JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Chocolate and Other Cocoa Products: Effects on Human Reproduction and Pregnancy

Eleonora Brillo* and Gian Carlo Di Renzo

Department of Obstetrics and Gynecology and Centre for Perinatal and Reproductive Medicine, S. Maria della Misericordia University Hospital, University of Perugia, 06132 Perugia, Italy

ABSTRACT: Chocolate and other cocoa products are not all alike. They differ between themselves in term of nutrients, calories, and bioactive constituents. Therefore, some of them are unhealthy foods, whereas others do not affect health and still others are healthy foods. One wonders which chocolate and other cocoa derivatives can be considered as biofunctional food products. This review explores the constituents of cocoa and chocolate and summarizes evidence about the role of cocoa and chocolate components on human health and particularly on reproduction. On the basis of the literature review, it can be asserted that some kinds of cocoa products have favorable effects on human health at different stages of life. Women seem to be particularly favored by consuming of cocoa products, and chocolate with specific features can also be a good supplementary source of energy for pregnant woman. However, many aspects remain to be investigated and others are still to be clarified. Future studies and systematic reviews will shed light on some preventive effects and health benefits of cocoa products.

KEYWORDS: *chocolate, reproduction, pregnancy, health*

INTRODUCTION

Chocolate and cocoa products are obtained from a long and complex work process to which cacao beans are subjected.

Nowadays, a new trend in the food market shows a steady increase in the consumption of cocoa products, because they are widely appreciated for their hedonic value and their multiple health benefits.[1] Cocoa products have increasingly become objects of scientific research mainly because of their interesting phytochemical composition. The scientific community wonders whether chocolate and other cocoa products can be considered as a super fruit or as biofunctional food products.[2−4]

The aim of this study is to conduct a systematic review of the literature on cocoa and chocolate constituents and on their effects on human health and particularly on reproduction.

MACRONUTRIENTS AND FATTY ACIDS PROFILE OF COCOA AND CHOCOLATE: QUANTITY AND EFFECTS ON HUMAN HEALTH

According to Directive 2000/36 EC,[5] chocolate is defined as a food obtained from cocoa and sugars, containing at least 35% total dry cocoa, composed of not less than 18% cocoa butter and not less than 14% degreased dry cocoa. In addition to the minimum percentages of cocoa butter and total dry cocoa, a maximum of 5% fats cocoa butter equivalents (CBE) by weight of the finished product is allowed. Therefore, chocolate is certainly composed of cocoa solids and cocoa butter; then other ingredients are usually added such as sugar to sweeten and lecithin to emulsify. However, chocolate now commercially available often contains several other foods such as cereals, dry nuts, and fruits. The three main categories of chocolate, white, milk, and dark, have different contents of cocoa solids, cocoa butter, and some components of milk. Dark chocolate does not contain milk solids, or at least should not, whereas they are contained in chocolate milk and white chocolate. The latter has only the fat part of cocoa (cocoa butter) to which, among other ingredients, is added a large quantity of sugar. Generally, milk chocolate contains a lesser amount of the nonfat part of cocoa than dark chocolate, which nonetheless contains variable proportions of the nonfat part of the cocoa bean. The content of nutrients in chocolate depends, in part, on the percentage of the nonfat portion of cocoa: the amount of carbohydrates decreases and the fats increase approximately linearly with the increasing percentage of cacao content.[6] As a result, a higher percentage of cocoa means a higher amount of calories.[6] However, the more cocoa content, the higher the minerals[6] and polyphenols contents are. Dark chocolate, even that at higher cocoa content, does not maintain the proportions of macronutrients found in cocoa beans.[77] In fact, the main component is not fats, as in the case of beans, but carbohydrates followed by total fats, and this is due to the choice of adding a larger proportion of sugar, which exceeds the amount of fats. The quantity of cocoa butter and minerals in chocolate also depends on the geographical origin of the cocoa beans, in particular on the growing conditions.[7] Even fatty acids, which in cocoa butter are mainly organized as triacylglycerols, have a pattern that is influenced by geographical origin.[7] As a result, it may happen that some chocolate types have a healthier fatty acid profile than others. Normally the fatty acids most represented are stearic acid (C18:0), oleic acid (C18:1), and palmitic acid (C16:0), which together constitute >90%.[8,9] Saturated fatty acids (SFA), with C18:0 and C16:0 as the main fractions, are present in the highest proportions.[9−11] Among the unsaturated

Special Issue: ISCHOM 1st International Congress on Chocolate and Cocoa in Medicine

Received: March 27, 2015
Revised: July 22, 2015
Accepted: July 28, 2015
Published: July 28, 2015

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935

fatty acids, monounsaturated fatty acids (MUFA) content is higher than polyunsaturated fatty acids (PUFA): C18:1, which represents about 30% of the fatty acids present in TAG, is quantitatively the most important unsaturated fatty acids, followed by linoleic acid (C18:2).[10,11] Trans fatty acids were found to be present in chocolate[11] but in variable amounts,[12] and seem to be contained in low concentrations.

Differently from most of the SFA (C16:0 included) evidence shows that C18:0 has a neutral effect and does not increase blood total and low-density lipoprotein (LDL) cholesterol levels;[13−22] it does not significantly influence blood coagulation, fibrinolysis, thrombotic tendency, and cardiovascular disease compared with C18:1 and C18:2.[23−27] Some doubt remains, however, concerning the effects of stearic acid on thrombosis, inflammation, and blood pressure.[20]

Also because of the high fat content, chocolate has a high caloric value. Nevertheless, no positive correlation has been shown between chocolate consumption and body mass index (BMI) and, contrariwise, chocolate intake appears to have an inverse impact on BMI in men and women.[28]

OTHER CONSTITUENTS OF COCOA AND CHOCOLATE: QUANTITY AND EFFECTS ON HUMAN HEALTH

Processing conditions do not affect quantitatively nutrients and fatty acids profile,[7] but those affect the amount of biogenic amines[29] and polyphenols:[30−40] fermentation, drying, roasting, Dutch processing, and baking with alkali-leavening agents reduce and change polyphenol contents in cocoa.[30−45] The origin of the cocoa bean also plays an important role in the variability of polyphenol contents of cocoa products,[43,46,47] and cacao cultivars significantly affect the levels of biogenic amines.[29] The different types of chocolate on the market have variable amounts of phenols, which is not always proportional to the amount of nonfat cocoa solid content.[48] The amount of phenols is usually higher in dark chocolate than in milk chocolate, and it is usually irrelevant in white chocolate[48,49] Flavanols, a subgroup of dietary polyphenols present in many fruits and vegetables, may be associated with health benefits, particularly with reducing the risk of coronary diseases. Cocoa and chocolate products are rich in flavonoids, the most represented group of polyphenols, and flavanol monomers, procyanidin oligomers, and polymers are the most numerous flavonoids.[48]

Even methylxanthines, which in cocoa are mainly theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine),[50] depend on the origin of cocoa beans: the theobromine/caffeine ratio and the amount of caffeine present in chocolate depend on the cocoa-growing geographical area.[47]

The amount of methylxanthines is also different in the different types of chocolate. In fact, using food composition data from the U.S. Department of Agriculture (USDA) databases,[6] it is possible to observe that the average caffeine content is 45 mg per 100 g of dark chocolate (45−59% cacao solids), 86 mg per 100 g of dark chocolate (60−69%), and 80 mg per 100 g of dark chocolate (70−85%). The average theobromine contents are 493, 632, and 802 mg, respectively.[6]

Flavonoids and methylxanthines are the most recognizable active components of cacao. Although concerns regarding the bioavailability and the extent to which phenols are biologically active have not been completely clarified,[51−57] numerous capabilities have been assigned to cocoa flavonoids. Several studies analyzing the biological properties of these molecules have provided considerable supportive evidence of flavonoid's role in improving vascular functions, preventing related endothelial dysfunctions and diseases,[58−66] reducing insulin-resistance indices[62,67] (probably as a result of a decrease in insulin secretion[67]), increasing insulin-sensitivity,[62,68] contributing to anti-inflammatory reactions,[69−73] inhibiting platelet aggregation and activation,[74−77] and decreasing blood pressure.[60,68,78−81] However, a meta-analysis of randomized trials does not confirm the flavonoid effect on systolic blood pressure,[67] whereas a second meta-analysis has found a small but statistically significant effect in lowering systolic and diastolic blood pressure in the short term.[82] Multiple trials have emphasized that cocoa has the ability to change the lipid fraction not only by limiting its oxidation but also by directly altering the lipid profile of plasma, increasing high-density lipoprotein (HDL)[83−86] and reducing LDL[71,83−85,87] and total cholesterol.[84] Flavonoid effects on human total plasma antioxidant capacity,[57,88−90] neurocognition, mood, and behavior remain controversial and limited.[91−96] Some studies have shown how cocoa intake is correlated with a low prevalence of hypertension, atherosclerosis, dyslipidemia, and diabetes[97,98] and cardiovascular disease[99,100] as well as mortality and morbidity due to common causes.[101] The protection of cardiovascular and endothelial tissue, as a consequence of chocolate consumption, may arise by the synergetic action of flavonoids, theobromine, and magnesium.[102] Cocoa flavonoids also seem to improve intestinal flora.[103,104] Even dark chocolate seems to change significantly gut microbial metabolism.[105,106]

The effects of methylxanthines contained in cocoa and chocolate have been summarized in a recent review by Franco et al.[107] Theobromine, the most abundant methylxanthine in cocoa and chocolate, can suppress cough[108] and influence in a positive way cognitive performance and mood together with caffeine.[109] In a study intended to probe the effects of theobromine on blood pressure, no change was observed on 24 h ambulatory or central systolic blood pressure after 3 weeks' intake of a natural dose of theobromine cocoa (106 mg), whereas intake of theobromine-enriched cocoa (979 mg) resulted in increased 24 h ambulatory systolic blood pressure and lower central systolic blood pressure.[110] According to the results of a study, the HDL cholesterol-raising effect of cocoa intake is mainly due to the theobromine content.[111]

Enhancements in psychological functioning induced by consuming chocolate with a high cocoa content are due, at least in part, to the combination of methylxanthines,[109] biogenic amines,[112] anandamide (*N*-arachidonoyl-ethanolamine), and *N*-acylethanolamines actions. The main monoamines contained in cocoa are phenylethylamine, tyramine, and tryptamine. Tyramine and phenylethylamine have the ability to act on different areas of the brain, which are responsible for mood control and the waking state. They are able to delay fatigue and induce the release of catecholamines (norepinephrine and dopamine) at the synaptic level, which consequently produces stimulation similar to that induced by amphetamine, including an attenuation of the sensation of hunger. Phenylethylamine, assisted by magnesium (found in abundance in chocolate) and small amounts of serotonin, acts on mood.

Anandamide, an endogenous lipid belonging to the class of endocannabinoids, acts as a cannabinoid receptor agonist, mimicking the central and peripheral action of cannabinoids such as Δ9-tetrahydrocannabinol (the active ingredient of cannabis extracts). Although an interesting double-blind trial has recognized a marginal role of all psychoactive substances

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935

apart from xanthine,[109] the biological activity of amines and anandamide may contribute to the feeling of reward derived from the act of consuming chocolate and the pleasant post-consumption euphoria. Studies carried out on the abuse of stimulants suggest a causal relationship to self-therapy for mood control resulting from cocoa intake.[113]

COCOA AND CHOCOLATE CONSTITUENTS: EFFECTS ON HUMAN REPRODUCTION

Cocoa consumption affects reproduction not only by its effect upon sexual appetite but also by possibly interfering with the etiology of subfertility. Cocoa polyphenols have been shown to be potent antioxidants, and oxidative stress (an imbalance between pro-oxidants and antioxidants due to decreased antioxidant defense mechanisms or an increase in reactive oxygen and/or reactive nitrogen species) is known to be involved with the causes of male and female infertility, reproductive diseases (including endometriosis, polycystic ovary syndrome, and unexplained infertility), and pregnancy complications (namely, pre-eclampsia and miscarriages).[114]

Although attempts have been made to prevent reproductive disorders through antioxidant supplementation, the effectiveness of this strategy has not been demonstrated.[115] For this reason, positive contributions of the phytochemical cocoa in preventing reproductive problems can only be speculated, but the possible correlation continues to fuel exploration through high-quality clinical trials. Moreover, the bioactive constituents of cocoa may contribute to reducing reproductive difficulties through actions directly exerted on the vascular endothelium and circulation.

In further regard of its effects on reproductive capacity, it must also be noted that even in low doses, cocoa contains caffeine, a molecule known to cross the placental barrier freely[116] and which is slowly metabolized during pregnancy. The fetus does not have sufficient enzymes to inactivate caffeine;[117,118] thus, its metabolites accumulate in the fetal brain.[116,119] Caffeine, if consumed in large amounts, may seemingly result in a reduced fecundability,[120,121] although other prospective studies have demonstrated either little or no effect[122,123] or even an increased fecundability.[124] Caffeine has also been positively correlated with spontaneous abortion, congenital malformation, fetal death, fetal growth restriction, preterm delivery, and decreased birth weight. Again, some studies have yielded conflicting results,[125−132] although caffeine has been conclusively demonstrated to decrease fetal weight and increase the risk of "small for gestational age" fetal development.[133] In any case, caffeine seems to compromise normal reproduction function and to increase embryo−fetal risks. Accordingly, the World Health Organization's recommended threshold of caffeine consumption (200 mg/day in Nordic countries[134] and 300 mg/day equivalent to 4.6 mg/kg of body weight/day in a 65 kg person in the United States[135]) should be well adhered to.[136] Chocolate's added contribution to caffeine intake should be noted, particularly when other drinks rich in caffeine are already included in a diet.

COCOA AND CHOCOLATE CONSTITUENTS: EFFECTS ON HUMAN PREGNANCY

During pregnancy so many hemodynamic and metabolic changes occur that considerable attention to maternal and fetal nutrition is warranted. From Prochownich's early 20th century publication about the relationship of diet to pregnancy, attention to maternal−fetal nutrition has grown in terms of improving the end point of fetal health globally. During gestation, biomolecular metabolism and cellular redox activity undergo changes that shift the balance in favor of oxidizing agents and pro-oxidants,[137−139] thereby reducing total plasma antioxidant capacity in advanced gestation.[140]

The oxidation−reduction imbalance leads to oxidative stress-linked pathological conditions concerning both the mother and the fetus and, relatedly, the susceptibility of embryonic and syncytiotrophoblastic cells to oxidative damage. The alteration of the redox status is a constant of gestation, but its extent is significantly higher in cases leading to spontaneous abortion and in pregnancies with pre-eclampsia conditions or with hypertension alone.[141] The oxidative imbalance[140] in pregnancy and the need for an additional caloric intake from the 10th to the 13th week of gestation[142] represent valid reasons to choose foods with antioxidant properties. The availability of foods with these characteristics and the inclusion of these foods in the diet of pregnant women are considered new strategies and lead to scrupulous food choices that combine a modest energetic intake (between 100 and 400 kcal, as a function of the index of prepregnancy body mass) with a high content of antioxidant molecules nutritional profiles. Available scientific evidence suggests that chocolate with a high cocoa content, consumed daily in modest quantities (30 g/day for 24 weeks), may fit properly into this nutritional strategy without entailing negative consequences in terms of weight during various trimesters.[143] Anyway, it is recommended not to overdo with consumption of chocolate and cocoa products because most of these are high-calorie foods. As a result, they should be consumed in moderation, especially during pregnancy, and the amount of chocolate to be consumed should be calculated on the basis of the specific calories of the chocolate chosen, the caloric intake derived from other foods, the physical activity level, and the maternal BMI.

Chocolate supplementation during pregnancy, from the end of the first trimester to term, appears to reduce systolic and diastolic blood pressure during the weeks of gestation (Table 1).[143] A second study, a pilot randomized controlled trial, found no association between chocolate intake (20 g/day for 12 weeks) and blood pressure levels and even between chocolate consumption and flow-mediated dilation (Table 1).[144] We believe that the diverging results on blood pressure are due, in part, to the intervention of control: in the first study,[143] women of the control group did not consume chocolate by protocol, whereas in the second study,[144] women in the control group consumed chocolate as in the experimental group but it had a lesser amount of flavanols (400 mg of total flavanols vs <60 mg). It could be that the absence of differences is due to similar effects of the two kinds of chocolate, which had the same nutrients and bioactive components, except for flavanols. It is reasonable to assume that the observed effects of chocolate are not solely and directly due to polyphenols content, but it is possible to assume the existence of synergistic interactions between bioactive compounds. In this way, the amount of polyphenols would not be so important. We think that the main reason for diverging results on blood pressure in the two studies[143,144] is that the two protocols consisted of interventions with very different timing: one of about 27 weeks (from the end of the first trimester to term)[143] and one of 12 weeks (from about the 12th week of gestation).[144] We hope other studies will be carried out on this topic in order to understand the real effect of chocolate consumption on maternal blood pressure in pregnancy.

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935

Table 1. Blood Pressure in Pregnant Women after Daily Consumption of Chocolate[a]

type of study	groups		chocolate composition	beginning of chocolate intake	duration of chocolate intake	results
			Di Renzo et al., 2012[143]			
RCT	46 pregnant women consumed 30 g daily of dark chocolate 70% NFCS + a program of nutrition education	44 pregnant women with no intervention + a program of nutrition education	dark chocolate 70% NFCS: c+e = 12.6 mg; theobromine = 194 mg; caffeine = 24 mg	12.1 ± 0.6 weeks of GA versus 12.0 ± 0.6 weeks of GA	entire second and third trimesters of pregnancy	↓ SBP; ↓ DBP
			Mogollon et al., 2013[144]			
pilot RCT	23 pregnant women consumed 20 g daily of HF chocolate	21 pregnant women consumed 20 g daily of LF chocolate	HF chocolate: flavanol = 400 mg; c+e = 64 mg; theobromine = 150 mg; caffeine = 23.6 mg LF chocolate: flavanol < 60 mg; c+e = 14 mg; theobromine = 150 mg; caffeine = 23.6 mg	21.13 ± 1.1 weeks of GA versus 21.10 ± 1.61 weeks of GA	12 weeks	↔ SBP; ↔ DBP; ↔ FMD

[a]RCT, randomized control trial; NFCS, nonfat cocoa solid; HF, high-flavanol; LF, low-flavanol; c+e, catechin + epicatechin; GA, gestational age; ↓ decrease; ↔ no change; SBP, systolic blood pressure; DBP, diastolic blood pressure; FMD, flow mediated dilation.

We propose that a placebo chocolate free of any bioactive compound be used in future studies.

It has been suggested that chocolate consumption in pregnancy could be a reasonable strategy to prevent pre-eclampsia. Considering the characteristics and risk factors of pre-eclampsia, including maternal hypertension, placental disease, endothelial dysfunction, oxidative stress, and lack of nitric oxide,[145,146] it is reasonable to think about the possibility of preventing pre-eclampsia by up-regulating nitric oxide (NO) availability due to antioxidant activity and the induction of NO-dependent vasodilatation by cocoa. In this regard, two recent studies, a cohort study and a control case one, have detected chocolate consumption's contribution in reducing risks of pre-eclampsia and gestational hypertension.[102,147] Anyway, a case-control study conducted on 2769 women found no association between chocolate consumption and reduced occurrence of pre-eclampsia.[148]

Probably the systematic review already planned[149] will provide more detailed answers about the possible connections between chocolate consumption and the risk of pre-eclampsia.

Although not all of cocoa's potential health benefits to pregnant women have been clearly confirmed, chocolate consumption during pregnancy has proven positive in some accounts and harmless in others.[143] Overall, there is a natural female preference for chocolate that becomes markedly apparent during pregnancy, with an increasing trend following the progress of gestation.[150] Chocolate in the balanced diet of a pregnant woman can instill psychological well-being to both the pregnant woman (typically during the time of high emotional lability) and the future child.[151] The mother's prenatal stress experience significantly predicted the infant's fear responses, but in this relationship, maternal chocolate consumption during pregnancy appears to have the role of modulator. In fact, chocolate consumption in pregnancy seems to reduce the negative effect of prenatal maternal stress on infant temperament.[151] Furthermore, daily consumption of chocolate during pregnancy seems to determine in infants at 6 months a greater positive reactivity and activity.[151]

■ CONCLUSIONS

Evidence shows that chocolate is able to produce beneficial effects for human health. However, some cocoa activities on humans should be investigated again. Currently it is possible to conclude that consuming chocolate in moderation is good for human health. Chocolate can also be used in the diet of pregnant women because no negative effect was found for either maternal or fetal health. Conversely, favorable effects were observed for mother, fetus, and future child. Future studies will reveal whether the cocoa products have also preventive effects of some complications of pregnancy.

Furthermore, because chocolate has many bioactive compounds, we hope that in the near future the labels of cocoa products will also report the kind and amount of bioactive substances. In this way everyone may consciously choose what to buy and consume; moreover, pregnant women could control the levels of intake of caffeine.

■ AUTHOR INFORMATION

Corresponding Author

*(E.B.) E-mail: eleonora.brillo@unipg.it.

Notes

The authors declare no competing financial interest.

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935

REFERENCES

(1) Belščak, A.; Komes, D.; Horžić, D.; Ganić, K. K.; Karlović, D. Comparative study of commercially available cocoa products in terms of their bioactive composition. *Food Res. Int.* **2009**, *42* (5−6), 707−716.

(2) Keen, C. L. Chocolate: food as medicine/medicine as food. *J. Am. Coll. Nutr.* **2001**, *20* (5 Suppl.), 436S−439S discussion 440S−442S..

(3) Crozier, S. J.; Preston, A. G.; Hurst, J. W.; Payne, M. J.; Mann, J.; Hainly, L.; Miller, D. L. Cacao seeds are a "Super Fruit": a comparative analysis of various fruit powders and products. *Chem. Cent. J.* **2011**, *5*, 5.

(4) Ackar, D.; Valek Lendić, K.; Valek, M.; Šubarić, D.; Miličević, B.; Babić, J.; Nedić, I. Cocoa polyphenols: can we consider cocoa and chocolate as potential functional food? *J. Chem.* **2013**, *2013*, 110.1155/2013/289392

(5) Directive 2000/36/EC of the European Parliament and the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption; pp OJ L 197, 19−25.

(6) U.S. Department of Agriculture (USDA) National Nutrient Database for Standard Reference, release 27; http://ndb.nal.usda.gov/ndb/ (accessed Nov 26, 2014).

(7) Torres-Moreno, M.; Torrescasana, E.; Salas-Salvadó, J.; Blanch, C. Nutritional composition and fatty acids profile in cocoa beans and chocolates with different geographical origin and processing conditions. *Food Chem.* **2015**, *166*, 125−32.

(8) Liendo, R.; Padilla, F.; Quintana, A. Characterization of cocoa butter extracted from Criollo cultivars of *Theobroma cacao* L. *Food Res. Int.* **1997**, *30* (9), 727−731.

(9) Buchgraber, M.; Ulberth, F.; Anklam, E. Cluster analysis for the systematic grouping of genuine cocoa butter and cocoa butter equivalent samples based on triglyceride patterns. *J. Agric. Food Chem.* **2004**, *52* (12), 3855−3860.

(10) Padilla, F. C.; Liendo, R.; Quintana, A. Characterization of cocoa butter extracted from hybrid cultivars of *Theobroma cacao* L. *Arch. Latinoam Nutr.* **2000**, *50* (2), 200−205.

(11) Suzuki, R. M.; Montanher, P. F.; Visentainer, J. V.; de Souza, N. E. Proximate composition and quantification of fatty acids in five major Brazilian chocolate brands. *Cienc. Tecnol. Aliment.* **2011**, *31* (2), 541−546.

(12) Çakmak, Y. S.; Güler, G. Ö.; Aktümsek, A. Trans fatty acid contents in chocolates and chocolate wafers in Turkey. *Czech J. Food Sci.* **2010**, *28* (3), 177−184.

(13) Tholstrup, T.; Marckmann, P.; Jespersen, J.; Sandström, B. Fat high in stearic acid favorably affects blood lipids and factor VII coagulant activity in comparison with fats high in palmitic acid or high in myristic and lauric acids. *Am. J. Clin. Nutr.* **1994**, *59* (2), 371−377.

(14) Louheranta, A. M.; Turpeinen, A. K.; Schwab, U. S.; Vidgren, H. M.; Parviainen, M. T.; Uusitupa, M. I. A high-stearic acid diet does not impair glucose tolerance and insulin sensitivity in healthy women. *Metab., Clin. Exp.* **1998**, *47* (5), 529−534.

(15) Bonanome, A.; Grundy, S. M. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N. Engl. J. Med.* **1988**, *318* (19), 1244−1248.

(16) Snook, J. T.; Park, S.; Williams, G.; Tsai, Y.-H.; Lee, N. Effect of synthetic triglycerides of myristic, palmitic, and stearic acid on serum lipoprotein metabolism. *Eur. J. Clin. Nutr.* **1999**, *53* (8), 597−605.

(17) Kelly, F. D.; Sinclair, A. J.; Mann, N. J.; Turner, A. H.; Abedin, L.; Li, D. A stearic acid-rich diet improves thrombogenic and atherogenic risk factor profiles in healthy males. *Eur. J. Clin. Nutr.* **2001**, *55* (2), 88−96.

(18) Kelly, F. D.; Sinclair, A. J.; Mann, N. J.; Turner, A. H.; Raffin, F. L.; Blandford, M. V.; Pike, M. J. Short-term diets enriched in stearic or palmitic acids do not alter plasma lipids, platelet aggregation or platelet activation status. *Eur. J. Clin. Nutr.* **2002**, *56* (6), 490−499.

(19) Mensink, R. P.; Zock, P. L.; Kester, A. D.; Katan, M. B. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr.* **2003**, *77* (5), 1146−1155.

(20) Mensink, R. P. Effects of stearic acid on plasma lipid and lipoproteins in humans. *Lipids* **2005**, *40* (12), 1201−1205.

(21) Thijssen, M. A.; Mensink, R. P. Small differences in the effects of stearic acid, oleic acid, and linoleic acid on the serum lipoprotein profile of humans. *Am. J. Clin. Nutr.* **2005**, *82* (3), 510−516.

(22) Kris-Etherton, P. M.; Griel, A. E.; Psota, T. L.; Gebauer, S. K.; Zhang, J.; Etherton, T. D. Dietary stearic acid and risk of cardiovascular disease: intake, sources, digestion, and absorption. *Lipids* **2005**, *40* (12), 1193−1200.

(23) Kris-Etherton, P. M.; Pearson, T. A.; Wan, Y.; Hargrove, R. L.; Moriarty, K.; Fishell, V.; Etherton, T. D. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *Am. J. Clin. Nutr.* **1999**, *70* (6), 1009−1015.

(24) Hunter, K. A.; Crosbie, L. C.; Weir, A.; Miller, G. J.; Dutta-Roy, A. K. A residential study comparing the effects of diets rich in stearic acid, oleic acid, and linoleic acid on fasting blood lipids, hemostatic variables and platelets in young healthy men. *J. Nutr. Biochem.* **2000**, *11* (7−8), 408−416.

(25) Hu, F. B.; Manson, J. E.; Willett, W. C. Types of dietary fat and risk of coronary heart disease: a critical review. *J. Am. Coll. Nutr.* **2001**, *20* (1), 5−19.

(26) Thijssen, M. A.; Hornstra, G.; Mensink, R. P. Stearic, oleic, and linoleic acids have comparable effects on markers of thrombotic tendency in healthy human subjects. *J. Nutr.* **2005**, *135* (12), 2805−2811.

(27) Hooper, L.; Thompson, R. L.; Harrison, R. A.; Summerbell, C. D.; Moore, H.; Worthington, H. V.; Durrington, P. N.; Ness, A. R.; Capps, N. E.; Davey Smith, G.; Riemersma, R. A.; Ebrahim, S. B. Omega 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database Syst. Rev.* **2004**, *4*, CD003177.

(28) Donfrancesco, C.; Lo Noce, C.; Brignoli, O.; Riccardi, G.; Ciccarelli, P.; Dima, F.; Palmieri, L.; Giampaoli, S. Italian network for obesity and cardiovascular disease surveillance: a pilot project. *BMC Fam. Pract.* **2008**, *9*, 53.

(29) Oracz, J.; Nebesny, E. Influence of roasting conditions on the biogenic amine content in cocoa beans of different *Theobroma cacao* cultivars. *Food Res. Int.* **2014**, *55*, 1−10.

(30) Hansen, C. E.; del Olmo, M.; Burri, C. Enzyme activities in cocoa beans during fermentation. *J. Sci. Food Agric.* **1998**, *77*, 273−281.

(31) Adamson, G. E.; Lazarus, S. A.; Mitchell, A. E.; Prior, R. L.; Cao, G.; Jacobs, P. H.; Kremers, B. G.; Hammerstone, J. F.; Rucker, R. B.; Ritter, K. A.; Schmitz, H. H. 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), 4184−4188.

(32) Wollgast, J.; Anklam, E. Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Res. Int.* **2000**, *33* (6), 423−447.

(33) Luna, F.; Crouzillat, D.; Cirou, L.; Bucheli, P. Chemical composition and flavor of Ecuadorian cocoa liquor. *J. Agric. Food Chem.* **2002**, *50* (12), 3527−3532.

(34) Counet, C.; Ouwerx, C.; Rosoux, D.; Collin, S. Relationship between procyanidin and flavor contents of cocoa liquors from different origins. *J. Agric. Food Chem.* **2004**, *52* (20), 6243−6249.

(35) Miller, K. B.; Stuart, D. A.; Smith, N. L.; Lee, C. Y.; McHale, N. L.; Flanagan, J. A.; Ou, B.; Hurst, W. J. Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States. *J. Agric. Food Chem.* **2006**, *54* (11), 4062−4068.

(36) Gu, L.; House, S. E.; Wu, X.; Ou, B.; Prior, R. L. Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. *J. Agric. Food Chem.* **2006**, *54* (11), 4057−4061.

(37) Cooper, K. A.; Campos-Giménez, E.; Jiménez Alvarez, D.; Nagy, K.; Donovan, J. L.; Williamson, G. Rapid reversed phase ultra-performance liquid chromatography analysis of the major cocoa polyphenols and inter-relationships of their concentrations in chocolate. *J. Agric. Food Chem.* **2007**, *55* (8), 2841−2847.

(38) Ortega, N.; Romero, M. P.; Macià, A.; Reguant, J.; Anglès, N.; Morelló, J. R.; Motilva, M. J. Obtention and characterization of

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935

phenolic extracts from different cocoa sources. *J. Agric. Food Chem.* **2008**, *56* (20), 9621−9627.
(39) Schinella, G.; Mosca, S.; Cienfuegos-Jovellanos, E.; Pasamar, M. Á.; Muguerza, B.; Ramón, D.; Ríos, J. L. Antioxidant properties of polyphenol-rich cocoa products industrially processed. *Food Res. Int.* **2010**, *43* (6), 1614−1623.
(40) Ioannone, F.; Di Mattia, C. D.; De Gregorio, M.; Sergi, M.; Serafini, M.; Sacchetti, G. Flavanols, proanthocyanidins and antioxidant activity changes during cocoa (*Theobroma cacao* L.) roasting as affected by temperature and time of processing. *Food Chem.* **2015**, *174*, 256−262.
(41) Andres-Lacueva, C.; Monagas, M.; Khan, N.; Izquierdo-Pulido, M.; Urpi-Sarda, M.; Permanyer, J.; Lamuela-Raventós, R. M. Flavanol and flavonol contents of cocoa powder products: influence of the manufacturing process. *J. Agric. Food Chem.* **2008**, *56* (9), 3111−3117.
(42) Miller, K. B.; Hurst, W. J.; Payne, M. J.; Stuart, D. A.; Apgar, J.; Sweigart, D. S.; Ou, B. Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders. *J. Agric. Food Chem.* **2008**, *56* (18), 8527−8533.
(43) Stahl, L.; Miller, K. B.; Apgar, J.; Sweigart, D. S.; Stuart, D. A.; McHale, N.; Ou, B.; Kondo, M.; Hurst, W. J. Preservation of cocoa antioxidant activity, total polyphenols, flavan-3-ols, and procyanidin content in foods prepared with cocoa powder. *J. Food Sci.* **2009**, *74* (6), C456−C461.
(44) Hurst, W. J.; Krake, S.; Bergmeier, S.; Payne, M.; Miller, K.; Stuart, D. Impact of fermentation, drying, roasting and Dutch processing on flavan-3-ol stereochemistry in cacao beans and cocoa ingredients. *Chem. Cent. J.* **2011**, *5*, 53.
(45) Kim, H.; Keeney, P. G. (−)-Epicatechin content in fermented and unfermented cocoa beans. *J. Food Sci.* **1984**, *49* (4), 1090−1092.
(46) Natsume, M.; Osakabe, N.; Yamagishi, M.; Takizawa, T.; Nakamura, T.; Miyatake, H.; Hatano, T.; Yoshida, T. Analyses of polyphenols in cacao liquor, cocoa, and chocolate by normal-phase and reversed-phase HPLC. *Biosci., Biotechnol., Biochem.* **2000**, *64* (12), 2581−2587.
(47) Carrillo, L. C.; Londoño-Londoño, J.; Gil, A. Comparison of polyphenol, methylxanthines and antioxidant activity in *Theobroma cacao* beans from different cocoa-growing areas in Colombia. *Food Res. Int.* **2014**, *60*, 273−280.
(48) Langer, S.; Marshall, L. J.; Day, A. J.; Morgan, M. R. Flavanols and methylxanthines in commercially available dark chocolate: a study of the correlation with nonfat cocoa solids. *J. Agric. Food Chem.* **2011**, *59* (15), 8435−8441.
(49) Vinson, J. A.; Proch, J.; Zubik, L. Phenol antioxidant quantity and quality in foods: cocoa, dark chocolate, and milk chocolate. *J. Agric. Food Chem.* **1999**, *47* (12), 4821−4824.
(50) Timbie, D. J.; Sechrist, L.; Keeney, P. G. Application of high-pressure liquid chromatography to the study of variables affecting theobromine and caffeine concentrations in cocoa beans. *J. Food Sci.* **1978**, *43* (2), 560−565.
(51) Richelle, M.; Tavazzi, I.; Enslen, M.; Offord, E. A. Plasma kinetics in man of epicatechin from black chocolate. *Eur. J. Clin. Nutr.* **1999**, *53* (1), 22−26.
(52) Hollman, P. C.; Katan, M. B. Dietary flavonoids: intake, health effects and bioavailability. *Food Chem. Toxicol.* **1999**, *37* (9−10), 937−942.
(53) Spencer, J. P.; Chaudry, F.; Pannala, A. S.; Srai, S. K.; Debnam, E.; Rice-Evans, C. Decomposition of cocoa procyanidins in the gastric milieu. *Biochem. Biophys. Res. Commun.* **2000**, *272* (1), 236−241.
(54) Wang, J. F.; Schramm, D. D.; Holt, R. R.; Ensunsa, J. L.; Fraga, C. G.; Schmitz, H. H.; Keen, C. L. A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. *J. Nutr.* **2000**, *130* (8S Suppl.), 2115S−2119S.
(55) Deprez, S.; Mila, I.; Huneau, J. F.; Tome, D.; Scalbert, A. Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxid. Redox Signal.* **2001**, *3* (6), 957−967.
(56) Rios, L. Y.; Bennett, R. N.; Lazarus, S. A.; Rémésy, C.; Scalbert, A.; Williamson, G. Cocoa procyanidins are stable during gastric transit in humans. *Am. J. Clin. Nutr.* **2002**, *76* (5), 1106−1110.
(57) Serafini, M.; Miglio, C.; Peluso, I.; Petrosino, T. Modulation of plasma non enzimatic antioxidant capacity (NEAC) by plant foods: the role of polyphenols. *Curr. Top. Med. Chem.* **2011**, *11* (14), 1821−1846.
(58) Karim, M.; McCormick, K.; Kappagoda, C. T. Effects of cocoa extracts on endothelium-dependent relaxation. *J. Nutr.* **2000**, *130* (8S Suppl.), 2105S−2108S.
(59) Fisher, N. D.; Hughes, M.; Gerhard-Herman, M.; Hollenberg, N. K. Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J. Hypertens.* **2003**, *21* (12), 2281−2286.
(60) Heiss, C.; Kleinbongard, P.; Dejam, A.; Perré, S.; Schroeter, H.; Sies, H.; Kelm, M. Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *J. Am. Coll. Cardiol.* **2005**, *46* (7), 1276−1283.
(61) Wang-Polagruto, J. F.; Villablanca, A. C.; Polagruto, J. A.; Lee, L.; Holt, R. R.; Schrader, H. R.; Ensunsa, J. L.; Steinberg, F. M.; Schmitz, H. H.; Keen, C. L. Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholesterolemic postmenopausal women. *J. Cardiovasc. Pharmacol.* **2006**, *47* (Suppl. 2), S177−S186 discussion S206−S209..
(62) Heiss, C.; Finis, D.; Kleinbongard, P.; Hoffmann, A.; Rassaf, T.; Kelm, M.; Sies, H. Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week. *J. Cardiovasc. Pharmacol.* **2007**, *49* (2), 74−80.
(63) Balzer, J.; Rassaf, T.; Heiss, C.; Kleinbongard, P.; Lauer, T.; Merx, M.; Heussen, N.; Gross, H. B.; Keen, C. L.; Schroeter, H.; Kelm, M. 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), 2141−2149.
(64) Grassi, D.; Desideri, G.; Necozione, S.; Ruggieri, F.; Blumberg, J. B.; Stornello, M.; Ferri, C. Protective effects of flavanol-rich dark chocolate on endothelial function and wave reflection during acute hyperglycemia. *Hypertension* **2012**, *60* (3), 827−832.
(65) Heiss, C.; Dejam, A.; Kleinbongard, P.; Schewe, T.; Sies, H.; Kelm, M. Vascular effects of cocoa rich in flavan-3-ols. *JAMA, J. Am. Med. Assoc.* **2003**, *290* (8), 1030−1031.
(66) Grassi, D.; Necozione, S.; Lippi, C.; Croce, G.; Valeri, L.; Pasqualetti, P.; Desideri, G.; Blumberg, J. B.; Ferri, C. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension* **2005**, *46* (2), 398−405.
(67) Hooper, L.; Kay, C.; Abdelhamid, A.; Kroon, P. A.; Cohn, J. S.; Rimm, E. B.; Cassidy, A. 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), 740−751.
(68) 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. *Am. J. Clin. Nutr.* **2005**, *81* (3), 611−614.
(69) Schramm, D. D.; Wang, J. F.; Holt, R. R.; Ensunsa, J. L.; Gonsalves, J. L.; Lazarus, S. A.; Schmitz, H. H.; German, J. B.; Keen, C. L. Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. *Am. J. Clin. Nutr.* **2001**, *73* (1), 36−40.
(70) Selmi, C.; Mao, T. K.; Keen, C. L.; Schmitz, H. H.; Eric Gershwin, M. The anti-inflammatory properties of cocoa flavanols. *J. Cardiovasc. Pharmacol.* **2006**, *47* (Suppl. 2), S163−S171 discussion S172−176..
(71) Selmi, C.; Cocchi, C. A.; Lanfredini, M.; Keen, C. L.; Gershwin, M. E. Chocolate at heart: the anti-inflammatory impact of cocoa flavanols. *Mol. Nutr. Food Res.* **2008**, *52* (11), 1340−1348.
(72) Khan, N.; Khymenets, O.; Urpí-Sardà, M.; Tulipani, S.; Garcia-Aloy, M.; Monagas, M.; Mora-Cubillos, X.; Llorach, R.; Andres-Lacueva, C. Cocoa polyphenols and inflammatory markers of cardiovascular disease. *Nutrients* **2014**, *6* (2), 844−880.

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935

(73) Kenny, T. P.; Keen, C. L.; Schmitz, H. H.; Gershwin, M. E. Immune effects of cocoa procyanidin oligomers on peripheral blood mononuclear cells. *Exp. Biol. Med. (Maywood)* **2007**, *232* (2), 293−300.
(74) Rein, D.; Paglieroni, T. G.; Pearson, D. A.; Wun, T.; Schmitz, H. H.; Gosselin, R.; Keen, C. L. Cocoa and wine polyphenols modulate platelet activation and function. *J. Nutr.* **2000**, *130* (8S Suppl.), 2120S−2126S.
(75) Pearson, D. A.; Paglieroni, T. G.; Rein, D.; Wun, T.; Schramm, D. D.; Wang, J. F.; Holt, R. R.; Gosselin, R.; Schmitz, H. H.; Keen, C. L. The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. *Thromb. Res.* **2002**, *106* (4−5), 191−197.
(76) Holt, R. R.; Schramm, D. D.; Keen, C. L.; Lazarus, S. A.; Schmitz, H. H. Chocolate consumption and platelet function. *JAMA, J. Am. Med. Assoc.* **2002**, *287* (17), 2212−2213.
(77) Murphy, K. J.; Chronopoulos, A. K.; Singh, I.; Francis, M. A.; Moriarty, H.; Pike, M. J.; Turner, A. H.; Mann, N. J.; Sinclair, A. J. Dietary flavanols and procyanidin oligomers from cocoa (*Theobroma cacao*) inhibit platelet function. *Am. J. Clin. Nutr.* **2003**, *77* (6), 1466−1473.
(78) Taubert, D.; Berkels, R.; Roesen, R.; Klaus, W. Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. *JAMA, J. Am. Med. Assoc.* **2003**, *290* (8), 1029−1030.
(79) Grassi, D.; Desideri, G.; Necozione, S.; Lippi, C.; Casale, R.; Properzi, G.; Blumberg, J. B.; Ferri, C. 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), 1671−1676.
(80) Alleyne, T.; Alleyne, A.; Arrindell, D.; Balleram, N.; Cozier, D.; Haywood, R.; Humphrey, C.; Pran, L.; Rampersad, K.; Reyes, D.; Bahall, S.; Holder, R.; Ignacio, D. Short term effects of cocoa consumption on blood pressure. *West Indian Med. J.* **2013**, *63* (4), 316−321.
(81) Taubert, D.; Roesen, R.; Lehmann, C.; Jung, N.; Schömig, E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. *JAMA, J. Am. Med. Assoc.* **2007**, *298* (1), 49−60.
(82) Ried, K.; Sullivan, T. R.; Fakler, P.; Frank, O. R.; Stocks, N. P. Effect of cocoa on blood pressure. *Cochrane Database Syst. Rev.* **2012**, *8*, CD008893.
(83) Wan, Y.; Vinson, J. A.; Etherton, T. D.; Proch, J.; Lazarus, S. A.; Kris-Etherton, P. M. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *Am. J. Clin. Nutr.* **2001**, *74* (5), 596−602.
(84) Baba, S.; Natsume, M.; Yasuda, A.; Nakamura, Y.; Tamura, T.; Osakabe, N.; Kanegae, M.; Kondo, K. Plasma LDL and HDL cholesterol and oxidized LDL concentrations are altered in normo- and hypercholesterolemic humans after intake of different levels of cocoa powder. *J. Nutr.* **2007**, *137* (6), 1436−1441.
(85) Fraga, C. G.; Actis-Goretta, L.; Ottaviani, J. I.; Carrasquedo, F.; Lotito, S. B.; Lazarus, S.; Schmitz, H. H.; Keen, C. L. Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. *Clin. Dev. Immunol.* **2005**, *12* (1), 11−17.
(86) Baba, S.; Osakabe, N.; Kato, Y.; Natsume, M.; Yasuda, A.; Kido, T.; Fukuda, K.; Muto, Y.; Kondo, K. Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. *Am. J. Clin. Nutr.* **2007**, *85* (3), 709−717.
(87) Mathur, S.; Devaraj, S.; Grundy, S. M.; Jialal, I. Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. *J. Nutr.* **2002**, *132* (12), 3663−3667.
(88) Rein, D.; Lotito, S.; Holt, R. R.; Keen, C. L.; Schmitz, H. H.; Fraga, C. G. Epicatechin in human plasma: in vivo determination and effect of chocolate consumption on plasma oxidation status. *J. Nutr.* **2000**, *130* (8S Suppl.), 2109S−2114S.
(89) Serafini, M.; Bugianesi, R.; Maiani, G.; Valtuena, S.; De Santis, S.; Crozier, A. Plasma antioxidants from chocolate. *Nature* **2003**, *424* (6952), 1013.
(90) Scheid, L.; Reusch, A.; Stehle, P.; Ellinger, S. Antioxidant effects of cocoa and cocoa products ex vivo and in vivo: is there evidence from controlled intervention studies? *Curr. Opin. Clin. Nutr. Metab. Care* **2010**, *13* (6), 737−742.
(91) Crews, W. D.; Harrison, D. W.; Wright, J. W. 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. *Am. J. Clin. Nutr.* **2008**, *87* (4), 872−880.
(92) Nurk, E.; Refsum, H.; Drevon, C. A.; Tell, G. S.; Nygaard, H. A.; Engedal, K.; Smith, A. D. Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. *J. Nutr.* **2009**, *139* (1), 120−127.
(93) Scholey, A. B.; French, S. J.; Morris, P. J.; Kennedy, D. O.; Milne, A. L.; Haskell, C. F. Consumption of cocoa flavanols results in acute improvements in mood and cognitive performance during sustained mental effort. *J. Psychopharmacol.* **2010**, *24* (10), 1505−1514.
(94) Sathyapalan, T.; Beckett, S.; Rigby, A. S.; Mellor, D. D.; Atkin, S. L. High cocoa polyphenol rich chocolate may reduce the burden of the symptoms in chronic fatigue syndrome. *Nutr. J.* **2010**, *9*, 55.
(95) Sokolov, A. N.; Pavlova, M. A.; Klosterhalfen, S.; Enck, P. Chocolate and the brain: Neurobiological impact of cocoa flavanols on cognition and behavior. *Neurosci. Biobehav. Rev.* **2013**, *37* (10, Part 2), 2445−2453.
(96) Pase, M. P.; Scholey, A. B.; Pipingas, A.; Kras, M.; Nolidin, K.; Gibbs, A.; Wesnes, K.; Stough, C. Cocoa polyphenols enhance positive mood states but not cognitive performance: a randomized, placebo-controlled trial. *J. Psychopharmacol.* **2013**, *27* (5), 451−8.
(97) McCullough, M. L.; Chevaux, K.; Jackson, L.; Preston, M.; Martinez, G.; Schmitz, H. H.; Coletti, C.; Campos, H.; Hollenberg, N. K. Hypertension, the Kuna, and the epidemiology of flavanols. *J. Cardiovasc. Pharmacol.* **2006**, *47* (Suppl. 2), S103−S109 discussion 119−121..
(98) K Hollenberg, N. Vascular action of cocoa flavanols in humans: the roots of the story. *J. Cardiovasc. Pharmacol.* **2006**, *47* (Suppl. 2), S99−S102 discussion S119−121..
(99) Buijsse, B.; Feskens, E. J.; Kok, F. J.; Kromhout, D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. *Arch. Intern. Med.* **2006**, *166* (4), 411−417.
(100) Janszky, I.; Mukamal, K. J.; Ljung, R.; Ahnve, S.; Ahlbom, A.; Hallqvist, J. Chocolate consumption and mortality following a first acute myocardial infarction: the Stockholm Heart Epidemiology Program. *J. Intern. Med.* **2009**, *266* (3), 248−257.
(101) Hollenberg, N. K.; Fisher, N. D.; McCullough, M. L. Flavanols, the Kuna, cocoa consumption, and nitric oxide. *J. Am. Soc. Hypertens.* **2009**, *3* (2), 105−112.
(102) Triche, E. W.; Grosso, L. M.; Belanger, K.; Darefsky, A. S.; Benowitz, N. L.; Bracken, M. B. Chocolate consumption in pregnancy and reduced likelihood of preeclampsia. *Epidemiology* **2008**, *19* (3), 459−464.
(103) Redovniković, I. R.; Delonga, K.; Mazor, S.; Dragović-Uzelac, V.; Carić, M.; Vorkapić-Furač, J. Polyphenolic content and composition and antioxidative activity of different cocoa liquors. *Czech J. Food Sci.* **2009**, *27* (5), 330−337.
(104) Tzounis, X.; Rodriguez-Mateos, A.; Vulevic, J.; Gibson, G. R.; Kwik-Uribe, C.; Spencer, J. P. Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *Am. J. Clin. Nutr.* **2011**, *93* (1), 62−72.
(105) Martin, F. P.; Rezzi, S.; Peré-Trepat, E.; Kamlage, B.; Collino, S.; Leibold, E.; Kastler, J.; Rein, D.; Fay, L. B.; Kochhar, S. Metabolic effects of dark chocolate consumption on energy, gut microbiota, and stress-related metabolism in free-living subjects. *J. Proteome Res.* **2009**, *8* (12), 5568−5579.

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935

(106) Martin, F. P.; Montoliu, I.; Nagy, K.; Moco, S.; Collino, S.; Guy, P.; Redeuil, K.; Scherer, M.; Rezzi, S.; Kochhar, S. Specific dietary preferences are linked to differing gut microbial metabolic activity in response to dark chocolate intake. *J. Proteome Res.* **2012**, *11* (12), 6252−6263.
(107) Franco, R.; Oñatibia-Astibia, A.; Martínez-Pinilla, E. Health benefits of methylxanthines in cacao and chocolate. *Nutrients* **2013**, *5* (10), 4159−4173.
(108) Usmani, O. S.; Belvisi, M. G.; Patel, H. J.; Crispino, N.; Birrell, M. A.; Korbonits, M.; Korbonits, D.; Barnes, P. J. Theobromine inhibits sensory nerve activation and cough. *FASEB J.* **2005**, *19*, 231−233.
(109) Smit, H. J.; Gaffan, E. A.; Rogers, P. J. Methylxanthines are the psycho-pharmacologically active constituents of chocolate. *Psychopharmacology (Berlin)* **2004**, *176* (3−4), 412−419.
(110) Van den Bogaard, B.; Draijer, R.; Westerhof, B. E.; van den Meiracker, A. H.; van Montfrans, G. A.; van den Born, B. J. 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), 839−846.
(111) Neufingerl, N.; Zebregs, Y. E.; Schuring, E. A.; Trautwein, E. A. Effect of cocoa and theobromine consumption on serum HDL-cholesterol concentrations: a randomized controlled trial. *Am. J. Clin. Nutr.* **2013**, *97* (6), 1201−1209.
(112) Pastore, P.; Favaro, G.; Badocco, D.; Tapparo, A.; Cavalli, S.; Saccani, G. Determination of biogenic amines in chocolate by ion chromatographic separation and pulsed integrated amperometric detection with implemented wave-form at Au disposable electrode. *J. Chromatogr. A* **2005**, *1098* (1−2), 111−115.
(113) Schifano, F.; Magni, G. MDMA ("ecstasy") abuse: psychopathological features and craving for chocolate: a case series. *Biol. Psychiatry* **1994**, *36* (11), 763−767.
(114) Agarwal, A.; Aponte-Mellado, A.; Premkumar, B. J.; Shaman, A.; Gupta, S. The effects of oxidative stress on female reproduction: a review. *Reprod. Biol. Endocrinol.* **2012**, *10*, 49.
(115) Showell, M. G.; Brown, J.; Clarke, J.; Hart, R. J. Antioxidants for female subfertility. *Cochrane Database Syst. Rev.* **2013**, *8*, CD007807.
(116) Aldridge, A.; Bailey, J.; Neims, A. H. The disposition of caffeine during and after pregnancy. *Semin. Perinatol.* **1981**, *5* (4), 310−314.
(117) Oesterheld, J. R. A review of developmental aspects of cytochrome P450. *J. Child Adolesc. Psychopharmacol.* **1998**, *8* (3), 161−174.
(118) Andersson, H.; Hallström, H.; Kihlman, B. A. *Intake of Caffeine and Other Methylxanthines during Pregnancy and Risk for Adverse Effects in Pregnant Women and Their Foetuses*; Nordic Council of Ministers: Copenhagen, Denmark, 2004.
(119) Wilkinson, J. M.; Pollard, I. Accumulation of theophylline, theobromine and paraxanthine in the fetal rat brain following a single oral dose of caffeine. *Dev. Brain Res.* **1993**, *75* (2), 193−199.
(120) Wilcox, A.; Weinberg, C.; Baird, D. Caffeinated beverages and decreased fertility. *Lancet* **1988**, *332* (8626−8627), 1453−1456.
(121) Jensen, T. K.; Henriksen, T. B.; Hjollund, N. H.; Scheike, T.; Kolstad, H.; Giwercman, A.; Ernst, E.; Bonde, J. P.; Skakkebaek, N. E.; Olsen, J. Caffeine intake and fecundability: a follow-up study among 430 Danish couples planning their first pregnancy. *Reprod. Toxicol.* **1998**, *12* (3), 289−295.
(122) Hakim, R. B.; Gray, R. H.; Zacur, H. Alcohol and caffeine consumption and decreased fertility. *Fertil. Steril.* **1998**, *70* (4), 632−637.
(123) Taylor, K. C.; Small, C. M.; Dominguez, C. E.; Murray, L. E.; Tang, W.; Wilson, M. M.; Bouzyk, M.; Marcus, M. Alcohol, smoking, and caffeine in relation to fecundability, with effect modification by NAT2. *Ann. Epidemiol.* **2011**, *21* (11), 864−872.
(124) Florack, E. I.; Zielhuis, G. A.; Rolland, R. Cigarette smoking, alcohol consumption, and caffeine intake and fecundability. *Prev. Med.* **1994**, *23* (2), 175−180.
(125) Cnattingius, S.; Signorello, L. B.; Annerén, G.; Clausson, B.; Ekbom, A.; Ljunger, E.; Blot, W. J.; McLaughlin, J. K.; Petersson, G.; Rane, A.; Granath, F. Caffeine intake and the risk of first-trimester spontaneous abortion. *N. Engl. J. Med.* **2000**, *343* (25), 1839−1845.
(126) Wen, W.; Shu, X. O.; Jacobs, D. R.; Brown, J. E. The associations of maternal caffeine consumption and nausea with spontaneous abortion. *Epidemiology* **2001**, *12* (1), 38−42.
(127) Giannelli, M.; Doyle, P.; Roman, E.; Pelerin, M.; Hermon, C. The effect of caffeine consumption and nausea on the risk of miscarriage. *Paediatr. Perinat. Epidemiol.* **2003**, *17* (4), 316−323.
(128) Bech, B. H.; Nohr, E. A.; Vaeth, M.; Henriksen, T. B.; Olsen, J. Coffee and fetal death: a cohort study with prospective data. *Am. J. Epidemiol.* **2005**, *162* (10), 983−990.
(129) Matijasevich, A.; Barros, F. C.; Santos, I. S.; Yemini, A. Maternal caffeine consumption and fetal death: a case-control study in Uruguay. *Paediatr. Perinat. Epidemiol.* **2006**, *20* (2), 100−109.
(130) Maconochie, N.; Doyle, P.; Prior, S.; Simmons, R. Risk factors for first trimester miscarriage − results from a UK-population-based case-control study. *BJOG* **2007**, *114* (2), 170−186.
(131) Weng, X.; Odouli, R.; Li, D. K. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. *Am. J. Obstet. Gynecol.* **2008**, *198* (3), 279.e1−279.e8.
(132) Peck, J. D.; Leviton, A.; Cowan, L. D. A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: a 2000−2009 update. *Food Chem. Toxicol.* **2010**, *48* (10), 2549−2576.
(133) Chen, L. W.; Wu, Y.; Neelakantan, N.; Chong, M.; Pan, A.; van Dam, R. M. Maternal caffeine intake during pregnancy is associated with risk of low birth weight: a systematic review and dose−response meta-analysis. *BMC Med.* **2014**, *12* (1), 174.
(134) NNR Project Group. *Nordic Nutrition Recommendations, Integrating Nutrition and Physical Activity*; Nordic Council of Ministers: Copenhagen, Denmark, 2004.
(135) American College of Obstetricians and Gynecologists (ACOG). ACOG Committee Opinion No. 462: Moderate caffeine consumption during pregnancy. *Obstet. Gynecol.* **2010**, *116* (2 Part1), 467−468.
(136) World Health Organization (WHO). *The World Health Report, Reducing Risks, Promoting Healthy Life*; World Health Organization: Geneva, Switzerland, 2002.
(137) Toescu, V.; Nuttall, S. L.; Martin, U.; Kendall, M. J.; Dunne, F. Oxidative stress and normal pregnancy. *Clin. Endocrinol. (Oxford, U. K.)* **2002**, *57* (5), 609−613.
(138) Ishihara, O.; Hayashi, M.; Osawa, H.; Kobayashi, K.; Takeda, S.; Vessby, B.; Basu, S. Isoprostanes, prostaglandins and tocopherols in pre-eclampsia, normal pregnancy and non-pregnancy. *Free Radical Res.* **2004**, *38* (9), 913−918.
(139) Palm, M.; Axelsson, O.; Wernroth, L.; Basu, S. F(2)-isoprostanes, tocopherols and normal pregnancy. *Free Radical Res.* **2009**, *43* (6), 546−552.
(140) Alberti-Fidanza, A.; Di Renzo, G. C.; Burini, G.; Antonelli, G.; Perriello, G. Diet during pregnancy and total antioxidant capacity in maternal and umbilical cord blood. *J. Matern.-Fetal Neonat. Med.* **2002**, *12*, 59−63.
(141) Jauniaux, E.; Poston, L.; Burton, G. J. Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. *Hum. Reprod. Update* **2006**, *12* (6), 747−755.
(142) Institute of Medicine (IOM). *DRI Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*; National Academy Press: Washington, DC, USA, 2002.
(143) Di Renzo, G. C.; Brillo, E.; Romanelli, M.; Porcaro, G.; Capanna, F.; Kanninen, T. T.; Gerli, S.; Clerici, G. Potential effects of chocolate on human pregnancy: a randomized controlled trial. *J. Matern.−Fetal Neonat. Med.* **2012**, *25* (10), 1860−1867.
(144) Mogollon, J. A.; Bujold, E.; Lemieux, S.; Bourdages, M.; Blanchet, C.; Bazinet, L.; Couillard, C.; Noël, M.; Dodin, S. Blood pressure and endothelial function in healthy, pregnant women after acute and daily consumption of flavanol-rich chocolate: a pilot, randomized controlled trial. *Nutr. J.* **2013**, *12* (1), 41.
(145) Magness, R. R.; Shaw, C. E.; Phernetton, T. M.; Zheng, J.; Bird, I. M. Endothelial vasodilator production by uterine and systemic

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935

arteries. II. Pregnancy effects on NO synthase expression. *Am. J. Physiol.* **1997**, *272* (4 Part 2), H1730−H1740.
(146) Widlansky, M. E.; Gokce, N.; Keaney, J. F.; Vita, J. A. The clinical implications of endothelial dysfunction. *J. Am. Coll. Cardiol.* **2003**, *42* (7), 1149−1160.
(147) Saftlas, A. F.; Triche, E. W.; Beydoun, H.; Bracken, M. B. Does chocolate intake during pregnancy reduce the risks of preeclampsia and gestational hypertension? *Ann. Epidemiol* **2010**, *20* (8), 584−591.
(148) Klebanoff, M. A.; Zhang, J.; Zhang, C.; Levine, R. J. Maternal serum theobromine and the development of preeclampsia. *Epidemiology* **2009**, *20* (5), 727−732.
(149) Mogollon, J. A.; Boivin, C.; Philippe, K.; Turcotte, S.; Lemieux, S.; Blanchet, C.; Bujold, E.; Dodin, S. Consumption of chocolate in pregnant women and risk of preeclampsia: a systematic review. *Syst. Rev.* **2013**, *2*, 114.
(150) Crozier, S. R.; Robinson, S. M.; Godfrey, K. M.; Cooper, C.; Inskip, H. M. Women's dietary patterns change little from before to during pregnancy. *J. Nutr.* **2009**, *139* (10), 1956−1963.
(151) Räikkönen, K.; Pesonen, A. K.; Järvenpää, A. L.; Strandberg, T. E. Sweet babies: chocolate consumption during pregnancy and infant temperament at six months. *Early Hum. Dev.* **2004**, *76* (2), 139−145.

DOI: 10.1021/acs.jafc.5b01045
J. Agric. Food Chem. 2015, 63, 9927−9935