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Casual Chocolate Consumption and Inhibition of Platelet Function

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Observational studies have associated reduced cardiovascular mortality with chocolate consumption. Feeding studies of high-dose, flavanol-rich chocolate show antiplatelet effects, but the effect of casual chocolate consumption on platelet function is unknown. Healthy adults (N=1535) were proscribed from consuming foods affecting platelet function, including chocolate, for 48 hours and completed a 24-hour dietary recall before ex vivo platelet testing with the Platelet Function Analyzer (PFA)-100 (Dade Behring, Inc, Deerfield, IL) test and in vivo testing with urinary 11-dehydro thromboxane B2 (Tx-M) measurements. Some participants (n=141) reported ignoring the prohibition of consuming chocolate before platelet testing. Despite having similar baseline characteristics, chocolate consumers had longer PFA closure times (130 vs 123 seconds, P=.005) and decreased Tx-M levels (175 vs 290 ng/mol creatinine, P=.03). Chocolate remained a significant independent predictor of both ex vivo and in vivo platelet function testing after adjusting for confounders. The authors concluded that even consuming modest amounts of commercial chocolate has important antiplatelet effects. (Prev Cardiol. 2007;10:175–180) ©2007 Le Jacq

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For thousands of years, indigenous people from Central and South America have used cocoa beans as a medicinal agent.[1] European settlers, and subsequently other cultures, also came to believe that cocoa products could prevent or heal a wide variety of ailments.[2] Recently, studies have addressed the health benefits of chocolate, the most commonly consumed cocoa product.

A recent 15-year prospective population-based observational study of elderly Dutch men, the Zutphen Elderly Study,[3] demonstrated the cardioprotective effect of long-term chocolate consumption. Coronary heart disease mortality was reduced by 45% to 50% among men whose cocoa consumption was in the highest tertile compared with those in the lowest tertile after adjusting for age, sex, biologic risk factors, and other dietary components.[3]

In laboratory-based studies, chocolate consumption has been associated with a number of potentially cardioprotective effects, including antiplatelet,[4] antioxidant, anti-inflammatory, antihypertensive, insulin-sensitizing, and vasodilatory effects.[5,6] With respect to platelet function, ingestion of known amounts of cocoa powder and/or flavanol-rich chocolate has been associated with several antiplatelet effects, including reduced adenosine diphosphate (ADP)–induced aggregation,[7] prolonged time to platelet plug formation under conditions of shear stress on the Platelet Function Analyzer (PFA)-100 test (Dade Behring, Inc, Deerfield, IL),[4] reduced epinephrine-induced glycoprotein IIb/IIIa membrane expression,[4] and reduced ADP-induced P-selectin membrane expression.[8]

Many beneficial effects of chocolate consumption are mediated, at least in part, through naturally occurring phenolic compounds, collectively known as flavanols. Flavanols are also commonly found in other foods and beverages including grapes and tea. Commercially available chocolate products in the United States have wide variation in the amount of measured flavanols because of variations in processing, preparation, and the extent to which flavanol-rich cocoa is used.

Table I. Sample Characteristics by Chocolate Consumption (N=1535)

	Chocolate (n=141)	No Chocolate (n=1394)	*P* Value
Age, y[a]	43±12	45±13	.16
Education, y[a]	13±2	13±2	.44
Systolic blood pressure, mm Hg[a]	121±17	121±16	.91
Fasting glucose, mg/dL[a]	96±30	96±28	.85
Body mass index, kg/m^2[a]	30±7	30±7	.47
Total cholesterol, mg/dL[a]	208±41	198±41	.005
Fibrinogen, mg/dL[a]	382±116	390±120	.44
von Willebrand's factor, %[a]	84±45	89±58	.27
Female, %	55	57	.76
African American, %	40	37	.45
Current smoking, %	29	25	.28
Diabetes mellitus,%	10	8	.42
Hypertension, %	33	32	.96
Obesity, %	46	38	.06

[a]Mean ± 1 SD.

Thus, because most prior studies of chocolate's antiplatelet effects have been conducted in small numbers of people (N=10–32) ingesting very large amounts of flavanol-rich chocolate in laboratory-based settings, we conducted this study to determine the impact of recently consumed, relatively small quantities of commercially available chocolate products on clinical measures of platelet activation among high-risk individuals, those with a family history of premature coronary artery disease (CAD).

METHODS

Study Sample

The study sample comprised unaffected, apparently healthy siblings of 454 patients with documented CAD events before 60 years of age, along with the index patient's and siblings' adult offspring and the offspring's other parent. Participants were recruited from a longitudinal family-based study of incident cardiovascular disease events, the Johns Hopkins Sibling and Family Heart Study. Participants were identified from the pool of eligible family members to participate in a substudy on the genetic determinants of aspirin responsiveness (GeneSTAR).[9] Evaluation of platelet function for this study involved only baseline native platelet function before initiation of the aspirin study.

Potential participants were excluded if they had known CAD, stroke, any vascular disease, any bleeding or hematologic disorder, a history of hemorrhage, or serious medical comorbidity (eg, renal or hepatic failure, AIDS, cancer, or autoimmune disease). Participants were also excluded if they had a baseline platelet count <100,000/mL or >500,000/mL, hematocrit level <30%, or white blood cell count >20,000/mL. Participants were free of aspirin for 4 weeks and nonsteroidal anti-inflammatory agents for 2 weeks before measurement of platelet reactivity and could not be taking any other anticoagulants, antiplatelet agents, or systemic glucocorticosteroids. The Johns Hopkins Institutional Review Board approved the study and all participants provided written informed consent.

Dietary and Lifestyle Instructions

Prior to their initial visit, participants were instructed by a nurse practitioner to refrain from consuming all chocolate, nuts, wine, grapes or grape juice, tea, coffee, caffeinated soda, fish, garlic, pineapple or pineapple juice, oats, and soy preferably for 2 weeks but for a minimum of 48 hours before baseline platelet measurement. They were also instructed to refrain from cigarette, cigar, or pipe smoking preferably for 4 hours before platelet measurement. Materials mailed to participants 3 to 4 weeks before the visit reinforced all proscribed medication, dietary, and smoking instructions. All participants were also called 48 hours before the initial visit to assure that they remained eligible (no recent bleeding or surgical event). At that time, all dietary and lifestyle instructions were again reinforced.

Risk Factor Assessment

Participants were seen in the Johns Hopkins General Clinical Research Center (GCRC), where they provided a complete cardiovascular history and underwent physical examination. All individuals were asked to bring current prescription medications, nutritional supplements, and over-the-counter medicines and to indicate which medications they had taken in the weeks preceding the visit. Blood pressure was measured at rest using the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines.[10] Hypertension was defined as a blood pressure level ≥140/90 mm Hg (average of 4 blood pressure determinations) and/or the use of an antihypertensive medication. Height and weight were measured, and body mass index (BMI) was calculated as weight (kg)/height^2

(m). Serum glucose and total cholesterol, high-density lipoprotein cholesterol, and triglyceride levels were measured after a 12-hour overnight fast using a Cholestech LDX analyzer (Cholestech Corporation, Hayward, CA). Low-density lipoprotein cholesterol was estimated using the Friedewald formula.[11] Diabetes was defined as having a fasting plasma glucose level ≥126 mg/dL and/or taking a hypoglycemic agent. Participants were classified as smokers if they reported smoking any cigarettes within the past month or had an exhaled carbon monoxide measurement of ≥8 parts per million on 2 successive readings.

Dietary Assessment

A 24-hour diet recall was conducted to identify proscribed foods that might affect the measurement of native platelet function. All dietary recalls were interviewer-administered by a GCRC dietitian, a trained nurse practitioner, or a research assistant trained and monitored by the dietitian. Participants were asked to specifically recall all foods and beverages consumed, including all meals and snacks commencing with the morning before the day of each visit. In addition, information on the use of all dietary supplements was also gathered. All data were coded for the actual foods consumed, quantity and portion size, and to the extent possible, the content of foods containing multiple components.

When food or beverage containing chocolate was reported, the nature of the item was probed for, including the brand name. The quantity of cocoa consumed (in grams) was determined using portion sizes, preparation, and data obtained from a known cocoa database.[12] For commercially prepared products like candy, cookies, ice cream, and some cakes, we searched each company's Web site to obtain the exact product weight and then multiplied that by the cocoa content from a database (standardized per 100 g) to calculate the amount of cocoa present in each item. For example, a typical milk chocolate and almond candy bar (41 g) has a standardized cocoa content of 22.5 g/100 g, yielding 9.225 g of cocoa (41 g × 22.5 g/100 g=9.225 g).

Platelet Function

After participants had fasted for 12 hours overnight, blood was collected via venipuncture into BD Vacutainer tubes (BD, Franklin Lakes, NJ) containing 3.2% sodium citrate. A single voided urine specimen was also collected at that time. Platelet function in whole blood under conditions of shear stress was assayed using the PFA-100 test, performed with prefabricated cartridges containing a combination of collagen and epinephrine as platelet agonists. Closure time was recorded in seconds, according to the manufacturer's instructions.

In vivo thromboxane production was measured as urinary 11-dehydro thromboxane B2 (Tx-M). Tx-M was quantified by commercially available enzyme-linked immunosorbent assay (ELISA) (Cayman Chemical Co, Ann Arbor, MI; coefficient of variation = 8%) and normalized to urinary creatinine levels. von Willebrand's factor (VWF) was measured to adjust for its potential influence on PFA measurement. Plasma VWF was quantified by a commercially available ELISA (DiaPharma Group, Inc, West Chester, OH; coefficient of variation = 4%), and plasma fibrinogen was measured by the Johns Hopkins Clinical Coagulation Laboratory.

Statistical Analyses

Means and SDs of quantitative variables were calculated. Tests for normality were performed using the Kolmogorov-Smirnov statistic and Wilk-Shapiro test. PFA and Tx-M were nonnormal and thus log-transformed for all significance testing. Simple bivariable relationships were examined using *t* test on the transformed quantitative outcome measures, and linear regression models were constructed to predict platelet function outcomes. Regression analyses were performed using the generalized estimating equation to adjust for nonindependence within families, and both outcomes, PFA and Tx-M, were log-transformed to achieve normal distributions. All significance testing was 2-tailed with an α of 0.05. Data were analyzed using SAS software version 9.1 (SAS Institute, Inc, Cary, NC) and SUDAAN version 9.0.1 (Research Triangle Institute, Research Triangle Park, NC).

RESULTS

Sample Characteristics

The sample consisted of 1535 persons, 37.5% of whom were African American. In total, 141 (9%) reported chocolate consumption in the 24 hours preceding the measurement of platelet function. Table I shows the characteristics of the sample population among both chocolate consumers and abstainers. Except for a relatively modest difference in total cholesterol levels, there were no significant differences in demographic or cardiac risk factors, including BMI, between those who consumed chocolate and those who did not. Overall, the entire sample was relatively young and well-educated and did not have an unusually high prevalence of cardiac risk factors, with the exception of obesity.

Dietary Outcomes

Chocolate was consumed from a variety of sources. Table II shows the calculated mean cocoa content, in grams, for each of the various categories of foods consumed and the frequency of consumption among participants. Overall, among chocolate consumers, the estimated mean amount of cocoa ingested per person was 5.9 g (SD 10 g) in the 24 hours before platelet measurement. Ice cream and commercial mixed-content candy bars contributed the largest number of grams of cocoa consumed, while cookies, mixed-content candy bars, and ice

Table II. Estimated Average Amounts of Cocoa in Chocolate-Containing Items Consumed (n=141)

Consumed (n=141)	Cocoa Estimates, g[a]	No.[b] [Ranking[c]]
Ice cream	11.2±22	21 [3]
Candy bars	10.0±7.9	25 [2]
Pudding	9.7±5.7	5 [8]
Chocolate (pure)	6.5±6.0	11 [5]
Beverages	5.4±2.3	6 [7]
Cookies	3.5±4.9	29 [1]
Cakes	3.4±3.6	18 [4]
Brownies	1.4±0.5	3 [9]
Donuts	0.4±0.4	5 [8]
Power bars	0.3±0.3	5 [8]
Other (cereal, frosting, hard candy)	0.1±0.2	8 [6]

[a]Mean ± 1 SD represents the average amount of cocoa in the average portion consumed for a given item. [b]No. represents the number of people who consumed the item. [c]Ranking represents the popularity of the chocolate sources consumed.

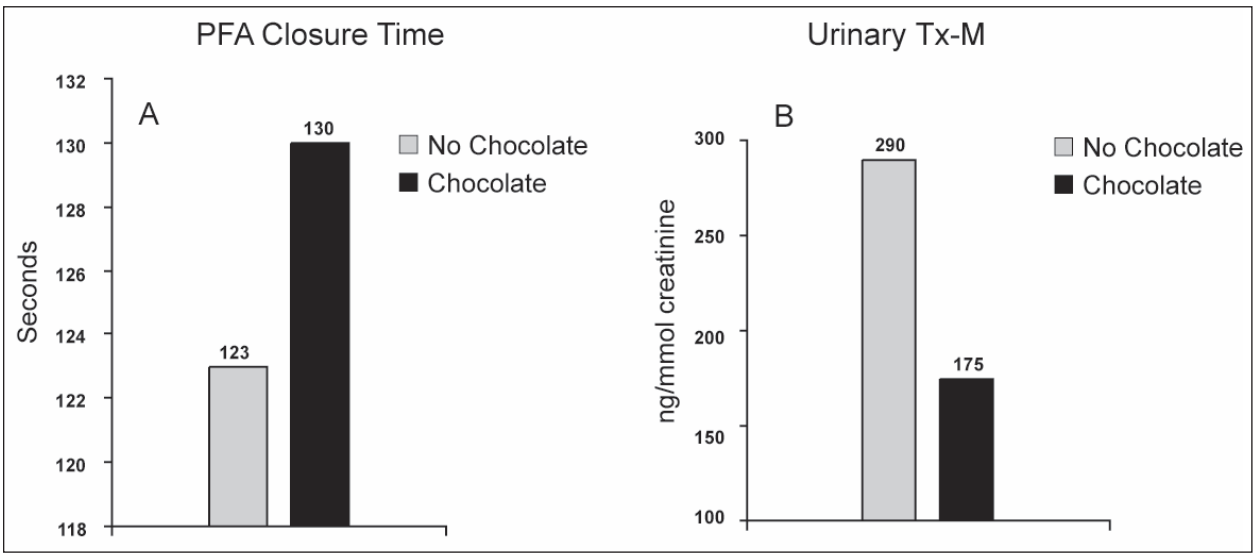

Figure. Mean platelet function analyzer (PFA) closure times (difference between groups, P=.005[a]) and mean urinary thromboxane metabolites (difference between groups, P=0.03[a]) among consumers of chocolate vs abstainers from chocolate. Significance testing was performed on log-transformed outcome variables.

cream were the chocolate-containing foods most frequently eaten.

Regarding the remaining proscribed foods, among chocolate consumers, 8.4% also consumed tea, 1.2% also consumed red wine, 3.8% also consumed grapes or grape juice, 14.3% also consumed coffee, 2.8% also consumed beer, 2% also consumed fish, 2.6% also consumed garlic, 5.9% also consumed nuts, 10.2% also consumed oats, 0.8% also consumed pineapple or pineapple juice, 15% also consumed any soda, and 0.7% also consumed soy. Of those who ate chocolate, 71.6% also ate one or more of the other proscribed foods.

Platelet Function and Chocolate Consumption

Panel A of the Figure shows the absolute difference in PFA closure time (in seconds) between those who consumed any chocolate vs those who abstained from chocolate intake. There was a statistically significant difference of 7 seconds in closure time between groups (P=.005) for the analysis using the log transformation for PFA. Panel B shows the absolute difference in Tx-M between those who consumed chocolate and those who did not (P=.03) using the log transformation of Tx-M. No specific form of chocolate consumed showed any tendency to be more or less associated with alterations in either PFA or Tx-M (data not shown).

Table III shows the results of multiple linear regression analyses for PFA and Tx-M, adjusting for age, sex, education level, race, cigarette smoking, and BMI and values of glucose, blood pressure, total cholesterol, fibrinogen, and VWF. While most risk factors, particularly systolic blood pressure and VWF levels, were statistically significant predictors of PFA closure time, chocolate consumption remained statistically significant even after adjustment. Similarly, in the Tx-M model, age, sex, and cigarette smoking were significant predictors, but chocolate consumption retained a

Table III. Multiple Linear Regression Models Predicting Platelet Function Outcomes[a] (N=1535)

	Model 1: PFA Closure Time, sec		Model 2: Tx-M, ng/mmol creatinine	
	β (SE)	*P* Value	β (SE)	*P* Value
Age, y	–0.002 (0.0005)	.001	0.009 (0.003)	.0005
Female	–0.001 (0.012)	.93	0.180 (0.061)	.006
Education, y	0.003 (0.002)	.18	–0.040 (0.013)	.002
White	–0.039 (0.016)	.01	0.050 (0.073)	.42
Current smoker	–0.039 (0.0143)	.007	0.375 (0.079)	<.0001
Body mass index, kg/m^2	0.002 (0.001)	.07	0.014 (0.005)	.006
Systolic blood pressure, mm Hg	0.002 (0.0004)	<.0001	0.001 (0.002)	.63
Fasting plasma glucose, mg/dL	0.0005 (0.0002)	.02	0.001 (0.001)	.31
Total cholesterol, mg/dL	0.0002 (0.0002)	.11	–0.001 (0.0007)	.13
Fibrinogen, mg/dL	–0.00006 (0.00006)	.32	0.00002 (0.0003)	.61
VWF, %	–0.0005 (0.0001)	<.0001	NA	
Chocolate consumption	0.049 (0.018)	.007	–0.224 (0.110)	.04

[a]Adjusted for nonindependence of families using the generalized estimating equations; both outcomes log-transformed.
Abbreviations: PFA, platelet function analyzer; Tx-M, urinary 11-dehydro thromboxane B_2; VWF, von Willebrand's factor.

statistically significant relationship when controlling for other variables. Consumption of other potentially proscribed flavanol-rich foods or other products known to affect platelet activation did not demonstrate any significant relationships with either PFA or Tx-M.

DISCUSSION

Although a number of small laboratory-based feeding studies have shown platelet suppression with large doses of chocolate and/or flavanols, no studies to date have examined platelet function in a free-living population consuming relatively modest and typically uncontrolled amounts of commercially available chocolate of different origins in different forms. In this healthy population, chocolate consumption in the past 24 hours was significantly and independently associated with a decrease in platelet function in both an ex vivo test of aggregation (PFA closure time) and an in vivo test of platelet activation (Tx-M), even after controlling for major potential confounding variables, including other possible platelet-modifying foods.

Laboratory studies examining the effect of chocolate consumption on platelet function have generally used doses 7 to 17 times higher than those reported by our study participants (5.9 g).[13,14] Our results suggest that a relatively modest consumption of chocolate in products with highly unpredictable flavanol levels and a variety of other ingredients can still achieve an antiplatelet effect. These results may provide insight into at least one of the putative mechanisms for cardiovascular risk reduction observed in the Zutphen Elderly Study.[3]

Among individuals who also consumed other flavanol-rich foods, none were significant alone or in combination. We only observed a significant relationship between chocolate consumption and platelet function. This is not surprising, because biochemical food analyses have demonstrated that in similar quantities, cocoa contains much higher levels of flavanols than most other foods.[15] Furthermore, in our study population, chocolate was consumed in larger quantities than other flavanol-containing foods such as grapes, wine, or teas. It is also worth noting that in this study, a substantial number of participants were unwilling to forego chocolate consumption for 2 days as required by the study protocol. Coffee and soda were 2 other proscribed items frequently consumed by study participants. Since these 2 items, along with chocolate, often contain mild mood- and energy-enhancing properties derived from sugar and caffeine, it is not surprising that these might have been the most difficult items for our participants to avoid. Because smoking was only proscribed for a short period, refraining from smoking may have been easier.

Given that chocolate consumption was measured by self-report, it is reasonable to expect that the actual number of individuals who consumed chocolate is somewhat larger because of both recall and social desirability biases. These would, if anything, underestimate chocolate's actual antiplatelet effect in our study. Because this was an observational study and flavanol levels in commercially available chocolates are highly variable and often unavailable because it is proprietary information, it was impossible to determine from our data either a cocoa- or flavanol-specific minimum dose required to achieve antiplatelet effects in our population. Future laboratory research may provide estimated "doses" of various commercially available chocolate products required to achieve significant antiplatelet effects. Furthermore, the extent to which these antiplatelet effects are cardioprotective in the context of the sugar and fat that are usually contained in commercial chocolate products needs to be determined.

CONCLUSIONS

Our study provides an important bridge between laboratory-based, tightly controlled chocolate feeding studies and epidemiologic data, such as that observed in the Zutphen Elderly Study,[3] where there was a notable decrement in cardiovascular mortality. Given the panoply of foods and beverages consumed in the average diet, it is remarkable that the small amounts of commercially available chocolate consumed in the average diet have an observable and significant impact on platelet inhibition in healthy adults at risk for cardiovascular events. It remains unclear, however, whether this effect imparts any cardioprotective benefit, although the possibility exists that the antiplatelet effect of chocolate may in part be responsible for the observed reduction in cardiovascular disease events in the Zutphen Elderly Study.[3] In an era when so many desirable foods are proscribed for health reasons, this study adds information further suggesting that there may be pro-health benefits from flavanol-rich chocolate that is not enriched with simple sugars and fats. Thus, consumption of healthier forms of chocolate without large amounts of sugar and fat may confer some cardioprotective benefits and may be included in moderation in a healthy diet.

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