High-flavanol and high-theobromine versus low-flavanol and low-theobromine chocolate to improve uterine artery pulsatility index: a double blind randomized clinical trial

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Condensation

Concentration of flavanol and theobromine in chocolate has no significant impact on uterine

artery pulsatility index changes between the first and second trimester of pregnancy.

Short version of title

Chocolate and uterine artery pulsatility index

ABSTRACT

OBJECTIVE: To evaluate the impact of high-flavanol and high-theobromine chocolate in women at risk of preeclampsia. **STUDY DESIGN:** We conducted a single-center randomized controlled trial including women with singleton pregnancy between 11 and 14 weeks gestation who had bilateral abnormal uterine artery (UtA) waveforms (notching) and elevated pulsatility index (PI). Participants were randomized to either high-flavanol and high-theobromine or low-flavanol and low-theobromine chocolate (30 grams daily for a total of 12 weeks). UtA PI, reported as multiple of medians (MoM) adjusted for gestational age, was assessed at baseline and 12 weeks after randomization. **RESULTS:** One hundred thirty-one women were randomized with mean gestational age of 12.4 ± 0.6 weeks and a mean UtA PI of 1.39 ± 0.31 MoM. UtA PI adjusted for gestational age significantly decreased from baseline to the second visit (12 weeks later) in the two groups (p<0.0001) but no significant difference was observed between the groups (p=0.16). **CONCLUSIONS:** Compared with low-flavanol and low-theobromine chocolate, daily intake of high-flavanol and high-theobromine chocolate was not associated with significant changes of UtA PI. Nevertheless, the improvement observed in both groups suggests that chocolate could improve placental function independently of flavanol and/or theobromine content.

Keywords : Pregnancy, preeclampsia, Doppler, chocolate, flavanol

INTRODUCTION

Preeclampsia (PE) is a multisystemic disorder that originates in early pregnancy and is one of the most common causes of maternal and fetal mortality and morbidity in the world, including developed countries [1-3]. PE, defined by new-onset gestational hypertension and proteinuria, is commonly associated with deep placentation disorders and is characterized by generalized maternal dysfunction of the endothelium [4, 5]. Despite intensive research, few prophylactic measures are available for PE [6]. Low-dose aspirin has been shown to reduce the risk of PE only when started in early pregnancy [7]. This situation underlines the importance to devote efforts in integrating methods aimed to detect pregnant women at risk of PE as part of usual antenatal care in early pregnancy. A recent meta-analysis has shown that uterine artery (UtA) Doppler, as early as the first-trimester, is a highly specific test for predicting early-onset PE with moderate sensitivity [8]. The UtA pulsatility index (PI) is typically increased in presence of PE and abnormal utero-placental circulation.

Some evidences suggest that therapeutic approaches focusing on up-regulating nitric oxide (NO) availability may be useful targets for PE prevention [9]. Flavanols, the most common flavonoids in dark chocolate, are potent antioxidants capable of inducing NO-dependent vasodilatation. Two recent meta-analyses of randomized controlled trials confirmed that flavanol-rich chocolate has a beneficial influence on endothelial function and reduces systolic and diastolic blood pressure [10, 11]. Theobromine, a major constituent of dark chocolate, also possesses vasodilatation and relaxing smooth tissue properties [12]. The objective of this study was therefore to assess the impact of high-flavanol and high-theobromine chocolate on UtA PI during pregnancy.

METHODOLOGY

Participants

Non-smoking, normotensive women between 20 and 38 years old, with a live singleton fetus were enrolled at the time of their 11-14 weeks' ultrasound. UtA Doppler was performed and women with bilateral diastolic notches and either a UtA PI>95th percentile on one side and/or bilateral UtA PI>50th percentile were considered as potentially eligible for randomization. Patients with essential hypertension requiring medication, using supplements or natural health products that potentially interfere with BP (fish oils, coenzyme Q10, garlic) were excluded from the study. Women that currently or previously used medication interfering with glucose or lipid metabolism, consumed ≥ 1 alcoholic beverage per day or presented an allergy or intolerance to nuts or chocolate were also excluded.

Recruitment and randomization

Women undergoing routine 11-14 weeks were informed about the project. Those found to be at high risk of PE according to UtA Doppler met the study coordinator for more details about the research project (**Figure 1**). An information and consent form, approved by the institutional Ethics Committee, was read and signed. Three BP measurements were taken at 3-minute intervals and averaged to obtain mean BP. Anthropometric variables were measured according to standardized procedures. Each woman fulfilled a questionnaire documenting social and demographic characteristics, consumption of alcohol and medications. Participants were asked to avoid consuming chocolate products seven days before their initial visit, as well as other foods

rich in flavonoids or theobromine such as tea, coffee, fruit juice and wine, 24 hours before the

visit. Foods rich in flavonoids consumed in the last month were assessed using a food frequency

questionnaire (FFQ). Eligible women were randomly assigned to either the high-flavanol and

high-theobromine (HFHT) or the low-flavanol and low-theobromine (LFLT) chocolate groups.

Computerized randomization was generated by blocks and stratified for parity and body mass

index (BMI) under the responsibility of an independent statistician. The higher risk of PE among

nulliparous women justified stratification by parity.

Incorporating instead of supplementing chocolate

The chocolate was supplied as chocolate bars by Barry-Callebaut (Zurich, Switzerland), a

company producing a chocolate bar with high polyphenol concentration. The company

developed a specific process that is used to preserve the flavanols from bean to chocolate. All

steps of the chocolate production including fermentation, drying, roasting and alkalization were

optimized to preserve the antioxidant content. Each 10g square contained 171 mg of flavanols

(see supplemental material). Chocolate bars were standardized for their flavanol and theobromine

content and matched for caloric load, nutrients and caffeine. All chocolate bars were similar in

taste and color and were supplied in individual, opaque packaging. Each 30g of chocolate

contained less than 18 mg of caffeine as Health Canada advises pregnant women to consume no

more than 300 mg caffeine per day. Women were advised to consume a 10g chocolate square

(about 50 kcal) provided by the study three times a day (i.e. at each meal) for a total of 30g/day

for 12 weeks. To avoid an increase in caloric intake, participants were instructed not to add

chocolate bars to their usual diet but instead to replace foods of similar energy and

macronutrients composition. To ensure support and motivation for substitution, pregnant women

were met individually by a dietitian and offered global counseling based on the Institute of Medicine guidelines.

Clinical follow-up

Participants were seen at the clinical facility of the Research Center of the Saint François d'Assise Hospital for the follow-up visit. Anthropometric, BP and Doppler measurements were taken at the randomization visit (visit 1) and at the follow-up visit 12 weeks after randomization (visit 2). At those visits, participants also had to complete a FFQ specifically evaluating their flavonoid consumption in the last month. Polyphenol intake results were analyzed using the USDA database for the flavonoid content of selected foods, release 2.1. To assess compliance, participants had to record their daily intake of chocolate bars on diary cards.

Ethics statement

The protocol and consent form for this study was reviewed and approved by the Institutional Ethics Committee of the Centre Hospitalier Universitaire de Québec. Each participant read and signed an informed consent form before participation to the study. This clinical trial was registered at www.clinicaltrials.gov as NCT01431443.

Doppler ultrasound

A technician certified by the *Fetal-Medicine Foundation* for UtA Doppler ultrasound technique carried out all UtA Doppler measurements, using Voluson E8 ultrasound (GE Healthcare, Milwaukee, WI) [13, 14]. Women were placed in the semi-recumbent position and a sagittal section of the uterus and cervical canal transabdominal ultrasound was obtained. The internal cervical os (opening of the cervix) were the first identified. Subsequently, the transducer was gently tilted

from side to side, and color flow mapping allowed identifying each UtA along the side of the cervix and uterus. Pulsed wave Doppler was used to obtain flow velocity waveforms from the ascending branch of the UtA at the point closest to the internal os. A sampling gate was set at 2 mm to cover the whole vessel. Precautions were taken to ensure that the angle of insonation was less than 50◦. After obtaining three similar consecutive waveforms, PI was measured and the mean PI of the left and right arteries was calculated. Mean PI was then reported in multiple of medians (MoM) adjusted for gestational age according to Gomez et al.[15]. The presence or absence of early diastolic notch in the waveform was recorded.

Plasma biomarkers of chocolate intake

The methylxanthines (caffeine, theobromine and theophylline) were simultaneously quantified by high-pressure liquid chromatography. Inter- and intra-assay coefficients of variation were less than 5%. Flavanol plasma concentrations were extracted and purified by solid extraction followed by high-pressure liquid chromatography with a fluorescence detection system.

Attrition, blinding and contamination bias

Participants were compared in the groups to which they were originally assigned. Analyses were based on an intention-to-treat method. The women, investigators, staff and all laboratory analyses were blinded to treatment assignment. To control for contamination bias, the consumption of flavonoids in the last month preceding the follow-up visit was measured by the FFQ.

Statistical Analysis

Statistical analyses and power calculations were performed using SAS 9.3 (SAS Institute Inc., USA). Baseline variables and pregnancy outcomes were compared between each treatment group

using a Student's *t* test for continuous variables or a Chi-Square test for categorical variables. Changes in the PI in the right and the left UtA, mean PI in UtA and MoM adjusted for gestational age variables were calculated as variations between baseline and 12 weeks after chocolate intake, and are expressed in tables as means ± standard deviations. The PROC MIXED procedure, which allow the inclusion of participants with missing data at some time points [16] was performed. MIXED procedures for repeated measurements were performed to determine group, time and group by time interaction effects on changes in dependent variables measured. In variables where a significant group by time interaction was observed, the Lsmeans procedure determine if a significant change occurred in outcomes over time within each treatment group. More specifically, the Tukey-Kramer adjustment further tested pairwise difference between and within each treatment group. For variables not normally distributed, a transformation was performed but these variables are presented as raw data in the tables. The probability level for significance used for the interpretation of all statistical analyses was set at a α level of $P \leq 0.05$. Sample size (130 participants) was estimated to detect an effect size of 0.287 (at least) between the 2 groups in endothelial function, our primary outcome. We calculated a marginal power of 86% to detect a significant difference of 0.2 MoM in UtA PI, our secondary outcome at the follow-up visit.

RESULTS

One hundred thirty-one women were randomized at a mean gestational age of 12.4 ± 0.6 weeks with a mean UtA PI of 2.22 ± 0.48 and a mean adjusted for gestational age of 1.39 ± 0.31 MoM. Women from both groups presented similar demographic, anthropometric and metabolic characteristics at baseline (**Table 1**) except for the proportion of women with regular alcohol

consumption in the last month, which was higher in HFHT chocolate group. Serum epicatechin and catechin concentrations did not increase significantly after 12-week intake of HFHT chocolate. Compared to baseline, theobromine concentrations were significantly higher (p<0.0001) in the HFHT compared to the LFLT chocolate group at the 12-week visit. No significant change was observed between groups for plasma caffeine concentrations, although similar significant increases were found in both groups (p<0.0001) over the treatment period. Overall, similar level of compliance was self-reported by women in both groups over the 12-week intervention (78% in LFLT and 82% in HFHT, p = 0.53).

Table 2 presents changes in UtA PI in response to the 12-week period of chocolate consumption. No group by time interaction was observed for PI in the left or right UtA and in mean UtA PI. Similar decreases were noted in those variables in both groups in response to 12 weeks of daily chocolate consumption as shown by significant time effects (p<0.0001) respectively for PI in the left and right UtA and for the mean UtA PI as well. Similarly, no significant group by time interaction was noted for UtA PI MoM adjusted for gestational age, but a significant time effect was found in both groups following 12 weeks of chocolate consumption (p<0.0001).

 No statistically significant difference was found for rate of gestational diabetes, gestational hypertension, preterm PE, intrauterine growth restriction, preterm birth and placental weight between the groups (data not shown). Mean gestational age at birth was greater in HFHT compared to LFLT chocolate group (40.1 ± 1.0 weeks vs 39.5 ± 1.3 weeks respectively, p=0.004).

DISCUSSION

We observed no significant difference in terms of UtA PI with 12 weeks consumption of HFHT compared to LFLT chocolate from 11-14 weeks of gestation. However, we observed a similar and significant improvement of UtA PI MoM adjusted for gestational age in both groups from 1.39 ± 0.31 MoM at a mean gestational age of 12 weeks to 1.03 ± 0.34 MoM at a mean gestational of 24 weeks. Finally, we observed a significant increase of gestational age at birth within HFHT compared to LFLT groups. These findings suggest that concentrations of flavanol or theobromine in chocolate had no significant impact on deep placentation, but chocolate could have a positive effect on deep placentation that would be independent to flavanol or theobromine content.

We did not find any clinical trial that specifically evaluated the impact of chocolate consumption on placental function or UtA Doppler during pregnancy. The association between chocolate intake and risk of PE has been previously explored. Saftias et al. observed that chocolate intake during first-trimester of pregnancy was more frequent in normotensive pregnancies than in women who developed preeclampsia (aOR: 0.56; 95% CI, 0.32-0.97) or gestational hypertension (aOR: 0.65; 95% CI, 0.45-0.87) [17]. In a prospective study using women self-reported consumption of chocolate, Triche et al. observed that women consuming 5+ servings of chocolate per week in the first 3 months of pregnancy had a non-significant decreased risk of preeclampsia (aOR: 0.81; 95% CI, 0.37-1.79) compared to women consuming under 1 serving of chocolate weekly[18]. In the non placebo, non blinded trial of Di Renzo et al., daily doses of 30 grams of chocolate (70% cocoa) was associated with improvement of blood pressure during pregnancy[19].

Our study has limitations. First, we selected women with bilateral UtA notches and elevated UtA PI, two surrogate markers of deep placentation with questionable predictive values for PE when performed in the first-trimester. Second, we used UtA PI references' chart that has been established using transvaginal ultrasound: UtA PI assessed through transvaginal ultrasound is typically associated with higher values than transabdominal ultrasound in the 1st trimester with the difference between the two approaches becoming smaller with gestational age[20, 21]. Therefore, we potentially underestimated the PI MoM values in the first-trimester and it is possible that the improvement of UtA PI MoM between the 2 visits was greater than what we reported. Third, chronic epicatechin intake from high-flavanol chocolate could have been too low to have an impact on placentation compared with low-flavanol chocolate group. Indeed, after daily intake of high-flavanol chocolate for 12 weeks, epicatechin serum concentrations did not increase significantly. However, we believe that a total of 30 grams of chocolate per day was a reasonable quantity that could be difficult to increase without better scientific evidence. Fourth, the absence of a group of women who would not have taken any chocolate is probably the most important limitation. The presence of a control group was originally planned but we had to remove it before the start of the study because of insufficient funding. A third-group of controls who would not have taken any chocolate during the study period would have supplied precious information to confirm that no other sources of bias were introduced and which could have helped to clarify our observations. Most participants were nulliparous and had elevated UtA PI (mean PI of 2.2 ± 0.5, 40% higher than the expected median) at randomization, two risk factors for PE and gestational hypertension: the positive impact of chocolate remains the best explanation related to the improvement of UtA PI and the low rate (<5%) of PE in both groups. Finally, regarding the impact of HFHT chocolate on pregnancy outcomes, we cannot exclude at

type 2 error. In regards to the improvement of UtA PI in both groups, we did not have the power to detect a significant difference of PE or gestational hypertension between the groups.

We believe that a positive impact of chocolate on placental function and blood pressure could be explained by other mechanisms than flavanol or theobromine content. Other components of chocolate, such as magnesium and caffeine, have vascular effects and could have played a role in placenta vascularization. Indeed, magnesium cleaves plasminogen activator inhibitor type 2 and therefore may reduce placental thrombosis. Moreover, PE is a disease of endothelial cell dysfunction. Magnesium has an in vitro protective effect on some endothelial cells. Also, PE is associated with an increase in inflammatory response demonstrated by leukocyte activation. Magnesium may have some anti-inflammatory properties because it reduces leukocyte activation[22]. Brillo and Di Renzo recently suggested that further mechanisms of action of chocolate on human reproduction and pregnancy could be explored[23].

In conclusion, our randomized trial suggests that high-flavanol and high-theobromine chocolate consumption has little impact on placental function, as evaluated by UtA Doppler, during pregnancy. However, we observed an improvement of UtA PI in both groups that could explain previous study findings suggesting a lower risk of PE with chocolate consumption in early pregnancy. Altogether, these observations provide a basis for a larger randomized trial to further evaluate the impact of chocolate on pregnancy.

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DISCLOSURE OF INTEREST

The authors report no conflicts of interest.

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