the high and intermediate cacao-flavanol groups were able to complete visual attention and task switching tests more quickly, and showed improved verbal fluency relative to the low cocoa-flavanol group. Likewise, insulin resistance, BP and lipid peroxidation were also reduced in the high and intermediate groups when compared with the low cocoa-flavanol group. Indeed, improvements in age-related cognitive dysfunction were connected to cocoa flavanol consumption, which was subsequently associated with reduced insulin resistance (Desideri et al., 2012; Mastroiacovo et al., 2015). Beneficial effects on dentate gyrus function have also been observed in healthy 50-69 year-old participants who consumed a high cocoa-flavanol diet (450 mg cocoa flavanols twice a day) for 3 months, relative to individuals who ingested a low daily dose of flavanols (45 mg daily divided into two doses) as mentioned in the previous section (Brickman et al., 2014). Sorond et al. (2013) included 60 participants (29 males and 31 females aged ≥ 65 years) who were randomly assigned to one of two groups. Each group stipulated a specific daily cocoa intake, as follows: two cups of cocoa per day containing either 609 or 13 mg per serving for 30 days. Consumption of the high cocoa dose increased neurovascular coupling and improved visual spatial attention in volunteers with impaired neurovascular coupling at baseline. This was related with improved cognitive function.

As mentioned above, cocoa could also exert beneficial effects on CVD. In this sense, improved FMD was reported in a randomized, controlled, double-blinded, parallel trial with 22 young healthy men and 20 elderly healthy men receiving either a cocoa-flavanol drink (containing 450 mg of cocoa flavanols) or placebo (cocoa flavanols-free drink) twice a day for 14 days. These volunteers also demonstrated decreased PWV and total peripheral resistance, and increased arteriolar and microvascular vasodilator capacity, red cell deformability and diastolic BP. In contrast, cardiac output remained unchanged (Heiss et al., 2015). Additionally, administration of a flavonoid-rich natural cocoa beverage (179 mg cocoa flavanol per serving) to 60 participants (55–70 years) improved glycemia, triglyceridemia, HDL-Cho, LDL-Cho,

Table 7
Human epidemiologic studies and interventional trials of the effects of cocoa intake on cancer[a].

Reference	Type of study	Participants	Time	Dose (day)	Main outcomes
(Bayard et al., 2007)	Mortality (death certificates)	77,375 (mainland Panama) + 558 (San Blas)	5 years	Cocoa beverages (>900 mg cocoa flavanols/day)	↓cancer deaths, ↓CVD deaths, ↓diabetes deaths
(Paganini-Hill et al., 2007)	Leisure World Cohort Study (observational)	13,978 (5101 males + 8877 females)	23 years	Times per month (FFQ)	↓ cancer deaths
(Arts et al., 2002)	Iowa Women's study (observational)	34651 postmenopausal women	3 years	25 mg/day catechin intake (dietary FFQ)	↓rectal cancer incidence, no effect for cancer upper digestive tract, pancreas and hematopoietic
(Thompson et al., 2010)	Iowa Women's study (observational)	41,836 women	18 years	Dietary FFQ (semi-quantitative)	= non-Hodgkin lymphoma
(Uccella et al., 2013)	Iowa Women's study (observational)	41836 women		Dietary FFQ (semi-quantitative)	= endometrial cancer
(Arts et al., 2001)	Zutphen Elderly study (prospective)	7280 males	10 years	Catechin intake, 72 mg/day; 3% chocolate contribution (dietary FFQ)	= epithelial cancer incidence, lung cancer incidence or epithelial cancers other than lung cancer
(McKelvey et al., 2000)	Case-control	234 cases (cancer) + 407 controls	2 years	FFQ	No ↑ adenoma prevalence
(Nkondjock and Ghadirian, 2005)	Case-control	616 case women (414 breast cancer + 202 colon cancer) + 429 control women	5 years	FFQ	= risk breast and colon cancer
(Boutron-Ruault et al., 1999)	Case-control (dietary intake)	171 cases (cancer) + 309 controls	1 year	FFQ	↑risk for colorectal cancer
(Roullier et al., 2005)	Case-control (dietary pattern)	1372 subjects	1 year	24.45 g/day chocolate (dietary FFQ)	= colon disease (polyps, adenoma-colorectal cancer)
(Chan et al., 2009)	Case-control	532 cases (cancer) + 1701 controls	5 years	FFQ	↑risk for pancreatic cancer in males and = risk for pancreatic cancer in females
(Malagoli et al., 2019)	Case-control	380 cases (cancer) + 719 controls	2 years	FFQ	↑risk for melanoma
(Russnes et al., 2016)	Case-control	1499 cases (cancer) + 1112 controls	2 years	2% chocolate contribution (Dietary FFQ)	↑risk for prostate cancer

CVD: cardiovascular disease; **FFQ:** food frequency questionnaire.
[a] The arrow indicates an increase (↑) or decrease (↓) in the levels of the different parameters analysed, " = " symbol designates unchanged parameters.

TG/HDL index and oxidative markers (Munguia et al., 2019). Flavanol-rich beverage intake also improved Up and Go test results, skeletal muscle indices and quality of life (positive effects on metabolic, oxidative stress, inflammatory endpoints, physical performance and frailty indicators) (Munguia et al., 2019). In line with this, a phase 2 randomized clinical trial conducted by McDermott et al. (2020) with 44 participants with peripheral artery disease found improved 6-min walk distance following 6-month daily intake of a cocoa beverage (15 g cocoa containing 75 mg of epicatechin).

In consideration of all of the studies presented above, cocoa consumption appears to improve general cognition and memory by modulating cerebral blood flow and other cardiovascular parameters (FMD, BP, etc.). This can ultimately contribute to improved quality of life within the elderly population.

8. Anti-carcinogenic effects of cocoa

Protective effects of cocoa/chocolate consumption against cancer in humans cannot be confirmed due to the fact that few epidemiological studies exist in this field, with evidence largely coming from a small number of short-duration interventional studies (Table 7). Some epidemiological studies support the protective role of cocoa in preventing cancer through evidence of reductions in mortality following intake. An example of such evidence is seen in the case of the Kuna tribe (Panama). A study examining death certificates over a period of 5 years (2000–2004) demonstrated lower rates of cancer deaths amongst Kuna Indians in comparison with the mainland population. This finding has been linked to consumption of flavanol-rich cocoa as a main beverage, providing more than 900 mg flavanol/day (Bayard et al., 2007) to this population. Mortality was also evaluated in the Leisure World Cohort Study. Here, it was observed that, despite the fact that mortality was not reduced within more frequent chocolate consumers (weekly to daily), occasional chocolate consumers (a few times/month or less frequently) did show lower mortality (Paganini-Hill et al., 2007). Likewise, the Iowa Women's Study reported an inverse relationship between catechin consumption and incidence of rectal cancer in post-menopausal women (55–69 years). However, no differences were found pertaining to cancer of the upper digestive tract, pancreatic cancer, hematopoietic cancers (Arts et al., 2002), the two most common non-Hodgkin lymphomas (follicular lymphoma and diffuse large B-cell) (Thompson et al., 2010) or endometrial cancer (Uccella et al., 2103). Further, no association between catechin intake and epithelial or lung cancer was also detected in the Zutphen study (Arts et al., 2001). Whilst, in a case-control study performed in North Carolina with adults (30–89 years), no relation was seen between chocolate candy consumption and lower prevalence of adenomatous polyps and colorectal cancer (McKelvey et al., 2000). Similar results were also obtained in two simultaneous case-control studies with French-Canadian women (35–79 years). Specifically, no significant associations with breast or colon cancer were detected pertaining to a chocolate-cereal dietary pattern characterized by a high intake of chocolate-based products, breakfast cereals, water and fruits (Nkondjock and Ghadirian, 2005).

On the contrary, some human studies have reported a detrimental impact of cocoa intake on cancer incidence. Indeed, in a case-control study in Burgundy (France), chocolate was identified as a risk factor for colorectal cancer. Further, it was linked with high intakes of sugar and harmful effects on insulin and insulin-like growth factor-I (Boutron-Ruault et al., 1999; Rouillier et al., 2005). Similarly, in another case-control study, this time in California, chocolate candy intake was associated with increased pancreatic cancer risk in men, whereas no associations between both parameters were observed in women (Chan et al., 2009). In contrast, positive associations between melanoma risk and chocolate consumption, especially within women, were reported in a case-control study in Northern Italy (Malagoli et al., 2019). Chocolate

Table 8
Human interventional trials of the effects of cocoa intake on colonic transit and microbiota, immunity and allergy, skin, fatigue, visual function, virus and bone density[a].

Reference	Type of study	Participants	Time	Dose (day)	Main outcomes
Colonic transit and microbiota					
(Castillejo et al., 2006)	RCDB, parallel	56 constipated children	4 weeks	Cocoa husk (5.2 g)	↑number of bowel movements, ↓transit time, better stool consistency
(Sarriá et al., 2012)	RCSB, crossover	44 healthy adults (21 males + 23 females)	4 weeks	Cocoa powder (15 g containing 2.26 NSP, and 30 g containing 6.60 g NSP)	↑number and frequency of bowel movements, ↑flatulence
(Fox et al., 2019)	RC, crossover	16 healthy adults (9 males + 7 females)	5 days	Dark chocolate (100 g, 72% cocoa, 250 mg flavanols)	↑colonic transit, ↑stool consistency, ↑cerebral function
(Tzounis et al., 2011)	RCDB, crossover	22 healthy adults (12 males + 10 females)	4 weeks	Cocoa beverage (494 mg flavanols)	↑*Lactobacillus spp*, ↑*Bifidobacterium spp*, ↓*Clostridium spp*, ↓TG, ↓CRP, No effect on BMI, BP, total-Cho, HDL-Cho, LDL-Cho, glucose
(Angiletta et al., 2018)	RCDB, crossover	20 obese (10 males+10 females, BMI˃27)	5 days	28 g cocoa powder (30–900 mg flavanols)	No effect on BMI, body mass, fat mass, lean mass, TMAO
Immunity and allergy					
Raguzzini et al. (2019)	Observational (pilot study)	13 celiac subjects (2 males + 11 females)	6 months	Chocolate consumers (1–3 times/week, FFQ)	↑lymphocyte-to-monocyte ratio. No effect on BMI, BP, PAL, platelet-to-lymphocyte ratio, haemoglobin, RBC, neutrophil, mean corpuscular volume
(Rodríguez-Lagunas et al., 2019)	Cross-sectional, observational (pilot study)	270 healthy students (71 males + 199 females)	5 months	FFQ (7 to >15 g cocoa/day)	↓chronic diseases, ↓allergy and allergic symptoms
Skin					
(Heinrich et al., 2006; Neukam et al., 2007)	RCDB, parallel	24 young-middle-aged healthy women	12 weeks	Cocoa powder (27 and 326 mg flavanols)	↑photoprotection, ↑dermal blood circulation, ↑oxygenation saturation, ↑skin density and hydration. No effect on smoothness and wrinkles
(Williams et al., 2009)	RCDB, parallel	30 healthy (8 males + 22 females)	12 weeks	Dark chocolate (20 g, 30 or ˃600 mg flavanols)	↑photoprotection
(Yoon et al., 2016)	RCDB, parallel	64 healthy women	24 weeks	Cocoa beverage (320 mg flavanols)	↓photo-aging (↓wrinkles, ↑photoprotection), ↑skin elasticity, = skin hydration, = barrier integrity
(Mogollon et al., 2014)	RCDB, parallel	74 healthy women	15 weeks	Dark chocolate (30 g, 30 or 600 mg flavanol)	↑photoprotection, ↑skin elasticity
Fatigue					
(Sathyapalan et al., 2010	RCDB, crossover	10 middle-aged subjects with CFS (4 males + 6 females)	8 weeks	Cocoa liquor polyphenol rich chocolate (165 mg flavanols)	↓fatigue, ↑residual function
(Coe et al., 2017)	RCDB, crossover	12 middle-aged subjects with MSF (2 males + 10 females)	Acute	Cocoa drink (120 or 350 mg GAE)	↓fatigue, = glucose, = 6-min-walk test
(Coe et al., 2019)	RCDB, crossover	40 middle-aged subjects with MSF (10 males + 20 females, 34–54 y)	6 weeks	Cocoa drink (120 or 350 mg GAE)	↓fatigue, ↓fatigability, = 6-min-walk test, = anxiety, = depression, = lipid oxidation, = TNF-α, = glutathione
(McDermott et al., 2020)	RCDB, parallel	44 elderly subjects with PAD	6 months	Cocoa drink (15 g cocoa+75 mg epicatechin)	↑6-min walking distance
Visual function					
Field et al. (2011)	RCSB, crossover	30 healthy young (8 males + 22 females)	Acute	Chocolate bars (35 g, 773 mg flavanols)	↑visual contrast sensitivity, ↑spatial memory and performance, ↓reaction time, = coherent motion
(Rabin et al., 2018)	RMDB, crossover	30 healthy young (9 males + 21 females)	Acute	Dark chocolate (47 g, 316.3 mg flavanols)	↑contrast sensitivity, ↑visual acuity
(Siedlecki et al., 2019)	RMDB, crossover	22 healthy young (9 males + 13 females)	Acute	Dark chocolate (20 g, 400 mg flavanols)	= visual acuity, = retinal blood flow
Virus					
Kamei et al. (2016)	RCSB, parallel	123 healthy young (89 males + 34 females)	3 weeks	Cocoa beverage (360 mg flavanols)	↑NK activity, = neutralizing antibody titters
Bone density					
Hodgson et al. (2008)	PRC trial	1510 elderly women	5 years	Cups or glasses per week of chocolate (cocoa) (FFQ)	↓bone density, ↓strength in the tibia, ↓BW, =BMI, =PAL.

BMI: body mass index; **BP:** blood pressure; **BW:** body weight; **CFS:** chronic fatigue syndrome; **Cho:** Cholesterol; **CRP:** C-reactive protein; **FFQ:** food frequency questionnaire; **GAE:** gallic acid equivalent; **HDL**: high-density lipoprotein; **LDL:** low-density lipoprotein; **MSF:** multiple sclerosis-related fatigue; **NK:** natural killer; **NSP:** non-starch polysaccharides; **PAD:** Peripheral Artery Disease; **PAL:** physical activity level; **PRC:** Prospective Randomized-Controlled; **RBC:** red blood cells; **RCDB**: Randomized Controlled Doble-Blind; **RCSB**: Randomized Controlled Single-Blind; **RMDB**: Randomized-Masked Doble-Blind; **TG:** triglycerides; **TMAO:** trimethylamine *N*-oxide; **TNF-α**: tumour necrosis factor α.

[a] The arrow indicates an increase (↑) or decrease (↓) in the levels or activity of the different parameters analysed, " = " symbol designates unchanged parameters.

intake has also been linked positively with prostate cancer in Sweden (Russnes et al., 2016). In addition, cocoa consumption data has been examined alongside worldwide testicular cancer and hypospadias incidence rates in offspring to establish positively relationships (Giannandrea, 2009), although effects were ultimately linked to the reproductive toxicity of cocoa theobromine.

It serves to highlight that no human intervention studies exist which have endeavored to demonstrate relationships between cocoa intake and cancer prevention. However, in order to support the anticarcinogenic effects of cocoa, a few human trials have shown beneficial outcomes of cocoa in relation to intermediary factors involved in cancer progression, such as biomarkers related with oxidative stress and inflammation (aspects reviewed in Martín et al., 2013; Maskarinec, 2009). In line with this, several mechanisms of action have been

proposed to explain the potential beneficial effects of cocoa intake on this disease. These mechanisms have been associated with the regulation of different signaling pathways such as cellular proliferation/survival (cell cycle), apoptosis, metastasis and angiogenesis, as well as to the inhibition of closely related processes that are relevant for the development and progression of cancer, namely, oxidative stress and inflammation (Martín et al., 2013a; Martin et al., 2013b; Ramos, 2008).

9. Other effects of cocoa on human health

Finally, although less documented, other effects on human health have been attributed to cocoa. These aspects are considered in the following sections and in Table 8.

9.1. Colonic transit and microbiota

Low dietary fiber intake has been associated with increased risk of different diseases (Sarriá et al., 2012). Indeed, fiber has both preventive and therapeutic actions that protect against common large-bowel diseases, such as chronic constipation. Thus, cocoa fiber exerts a beneficial role. In a parallel, randomized, double-blind, controlled trial with 3–10 year-old children suffering chronic idiopathic constipation, participants were instructed to consume either one (3–6 years) or two (7–10 years) sachets of cocoa husks before lunch and/or dinner for a period of 4 weeks (Castillejo et al., 2006). Relative to the placebo group, the cocoa fiber supplement (5.2 g of fiber/sachet) decreased total transit time and time spent in the right colon. In addition, this cocoa husk supplement increased the frequency of bowel movements and improved subjective stool consistency (Castillejo et al., 2006). Similar results were obtained in 44 healthy adults (18–55 years) in a two-stage, randomized, crossover, single-blind intervention in which volunteers received two servings of fiber-enriched cocoa (2.26 and 6.60 g/day of non-starch polysaccharides) for four weeks (Sarriá et al., 2012). More frequent daily bowel movements were reported, as well as shorter time intervals between bowel movements and less feelings of constipation. Greater flatulence was the only adverse gastrointestinal symptom (Sarriá et al., 2012). In contrast, no effects on upper gastrointestinal function were seen in 16 healthy volunteers (18–65 years) who ingested 100 g of dark (72% cocoa, 250 mg flavanols) or white (0% cocoa) chocolate for 5 days, although dark chocolate tended to slow colonic transit and increase stool consistency (Fox et al., 2019). This unexpected outcome was explained by the fact that, at high levels, cocoa methylxanthines can accelerate colonic transit, whereas the low content found in dark chocolate may not trigger this response (Fox et al., 2019). In addition, effects observed by Fox et al. (2019) were related to the inhibition of chloride channels induced by cocoa flavanols and leading to reduced water transport across the colonic epithelium.

At present, it is clear that dietary modification of microbiota, for instance via cocoa ingestion, provides an approach that could help to prevent many diseases. Nonetheless, evidence about the effects of cocoa intake on microbiota composition in humans is very limited. Effects of high-cocoa flavanol consumption on microbiota composition in healthy volunteers (20–40 years) has been studied in an interventional trial (Tzounis et al., 2011). Daily ingestion over a 4-week period of a high-cocoa flavanol beverage containing 494 mg flavanols, increased growth of *Lactobacillus* spp., and *Bifidobacterium* spp. when compared to ingestion of a control low-cocoa flavanol drink (29 mg flavanols). This outcome was accompanied by reduced values of triacylglycerol and CRP, pointing to a potential prebiotic beneficial effect of cocoa (Tzounis et al., 2011). Importantly, microbial metabolites may also contribute towards health and disease. In this sense, TMAO has been associated with an increased risk of CVD (Ramos and Martín, 2021), although, as previously mentioned, 5-day cocoa intake (180–900 mg flavanols/day) did not alter TMAO values in obese individuals (Angiletta et al., 2018). Overall, these findings suggest that cocoa might have significant effects on the growth of selected gut microbes, which could positively impact on health and disease (Sorrenti et al., 2020). These novel approaches warrant further investigation.

9.2. Immunity and allergy response

To the best of our knowledge, no human interventional studies have been conducted to examine the administration of cocoa during immune compromised or allergic conditions. Nevertheless, it is important to note that a pilot trial examining 26 celiac volunteers (25–55 years) who had been classified as chocolate consumers (13 participants, 1–3 times/week consumption) or non-consumers (13 volunteers) found that consumers had a high lymphocyte-to-monocyte ratio (inflammatory marker) (Raguzzini et al., 2019). No effects were observed in relation with other blood parameters (platelet-to-lymphocyte ratio, hemoglobin, red blood cells, neutrophils, mean corpuscular volume), or with BMI, BP or physical activity levels. In contrast, another pilot study of 270 university students (19–25 years) classified according to their cocoa consumption reported contrasting outcomes. Students were classified as being low (LC, 5 g cocoa/day), moderate (MC, 5–15 g cocoa/day) or high (HC, $\geq$15 g cocoa/day) consumers. The MC group reported significantly lower rates of chronic disease than the LC group, whilst a lower percentage of volunteers reported allergies the MC and HC groups than in the LC group (Rodríguez-Lagunas et al., 2019). Indeed, cocoa intake, especially at moderate levels, was also associated with a lower presence of allergic symptoms (Rodríguez-Lagunas et al., 2019). These results indicate the importance of establishing cocoa dosages that could lead to the modulation of symptom markers associated with celiac disease and allergies. Moreover, the molecular mechanisms behind these actions are not yet fully clear, although pre-clinical studies have demonstrated that a cocoa-enriched diet might modify T cell functions, leading to the regulation of both systemic and gut antibody synthesis. This, in turn, has been linked to preventive effects on IgE synthesis in an allergy model, and to the modulation of gut IgA secretion and microbiota (Pérez-Cano et al., 2013).

9.3. Skin

There is a scarcity of studies examining cocoa and its effects on skin in humans, however, some specific works have shown beneficial effects on this organ. In this sense, cocoa has been demonstrated to have photoprotective effects. Concretely, 12-week consumption of a high flavanol cocoa (326 mg/day) decreased UV-induced erythema, improved cutaneous and subcutaneous blood circulation and oxygen saturation, increased skin density and hydration, and diminished skin roughness and scaling (Heinrich et al., 2006; Neukam et al., 2007). In this study, no changes in the mentioned parameters were detected when a low flavanol cocoa (27 mg/day) was ingested (Heinrich et al., 2006; Neukam et al., 2007). Similarly, 12-week administration of chocolate (20 g) with a high flavanol content (>600 mg flavanols) in 32–52 years-old women doubled the minimal erythema dose (MED). In contrast, a low flavanol chocolate group (<30 mg flavanols) did not experience any changes (Williams et al., 2009). A study examining photo-aged Korean women (43–86 years) found that visible facial wrinkles and roughness values (gross elasticity) were reduced in a group receiving a daily cocoa beverage (320 mg flavanols) for 24 weeks in comparison to a placebo group (Yoon et al., 2016). These results demonstrated a preventive effect of cocoa on the progression of photo-aging. However, no differences in skin hydration and barrier integrity were detected between the two study groups (Yoon et al., 2016). Finally, a study performed with chocolate containing high and low flavanol amounts (600 and 30 mg/day, respectively) demonstrated that daily consumption of 30 g of chocolate with a high flavanol content for 12 weeks, increased MED and skin elasticity in 20–65 year-old women (Mogollon et al., 2014). Overall, these studies demonstrate that regular consumption of cocoa rich in flavanols can confer substantial photoprotection and may help to maintain skin health by improving structure and function. These

beneficial effects have been linked with the antioxidant and anti-inflammatory properties of cocoa flavanols, as well as their ability to improve blood flow, and influence glycosaminoglycans and collagen expression in *in vitro* and *in vivo* studies (Gasser et al., 2008; Karim et al., 2014; Scapagnini et al., 2014).

9.4. Fatigue

Chronic fatigue syndrome (CFS) is a debilitating condition associated with high morbidity and poor quality of life. Despite this, few studies have examined whether this syndrome could be alleviated by cocoa ingestion. Sathyapalan et al. (2010) reported that 10 individuals suffering from chronic fatigue syndrome (6 females and 4 males, aged 44–60 years) who received a-15 g bar of high cocoa liquor/polyphenol rich chocolate (55 mg flavanols) three times a day for 8 weeks improved fatigue and residual function, in comparison to individuals given iso-caloric cocoa mass free/low polyphenol chocolate. Similar results were also obtained in a pilot study with 12 subjects (44–64 years), which administered a high flavonoid cocoa drink containing 350 mg gallic acid equivalents (GAE)/g to some of the sample and a low flavonoid control powder (120 mg GAE/g) (Coe et al., 2017) to the rest. In line with this, 40 individuals suffering relapsing and remitting multiple sclerosis and fatigue, received a daily supplementation of the same cocoa beverages used in the previous study for 6 weeks. Those receiving the highest dose of flavonoids experienced improved fatigue and fatigability as measured by a 6-min walk test (distance walked during 6 min) (Coe et al., 2019). No changes were detected in parameters related with mood (anxiety and depression), oxidative stress (lipid oxidation and glutathione), or inflammation (TNF-α). Finally, the same double-blinded, placebo-controlled, randomized study mentioned in section 7 (McDermott et al., 2020), in which a cocoa beverage (15 g cocoa and 75 mg of epicatechin) was administrated daily to elderly participants (˃60 years) with peripheral artery disease found improved 6-min walk distance outcomes.

The mechanisms underlying these outcomes have scarcely been explored, however, beneficial effects have been linked to the antioxidant (prevention of oxidative stress) and anti-inflammatory activities of cocoa flavanols (Coe et al., 2019; McDermott et al., 2020; Sathyapalan et al., 2010).

9.5. Visual function

Several interventional studies have identified an inverse association between regular cocoa consumption and visual function. In this sense, an acute supplementation study, described in section 6, showed that cocoa flavanols improved visual and cognitive functions (Field et al., 2011). In this trial, 30 healthy young adults (18–25 years) consumed either 35 g of dark chocolate (containing 720 mg cocoa flavanols) or 35 g of white chocolate, with a one-week interval between testing sessions. Visual contrast sensitivity and spatial memory improved 2 h after cocoa intake, whilst the time required to detect motion direction and to react and perform a selected task was reduced. All of these acute effects were associated with increased cerebral blood flow, whilst contrast sensitivity was linked with the enhanced retinal blood flow induced by cocoa flavanols (Field et al., 2011). Likewise, Rabin et al. (2018) evaluated visual acuity and contrast sensitivity in 30 young adults 2 h after consuming dark chocolate (47 g, containing 316.3 mg flavanols) or milk chocolate (40 g, containing 40 mg flavanols). Following dark chocolate ingestion, improvements were reported in small letter contrast sensitivity, although visual acuity and large letter contrast sensitivity improvements were less evident. In contrast, another clinical trial with 22 healthy young participants who ingested 20 g of dark chocolate (400 mg flavanols) or 7.5 g of milk chocolate failed to detect differences in visual acuity or retinal blood flow 2 h following cocoa intake (Siedlecki et al., 2019). Altogether, study outcomes indicate that the role of cocoa in relation to visual function remains unclear and more trials with longer follow-ups are needed.

9.6. Virus and bone density

Despite both *in vitro* and *in vivo* studies seeming to indicate that cocoa possesses antivirus effects and modulates bone density (Nijveldt et al., 2001; Seem et al., 2019), evidence in humans is very scarce. In this sense, inhibitory effects on influenza virus infection after 3 weeks of consuming a cocoa beverage (360 mg flavanols) have been detected (Kamei et al., 2016). Further, participants (aged 29–49 years) who were vaccinated against A(H1N1)pdm2009 influenza virus and ingested cocoa demonstrated the same increase in neutralizing antibody titers against the virus as those in a control group. However, increases in natural killer cell activity were greater in the cocoa group in comparison to the non-consumer control group.

With regards to bone density, a 5-year prospective, randomized, controlled trial of oral calcium supplements including 1510 Western Australian women (˃70 years) reported that daily (≥1 times/d) chocolate consumption was associated with lower whole-body bone density, as well as lower total hip, femoral neck, tibia and heel density relative to less frequent consumption (˂1 time/wk.) (Hodgson et al., 2008). Additional cross-sectional and longitudinal studies are needed to confirm the effects of daily chocolate consumption on bone density and strength in older women and examine implications pertaining to osteoporosis.

10. Future perspectives and conclusions

Based on evidence presented by most of the epidemiological and interventional studies presented in this review, cocoa and its flavanols are demonstrated to play a prominent role in protecting against relevant diseases and improving health. Taken together, evidence suggests that these natural compounds could be considered as potential chemopreventive tools that are useful for the nutritional management of chronic disorders, such as cardiovascular disease, obesity, diabetes, etc. (Fig. 1). However, certain observational and interventional approaches have failed to show that cocoa flavanols exert any effects on the mentioned pathologies, with negative outcomes even being reported in some cases. These controversial results show the difficulty when it comes to performing these trials, as many factors and confounders need to be considered. In line with this, different doses of cocoa flavanols from different types of cocoa/chocolates should be considered, alongside their additive or synergistic effects (for instance, effects occurring between flavanols and other polyphenols or antioxidants present in foods). Interindividual differences in bioavailability and metabolism should also be considered. In addition, methodological considerations such as dietary pattern differences between populations, measurement

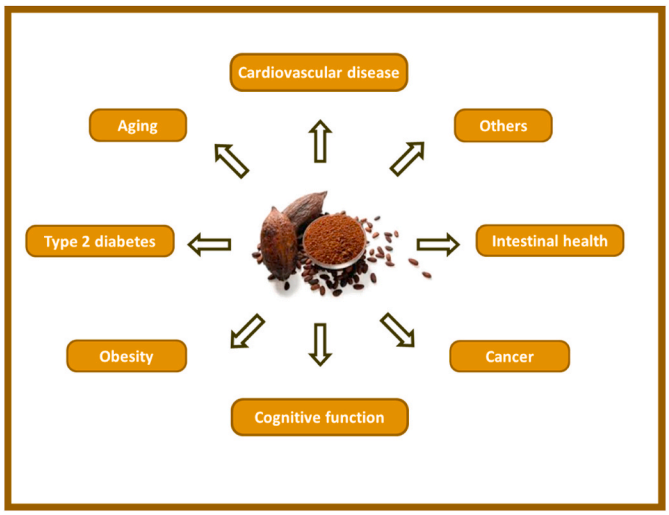


Fig. 1. Potential effect of cocoa flavanols on health and diseases.

accuracy in relation to food intake, genetic variations, diversity in microbiota composition and other confounding factors, even mild ones, may have contributes to the overall conclusions reached. This requires further studies to be conducted and, especially, large controlled clinical trials with long follow-ups. Such trials are needed to: (i) reveal and confirm the real efficacy of cocoa flavanols/chocolate in relation to health and protection from relevant human disorders such as the ones considered in the present review, (ii) elucidate the molecular mechanisms and targets of the natural compounds abundant in cocoa/chocolate, and (iii) define optimal doses and treatment durations for achieving final positive effects on health and disease.

CRediT authorship contribution statement

María Ángeles Martin: Conceptualization, Writing – review & editing. **Sonia Ramos:** Conceptualization, Writing – review & editing.

Declaration of competing interest

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

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