

7-2016

Relation of habitual chocolate consumption to arterial stiffness in a community-based sample: Preliminary findings

Georgina E. Crichton

Merrill F. Elias

University of Maine - Main, mfelias@maine.edu

Ala'a Alkerwi

Walter P. Abhayaratna

Follow this and additional works at: https://digitalcommons.library.umaine.edu/longitudinal_papers

 Part of the [Cardiovascular Diseases Commons](#), and the [Health Psychology Commons](#)

Repository Citation

Crichton, Georgina E.; Elias, Merrill F.; Alkerwi, Ala'a; and Abhayaratna, Walter P., "Relation of habitual chocolate consumption to arterial stiffness in a community-based sample: Preliminary findings" (2016). *Maine-Syracuse Longitudinal Papers*. 23.
https://digitalcommons.library.umaine.edu/longitudinal_papers/23

This Article is brought to you for free and open access by DigitalCommons@UMaine. It has been accepted for inclusion in Maine-Syracuse Longitudinal Papers by an authorized administrator of DigitalCommons@UMaine. For more information, please contact um.library.technical.services@maine.edu.

Original Paper

Relation of Habitual Chocolate Consumption to Arterial Stiffness in a Community-Based Sample: Preliminary Findings

Georgina E. Crichton^a Merrill F. Elias^{b, c} Ala'a Alkerwi^d
Saverio Stranges^d Walter P. Abhayaratna^e

^aAlliance for Research in Exercise, Nutrition and Activity (ARENA), Sansom Institute for Health Research, University of South Australia, Adelaide, S.A., Australia; ^bDepartment of Psychology and ^cGraduate School of Biomedical Sciences and Engineering, University of Maine, Orono, Maine, USA; ^dEpidemiology and Public Health Research Unit (EPHRU), Luxembourg Institute of Health (LIH), Strassen, Luxembourg; ^eCollege of Medicine, Biology, and Environment, Australian National University, Canberra, A.C.T., Australia

Key Words

Arterial stiffness · Chocolate · Cocoa · Pulse wave velocity

Abstract

Background: The consumption of chocolate and cocoa has established cardiovascular benefits. Less is known about the effects of chocolate on arterial stiffness, a marker of subclinical cardiovascular disease. The aim of this study was to investigate whether chocolate intakes are independently associated with pulse wave velocity (PWV), after adjustment for cardiovascular, lifestyle and dietary factors. **Methods:** Prospective analyses were undertaken on 508 community-dwelling participants (mean age 61 years, 60% women) from the Maine-Syracuse Longitudinal Study (MSLS). Habitual chocolate intakes, measured using a food frequency questionnaire, were related to PWV, measured approximately 5 years later. **Results:** Chocolate intake was significantly associated with PWV in a non-linear fashion with the highest levels of PWV in those who never or rarely ate chocolate and lowest levels in those who consumed chocolate once a week. This pattern of results remained and was not attenuated after multivariate adjustment for diabetes, cardiovascular risk factors and dietary variables ($p = 0.002$). **Conclusions:** Weekly chocolate intake may be of benefit to arterial stiffness. Further studies are needed to explore the underlying mechanisms that may mediate the observed effects of habitual chocolate consumption on arterial stiffness.

© 2016 S. Karger AG, Basel

Dr. Georgina E. Crichton
ARENA, Sansom Institute for Health Research
University of South Australia, GPO Box 2471
Adelaide, SA 5001 (Australia)
E-Mail georgina.crichton@unisa.edu.au

Introduction

The consumption of chocolate is widespread throughout the world, with particularly high intakes in the United States [1]. With a rich natural complexity, it is commonly associated with pleasure and enjoyment, as well as having a wide-ranging number of medicinal benefits [2, 3]. More recent scientific interest has been directed at the cardiovascular benefits derived from chocolate and cocoa consumption [4–6]. Flavonoids, naturally occurring polyphenolic compounds present in plant-based foods, represent up to 20% of the compounds present in cocoa beans [7] and may be responsible for the benefits to cardiovascular function [4]. Flavanols, in particular epicatechin, are the most common subgroup of flavonoids, and are the most common cocoa flavonoids [7]. High levels of flavanols are also found in tea, red wine and fruits such as grapes and apples [8, 9].

The majority of vascular studies examining cocoa/chocolate have focused on their associations with endothelial function. The antioxidant properties of flavonoids [4] have been used to explain the demonstrated improvements in flow-mediated dilation of the brachial artery as a result of cocoa [10–13] or chocolate [14] consumption in healthy adults. Fewer studies have examined associations between chocolate or cocoa products and arterial stiffness. Arterial stiffness is higher in persons with diabetes mellitus, obesity and other cardiovascular risk factors and increases with blood pressure (BP) and age [15, 16]. It is a major risk factor for myocardial infarction, stroke, end-stage renal disease and other cardiovascular diseases (CVD) [17–19]. Observational cross-sectional findings with regard to cocoa/chocolate intakes and arterial stiffness have been inconsistent to date [20, 21]. Similarly, a few randomized trials examining the short-term effects on arterial stiffness from chocolate/cocoa consumption ranging from a single dose to daily consumption over a number of weeks have shown mixed results [22–25]. We have not identified any cohort studies that have examined associations between longer-term habitual chocolate intake over a period of years and measures of arterial stiffness. Using data collected from participants of wave 6 and 7 in the Maine-Syracuse Longitudinal Study (MSLS), the aim of the present study was to determine whether habitual chocolate intakes were associated with arterial stiffness, with control for cardiovascular, lifestyle and dietary factors.

Materials and Methods

Participants

The MSLS was a community-based study of cardiovascular risk factors and cognitive functioning in community-living adults [26, 27]. The MSLS consists of five cohorts defined by time of entry into the study (1975–2000). At initial recruitment, participants were living independently in Syracuse, N.Y., and were not being seen as patients. All subjects were accepted into the study regardless of health status. The only exclusions at recruitment were diagnosis of or treatment for psychiatric illness, alcoholism and inability to comprehend English.

Dietary data were collected for the first time at wave 6 (2001–2006), and pulse wave velocity (PWV) data were obtained for the first time at wave 7 (2006–2010). This allowed for a prospective design in which chocolate intakes at wave 6 (baseline) were used to predict PWV at wave 7. The mean time between waves 6 and 7 was 4.7 ± 0.6 years. Eight-hundred and twenty-two subjects were invited back to the laboratory for testing at wave 7. Six-hundred and nine participants returned and completed PWV data collection. Participants were excluded for the following reasons: before or at wave 6 having missing data for health variables ($n = 58$), history of acute stroke ($n = 28$), probable dementia ($n = 8$), undertaking renal dialysis treatment ($n = 5$), inability to read English ($n = 1$) and prior alcohol abuse ($n = 1$), leaving a sample of 508 study participants.

Stroke, defined as a focal neurological deficit of acute onset persisting for more than 24 h, was based on self-report and was confirmed by a record review indicating a diagnosis of acute stroke. Clinical diagnoses

of dementia were determined from cognitive data and medical records using the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [28] and confirmed using the ICD-10 Guidelines [29].

This study was conducted according to the guidelines established by the Declaration of Helsinki, and all procedures were approved by the University of Maine Institutional Review Board. Written informed consent was obtained from all subjects.

Assessment of Dietary Intake

Dietary intake was assessed using the Nutrition and Health Questionnaire, including a widely validated food frequency questionnaire [30, 31]. Participants were required to stipulate how frequently they consume a list of foods, including meat, fish, eggs, breads, cereals, rice and pasta, fruit, vegetables, dairy foods, chocolate, nuts, other snack-type foods and beverages, including tea, coffee, water, fruit juice and alcohol. Participants were required to stipulate how frequently they consume each food, from six response options: never, seldom, once/week, 2–4 times/week, 5–6 times/week and once or more per day. Chocolate was not differentiated according to type, i.e. dark, milk, or white chocolate.

In order to estimate mean intakes of the major food groups and total energy intake, the median score within each response option was used to estimate total intakes per week; for example, 2–3 times per week was estimated at 2.5. The mean number of times each food was consumed on a weekly and then daily basis was calculated for all foods in the questionnaire. As portion sizes were not stipulated to participants, these totals are an estimate of the number of *times* each food was consumed on a daily basis. Individual foods were categorized into five major food groups – grains, fruits, vegetables, protein foods and dairy foods – based on the USDA Food Guide Pyramid [32]. Intakes of individual foods and beverages within each food group were summed to give an estimate of total intake for each group. An estimation of total energy intake was calculated by adding intakes of all food groups, and was used to control for energy intake in subsequent analyses.

BP and PWV Assessment

Automated BP measures (GE DINAMAP 100DPC-120XEN, GE Healthcare) were taken in the right arm five times each in recumbent, standing and sitting position after a supine rest for 15 min, and the 15 values were averaged for systolic BP (SBP) and diastolic BP (DBP). Mean arterial pressure (MAP) was calculated using the following: $DBP + (1/3 \text{ pulse pressure})$. Following these BP measurements, PWV was assessed non-invasively in the supine position following the SphygmoCor[®] protocol which required an additional 5 reclining BPs immediately before the PWV assessment (AtCor Medical, Sydney, Australia). The PWV technician was trained to a high level of proficiency by a cardiologist who, as part of the training, supervised the procedure. Electrocardiogram-gated carotid and femoral waveforms were recorded using applanation tonometry. Carotid-femoral path length was measured as the difference between the surface distances joining: (a) the suprasternal notch, the umbilicus and the femoral pulse, and (b) the suprasternal notch and the carotid pulse. Carotid-femoral transit time was estimated in 8–10 sequential femoral and carotid waveforms as the average time difference between the onset of the femoral and carotid waveforms. The foot of the pulse wave was identified using the intersecting tangent method. The distance measurements were entered into the software in millimeters. Carotid-femoral PWV was calculated as the carotid-femoral path length divided by the carotid-femoral transit time, and expressed in meters per second. This is an established, widely employed, non-invasive and reproducible method to determine arterial stiffness [33]. The coefficient of variation (1.79%) for serial measurements of PWV in our laboratory indicates high reproducibility of the PWV measurements.

Demographics and Physical Health Assessment

Demographic, socioeconomic and lifestyle characteristics were obtained from the Nutrition and Health Questionnaire [30, 31]. Data obtained included smoking history, marital status and medical history. Physical activity was measured with the Nurses' Health Study Activity Questionnaire, a validated measure of time spent engaging in various physical activities [34]. Education level was obtained through self-report and ranged from 4 to 20 years.

Standard assay methods were employed [35] to obtain fasting plasma glucose (mg/dl), total cholesterol (mg/dl), low-density lipoprotein cholesterol (LDL, mg/dl), high-density lipoprotein cholesterol (HDL, mg/dl), triglycerides (mg/dl) and C-reactive protein (mg/l), following an overnight fast. Body weight was measured with participants wearing light clothing to the nearest 0.1 kg, and height was measured with a vertical ruler to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kilograms divided by

height in meters squared. Waist circumference (in centimeters) was taken over light clothing using a non-extendable measuring tape, at the level of the iliac crest. Diabetes mellitus was defined as a fasting glucose level of ≥ 126 mg/dl, or being treated with antidiabetic medication, obesity as BMI ≥ 30 , and CVD was based upon self-reported history of coronary artery disease, myocardial infarction, congestive heart failure, transient ischemic attack or angina pectoris, confirmed by medical records.

Statistical Analyses

Participant demographics, health and dietary variables, and BP measures were compared according to chocolate consumption (<1 serve/week, 1 serve per week, >1 serve/week). Independent samples t tests were used for continuous variables and χ^2 for categorical variables for the analyses involving demographic variables.

Analysis of variance (categorical regression analyses) was used to relate chocolate intakes with PWV using three covariate models. After eliminating CVD variables unrelated to both chocolate intake and PWV, the following models were employed to adjust for confounding in the primary analysis:

Model 1 – Basic: age (years) + gender + education (years) + ethnicity;

Model 2 – PWV model: Basic + heart rate (bpm) + MAP (mm Hg) + height (cm) + weight (kg);

Model 3 – Full model: Basic + PWV model + smoking (cigarettes/day), physical activity (MET hours/week), total cholesterol (mg/dl), LDL cholesterol (mg/dl), diabetes mellitus, CVD, estimated energy intake (total daily intakes of all food groups as previously described), intakes of alcohol, meats, vegetables and dairy foods (all serves/day).

The predictor and the covariates were taken from wave 6 data (prospectively). The PWV-related variables in Model 2 (heart rate, height, weight and MAP) were taken from wave 7 as it is essential that they be taken at the time of PWV assessment.

Analysis of variance (SPSS) was employed to compare mean values for the three chocolate intake groups. Linear and quadratic trend analyses across values for the groups were performed when the omnibus test was statistically significant at $\alpha = 0.05$, in addition to individual contrasts between the groups. Distributional requirements were met. All statistical analyses were performed with the PASW for Windows[®] version 21.0 software (formerly SPSS Statistics; SPSS Inc. Chicago, Ill., USA); $p < 0.05$ was considered statistically significant.

Results

Participant Characteristics and Chocolate Consumption

Table 1 shows the demographic and health-related variables, PWV and dietary intakes for MSLs participants ($n = 508$) according to chocolate consumption (<1 serve/week, 1 serve per week, >1 serve/week). A significantly higher proportion of women than men consumed chocolate more than once per week. Those who consumed chocolate more frequently also had greater intakes of total meats, vegetables and dairy foods, and total energy intakes, but consumed significantly less alcohol. The levels of total cholesterol and LDL cholesterol increased significantly across the three chocolate intake categories, but fasting plasma glucose levels decreased.

Preliminary Test of Interactions

Preliminary tests for interactions (multiplicative effects) with age, sex, BMI and diabetes were done prior to determining the final models. In each case, the main effect of the risk factor was included in the model with the interaction term, and the Basic model was employed. Interaction tests were not statistically significant ($p > 0.05$).

Main Analyses: Chocolate Consumption and Arterial Stiffness in the MSLs

Table 2 shows the associations between chocolate intake at wave 6 and PWV at wave 7. The omnibus comparisons among PWV means for the three chocolate intake groups, and the tests of quadratic trend across the three PWV means, were all statistically significant for all three models ($p < 0.01$).

Table 1. Baseline demographic, health and PWV factors according to chocolate intake in the MSLS sample (n = 508)

Variable	Chocolate intake			p value
	<1 serve/week (n = 174, 34.3%)	1 serve/week (n = 141, 27.8%)	>1 serve/week (n = 193, 38.0%)	
Age, years	61.6±11.4	61.9±11.9	59.8±11.5	0.2
Education, years	14.7±2.9	14.5±2.6	15.0±2.6	0.2
Smoking, cigarettes/day	2.1±6.7	1.0±4.8	1.0±4.3	0.09
BMI	29.5±6.4	29.8±6.4	28.8±5.0	0.3
Physical activity, MET-h/week	18.5±22.4	21.4±22.8	22.6±29.0	0.3
Total cholesterol, mg/dl	198.4±42.0	200.4±32.4	209.4±4.8	0.02
HDL cholesterol, mg/dl	54.7±16.7	54.3±14.3	55.5±15.8	0.8
LDL cholesterol, mg/dl	118.0±35.8	120.5±30.6	127.7±33.2	0.02
SBP, mm Hg	130.8±21.3	130.5±23.0	126.4±19.5	0.09
DBP, mm Hg	69.9±9.9	70.4±10.3	69.2±9.3	0.5
Fasting blood glucose, mg/dl	102.1±36.6	96.6±24.2	95.2±17.5	0.04
Triglycerides, mg/dl	143.8±133.0	134.2±99.8	131.5±97.0	0.6
C-reactive protein, mg/l	0.37±0.38	0.40±0.48	0.45±0.41	0.3
PWV-related variables (at wave 7)				
PWV, m/s	11.2±3.5	10.2±2.5	10.3±3.0	0.004
Pulse pressure, mm Hg	54.2±16.7	53.9±15.8	51.3±15.8	0.2
Augmentation index, %	33.1±10.1	32.7±9.7	33.3±10.1	0.5
Heart rate, bpm	60.1±9.4	59.6±9.8	60.2±8.7	0.8
MAP, mm Hg	95.1±11.4	96.0±13.1	93.3±11.5	0.1
Height, cm	168.3±9.7	167.7±11.7	166.1±9.7	0.1
Weight, kg	83.5±19.9	85.1±20.1	79.5±16.6	0.02
Dietary variables				
Total grains, serves/day	3.7±2.1	4.0±2.0	3.7±1.0	0.4
Total meats, serves/day	1.9±0.8	2.1±0.9	2.2±0.9	0.007
Total fruit, serves/day	1.5±1.0	1.7±1.0	1.6±0.9	0.2
Total vegetables, serves/day	2.6±1.2	2.9±1.1	2.8±1.2	0.05
Total dairy foods, serves/day	1.9±1.0	1.9±1.1	2.1±1.2	0.05
Alcohol, g/week	45.6±75.6	30.3±50.4	25.8±44.2	0.004
Total intake, total serves/day all food groups	13.9±4.1	15.1±4.8	15.6±4.2	0.001
Gender				
Male	81 (46.6)	64 (45.4)	58 (30.1)	0.002
Female	93 (53.4)	77 (54.6)	135 (69.9)	
Ethnicity				
African-American	19 (10.9)	11 (7.8)	8 (4.1)	0.047
Other	155 (89.1)	130 (92.2)	185 (95.9)	
CVD ^a	57 (16.9)	29 (10.9)	53 (14.3)	0.1
Diabetes mellitus ^b	59 (17.5)	31 (11.7)	31 (8.4)	0.001
Obesity ^c	131 (39.5)	106 (40.9)	132 (36.2)	0.4

Data are presented as mean ± SD or n (%). All variables taken at wave 6, with the exception of PWV-related variables, taken from wave 7. p values were obtained by analysis of variance for continuous variables or the χ^2 test for categorical variables. ^a Based upon self-reported history of coronary artery disease, myocardial infarction, congestive heart failure, transient ischemic attack, or angina pectoris, confirmed by medical records. ^b Defined as fasting glucose level of ≥ 126 mg/dl, or being treated with antidiabetic medication. ^c Defined as BMI ≥ 30 .

Table 2. Multivariate-adjusted means \pm standard error for PWV according to chocolate intake (n = 508) at wave 6, predicting wave 7 PWV

Model	<1 serve/week (n = 174, 34.3%)	1 serve/week (n = 141, 27.8%)	>1 serve/week (n = 193, 38.0%)	Model R ²	p overall	p quadratic trend
Basic ^a	11.0 \pm 0.18**	10.0 \pm 0.22	10.6 \pm 0.19*	0.316	0.002	0.002
Model 2 ^b	11.0 \pm 0.18***	10.0 \pm 0.19	10.7 \pm 0.17**	0.457	<0.0001	<0.0001
Model 3 ^c	11.0 \pm 0.18***	10.0 \pm 0.19	10.7 \pm 0.17*	0.508	0.002	0.001

* p < 0.05, ** p < 0.01, *** p < 0.0001, significantly different from the 1 serve/week group. ^a Basic model: adjusted for age, education, gender, ethnicity. ^b Model 2: adjusted for Basic covariates + height, weight, heart rate, MAP (all at wave 7). ^c Model 3: adjusted for Basic + Model 2 covariates + smoking, physical activity, total cholesterol, LDL cholesterol, diabetes, CVD, intakes of total alcohol, meats, vegetables, dairy foods and total energy.

Those who never or rarely ate chocolate (less than once per week) had the highest PWV of the three consumption groups. The mean PWV of 11 m/s observed in this consumption group is above reported normative mean values in both men and women [15]. Participants who consumed chocolate once per week had significantly lower PWV than those who consumed chocolate less frequently (never or seldom) and more frequently (more than once per week) than this (p values <0.05). This pattern of results was significant for the Basic model and remained with the addition of PWV-related variables (Model 2, p < 0.0001). Similarly, there was no attenuation observed with the addition of multiple cardiovascular risk factors and dietary variables to the model (Model 3, p = 0.002).

Attrition

In order to evaluate the effects of attrition on the results, health variable values for those who were participants at both wave 6 and wave 7 were compared with values for participants who did not return for wave 7 testing. Those who dropped out of the study were older, exhibited more depressive symptoms, had higher SBP and DBP, and lower HDL cholesterol (all p < 0.05, data not shown). However, there was no significant difference in chocolate intakes between those who did and did not complete wave 7 testing (p = 0.3, data not shown).

Tests of Interactions and Sensitivity Analyses

The PWV-related variables taken from wave 7 in Model 2 (heart rate, height, weight and MAP) were taken from wave 6 in a sensitivity analysis, and the results remain unchanged. Moreover, when weight and height (from wave 7) were replaced with waist circumference and then BMI (from wave 7), similarly, no change in the results was observed. In additional sensitivity analyses, replacing diabetes with fasting plasma glucose in Model 3 did not change the results, nor did the addition of triglycerides to the final model. We performed further analyses excluding both those with diabetes and CVD, and the results remained unchanged. The same significant non-linear findings were observed when we stratified the sample according to obesity status (BMI <30 and \geq 30). Results were the same for the obese and non-obese groups. Further, replacing MAP (at wave 7) with SBP and DBP (taken at wave 7) did not alter the results, and reanalyses following stratification according to sex did not change the pattern of results.

Discussion

In this prospective study, chocolate intake was significantly associated with PWV in a non-linear fashion. Those who never or rarely consumed chocolate had the highest PWV. Those with a moderate chocolate intake (once per week) had significantly lower mean PWV values than those who never or rarely consumed chocolate, and those who consumed more frequently than once per week. Importantly, this pattern remained significant after adjustment for a number of cardiovascular risk factors, physical activity, smoking, CVD and diabetes mellitus. Associations were not attenuated with the addition of dietary variables (alcohol, meats, vegetables and dairy foods), indicating that chocolate may be associated with arterial stiffness, irrespective of other dietary habits. Although there was a statistically significant difference between the moderate intake group (1 serve/week) and the highest intake group (>1 serve/week), the difference is not of notable clinical importance in terms of the magnitude of the difference between the two groups [15].

Our findings are consistent with previous studies. A higher cocoa intake (from dark and milk chocolate) was associated with lower PWV in a cross-sectional study of 198 adults [21], one of the first studies to suggest that habitual cocoa consumption (from chocolate) may be associated with lower arterial stiffness. Vlachopoulous et al. [21] showed that the lowest PWV (mean 6.2 ± 1.2 m/s) was observed in those with low cocoa intakes (<4.63 g cocoa/day), and PWV was higher in both the non-consumer group (mean 7.0 ± 6.2 m/s) and the high consumption (≥ 4.63 g cocoa/day) group (mean 6.9 ± 1.0 m/s), which is in line with our results.

A more recent cross-sectional study of 351 adults from a primary care setting with some cardiovascular risk factors (hypertension, diabetes or dyslipidemia) showed that higher PWV was higher in non-cocoa consumers as compared to high consumers (>1 serving/week); however, these differences were no longer significant following adjustment for age, sex, presence of diabetes, SBP and use of antihypertensive and lipid-lowering drugs [20]. It is unknown whether a threshold effect as in our study may have been observed in the study by Recio-Rodriguez et al. [20] as there was no further classification of cocoa consumers beyond more than one serving per week. Findings from dietary intervention studies have also been mixed. In a randomized, cross-over study, Vlachopoulous et al. [23] examined the immediate effects of the consumption of 100 g of dark chocolate in 17 young healthy adults and found no significant change in PWV, despite increased flow-mediated dilation. In contrast, West et al. [24] examined the effect of daily cocoa and dark chocolate consumption (22 g/day of natural cocoa) for 4 weeks on endothelial function and arterial stiffness in 30 overweight adults in a placebo-controlled, randomized, crossover study. Significant reductions in peripheral arterial stiffness were observed in women only. Pereira et al. [36] reported that the consumption of 10 g of dark chocolate daily for 1 month in 30 young, healthy individuals significantly decreased aortic PWV compared to a control group. In a subsequent study, Grassi et al. [22] reported that cocoa dose-dependently decreased PWV following daily intakes of cocoa (with differing quantities of cocoa flavonoids) over a 1-week period. In contrast to these studies, another placebo-controlled, 3-week cross-over trial in 42 healthy adults showed that a theobromine-enriched cocoa powder drink increased PWV, but decreased central SBP [25]. However, these short-term dietary intervention studies are difficult to interpret in terms of clinical and public health relevance since atherosclerosis and CVD are chronic conditions; therefore, their natural history is more likely to be affected by long-term exposures.

The possible beneficial actions of cocoa on BP have largely been attributed to flavanols and their antioxidant properties, mainly by improving nitric oxide bioavailability [10, 37, 38]. Flavanols and their metabolites may reduce BP by angiotensin-converting enzyme inhibition [39], nicotinamide adenine dinucleotide phosphate-oxidase activity inhibition [40] and stimulating the release of nitric oxide [10]. The anti-inflammatory properties of cocoa may also

play a role as inflammation is associated with increased arterial stiffness [41]. Additionally, theobromine, present in cocoa in high concentrations, could also contribute to the antihypertensive effect of cocoa [42, 43], as it is thought to have vasodilating properties by inhibiting phosphodiesterase [44].

Several limitations of the present study must be acknowledged. Chocolate intake was self-reported, and therefore subject to inherent reporting error. The dietary questionnaire used did not require the respondent to differentiate between dark, milk or white chocolate. Most clinical trials have used dark chocolate as the source of cocoa flavanols. In 2012, the distribution share of chocolate in the United States by favorite chocolate type was 57% milk chocolate, 35% dark chocolate and 8% white chocolate [1]. We can therefore make the assumption that the majority of chocolate consumed in this sample was dark or milk, both containing cocoa flavanols to varying degrees. We are unable to calculate the actual quantities of chocolate and cocoa associated with higher or lower PWV due to the nature of the questionnaire. There are a number of reasons why a linear dose-response relationship was not observed. There are many examples in medicine whereby a low dose may be equally or more effective than a high dose; for example, the use of low-dose aspirin in preventing future stroke [45]. Secondly, there may be components in chocolate that may 'override' the potentially beneficial effects from small intakes. Clearly, further studies are needed, particularly longitudinal studies and clinical trials in which specific quantities of chocolate, across a wider range of consumption levels are investigated to estimate optimal quantities associated with cardiovascular benefits.

We were able to statistically control for a number of cardiovascular, lifestyle and dietary variables that may impact upon a relationship between habitual chocolate consumption and arterial stiffness. Interestingly, with the addition of these variables to the model, the multivariable adjusted mean PWV for the highest chocolate intake group increased (from an unadjusted 10.3 to 10.7 m/s in Model 3). There was less change in the lower intake groups; for the non-consumer, the mean PWV of 11.2 m/s became 11.0 m/s after adjustment, and for the moderate consumer group, the mean PWV of 10.3 m/s became 10.0 m/s after adjustment. We speculate that this may relate to the ranges of intake levels within each group. For example, in the lowest consumption group, intakes ranged from 0 to 0.5 serves per week. However, the highest consumption group had intakes ranging from 1.9 to 7 serves per week. Perhaps the multivariable adjustment may have had a larger effect on this group due to the wider range of intakes. This reinforces the need for further studies examining a wider range of chocolate intakes.

This is the first longer-term study (5 years) that we are aware of to show that chocolate consumption in adults may impact upon measures of arterial stiffness up to 5 years later. Our study also had a larger sample size than previous observational studies [20, 21], which may contribute to the different findings.

Conclusion

Large artery stiffness plays a causal role in systolic hypertension and is an independent marker of cardiovascular risk. The present findings support recent clinical trials and other cross-sectional research suggesting that a regular intake of a moderate amount of chocolate may have a beneficial effect on arterial stiffness. These relationships were independent of cardiovascular risk factors, diabetes, BMI and other dietary factors. Our findings are novel in that they are suggestive of a threshold effect, whereby chocolate in moderate amounts may be more beneficial than not consuming any chocolate, but higher intakes may not. The findings suggest that future studies are warranted to investigate optimal quantities of chocolate/cocoa consumption to produce short or longer-term effects while taking into account overall dietary patterns.

Acknowledgments

The Maine Syracuse Longitudinal Study was supported by grants R01HL067358, and R01HL081290 from the National Heart, Lung and Blood Institute, National Institutes of Health (USA), and research grant R01AG03055 from the National Institute on Aging, National Institutes of Health (USA). G.E.C. is supported by a National Health and Medical Research Council (NHMRC) Sidney Sax Research Fellowship (GNT1054567) (Australia). A.A. is supported by a grant from the Fond National de Recherche for the project DIQUA-LUX (5870404) (Luxembourg). The funding sources had no involvement in the study design, data collection, writing or decision to submit for publication.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

- 1 Statista: Statistics and facts on the chocolate industry. www.statista.com/statistics/238849/global-chocolate-consumption/ (accessed December 7, 2015).
- 2 Macht M, Mueller J: Immediate effects of chocolate on experimentally induced mood states. *Appetite* 2007;49:667–674.
- 3 Wilson PK: Centuries of seeking chocolate's medicinal benefits. *Lancet* 2010;376:158–159.
- 4 Grassi D, Desideri G, Ferri C: Flavonoids: antioxidants against atherosclerosis. *Nutrients* 2010;2:889–902.
- 5 Heiss C, Keen CL, Kelm M: Flavonols and cardiovascular disease prevention. *Eur Heart J* 2010;31:2583–2592.
- 6 Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, 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;95:740–751.
- 7 Sokolov AN, Pavlova MA, Klosterhalfen S, Enck P: Chocolate and the brain: neurobiological impact of cocoa flavanols on cognition and behavior. *Neurosci Biobehav Rev* 2013;37:2445–2453.
- 8 Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL: Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J Nutr* 2004;134:613–617.
- 9 Hartley L, Flowers N, Holmes J, Clarke A, Stranges S, Hooper L, Rees K: Green and black tea for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2013;6:CD009934.
- 10 Fisher ND, Hughes M, Gerhard-Herman M, Hollenberg NK: Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens* 2003;21:2281–2286.
- 11 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;49:74–80.
- 12 Monahan KD, Feehan RP, Kunselman AR, Preston AG, Miller DL, Lott ME: Dose-dependent increases in flow-mediated dilation following acute cocoa ingestion in healthy older adults. *J Appl Physiol* (1985) 2011;111:1568–1574.
- 13 Njike VY, Faridi Z, Shuval K, Dutta S, Kay CD, West SG, Kris-Etherton PM, Katz DL: Effects of sugar-sweetened and sugar-free cocoa on endothelial function in overweight adults. *Int J Cardiol* 2011;149:83–88.
- 14 Engler MB, Engler MM, Chen CY, Malloy MJ, Browne A, Chiu EY, Kwak HK, Milbury P, Paul SM, Blumberg J, Mietus-Snyder ML: Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J Am Coll Nutr* 2004;23:197–204.
- 15 Elias MF, Dore GA, Davey A, Abhayaratna WP, Goodell AL, Robbins MA: Norms and reference values for pulse wave velocity: one size does not fit all. *J Biosci Med* DOI: 10.5780/jbm2011.4.
- 16 Khoshdel AR, Thakkinian A, Carney SL, Attia J: Estimation of an age-specific reference interval for pulse wave velocity: a meta-analysis. *J Hypertens* 2006;24:1231–1237.
- 17 Benetos A, Waeber B, Izzo J, Mitchell G, Resnick L, Asmar R, Safar M: Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. *Am J Hypertens* 2002;15:1101–1108.
- 18 Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S: Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension* 2002;39:10–15.
- 19 Vlachopoulos C, Aznaouridis K, Stefanadis C: Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010;55:1318–1327.
- 20 Recio-Rodriguez JI, Gomez-Marcos MA, Patino-Alonso MC, Agudo-Conde C, Rodriguez-Sanchez E, Garcia-Ortiz L; Vaso-Risk Group: Cocoa intake and arterial stiffness in subjects with cardiovascular risk factors. *Nutr J* 2012;11:8.

- 21 Vlachopoulos CV, Alexopoulos NA, Aznaouridis KA, Ioakeimidis NC, Dima IA, Dagre A, Vasiliadou C, Stefanadi EC, Stefanadis CI: Relation of habitual cocoa consumption to aortic stiffness and wave reflections, and to central hemodynamics in healthy individuals. *Am J Cardiol* 2007;99:1473–1475.
- 22 Grassi D, Desideri G, Necozione S, di Giosia P, Barnabei R, Allegaert L, Bernaert H, Ferri C: Cocoa consumption dose-dependently improves flow-mediated dilation and arterial stiffness decreasing blood pressure in healthy individuals. *J Hypertens* 2015;33:294–303.
- 23 Vlachopoulos C, Aznaouridis K, Alexopoulos N, Economou E, Andreadou I, Stefanadis C: Effect of dark chocolate on arterial function in healthy individuals. *Am J Hypertens* 2005;18:785–791.
- 24 West SG, McIntyre MD, Piotrowski MJ, Poupin N, Miller DL, Preston AG, Wagner P, Groves LF, Skulas-Ray AC: Effects of dark chocolate and cocoa consumption on endothelial function and arterial stiffness in overweight adults. *Br J Nutr* 2014;111:653–661.
- 25 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* 2010;56:839–846.
- 26 Elias MF, Robbins MA, Budge MM, Abhayaratna WP, Dore GA, Elias PK: Arterial pulse wave velocity and cognition with advancing age. *Hypertension* 2009;53:668–673.
- 27 Robbins MA, Elias MF, Elias PK, Budge MM: Blood pressure and cognitive function in an African-American and a Caucasian-American sample: the Maine-Syracuse Study. *Psychosom Med* 2005;67:707–714.
- 28 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939–944.
- 29 World Health Organization: The ICD 10 Classification of Mental and Behavioral Disorders: Clinical Descriptions and Diagnostic Guidelines. Geneva, World Health Organization, 1992.
- 30 Kaaks R, Riboli E: Validation and calibration of dietary intake measurements in the EPIC project: methodological considerations. *European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol* 1997;26(suppl 1):S15–S25.
- 31 Riboli E, Kaaks R: The EPIC Project: rationale and study design. *European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol* 1997;26(suppl 1):S6–S14.
- 32 United States Department of Agriculture: MyPyramid. Washington, United States Department of Agriculture, 2011.
- 33 Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H: Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006;27:2588–2605.
- 34 Wolf AM, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Corsano KA, Rosner B, Kriska A, Willett WC: Reproducibility and validity of a self-administered physical-activity questionnaire. *Int J Epidemiol* 1994;23:991–999.
- 35 Elias MF, Robbins MA, Budge MM, Elias PK, Brennan SL, Johnston C, Nagy Z, Bates CJ: Homocysteine, folate, and vitamins B6 and B12 blood levels in relation to cognitive performance: the Maine-Syracuse Study. *Psychosom Med* 2006;68:547–554.
- 36 Pereira T, Maldonado J, Laranjeiro M, Coutinho R, Cardoso E, Andrade I, Conde J: Central arterial hemodynamic effects of dark chocolate ingestion in young healthy people: a randomized and controlled trial. *Cardiol Res Pract* 2014;2014:945951.
- 37 Flammer AJ, Hermann F, Sudano I, Spieker L, Hermann M, Cooper KA, Serafini M, Luscher TF, Ruschitzka F, Noll G, Corti R: Dark chocolate improves coronary vasomotion and reduces platelet reactivity. *Circulation* 2007;116:2376–2382.
- 38 Hermann F, Spieker LE, Ruschitzka F, Sudano I, Hermann M, Binggeli C, Luscher TF, Riesen W, Noll G, Corti R: Dark chocolate improves endothelial and platelet function. *Heart* 2006;92:119–120.
- 39 Actis-Goretta L, Ottaviani JJ, Fraga CG: Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. *J Agric Food Chem* 2006;54:229–234.
- 40 Schewe T, Steffen Y, Sies H: How do dietary flavanols improve vascular function? A position paper. *Arch Biochem Biophys* 2008;476:102–106.
- 41 Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, Aggeli C, Toutouza M, Stefanadis C: Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation* 2005;112:2193–2200.
- 42 Cooper KA, Campos-Gimenez E, Jimenez Alvarez D, Rytz A, Nagy K, Williamson G: Predictive relationship between polyphenol and nonfat cocoa solids content of chocolate. *J Agric Food Chem* 2008;56:260–265.
- 43 Kelly CJ: Effects of theobromine should be considered in future studies. *Am J Clin Nutr* 2005;82:486–487; author reply 7–8.
- 44 Kamphuis J, Smits P, Thien T: Vascular effects of pentoxifylline in humans. *J Cardiovasc Pharmacol* 1994;24:648–654.
- 45 Hall HM, de Lemos JA, Enriquez JR, McGuire DK, Peng SA, Alexander KP, Roe MT, Desai N, Wiviott SD, Das SR: Contemporary patterns of discharge aspirin dosing after acute myocardial infarction in the United States: results from the National Cardiovascular Data Registry (NCDR). *Circ Cardiovasc Qual Outcomes* 2014;7:701–707.