

Cocoa and Chocolate their clinical benefits: Insights in study design

Mellor, D, Georgousopoulou, E & Naumovski, N

Author post-print (accepted) deposited by Coventry University's Repository

Original citation & hyperlink:

Mellor, D, Georgousopoulou, E & Naumovski, N 2017, 'Cocoa and Chocolate their clinical benefits: Insights in study design' *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, vol 12, no. 018, pp. 1-7

<https://dx.doi.org/10.1079/PAVSNNR201712018>

DOI 10.1079/PAVSNNR201712018

ISSN 1749-8848

Publisher: CABI

This material has been published in CAB Reviews, Vol 12, Issue 018, 1-7, the only accredited archive of the content that has been certified and accepted after peer review. Copyright and all rights therein are retained by CABI.

www.cabi.org/CABReviews

Copyright © and Moral Rights are retained by the author(s) and/ or other copyright owners. A copy can be downloaded for personal non-commercial research or study, without prior permission or charge. This item cannot be reproduced or quoted extensively from without first obtaining permission in writing from the copyright holder(s). The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the copyright holders.

This document is the author's post-print version, incorporating any revisions agreed during the peer-review process. Some differences between the published version and this version may remain and you are advised to consult the published version if you wish to cite from it.

COCOA AND CHOCOLATE THEIR CLINICAL BENEFITS: INSIGHTS IN STUDY DESIGN

Duane D. Mellor^{1,2}, Ekavi N. Georgousopoulou^{1,3}, Nenad Naumovski¹

¹ Faculty of Health, University of Canberra, Canberra, Australia

² Faculty of Health and Life Sciences, Coventry University, Coventry, UK

³Department of Nutrition-Dietetics, School of Health and Education, Harokopio University, Athens, Greece

Correspondence

Duane D. Mellor, PhD, APD

Associate Professor in Nutritional Sciences

School of Public Health and Nutrition, Faculty of Health

University of Canberra, Canberra, ACT, Australia

Tel: 02 6206 8746

Email: duane.mellor@canberra.edu.au

Abstract

Randomised controlled trials and meta-analyses have demonstrated the potential protective effect of cocoa and chocolate consumption with respect to Cardiovascular Disease (CVD) risk markers. Findings from experimental studies are in concordance with observational data, which includes reduction in clinical disease (especially stroke) being associated with chocolate consumption. However, the effect size of any benefit, and the exact mechanism of action due to variability in reporting of dose and type potential bioactive compounds remains unclear. Thus, the present review aimed to analyse the published work where cocoa and chocolate has been assessed for its potential to protect against CVD and highlight the role of study design and type of product used in the variances of outcomes and how that might be used in formulating health advice.

Keywords: chocolate; cocoa; cardiovascular disease; risk factors; review

Introduction

Christopher Columbus in early 1500s was the first European to encounter cacao seeds (cocoa) which are obtained from the tree – *Theobroma cacao* and he described them as a “mysterious-looking almond”, but he soon realized that the “cocoa beans” were already an established Mesoamerican currency (1). Nevertheless, the word “cacao” or “cocoa” is believed to be used to describe the native to America plant seeds, prior even to the Aztecs arrival in Mexico thought to date back to the Olmec possibly as early as 1000-1500 BCE (2). The culinary and ritual uses of the *Theobroma cacao* beans can be traced historically in ancient Mayan texts that referred to cocoa as “*a gift of the gods*”, whilst pre-Columbian societies were known to use cocoa often in a beverage form as medicine (2). The Aztecs believed that cocoa had nourishing, fortifying and aphrodisiac properties and thus, represented fertility, wisdom and power; so they reserved the use of cocoa as a beverage for high status adult males, who were the priests, highest government officials, military officers and distinguished warriors. In the classic period of Mayan civilization (250-900 CE.), ground cocoa seeds were mixed with seasonings to make a bitter, spicy drink that was believed to be a health-promoting elixir (2). The aforementioned medicinal use of cacao soon spread to Europe by the mid-1500s. Within a century, the culinary and medical uses of chocolate was being adopted in Western Europe through colonial era documents including instructions for the medicinal use of cacao (2). However, during the Enlightenment, the therapeutical use began to be separated from taste and potentially leading to chocolate becoming its main excipient, bearing the burden, over time, of a negative valence due to being associated with obesity, dental problems and unhealthy lifestyle (3). Since then into the present day chocolate is a food that is associated with gluttony, excess and frequently guilt.

Recently, evidence has emerged revealing protective aspects of chocolate, this has started to overshadow chocolate’s negative reputation, suggesting it could have potential positive health benefits when eaten in moderation and as part of a balanced diet (4). Firstly, it has been suggested that chocolate could help manage cardiovascular disease (CVD) and type 2 diabetes risk through a number of proposed pathways which have been elucidated via *in vitro* and animal studies. The candidate mechanisms include the moderating insulin resistance, the induction of nitric oxide synthesis as a vasodilator, inhibition of angiotensin converting enzyme and facilitation of the formation of the nascent particle High Density Lipoprotein (5), which have been associated with the vitamin, mineral and other bioactive compound content of the cacao bean. This evidence is based on studies that tend to report on extremely small sized trials, with short follow-up period (56). These studies have been followed by the initial clinical trials (6-87-9), but the issue remains controversial and debatable even today. Advocates support that chocolate, offers important benefits in terms of regulation of arterial blood pressure and diameter (910) per se as a food, whilst opponents like Ellinger et al., (11) using Bayesian inference stated that recommending chocolate is economically sound as a blood pressure intervention, but not evidence-based. The latter is based on the assumption that epicatechin is the primary active compound, which is found in other foods including apples, tea and flava (broad) beans (12) and thus, epicatechin-rich foods not chocolate alone, should be the focus of such public health recommendations. However, the evidence that any health effect is largely due to epicatechin content of chocolate is not perhaps plausible, due to variable quantities of

epicatechin delivered in many of the studies including a large number of studies which do not specify subgroups and individual polyphenols or omit these all together (5). Therefore, this issue of dose warrants further study, as does the potential beneficial effects of chocolate beyond its epicatechin content including theobromine, amino acid and lipid contents.

This review aims to look holistically at chocolate and cocoa consumption, in the process acknowledging the limitation that this defined doses of individual potential bioactives such as epicatechins will not be explored within the scope of the review. Instead, an approach considering the potential clinical benefits of whole food products will be considered. Therefore, although the antioxidant-like and anti-inflammatory mechanisms have been linked chocolate consumption to favourable health outcomes (13), and as such support the ancient beliefs regarding cocoa, it is important to consider the food product as consumed and not only individual components. Nevertheless issues about formulation of the cocoa products vary between studies, making it difficult to reach a safe conclusion. This review aims to describe the potential role of chocolate and cocoa products in CVD risk and risk markers, under the context of product formulation and by taking into account the study design.

Review Methodology

The search terms were decided according to the PICOS (Participants, Interventions, Comparisons, Outcomes, and Study design) from scoping the literature concerning chocolate and cocoa consumption and its association with Cardiovascular Disease (CVD) risk and related markers such as CVD mortality, Myocardial Infarction (MI), Type 2 Diabetes Mellitus, stroke, endothelial function, arterial blood pressure, blood lipids and quality of life aspects. Both observational studies as well as clinical trials were included in this systematic review.

All clinical trials that lasted at least two weeks and were placebo-controlled met the inclusion criteria. Key terms, related to the inclusion criteria, used in the titles and abstracts of these papers were consolidated in order to produce the minimum number of search terms required to retrieve the maximum number of relevant articles, e.g. cacao and cocoa were found to deliver the same search results. Key terms related to the inclusion criteria included chocolate (e.g. raw cocoa, chocolate), CVD mortality and morbidity (MI, stroke) and terms related to CVD risk factors such as (type 2) diabetes mellitus, blood pressure and endothelial function. Other key terms included quality of life (e.g. mood, depression, anxiety) and terms related to side-effects (e.g. weight change, energy content, fat mass, sugar, caffeine). Boolean operators (AND and OR) were used to construct and refine the search in *MEDLINE* and *CAB reviews*, but only papers published in English were included in the review.

Cocoa/Chocolate, endothelial function and arterial blood pressure

Hooper et al. (14) conducted a meta-analysis of 11 randomised clinical trials to investigate the effects of chocolate/cocoa consumption on endothelial function, measured as FMD (Flow-mediated dilatation) and found that chronic intake of chocolate/cocoa improved the FMD per 1.34%, p -value <0.05 . Moreover, chocolate/cocoa consumption lead to a significant reduction in diastolic blood pressure per 1.60 mm Hg, $p<0.05$, but no difference

was detected in systolic blood pressure, $p > 0.05$. Published in the same year, Ried et al. (15) meta-analysis of same study designs, focused on arterial blood pressure only and found significant reduction in systolic blood pressure by 2.77 mm Hg, $p < 0.05$ and in diastolic blood pressure by 2.2 mm Hg, $p < 0.05$. Just two year after these two meta-analyses were published, Mastroiacovo et al. reported a significant reduction only in systolic (4.3mmHg; $p = 0.028$) but not in diastolic blood pressure (2.0mmHg; $p = 0.37$) after an 8-week supplementation of high-flavanols cocoa drink. Heiss et al. (16) found a different effect with a significant decrease in diastolic blood pressure ($p < 0.05$) following a 2-weeks high-flavanol cocoa drink supplementation in males, but no difference was reported in systolic blood pressure ($p > 0.05$) across clinic and ambulatory readings. More recently, Sansone et al. (17) in a 4-week randomised trial reported significant reduction in both systolic (4.4mmHg) and diastolic (3.9mmHg) arterial blood pressure after the consumption of study-designed high in flavanols cocoa drink.

Cocoa/Chocolate and Diabetes

Hooper et al. (14) summarised the effect of cocoa/chocolate supplementation as significant reduction of fasting insulin ($-2.65 \mu\text{U/mL}$, $p < 0.05$), but reported no effect on fasting serum glucose levels in clinical trials. However, data from two observational prospective studies (18, 19) and a meta-analysis of prospective studies (20) have suggested that frequent chocolate consumption is linked to a significant potential reduction in risk of developing type 2 diabetes mellitus by approximately 30%, $p < 0.05$ based on the reduction in insulin resistance calculated from fasting insulin and glucose.

Cocoa/Chocolate and Cholesterol

As regards cholesterol levels, Hooper et al. (14) meta-analysis revealed a significant reduction in LDL-cholesterol (0.07mmol/l; $p = 0.05$) as a result of cocoa/chocolate supplementation, but no impact on total cholesterol, ($p > 0.05$), suggestive of a modest improvement with respect to atherogenic lipid profile. Sansone et al. (17) found significant reduction in both total and LDL-cholesterol as a result of the cocoa drink supplementation (0.20mmol/l and 0.17mmol/l respectively; $p < 0.05$), whilst Mastroiacovo with a longer intervention, that was double the length of Sansone et al. (17) and higher dosage reported favourable difference only in LDL-cholesterol levels (0.44mmol/l; $p = 0.02$). It should perhaps also be noted that LDL cholesterol is typically calculated from Friedewald equation and represents a composite of HDL cholesterol, total cholesterol and triglycerides, and as such could be prone to type 1 error due to non-significant clinically, irrelevant changes observed in studies.

Cocoa/Chocolate and CVD risk

The effect of chocolate consumption on fatal CVD risk was assessed in a systematic review by Kwok et al. (21), reporting the findings of nine studies with follow-up time ranging from 8-16 years, found an independent protective effect of frequent chocolate consumption

(45% risk reduction as compared to no consumption, $p=0.005$), additionally chocolate consumption was protective against non-fatal stroke events. In the same systematic review, chocolate consumption was favourable only against stroke among the non-fatal CVD clinical outcomes reported(21). The latter was also confirmed by Larsson et al. (22) in a meta-analysis of five prospective trials, where chocolate consumption was proven protective against stroke by 20%, $p<0.05$. Finally, Khawaja et al. systematically reviewed seven prospective studies and found an overall protective effect of chocolate consumption against (23) myocardial infarction risk. It is perhaps a limitation that many of these studies assessing clinical disease endpoints do not report the clinical CVD risk factors such as lipid profiles and blood pressure. This has the effect that comparison of clinical trials, which assessed the effect of chocolate, or cocoa on biomarkers of CVD and observational studies that reported CVD incidence is not possible without extrapolating data and risking introducing considerable confounding.

Commercial vs. Study designed product

Out of the 30 Randomized, placebo-controlled clinical trials included in this review (6, 17, 24, 51), only 10 used commercial chocolate (6, 24, 31, 33, 36, 39, 46, 47), and 20 used research tailor-made products designed by collaborating industry partners or in the case of four have limited availability as a specialist product or supplement (17, 25-30, 32, 37, 39, 40-45, 48-51). With the nature of many of the studies attempting to match the control product to the investigational one, it meant that in 21 studies (17, 24-32, 38, 40-45, 48-51) the control chocolate was specially designed. This needs to be considered, as it is plausible that the health effects of these types of product may not represent those of typical commercially available chocolates.

Composition of the product/placebo

Among the 30 reviewed trials, only 12 used solid chocolate (75% of them commercial) and the remaining studies used cocoa beverages (usually made with milk). Although not a direct focus of this review, it was clear the detail of reporting of the content of the chocolate with respect to polyphenol content and the fractions of polyphenols contained in the products were varied. One paper provided no data on the polyphenol content (39) with only four papers stating the profile of compounds and the methodology used to measure them was by HPLC (25, 26, 35, 49). There also seemed to be a lack of concordance in the data with several papers reporting the use of the same commercially available white chocolate used as a control was polyphenol free (46, 47) this product was used by another group and described as flavanol-free (6) in one study and presumed to contain no polyphenols (34) in a separate study. However, the later group in a subsequent study reported that this product did contain 1.3mg per g of polyphenols (35), however the same studies supplementary data suggested the polyphenol content of this product were not detectible. Overall the reporting of potential bioactive compounds in many chocolate and cocoa studies, in particular related to the proposed placebo is a limitation, and the polyphenol content of a number of the control products used, means it is difficult to conclude that either epicatechins or other polyphenols are the primary bioactive compound in clinical studies. Reporting of a significant polyphenol content in the white chocolate placebo

of some studies (35), which are inconsistent within the same report and contradict the reporting of the same product in earlier publications further challenges the data with respect to quality and risk of bias. Other groups have reported polyphenols being present in white chocolate (40, 43), and although polyphenols are more concentrated in the cocoa mass, they are not absent in cocoa butter and white chocolate (52).

Concordance between intervention product and placebo composition

Out of the 30 reviewed trials, five of them used a placebo of different source (commercial vs. study designed product) as compared to the intervention supplement. However, in all studies the placebo composition (liquid cocoa vs. solid chocolate) was in agreement with the intervention product. With respect to matching control product to the active, only 10 out of 30 trials described how they matched the placebo to the intervention product in for its appearance, eight matched the products for taste and 26 matched for macro-nutrients in the consumed products. This means that challenges are apparent in the interpretation of the data, as high levels of heterogeneity between studies exist. This together with the frequent use of study specific products which are typically supplemented with additional flavanols to produce products that are not available to the public health recommendations based on the published data are not possible. Therefore, it is plausible to recommend that the use of the term placebo is not really valid in most chocolate and cocoa studies, and terms such as comparator products should be used. This approach perhaps should be considered for most food based intervention studies where a true placebo, where only the proposed active compound is removed is rarely possible.

Discussion

The potential of cocoa and chocolate to improve markers of cardiovascular disease has been shown in a number of intervention trials, which have reported significant independent protective effects of chocolate/cocoa consumption against CVD risk markers (i.e. blood pressure, LDL-cholesterol levels and insulin resistance). However, the challenges in results interpretation still remain because of the high level of heterogeneity between study designs. Specifically, the majority of the existing studies have used tailor-made products, high in flavanols, which are not readily available to the public, whilst the few studies that did use commercial products failed to match the placebos in taste and appearance. Thus, it is still hard to formulate safe clinical recommendation regarding chocolate consumption for reducing CVD risk for public health purposes. Moreover, observational studies have shown promising protective results mainly with respect to stroke risk, but there is lack of robust results for other CVD clinical outcomes (21). Moving from statistical significance to clinical benefits seems to be the most important challenge, as trials' results showing a significant decrease in many CVD risk markers are not consistently in concordance with findings from observational studies.

There is a considerable amount of variation in the amount of flavanols, that many of the studies report are contained in the product. Manufacturers further challenge the reliability of this data but the lack of reporting of methods of analysis and frequent reliance on the provision of data. The lack of clarity across a number of studies which used a commercially available white chocolate which appears across a number of studies to be assumed to be polyphenol free (6, 34, 46, 47) but when latter tested (35) and found to contained 10-20% the

polyphenol content of the active means data needs to be treated with caution. This points to the need for consistent reporting of the content of chocolate and cocoa in clinical studies and clearer description with respect to the availability of product and the applicability of findings to the public. This later point may create challenges due to the commercial sensitivities and often funding of many of these studies, so therefore independently designed studies with standardised products may be useful in elucidating the potential beneficial impacts of chocolate and cocoa on human health.

The favourable effect of chocolate and cocoa consumption with respect to CVD risk factors could be explained by several mechanisms that have been linked to its antioxidant-like and anti-inflammatory properties (5). The proposed mechanism varies, from induction of endothelial nitric oxide synthase as a mechanism for the improvement in endothelial function, through to induction of endogenous antioxidant systems including superoxide dismutase to explain the antioxidant-like effects seen (53). With the previously held view that the antioxidant effects observed *in vitro* simply could be translated to *in vivo*, being widely dismissed due to low circulating levels of these compounds due to poor absorption and bioavailability. Further mechanisms have been explored to explain the blood pressure lowering effects possibly being linked to inhibition of Angiotensin Converting Enzyme (ACE) and insulin resistance via modulation of AMP-kinase pathway in skeletal muscle and liver (53-55). However, critics have suggest that the same amount of flavanols and epicatechin could be met though the consumption of other foods and food products (e.g. via tea) (11). Furthermore, anxiety and depressive symptoms are tightly linked to CVD development (56) and as chocolate is known to improve positive mood (57), this could be an interesting mediating path that needs further investigation.

Conclusion/Summary

Habitual chocolate consumption has shown protective effects against CVD risk markers including arterial blood pressure, insulin resistance in clinical trials, findings which are also supported by epidemiological studies. Nevertheless, the effect size is significantly higher in clinical trials as compared to prospective studies, which needs attention when forming guidelines. Furthermore, promising results from short-term clinical trials cannot necessarily mean it can be considered clinically relevant as a potential therapeutic option, but the combined results from this review suggest that chocolate consumption need not to be avoided and should not be regarded as an unhealthy food of sin.

References

1. Rusconi M, Conti A. Theobroma cacao L., the Food of the Gods: a scientific approach beyond myths and claims. *Pharmacological research*. 2010;61(1):5-13.
2. Dillinger TL, Barriga P, Escarcega S, Jimenez M, Salazar Lowe D, Grivetti LE. Food of the gods: cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *The Journal of nutrition*. 2000;130(8S Suppl):2057s-72s.
3. Lippi D. Sin and pleasure: the history of chocolate in medicine. *Journal of agricultural and food chemistry*. 2015;63(45):9936-41.
4. Lippi D. Chocolate and medicine: dangerous liaisons? *Nutrition (Burbank, Los Angeles County, Calif)*. 2009;25(11-12):1100-3.
5. Mellor DD, The effects of polyphenol rich chocolate on cardiovascular risk and glycaemic control in type 2 diabetes mellitus. PhD Thesis. University of Hull, UK. 2013
6. Grassi D, Lippi C, Necozione S, Desideri G, Ferri C. Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *The American journal of clinical nutrition*. 2005;81(3):611-4.
7. Davidsson L, Walczyk T, Morris A, Hurrell RF. Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. *The American journal of clinical nutrition*. 1998;67(5):873-7.
8. Gibson EL, Desmond E. Chocolate craving and hunger state: implications for the acquisition and expression of appetite and food choice. *Appetite*. 1999;32(2):219-40.
9. Kris-Etherton PM, Derr JA, Mustad VA, Seligson FH, Pearson TA. Effects of a milk chocolate bar per day substituted for a high-carbohydrate snack in young men on an NCEP/AHA Step 1 Diet. *The American journal of clinical nutrition*. 1994;60(6 Suppl):1037s-42s.
10. Ried K, Sullivan TR, Fakler P, Frank OR, Stocks NP. Effect of cocoa on blood pressure. *The Cochrane database of systematic reviews*. 2012(8):Cd008893.
11. Ellinger S, Reusch A, Stehle P, Helfrich HP. Epicatechin ingested via cocoa products reduces blood pressure in humans: a nonlinear regression model with a Bayesian approach. *The American journal of clinical nutrition*. 2012;95(6):1365-77.
12. Rothwell JA, Pérez-Jiménez J, Neveu V, Medina-Ramon A, M'Hiri N, Garcia Lobato P, Manach C, Knox K, Eisner R, Wishart D, Scalbert A. (2013) Phenol-Explorer 3.0: a major update of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. *Database*, 10.1093/database/bat070
13. De Araujo QR, Gattward JN, Almoosawi S, Silva M, Dantas PA, De Araujo Junior QR. Cocoa and Human Health: From Head to Foot--A Review. *Critical reviews in food science and nutrition*. 2016;56(1):1-12.
14. Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. *The American journal of clinical nutrition*. 2012;95(3):740-51.
15. Ried K, Sullivan T, Fakler P, Frank OR, Stocks NP. Does chocolate reduce blood pressure? A meta-analysis. *BMC medicine*. 2010;8:39.
16. Heiss C, Sansone R, Karimi H, Krabbe M, Schuler D, Rodriguez-Mateos A, et al. Impact of cocoa flavanol intake on age-dependent vascular stiffness in healthy men: a randomized, controlled, double-masked trial. *Age (Dordrecht, Netherlands)*. 2015;37(3):9794.
17. Sansone R, Rodriguez-Mateos A, Heuel J, Falk D, Schuler D, Wagstaff R, et al. Cocoa flavanol intake improves endothelial function and Framingham Risk Score in healthy men and women: a randomised, controlled, double-masked trial: the Flaviola Health Study. *The British journal of nutrition*. 2015;114(8):1246-55.

18. Matsumoto C, Petrone AB, Sesso HD, Gaziano JM, Djousse L. Chocolate consumption and risk of diabetes mellitus in the Physicians' Health Study. *The American journal of clinical nutrition*. 2015;101(2):362-7.
19. Greenberg JA. Chocolate intake and diabetes risk. *Clinical nutrition (Edinburgh, Scotland)*. 2015;34(1):129-33.
20. Larsson SC, Akesson A, Gigante B, Wolk A. Chocolate consumption and risk of myocardial infarction: a prospective study and meta-analysis. *Heart (British Cardiac Society)*. 2016;102(13):1017-22.
21. Kwok CS, Boekholdt SM, Lentjes MA, Loke YK, Luben RN, Yeong JK, et al. Habitual chocolate consumption and risk of cardiovascular disease among healthy men and women. *Heart (British Cardiac Society)*. 2015;101(16):1279-87.
22. Larsson SC, Virtamo J, Wolk A. Chocolate consumption and risk of stroke: a prospective cohort of men and meta-analysis. *Neurology*. 2012;79(12):1223-9.
23. Khawaja O, Gaziano JM, Djousse L. Chocolate and coronary heart disease: a systematic review. *Current atherosclerosis reports*. 2011;13(6):447-52.
24. Allgrove J, Farrell E, Gleeson M, Williamson G, Cooper K. Regular dark chocolate consumption's reduction of oxidative stress and increase of free-fatty-acid mobilization in response to prolonged cycling. *International journal of sport nutrition and exercise metabolism*. 2011;21(2):113-23.
- 25.. Baba S, Natsume M, Yasuda A, Nakamura Y, Tamura T, Osakabe N, et al. Plasma LDL and HDL cholesterol and oxidized LDL concentrations are altered in normo- and hypercholesterolemic humans after intake of different levels of cocoa powder. *The Journal of nutrition*. 2007;137(6):1436-41.
26. Baba S, Osakabe N, Kato Y, Natsume M, Yasuda A, Kido T, et al. Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. *The American journal of clinical nutrition*. 2007;85(3):709-17.
27. Balzer J, Rassaf T, Heiss C, Kleinbongard P, Lauer T, Merx M, et al. Sustained benefits in vascular function through flavanol-containing cocoa in medicated diabetic patients a double-masked, randomized, controlled trial. *Journal of the American College of Cardiology*. 2008;51(22):2141-9.
28. Berry NM, Davison K, Coates AM, Buckley JD, Howe PR. Impact of cocoa flavanol consumption on blood pressure responsiveness to exercise. *The British journal of nutrition*. 2010;103(10):1480-4.
29. Crews WD, Jr., Harrison DW, Wright JW. A double-blind, placebo-controlled, randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health: clinical findings from a sample of healthy, cognitively intact older adults. *The American journal of clinical nutrition*. 2008;87(4):872-80.
30. Davison K, Berry NM, Misan G, Coates AM, Buckley JD, Howe PR. Dose-related effects of flavanol-rich cocoa on blood pressure. *Journal of human hypertension*. 2010;24(9):568-76.
31. Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, et al. Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *Journal of the American College of Nutrition*. 2004;23(3):197-204.
32. Farouque HM, Leung M, Hope SA, Baldi M, Schechter C, Cameron JD, et al. Acute and chronic effects of flavanol-rich cocoa on vascular function in subjects with coronary artery disease: a randomized double-blind placebo-controlled study. *Clinical science (London, England : 1979)*. 2006;111(1):71-80.

33. Fraga CG, Actis-Goretta L, Ottaviani JI, Carrasquedo F, Lotito SB, Lazarus S, et al. Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. *Clinical & developmental immunology*. 2005;12(1):11-7.
34. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, et al. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension (Dallas, Tex : 1979)*. 2005;46(2):398-405.
35. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, et al. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *The Journal of nutrition*. 2008;138(9):1671-6.
36. Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M. Vascular effects of cocoa rich in flavan-3-ols. *Jama*. 2003;290(8):1030-1.
37. Heiss C, Finis D, Kleinbongard P, Hoffmann A, Rassaf T, Kelm M, et al. Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week. *Journal of cardiovascular pharmacology*. 2007;49(2):74-80.
38. Heiss C, Jahn S, Taylor M, Real WM, Angeli FS, Wong ML, et al. Improvement of endothelial function with dietary flavanols is associated with mobilization of circulating angiogenic cells in patients with coronary artery disease. *Journal of the American College of Cardiology*. 2010;56(3):218-24.
39. Hermann F, Spieker LE, Ruschitzka F, Sudano I, Hermann M, Binggeli C, et al. Dark chocolate improves endothelial and platelet function. *Heart (British Cardiac Society)*. 2006;92(1):119-20.
40. Mellor DD, Sathyapalan T, Kilpatrick ES, Beckett S, Atkin SL. High-cocoa polyphenol-rich chocolate improves HDL cholesterol in Type 2 diabetes patients. *Diabetic medicine : a journal of the British Diabetic Association*. 2010;27(11):1318-21.
41. Muniyappa R, Hall G, Kolodziej TL, Karne RJ, Crandon SK, Quon MJ. Cocoa consumption for 2 wk enhances insulin-mediated vasodilatation without improving blood pressure or insulin resistance in essential hypertension. *The American journal of clinical nutrition*. 2008;88(6):1685-96.
42. Njike VY, Faridi Z, Shuval K, Dutta S, Kay CD, West SG, et al. Effects of sugar-sweetened and sugar-free cocoa on endothelial function in overweight adults. *International journal of cardiology*. 2011;149(1):83-8.
43. Sathyapalan T, Beckett S, Rigby AS, Mellor DD, Atkin SL. High cocoa polyphenol rich chocolate may reduce the burden of the symptoms in chronic fatigue syndrome. *Nutrition journal*. 2010;9:55.
44. Schnorr O, Brossette T, Momma TY, Kleinbongard P, Keen CL, Schroeter H, et al. Cocoa flavanols lower vascular arginase activity in human endothelial cells in vitro and in erythrocytes in vivo. *Archives of biochemistry and biophysics*. 2008;476(2):211-5.
45. Sorond FA, Lipsitz LA, Hollenberg NK, Fisher ND. Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans. *Neuropsychiatric disease and treatment*. 2008;4(2):433-40.
46. Taubert D, Berkels R, Roesen R, Klaus W. Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. *Jama*. 2003;290(8):1029-30.
47. Taubert D, Roesen R, Lehmann C, Jung N, Schomig E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. *Jama*. 2007;298(1):49-60.
48. Tzounis X, Rodriguez-Mateos A, Vulevic J, Gibson GR, Kwik-Urbe C, Spencer JP. Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *The American journal of clinical nutrition*. 2011;93(1):62-72.

49. van den Bogaard B, Draijer R, Westerhof BE, van den Meiracker AH, van Montfrans GA, van den Born BJ. Effects on peripheral and central blood pressure of cocoa with natural or high-dose theobromine: a randomized, double-blind crossover trial. *Hypertension (Dallas, Tex : 1979)*. 2010;56(5):839-46.
50. Wang-Polagruto JF, Villablanca AC, Polagruto JA, Lee L, Holt RR, Schrader HR, et al. Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholesterolemic postmenopausal women. *Journal of cardiovascular pharmacology*. 2006;47 Suppl 2:S177-86; discussion S206-9.
51. Mastroiacovo D, Kwik-Urbe C, Grassi D, Necozione S, Raffaele A, Pistacchio L, et al. Cocoa flavanol consumption improves cognitive function, blood pressure control, and metabolic profile in elderly subjects: the Cocoa, Cognition, and Aging (CoCoA) Study--a randomized controlled trial. *The American journal of clinical nutrition*. 2015;101(3):538-48.
52. Meng CC, Jalil AM, Ismail, A. Phenolic and theobromine content of commercial dark, milk and white chocolates on the Malaysian market. *Molecules*. 2009, Jan 5; 14 (1): 200-9.
53. Mellor DD, Naumovski N. Effect of cocoa in diabetes: the potential of the pancreas and liver as key target organs, more than an antioxidant effect? *International Journal of Food Science and Technology*. 2016; 51 (4): 829-841
54. Vlachojannis J, Erne P, Zimmermann B, Chrubasik-Hausmann S. The Impact of Cocoa Flavanols on Cardiovascular Health. *Phytotherapy research : PTR*. 2016;30(10):1641-57.
55. Lin X, Zhang I, Li A, Manson JE, Sesso HD, Wang L, et al. Cocoa Flavanol Intake and Biomarkers for Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *The Journal of nutrition*. 2016;146(11):2325-33.
56. Kyrou I, Kollia N, Panagiotakos D, Georgousopoulou E, Chrysohoou C, Tsigos C, et al. Association of depression and anxiety status with 10-year cardiovascular disease incidence among apparently healthy Greek adults: The ATTICA Study. *European journal of preventive cardiology*. 2016.
57. Meier BP, Noll SW, Molokwu OJ. The sweet life: The effect of mindful chocolate consumption on mood. *Appetite*. 2016;108:21-7.