Cocoa, blood pressure, and vascular function

Sudano, I ; Flammer, A J ; Roas, S ; Enseleit, F ; Ruschitzka, F ; Corti, R ; Noll, G

Abstract: The consumption of a high amount of fruits and vegetables was found to be associated with a lower risk of coronary heart disease and stroke. Epidemiologically, a similar relationship has been found with cocoa, a naturally polyphenol-rich food. Obviously, double blind randomized studies are difficult to perform with cocoa and chocolate, respectively. However, intervention studies strongly suggest that cocoa has several beneficial effects on cardiovascular health, including the lowering of blood pressure, the improvement of vascular function and glucose metabolism, and the reduction of platelet aggregation and adhesion. Several potential mechanisms through which cocoa might exert its positive effects have been proposed, among them activation of nitric oxide synthase, increased bioavailability of nitric oxide as well as antioxidant, and anti-inflammatory properties. It is the aim of this review to summarize the findings of cocoa and chocolate on blood pressure and vascular function.

DOI: https://doi.org/10.1007/s11906-012-0281-8

Cocoa, Blood Pressure and Vascular Function

Isabella Sudano[1], MD; Andreas J Flammer[1,2], MD; Susanne Roas[1], MD; Frank Enseleit[1], MD; Frank Ruschitzka[1,3], MD; Roberto Corti[1,3], MD; Georg Noll[1,3], MD

[1]Cardiovascular Center Cardiology, University Hospital Zurich, Switzerland; [2]Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic and College of Medicine, Rochester, MN, USA; [3]Center for Integrative Human Physiology, University Zurich, Switzerland

Correspondence

Prof. Dr. med. Georg Noll

Cardiovascular Center, Cardiology

University Hospital

Raemistrasse 100

CH-8091 Zurich

Tel: +41-44-255 2266

Fax: +41-44-255 4859

eMail: georg.noll@usz.ch

ABSTRACT

The consumption of a high amount of fruits and vegetables was found to be associated with a lower risk of coronary heart disease and stroke. Epidemiologically, a similar relationship has been found with cocoa, a naturally polyphenol-rich food. Obviously, double blind randomized studies are difficult to perform with cocoa and chocolate, respectively. However, intervention studies strongly suggest that cocoa has several beneficial effects on cardiovascular health, including the lowering of blood pressure, the improvement of vascular function and glucose metabolism, and the reduction of platelet aggregation and adhesion. Several potential mechanisms through which cocoa might exert its positive effects have been proposed, among them activation of nitric oxide synthase, increased bioavailability of nitric oxide as well as antioxidant, and anti-inflammatory properties. It is the aim of this review to summarize the findings of cocoa and chocolate on blood pressure and vascular function.

Key words: cocoa, flavanols, polyphenols, antioxidants, endothelial function, blood pressure, nitric oxide, vascular compliance.

INTRODUCTION

In epidemiological studies, regular dietary intake of plant-derived foods and beverages was found to be associated with a reduced risk of coronary heart disease (CAD)[1-4] and stroke[5], and to be inversely associated with the risk of cardiovascular disease in general.[2, 4]

The Iowa Women's Health Study is a prospective study in 34'489 postmenopausal women free of cardiovascular disease who were followed for up to 16 years.[6] In this population regular consumption of food rich in flavonoids was associated with a decreased risk of death due to CAD and the inverse association between chocolate intake and cardiovascular mortality remained significant after multivariate adjustment.[6] Moreover, the Zutphen Elderly Study, involving 470 elderly men free of chronic disease, suggest that habitual cocoa intake per se might reduce cardiovascular risk and is inversely related to cardiovascular and all-cause mortality).[7] Moreover, a retrospective analysis of the Potsdam arm of the "European Prospective Investigation into Cancer and Nutrition" recently showed that high consumption of cocoa was associated with a lower prevalence of stroke and myocardial infarction.[8]

It has been proposed that polyphenols may play an important role in cardiovascular protection.

Several food sources are exceptionally rich in polyphenols, among them green and black teas, wine, grape juices, berries and cocoa, the later with particularly high amounts.[9, 10]**(Table 1)** Several groups of polyphenols are found in fruits, whereas the most important are the flavanols which can be further subdivided into the monomers epicatechin and catechin,[11, 12] and their dimers, oligomers and polymers, the so-called procyanidins,[13, 14] responsible for the bitterness of cacao, through the formation of the complexes with salivary proteins.[15] Although the flavanols are likely responsible for the beneficial health effects, conventional chocolate

manufacturing processes, such as fermentation and roasting markedly decrease the concentration of these substances. [10, 16]

In humans, flavanol plasma concentration dose-dependently increases after ingestion, reaching its peak usually after 2-3h[17, 18] after cocoa ingestion and are still measurable in plasma 8 hours after cocoa ingestion.[19]

Cocoa and its flavanols might increase nitric oxide (NO) bioavailability, activate nitric oxide synthase (NOS), and exert antioxidative, anti-inflammatory, and anti-platelet effects, which in turn might improve vascular function, reduced blood pressure and therefore, explain the positive impact on clinical outcome proposed by epidemiological studies.[10, 16, 20, 21]

This review will focus on the effect of cocoa on blood pressure and vascular function.

Effect of cocoa on blood pressure

Initial findings, which suggested possible antihypertensive effects of cocoa, came from observations of the Kuna Indians, a native population living on islands off the Panama coast, which have a very low incidence of hypertension and, remarkably, do not show an age-dependent increase in blood pressure. These effects are likely environmental because they are lost upon migration to urban Panama City and are likely linked to the reduction in intake of natural cocoa drinks rich in flavanols.[22]

A relationship between cocoa consumption and reduced blood pressure was first observed in a cross sectional analysis of the Zutphen Elderly Study[7] .The association of chocolate consumption with blood pressure and the incidence of cardiovascular disease was further evaluated in the population included in a Potsdam arm of the "European Prospective Investigation into Cancer".[8] The later study showed over a follow up p to 8 years that a high consumption of chocolate was associated with a lower cardiovascular risk with a strong inverse association for stroke (more than for myocardial infarction). The authors emphasized that this positive effect could be explained at least in part by a reduction in blood pressure, observed in the group with high as compared to low chocolate-consumption.[8]

Randomized controlled trials have confirmed this epidemiological association mostly in patients with concurrent arterial hypertension or other cardiovascular risk factors.[23-26]

A meta-analysis of ten such trials[27] found that cocoa consumption was associated with significant reductions in systolic (-4.5±1.35 mmHg) and diastolic (-2.5±1.36 mmHg) blood pressures confirming the results of a previous meta-analysis published 2007.[28]

A number of mechanisms have been proposed to explain the cocoa's effects on blood pressure. Because of their importance in blood pressure maintenance, the improvements in nitric oxide availability and endothelial function associated with cocoa consumption may explain much, of its

antihypertensive effects. However, there is some evidence that flavanols and flavanol-rich foods including cocoa can inhibit angiotensin-converting enzyme (ACE) activity in vitro.[29, 30] ACE regulates the renin−angiotensin system; it cleaves angiotensin-I into angiotensin-II, which stimulates the release of vasopressin or aldosterone and antidiuretic hormone, increasing sodium and water retention. It also inactivates vasodilators bradykinin and kallidin. Whether ACE inhibition mediates the antihypertensive activity of cocoa flavanols in humans is not yet completely clear.[31]

One study also looked at the blood pressure responsiveness after exercise showing as an high flavonols drink reduced the BP response to exercise.[32]

Cocoa and vascular function

The vascular endothelium plays a fundamental role in modulating vascular tone and structure. Physiological production of vascular relaxing factors, including “nitric oxide”, “prostacyclin” and “hyperpolarizing relaxing factor” protect the vessel wall by antagonizing the initial pathological steps of atherosclerosis and thrombosis[33].(**Figure 1**) Cardiovascular risk factors and disease are associated with endothelial dysfunction or damage.[34, 35] Endothelial dysfunction in the forearm circulation correlates with the presence of coronary vascular dysfunction and is predictive of future coronary events.[36-38]

A meta-analysis published in 2008 showed that consumption of polyphenol-rich foods mostly was associated with an improvement in endothelial function in the short- and long-term[39] as exemplified with the effect of tea[40] and other flavanoid-rich food such as red wine, grape juice, dealcoholized red wine extract from grape seeds [41, 42] and orange juice.[43],

As cocoa is particularly rich in polyphenols it is not surprising that cocoa induces NO-dependent vasodilatation in rat[44] and improve endothelial function in healthy humans and in patients with cardiovascular risk factors or disease.[10, 16]

Studies evaluating the effect of cocoa/chocolate an endothelial function are summarized in **Table 2.**

A cocoa drink high in flavanols content enhances the circulating pool of bioactive NO by a third and in turn improves flow-mediated vasodilation in patients with cardiovascular risk factors.[45, 46] The increase in NO and the improvement of endothelial function induced by cocoa intake was inhibited by the infusion of L-NMMA, an inhibitor of NO-synthesis.[46] Commercially available dark chocolate (74% cocoa), but not white chocolate, improves flow-mediated vasodilatation (FMD) by 80% in young healthy smokers. This effect was seen two hours after chocolate ingestion and lasted for up to 8 hours. Because plasma antioxidant status, was

significantly improved 2 hours after ingestion, it is likely that not only an induction of endothelial nitric oxide synthase (NOS) and in turn elevated NO levels, but also a reduction in oxidative stress and in turn a reduced breakdown of NO by reactive oxidant species, contributes to the enhanced endothelial function, especially under conditions with a high oxidative stress burden, such as in smokers.[47] Indeed, antioxidants may prevent NO transformation into peroxynitrite and in turn protect against vasoconstriction and vascular damage.[48] Oxidative stress and reduced antioxidant defenses play also a crucial role in the pathogenesis of atherosclerosis and in in transplant vasculopathy. Indeed, we were able to demonstrate that flavonoid-rich dark chocolate improved epicardial coronary vasomotion in cardiac transplant recipients. [49] Interestingly 40g dark chocolate induced coronary vasodilatation, improved coronary vascular function, and decreased platelet adhesion two hours after consumption.

As outlined in **Table 2**, cocoa consistently improved endothelial function in patients with atherosclerosis and/or cardiovascular risk factors such as in patients with arterial hypertension,[24] diabetes mellitus,[50] overweight and obesity,[51] coronary artery disease,[52] and hart failure.[53] Not only endothelial function improved after consumption of cocoa or chocolate. Vlachopoulos and colleagues showed that chocolate acutely decrease augmentation index of the central (aortic) pressure waveform suggesting dilation of small and medium-sized peripheral arteries and arterioles.[54] Moreover, an observational study in 198 healthy subjects showed that habitual cocoa consumption is associated with decreased aortic stiffness and wave reflections and with improved central hemodynamics in healthy subjects.[55]

A possible mechanism explaining the effects of cocoa on the vasculature is the antioxidative effect of the flavanols and procyanidins contained in cocoa which may reduce the production of oxygen free-radicals and therefore improve nitric oxide bioavailability and a eNOS activation.[10, 16](**Figure 1**) However, the antioxidative effect of cocoa is discussed

controversial because in addition to flavanols,[56] macro- and micronutrients, as well as the increased uric-acid levels resulting from fructose metabolism[57] could affect antioxidative capacity of plasma.

Ramirez and coauthors showed that epicatechins increase the synthesis of NO via eNOS activation in human coronary artery endothelial cells.[58] Furthermore, this epicatechin-induced NO production in human endothelial cells can be obtained through both Ca^{2+}-dependent and Ca^{2+}-independent eNOS phosphorylation,[59] suggesting that epicatechin may act to retain vascular function in diseases where NO production is limited.

However, further studies are still needed to clarify the exact mechanisms underlying the beneficial vascular effects due to cocoa consumption.

CONCLUSION

For many centuries, cocoa has been loved for its good taste and praised for its beneficial effects on health. In the last ten years many research studies confirmed that cocoa does indeed exert beneficial effects on vascular and platelet function, probably mainly mediated by its high polyphenol content, a heterogeneous group of molecules mainly found in fruits and vegetables. The beneficial effects of cacao are most likely due to a decrease in oxidative stress, induction of NOS and in turn an increased bioavailability of NO.

ACKNOWLEDGMENTS

Work of the authors was partly supported by the Swiss National Science Foundation (grant Nr. 32000BO-109905/1 to R.C.) The authors received a unrestricted research grant by Nestlé.

Table and Figure legend

Table 1 Catechin/Epicatechin Concentrations found in foods. Modified from Manach et al[15]

Table 2 Studies Investigating Cocoa and Endothelial Function. LDL, low density lipoprotein; NO, nitric oxide; FMD, flow mediated dilatation; CAD, coronary artery disease. Modified from[10, 16]

Figure 1 Endothelium-dependent effect of cocoa polyphenols. AII indicates angiotensin II; AI, angiotensin I; PKC, protein kinase C; SOD, superoxide dismutase; PGI2, prostacyclin; ACE, angiotensin-converting enzyme; ECE, endothelin-converting enzyme; AT1, angiotensin receptor; ET-1, endothelin 1; bET-1, big endothelin 1; ETa/b, endothelin receptor a and b; cGMP, cyclic guanosine monophosphate; and ROS, reactive oxygen species. Modified from Corti R et al.[10]

Table 1:

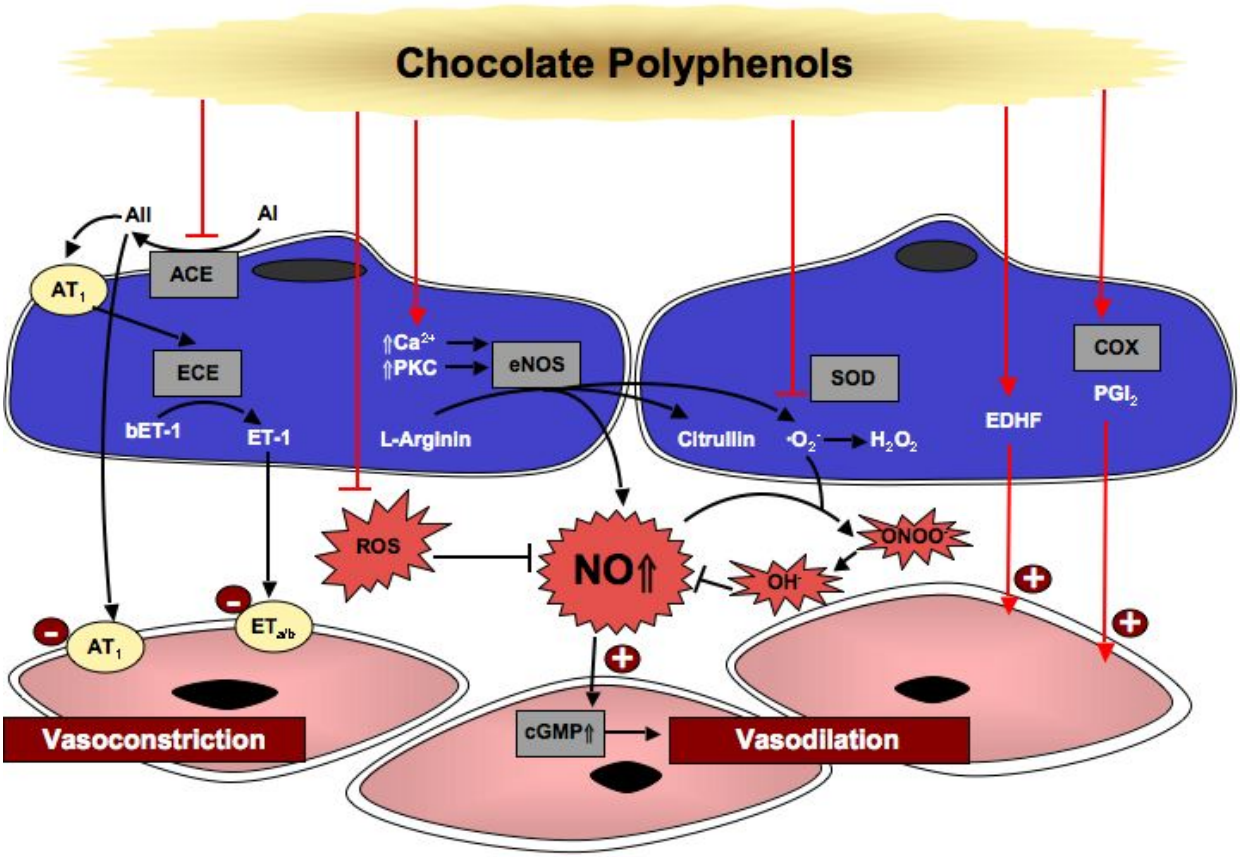
Source	**Flavanol content per mg/kg or mg/L**
Chocolate	460-610
Beans	350-550
Apricot	100-250
Cherry	50-220
Peach	50-140
Blackberry	130
Apple	20-120
Green tea	100-800
Black tea	60-500
Red wine	80-300
Cider	40

Table 2

Author	Year	No	Population	Duration	Intervention	Outcome
Heiss[45]	2003	26	Patients with at least 1 CV risk factor	2 hours (crossover)	Flavanol rich cocoa drink (100ml)	Improvement of FMD and increased levels of nitrosated and nitrosylated species.
Fisher[60]	2003	27	Healthy people	5 days	Flavanol rich cocoa (821mg/d)	Peripheral vasodilatation, improved vasodilator response to ischemia assessed by pulse wave amplitude on the finger
Engler[61]	2004	21	Healthy subjects	2 weeks	High flavonoid chocolate (213mg procyanidins, 46mg epicatechin) vs. low flavonoid chocolate	Improvement of FMD of the brachial artery, increased epicatechin concentrations
Grassi[24]	2005	20	Untreated essential hypertension	15 days (crossover)	100 g dark chocolate (21.91mg catechin, 65,97 mg epicatechins) vs. flavanol free white chocolate	Increased FMD of the brachial artery. Decrease in blood-pressure and LDL cholesterol, increase of insulin sensitivity
Heiss[46]	2005	11	Smokers	2 hours (crossover)	100ml cocoa drink with high (176-18mg) or low (<11mg) flavanol content	Increase of FMD and circulating NO pool. Increase of flavanol metabolites
Hermann[47]	2006	20	Healthy smokers	2 hours	40g commercially available dark chocolate vs. white chocolate	Increase in FMD of the brachial artery. Improvement of antioxidant status and improvement of platelet function.
Schroeter[62]	2006	16	Healthy subjects, isolated rabbit rings	2 hours	Drink with high flavonoid content	Improvement of FMD, paralleled the appearance of flavanoles in plasma. Concentrations in plasma enough to mediate ex vivo vasodilatation. Pure epicatechins mimics vascular effects of cocoa. High flavanol diet is associated with high urinary excretion of NO metabolites
Flammer[49]	2007	22	Heart transplant patients	2 hours	40g commercially available dark chocolate vs. flavonoid free placebo chocolate	Inducing coronary vasodilation, improvement in coronary endothelial function and improvement of platelet function.
Balzer[50]	2008	41	diabetics	30 days	flavanol-rich cocoa (321 mg flavanolsx3) or a nutrient-matched control (25 mg flavanolsx3)	Improvement in brachial FMD
Shiina[63]	2009	39	Healthy	2 weeks	45g commercially available dark chocolate vs. white chocolate	Improvement in coronary circulation as measured by coronary velocity flow reserve

Davison[51]	2008		Obese and overweight patients	12 weeks	Dietary high (902 mg) vs. low (36 mg) flavanol intake	Improvement in brachial FMD
Heiss[52]	2010	16	CAD	30 days	Dietary high (375 mgx2) vs. low (9 mgx2) flavanol intake	Improvement in FMD and mobilization of endothelial progenitor cells
Njike[64]	2011	44	Healthy BMI 25-35	6 weeks	sugar-free cocoa beverage or placebo, sugar-sweetened cocoa beverage or placebo	Improvement in FMD, no change in weight
Flammer[53]	2011	22	Heart Failure	2 hours and 30 days	40g commercially available dark chocolate vs. flavonoid free placebo chocolate	Inducing brachial vasodilation, improvement in endothelial function and acute improvement of platelet function.

Figure 1


Chocolate Polyphenols
AII
AI
ACE
AT1
ECE
bET-1
ET-1
⇑Ca2+
⇑PKC
eNOS
L-Arginin
SOD
Citrullin
·O2-
H2O2
EDHF
COX
PGI2
ROS
NO⇑
ONOO-
OH·
AT1
ETa/b
Vasoconstriction
cGMP⇑
Vasodilation

Literature

1. Hertog, M.G., D. Kromhout, C. Aravanis, et al., *Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study.* Arch Intern Med, 1995. **155**(4): p. 381-6.
2. Hertog, M.G., E.J. Feskens, P.C. Hollman, et al., *Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study.* Lancet, 1993. **342**(8878): p. 1007-11.
3. Knekt, P., R. Jarvinen, A. Reunanen, et al., *Flavonoid intake and coronary mortality in Finland: a cohort study.* BMJ, 1996. **312**(7029): p. 478-81.
4. Joshipura, K.J., F.B. Hu, J.E. Manson, et al., *The effect of fruit and vegetable intake on risk for coronary heart disease.* Ann Intern Med, 2001. **134**(12): p. 1106-14.
5. Keli, S.O., M.G. Hertog, E.J. Feskens, et al., *Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study.* Arch Intern Med, 1996. **156**(6): p. 637-42.
6. Mink, P.J., C.G. Scrafford, L.M. Barraj, et al., *Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women.* Am J Clin Nutr, 2007. **85**(3): p. 895-909.
7. Buijsse, B., E.J. Feskens, F.J. Kok, et al., *Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study.* Arch Intern Med, 2006. **166**(4): p. 411-7.

*8. Buijsse, B., C. Weikert, D. Drogan, et al., *Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults.* Eur Heart J, 2010. **31**(13): p. 1616-23.

Recent study showing the relationship between chocolate and blood pressure

9. Schroeter, H., C. Heiss, J.P.E. Spencer, et al., *Recommending flavanols and procyanidins for cardiovascular health: Current knowledge and future needs.* Molecular Aspects of Medicine, 2010. **31: p. 546-557.

Review about flavanols and cardiovascular health

10. Corti, R., A.J. Flammer, N.K. Hollenberg, et al., *Cocoa and cardiovascular health.* Circulation, 2009. **119(10): p. 1433-41.

Very important review summarizing the effect of cocoa and chocolate

11. Arts, I.C., B. van De Putte, and P.C. Hollman, *Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices, and chocolate milk.* J Agric Food Chem, 2000. **48**(5): p. 1752-7.
12. Arts, I.C., B. van de Putte, and P.C. Hollman, *Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods.* J Agric Food Chem, 2000. **48**(5): p. 1746-51.
13. Lazarus, S.A., G.E. Adamson, J.F. Hammerstone, et al., *High-performance liquid Chromatography/Mass spectrometry analysis of proanthocyanidins in foods and beverages.* J Agric Food Chem, 1999. **47**(9): p. 3693-701.
14. Adamson, G.E., S.A. Lazarus, A.E. Mitchell, et al., *HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity.* J Agric Food Chem, 1999. **47**(10): p. 4184-8.

15. Manach, C., A. Scalbert, C. Morand, et al., *Polyphenols: food sources and bioavailability.* Am J Clin Nutr, 2004. **79**(5): p. 727-47.
16. Sudano, I., A.J. Flammer, G. Noll, et al., *Vascular and Platelet Effects of Cocoa*, in *CHOCOLATE AND HEALTH*, R. Paoletti, A. Poli, A. Conti, and F.E. Visioli, Editors. 2012, Springer Verlag Italia: Milan.
17. Rein, D., S. Lotito, R.R. Holt, et al., *Epicatechin in human plasma: in vivo determination and effect of chocolate consumption on plasma oxidation status.* J Nutr, 2000. **130**(8S Suppl): p. 2109S-14S.
18. Serafini, M., R. Bugianesi, G. Maiani, et al., *Plasma antioxidants from chocolate.* Nature, 2003. **424**(6952): p. 1013.
19. Richelle, M., I. Tavazzi, M. Enslen, et al., *Plasma kinetics in man of epicatechin from black chocolate.* Eur J Clin Nutr, 1999. **53**(1): p. 22-6.
20. Shrime, M.G., S.R. Bauer, A.C. McDonald, et al., *Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies.* J Nutr, 2011. **141**(11): p. 1982-8.
21. Hooper, L., C. Kay, A. Abdelhamid, et al., *Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials.* Am J Clin Nutr, 2012. **95(3): p. 740-51.

Last appeared systematic review and meta-analysis about cardiovascular system and cocoa, chocolate and flavanols.

22. McCullough, M.L., K. Chevaux, L. Jackson, et al., *Hypertension, the Kuna, and the epidemiology of flavanols.* J Cardiovasc Pharmacol, 2006. **47 Suppl 2**: p. S103-9; discussion 119-21.
23. Grassi, D., G. Desideri, S. Necozione, et al., *Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate.* J Nutr, 2008. **138**(9): p. 1671-6.
24. Grassi, D., S. Necozione, C. Lippi, et al., *Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives.* Hypertension, 2005. **46**(2): p. 398-405.
*25. van den Bogaard, B., R. Draijer, B.E. Westerhof, et al., *Effects on peripheral and central blood pressure of cocoa with natural or high-dose theobromine: a randomized, double-blind crossover trial.* Hypertension, 2010. **56**(5): p. 839-46.

Study showing the effect of different dosis of theobromine on peripheral and central blood pressure.

26. Desch, S., D. Kobler, J. Schmidt, et al., *Low vs. higher-dose dark chocolate and blood pressure in cardiovascular high-risk patients.* Am J Hypertens, 2010. **23**(6): p. 694-700.
27. Desch, S., J. Schmidt, D. Kobler, et al., *Effect of cocoa products on blood pressure: systematic review and meta-analysis.* Am J Hypertens. **23**(1): p. 97-103.
28. Taubert, D., R. Roesen, and E. Schomig, *Effect of cocoa and tea intake on blood pressure: a meta-analysis.* Arch Intern Med, 2007. **167**(7): p. 626-34.
29. Actis-Goretta, L., J.I. Ottaviani, C.L. Keen, et al., *Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins.* FEBS Lett, 2003. **555**(3): p. 597-600.
30. Actis-Goretta, L., J.I. Ottaviani, and C.G. Fraga, *Inhibition of angiotensin converting enzyme activity by flavanol-rich foods.* J Agric Food Chem, 2006. **54**(1): p. 229-34.
*31. Persson, I.A., K. Persson, S. Hagg, et al., *Effects of cocoa extract and dark chocolate on angiotensin-converting enzyme and nitric oxide in human endothelial cells and healthy volunteers--a nutrigenomics perspective.* J Cardiovasc Pharmacol, 2011. **57**(1): p. 44-50.

New study suggesting an effect of cocoa on angiotensin converting

32. Berry, N.M., K. Davison, A.M. Coates, et al., *Impact of cocoa flavanol consumption on blood pressure responsiveness to exercise.* Br J Nutr, 2010. **103**(10): p. 1480-4.
33. Flammer, A.J. and T.F. Luscher, *Human endothelial dysfunction: EDRFs.* Pflugers Arch, 2010. **459**(6): p. 1005-13.
34. Sudano, I., A.J. Flammer, J. Steffel, et al., *The vascular endothelium in hypertension: target and promoter?* Hot Topic in Cardiology, 2009(15).
35. Deanfield, J.E., J.P. Halcox, and T.J. Rabelink, *Endothelial function and dysfunction: testing and clinical relevance.* Circulation, 2007. **115**(10): p. 1285-95.
36. Celermajer, D.S., K.E. Sorensen, V.M. Gooch, et al., *Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis.* Lancet, 1992. **340**(8828): p. 1111-5.
37. Schachinger, V., M.B. Britten, and A.M. Zeiher, *Prognostic impact of coronary vasodilator dysfunction on adverse long- term outcome of coronary heart disease.* Circulation, 2000. **101**(16): p. 1899-906.
38. Suwaidi, J.A., S. Hamasaki, S.T. Higano, et al., *Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction.* Circulation, 2000. **101**(9): p. 948-54.
39. Hooper, L., P.A. Kroon, E.B. Rimm, et al., *Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials.* Am J Clin Nutr, 2008. **88**(1): p. 38-50.
40. Ras, R.T., P.L. Zock, and R. Draijer, *Tea consumption enhances endothelial-dependent vasodilation; a meta-analysis.* PLoS One, 2011. **6**(3): p. e16974.
41. Dell'Agli, M., A. Busciala, and E. Bosisio, *Vascular effects of wine polyphenols.* Cardiovasc Res, 2004. **63**(4): p. 593-602.
42. Schini-Kerth, V.B., C. Auger, J.H. Kim, et al., *Nutritional improvement of the endothelial control of vascular tone by polyphenols: role of NO and EDHF.* Pflugers Arch. **459**(6): p. 853-62.
43. Morand, C., C. Dubray, D. Milenkovic, et al., *Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers.* Am J Clin Nutr, 2011. **93**(1): p. 73-80.
44. Karim, M., K. McCormick, and C.T. Kappagoda, *Effects of cocoa extracts on endothelium-dependent relaxation.* J Nutr, 2000. **130**(8S Suppl): p. 2105S-8S.
45. Heiss, C., A. Dejam, P. Kleinbongard, et al., *Vascular effects of cocoa rich in flavan-3-ols.* JAMA, 2003. **290**(8): p. 1030-1.
46. Heiss, C., P. Kleinbongard, A. Dejam, et al., *Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers.* J Am Coll Cardiol, 2005. **46**(7): p. 1276-83.
47. Hermann, F., L.E. Spieker, F. Ruschitzka, et al., *Dark chocolate improves endothelial and platelet function.* Heart, 2006. **92**(1): p. 119-20.
48. Wever, R.M., T.F. Luscher, F. Cosentino, et al., *Atherosclerosis and the two faces of endothelial nitric oxide synthase.* Circulation, 1998. **97**(1): p. 108-12.
49. Flammer, A.J., F. Hermann, I. Sudano, et al., *Dark chocolate improves coronary vasomotion and reduces platelet reactivity.* Circulation, 2007. **116**(21): p. 2376-82.
50. Balzer, J., T. Rassaf, C. Heiss, et al., *Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-masked, randomized, controlled trial.* J Am Coll Cardiol, 2008. **51**(22): p. 2141-9.

51. Davison, K., A.M. Coates, J.D. Buckley, et al., *Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects.* Int J Obes (Lond), 2008. **32**(8): p. 1289-96.
52. Heiss, C., S. Jahn, M. Taylor, et al., *Improvement of endothelial function with dietary flavanols is associated with mobilization of circulating angiogenic cells in patients with coronary artery disease.* J Am Coll Cardiol, 2010. **56**(3): p. 218-24.
**53. Flammer, A.J., I. Sudano, M. Wolfrum, et al., *Cardiovascular effects of flavanol-rich chocolate in patients with heart failure.* Eur Heart J, 2011.
First study evaluating the effect of cocoa on endothelial function
54. Vlachopoulos, C., K. Aznaouridis, N. Alexopoulos, et al., *Effect of dark chocolate on arterial function in healthy individuals.* Am J Hypertens, 2005. **18**(6): p. 785-91.
55. Vlachopoulos, C.V., N.A. Alexopoulos, K.A. Aznaouridis, et al., *Relation of habitual cocoa consumption to aortic stiffness and wave reflections, and to central hemodynamics in healthy individuals.* Am J Cardiol, 2007. **99**(10): p. 1473-5.
56. Heiss, C., D. Finis, P. Kleinbongard, et al., *Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week.* J Cardiovasc Pharmacol, 2007. **49**(2): p. 74-80.
57. Lotito, S.B. and B. Frei, *Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon?* Free Radic Biol Med, 2006. **41**(12): p. 1727-46.
58. Ramirez-Sanchez, I., L. Maya, G. Ceballos, et al., *(-)-epicatechin activation of endothelial cell endothelial nitric oxide synthase, nitric oxide, and related signaling pathways.* Hypertension. **55**(6): p. 1398-405.
59. Ramirez-Sanchez, I., L. Maya, G. Ceballos, et al., *(-)-Epicatechin induces calcium and translocation independent eNOS activation in arterial endothelial cells.* Am J Physiol Cell Physiol. **300**(4): p. C880-7.
60. Fisher, N.D., M. Hughes, M. Gerhard-Herman, et al., *Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans.* J Hypertens, 2003. **21**(12): p. 2281-6.
61. Engler, M.B., M.M. Engler, C.Y. Chen, et al., *Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults.* J Am Coll Nutr, 2004. **23**(3): p. 197-204.
62. Schroeter, H., C. Heiss, J. Balzer, et al., *(-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans.* Proc Natl Acad Sci U S A, 2006. **103**(4): p. 1024-9.
63. Shiina, Y., N. Funabashi, K. Lee, et al., *Acute effect of oral flavonoid-rich dark chocolate intake on coronary circulation, as compared with non-flavonoid white chocolate, by transthoracic Doppler echocardiography in healthy adults.* Int J Cardiol, 2009. **131**(3): p. 424-9.
64. Njike, V.Y., Z. Faridi, K. Shuval, et al., *Effects of sugar-sweetened and sugar-free cocoa on endothelial function in overweight adults.* Int J Cardiol, 2011. **149**(1): p. 83-8.