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Regular Consumption of Dark Chocolate Is Associated with Low Serum Concentrations of C-Reactive Protein in a Healthy Italian Population[1,2]

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Abstract

Dark chocolate contains high concentrations of flavonoids and may have antiinflammatory properties. We evaluated the association of dark chocolate intake with serum C-reactive protein (CRP). The Moli-sani Project is an ongoing cohort study of men and women aged ≥35 y randomly recruited from the general population. By July 2007, 10,994 subjects had been enrolled. Of 4849 subjects apparently free of any chronic disease, 1317 subjects who declared having eaten any chocolate during the past year (mean age 53 ± 12 y; 51% men) and 824 subjects who ate chocolate regularly in the form of dark chocolate only (50 ± 10 y; 55% men) were selected. High sensitivity-CRP was measured by an immunoturbidimetric method. The European Prospective Investigation into Cancer and Nutrition FFQ was used to evaluate nutritional intake. After adjustment for age, sex, social status, physical activity, systolic blood pressure, BMI, waist:hip ratio, food groups, and total energy intake, dark chocolate consumption was inversely associated with CRP ($P = 0.038$). When adjusted for nutrient intake, analyses showed similar results ($P = 0.016$). Serum CRP concentrations [geometric mean (95% CI)] univariate concentrations were 1.32 (1.26–1.39 mg/L) in nonconsumers and 1.10 (1.03–1.17 mg/L) in consumers ($P < 0.0001$). A J-shaped relationship between dark chocolate consumption and serum CRP was observed; consumers of up to 1 serving (20 g) of dark chocolate every 3 d had serum CRP concentrations that were significantly lower than nonconsumers or higher consumers. Our findings suggest that regular consumption of small doses of dark chocolate may reduce inflammation. J. Nutr. 138: 1939–1945, 2008.

Introduction

Cocoa, the seed of the cocoa tree, *Theobroma cacao*, was cultivated over 3000 y ago by the original inhabitants of Central America and Northern South America. The cocoa beans contain different types of physiologically active compounds, among others monomeric flavanols (epicatechin and catechin) and oligomeric procyanidins (1). The antioxidant properties of flavonoids (including cocoa flavanols) have generated great interest in their potential role in lowering the risk of cardiovascular disease (CVD).[5] Vinson et al. (2) showed that chocolate had a higher flavonoid antioxidant quantity-quality index than did fruit, vegetables, red wine, and black tea. Lee et al. (3) reported that cocoa contains higher levels of total phenolic phytochemicals and flavonoids per serving than black tea, green tea, and red wine, suggesting that cocoa might be more beneficial to health than tea and red wine because of its higher antioxidant capacity. Clinical studies showed that consumption of dark chocolate increases the serum concentration of HDL cholesterol and did not affect serum total and LDL cholesterol (4).

In vitro studies showed that cocoa flavanols may induce endothelium-dependent vessel relaxation (5) and modulate cytokines and eicosanoids involved in the inflammatory response (6,7). Small, short-term intervention studies indicate that cocoa-containing foods improve endothelial function and reduce blood pressure (BP) in hypertensive subjects (8). A recent observational study found that habitual cocoa intake was inversely related with

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[5] Abbreviations used: BP, blood pressure; CRP, C-reactive protein; CVD, cardiovascular disease; MET, metabolic equivalent.

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BP in cross-sectional analysis and with cardiovascular and all-cause mortality in prospective analysis (9), whereas supplementation with cocoa products in humans affects LDL oxidability but not markers of inflammation (10).

We hypothesized that dark chocolate consumption, which significantly contributes to the antioxidant potential of the diet, could be inversely related to the level of C-reactive protein (CRP), a marker of inflammation considered as an independent indicator of coronary heart disease (11).

For this purpose, we evaluated the association between serum CRP and chocolate consumption in a healthy population selected from the Moli-sani project (12).

Materials and Methods

The cohort of the Moli-sani Project was recruited from city hall registries by a multistage sampling. First, townships were sampled in major areas or by cluster sampling; then, within each township, participants aged ≥35 y were selected by simple random sampling using electronically generated numbers. Exclusion criteria were pregnancy at the time of recruitment, disturbances in understanding or willingness, current poly-traumas or coma, or refusal to sign the informed consent. Twenty percent of subjects refused to participate; these individuals were generally older and had a higher prevalence of CVD.

Trained interviewers administered structured questionnaires to collect personal and clinical information, including socioeconomic status, physical activity, physiopathological medical history, risk factors for CVD and/or tumor, family/personal history for CVD and/or tumor and drug use, and dietary habits (12).

Dietary assessment. The European Prospective Investigation into Cancer and Nutrition FFQ was used to determine daily nutritional intakes consumed in the past year (13). This questionnaire was designed and validated in a multicenter study performed in 10 European countries, with the aim to evaluate the relation between diet and cancer. The questionnaire, computerized with tailor-made software, allowed researchers to interview participants in a fully interactive way, including illustrations of sample dishes of definite sizes or by reference to standard portion sizes. All the answers provided by the participants were registered in a database in real time. Chocolate consumption was investigated by asking participants about frequency (daily, weekly, or monthly) of a standard dose (20 g) and about type of chocolate consumed (dark, milk, nut chocolate, or any type). A person consuming more than 1 type of chocolate was classified as any type.

The NAF software (Nutritional Analysis of Food Frequency Questionnaires, National Cancer Institute, Milan, Italy) (14) was used to transform information about food composition into daily intake of food items (g/d), energy (kcal or kJ/d), and macro- and micronutrients (g or mg/d). Nutrient data for specific foods were obtained from the food composition database for epidemiological studies in Italy (15).

BP and anthropometric measurements. Trained research personnel took BP and anthropometric measurements using methods standardized beforehand during preliminary training sessions. BP was measured by an automatic device (OMRON-HEM-705CP) (16) 3 times on the nondominant arm and the last 2 values were taken as the BP (17). Measurements were made in a quiet room with comfortable temperature with the participants lying down for at least 5 min. Body weight and height were measured on a standard beam balance scale with an attached ruler with subjects wearing no shoes and only light indoor clothing. BMI was calculated as kg/m^2. Waist circumferences were measured according to the NIH, Heart, Lung, and Blood guidelines (18).

Definition of risk factors. Subjects were classified as nonsmokers if they had never smoked cigarettes, ex-smokers if they had smoked cigarettes in the past and had stopped smoking for at least 1 y, and current smokers if they were currently smoking 1 or more cigarettes per day on a regular basis. Social status was classified as a score based on education, job, income, and housing; the higher the score, the higher the social level. Total physical activity (leisure and working time) was classified in metabolic equivalent (MET)/d (19).

Biochemical measurements. Blood samples were obtained between 0700 and 0900 from participants who had fasted overnight and had refrained from smoking for at least 6 h. Biochemical analyses were performed in the centralized Moli-sani laboratory on fresh samples. Serum lipids and blood glucose were assayed by enzymatic reaction methods using an automatic analyzer (ILab 350). LDL-cholesterol was calculated according to Friedewald (20).

High sensitivity CRP was measured in fresh serum samples within 3 h from collection. A latex particle-enhanced immunoturbidimetric assay (IL Coagulation Systems on ACL9000) was used. Quality control was maintained using in-house serum pool and internal laboratory standard at 1.5 mg/L. Inter- and intra-day CV were 5.5% and 4.17%, respectively.

Population for analysis. Between March 2005 and July 2007, a total of 10,994 persons were recruited. Among them, 34% did not consume any type of chocolate and 18% consumed chocolate only in the form of dark chocolate. Subjects who reported a diagnosis of CVD ($n = 534$), were undergoing pharmacological treatment for hypertension, diabetes, or dyslipidemia ($n = 3513$), or were consuming a special diet ($n = 1676$) were excluded. Another 263 subjects were excluded because of missing values for 1 or more of the eligibility criteria and 159 subjects because of serum CRP concentrations >10 mg/L (4.7% and 3.6% of nonconsumers and dark chocolate consumers, respectively) to avoid introducing confounding due to an acute inflammatory condition.

In total, we selected a sample of 4849 subjects; among these we extracted 2 subpopulations based on the consumption of dark chocolate: a control group of 1317 (27.2%) subjects who never ate any type of chocolate and a test group of 824 (17.0%) subjects who regularly ate dark chocolate only.

The Moli-sani study was approved by the Catholic University ethics committee and is conducted under the supervision of both the Bioethics Institute of our university and the Istituto Superiore di Sanità, Rome. All participants enrolled provided written informed consent to giving blood samples for biochemical measurements.

Statistical analysis. Serum triglyceride, blood glucose, and serum CRP concentrations were transformed into natural logarithms to reduce their positive skewness and data were reported as geometric means and 95% CI. Values for continuous no-skewed variables are means ± SEM. Differences between chocolate nonconsumers and dark chocolate consumers were evaluated using multivariate analysis of variance or multivariate binomial (Poisson) regression with the log link function (21). When the association between continuous variables, in particular serum CRP, and dark chocolate consumption was evaluated, the results obtained with Poisson regression were largely comparable with that of multivariate ANOVA.

The covariates included sociodemographic variables (age, sex, smoking habit, and social status), serum lipid concentrations (total-, HDL-, and LDL-cholesterol, and triglycerides), systolic and diastolic BP, blood glucose, BMI (computed as kg/m^2), waist:hip ratio, and physical activity (expressed as MET/d). All dietary covariates were adjusted for total energy intake according to the residual method and total energy intake was included in all regression models (22). To build the multivariate models, the following strategy was applied: *1*) simple univariate analysis was used to identify variables associated with binary outcome (nonconsumers and dark chocolate consumers) and serum CRP at the level $P < 0.10$; *2*) all the variables identified in the univariate analysis were inserted in a full model together. For the purpose of this study, several multivariate models were performed as follows: a basic model, in which the association between serum CRP and exposure groups were controlled for confounding factors, in particular age, sex, social status, and physical activity; the nutrient-adjusted model; the food group-adjusted model; and a full multivariate model adjusted for all those variables used in the basic model and alternatively in the nutrient and food group models, and for all covariates associated with exposure group but that are well-known determinants of serum CRP: BMI, waist:hip ratio, and systolic BP. Different associations of

serum CRP with chocolate consumption according to principal cardiovascular risk factors or indicators of healthy status were evaluated inserting in the multivariate analyses interaction terms.

To explore the data for a nonlinear dose-response relation, both a linear and a quadratic term for chocolate intake were inserted in ANOVA.

The presence of colinearity among independent variables was tested measuring the variance inflation factor in linear regression analysis. A variance inflation factor ≥10 indicates that the variable under consideration is almost a perfect linear combination of the independent variables already in the equation and that it should not be added to the regression equation (23). Two-sided 95% CI and *P*-values were calculated; *P*-value < 0.05 was chosen as the level of significance. All analyses were carried out using SAS (SAS Institute, 8.12 for Windows, 1989).

Results

Chocolate intake and population characteristics. Among consumers, the median intake of dark chocolate was 5.7 g/d. In univariate analysis, dark chocolate consumers were younger and had lower BMI and systolic BP than nonconsumers. Moreover, they had a higher social status but were less likely to practice physical activity (**Table 1**).

Dark chocolate users consumed less cereal, meat, and alcoholic beverages but more nuts and seeds, dairy products, fish, sweets and confectionary, sweet beverages, coffee, and tea.[6] Moreover, chocolate consumption was positively associated with total energy intake (**Table 2**). No association was found with the consumption of potatoes, legumes and vegetables, fruits, vegetable oils, or fat (Table 2). When adjusted for total energy intake, macro- and micro-nutrients were all positively associated with dark chocolate consumption, with the exception of total carbohydrates, dietary fibers, and protein intake and zinc intake. Only iron and sodium intake were inversely related to dark chocolate consumption (**Table 3**).

After multivariate analysis, however, age, male sex, social status, physical activity, total energy, cereals, nuts and seeds, fish, dairy products, sweets, tea, alcohol, calcium, and zinc were still independently associated with dark chocolate consumption (Tables 1–3).

Chocolate intake and CRP. The percentage of subjects with serum concentrations of CRP >3 mg/L was higher in nonconsumers (19%) than in dark chocolate consumers (14%), whereas the percentage of subjects with serum concentrations of CRP <1 mg/L was lower (38 vs. 45%; $P = 0.0006$).

Age-adjusted serum concentrations of CRP were lower in dark chocolate users [1.30 (1.24–1.36) mg/L] than in the chocolate nonconsumers [1.13 (1.06–1.20) mg/L] ($P < 0.0005$) (**Table 4**).

After adjustment for age, sex, social status, and physical activity (model 1) (Table 4), dark chocolate consumption was still associated with serum CRP. In Model 2, in which the adjustment, besides the variables in model 1, also included nutrient intake, dark chocolate consumption remained inversely associated with serum CRP. The geometric mean serum CRP in dark chocolate consumers was 1.14 mg/L compared with 1.29 mg/L in nonconsumers. When the adjustment was conducted according to food group intake instead of nutrient intake (model 3), geometric mean serum concentrations of CRP were 0.12 mg/L lower in the dark chocolate consumer group than in nonconsumers. The last 2 models, 4 and 5 (Table 4), were fully adjusted for food group intake or nutrient intake, respectively (models 2 and 3), and for BMI, waist:hip ratio, and systolic BP, covariates associated with both binary outcome and serum CRP that are well-known determinants of this inflammatory marker. In both models, the inverse association between dark chocolate consumption and serum CRP was confirmed. In the food group fully adjusted model (model 4), the geometric mean serum CRP concentration was 0.09 mg/L lower in dark chocolate consumers compared with nonconsumers. This difference was −0.11 mg/L after full adjustment according to model 5, which included intake of nutrients rather than food.

Among the variables associated with dark chocolate consumption, only age, sex, and sweets were also independently associated with serum CRP concentrations in multivariate analysis. Stratified analysis for factors influencing serum CRP concentrations and associated with dark chocolate consumption was then performed. The association between dark chocolate intake and serum CRP did not differ between strata of sex, age,

TABLE 1 Characteristics of dark chocolate consumers and nonconsumers in a Moli-sani population[1]

Variable	Nonconsumers	Dark chocolate consumers	*P*-value[3]	
Median chocolate intake (interquartile range), *g/d*	0	5.7 (0.7–20.0)	—	—
n	1317	824	—	—
Men, *%*	50.9	54.8	0.073	0.001
Age, *y*	53.4 ± 0.3	50.3 ± 0.4	<0.0001	0.029
BMI, kg/m^2	27.1 ± 0.1	26.6 ± 0.1	0.003	0.461
Waist:hip ratio	0.91 ± 0.002	0.90 ± 0.003	0.061	0.584
Systolic BP, *mm Hg*	139.1 ± 0.5	135. 9 ± 0.7	<0.001	0.633
Diastolic BP, *mm Hg*	82.4 ± 0.3	82.3 ± 0.3	0.772	—
Serum total-cholesterol, *mmol/L*	5.52 ± 0.03	5.52 ± 0.03	0.896	—
Serum HDL-cholesterol, *mmol/L*	1.49 ± 0.01	1.48 ± 0.01	0.597	—
Serum LDL-cholesterol, *mmol/L*	3.34 ± 0.02	3.37 ± 0.01	0.411	—
Serum triglycerides, *mmol/L*	1.22 (1.18–1.25)	1.18 (1.14–1.23)	0.246	—
Blood glucose, *mmol/L*	5.30 (5.26–5.34)	5.25 (5.20–5.30)	0.142	—
Serum CRP, *mg/L*	1.32 (1.26–1.39)	1.10 (1.03–1.17)	<0.0001	0.025
Physical activity, *MET/d*	44.1 ± 0.3	42.6 ± 0.3	<0.0001	0.029
High social status, *%*	27.6	41.8	<0.0001	<0.001
Current smoker, *%*	25.6	23.2	0.890	—
White blood cells,[2] $n \times 10^{-3}/\mu L$	6.09 ± 0.05	6.20 ± 0.05	0.149	—
Lymphocytes,[2] $n \times 10^{-3}/\mu L$	1.90 ± 0.02	1.95 ± 0.03	0.245	—
Neutrophils,[2] $n \times 10^{-3}/\mu L$	3.46 ± 0.05	3.55 ± 0.07	0.311	—

[1] Values are means ± SEM or geometric means (95% CI).
[2] $n = 2027$, 821 and 821 for white blood cells, lymphocytes, and neutrophils, respectively.
[3] *P*-values from univariate analysis in first column. *P*-values from multivariate analysis adjusted for all the variables associated with chocolate consumption at the level $P <$ 0.10 in the univariate analysis in the 2nd column.

[6] Foods were grouped as follows (9): cereals (pasta and other grain, rice, bread, crispbread, rusks, salty biscuits, aperitifs biscuits, crackers, pizza); potatoes; legumes (peas, beans, lentils, chickpeas); vegetables raw and cooked (tomatoes, cabbage, broccoli, cauliflower, brussels sprout, carrot, beet root, beet, spinach, mushroom, pumpkin, squash, eggplant, lettuce, fennel, celery, capsicum, onion, garlic, mixed vegetables, soups, bouillon); fruits (apple, pear, banana, kiwi, grape, orange, tangerine, peach, apricot, prune, strawberry, fig, kaki, cherry, yellow melon); nuts and seeds; fish (shellfish, mollusk, tuna, cod, and other fish); meats (beef, veal, hamburger, pork, sausage, mutton, lamb, goat, game, horse, rabbit, chicken, turkey, offal, processed meat); low-, medium-, and high-fat dairy products; sweets (candies, sugar, honey, jam, Nutella, cakes, pies, pastries, puddings, dry cakes, biscuits); vegetable oils (olive oil, seed oils); fats (butter, margarine, other animal fat, mayonnaise, etc.); coffee, tea, alcoholic beverages (white, red, and rosé wine, beer, spirits); sweet beverages (fruit juices, carbonated/soft/isotonic drinks, diluted syrups).

TABLE 2 Estimated daily food group intakes of dark chocolate consumers and nonconsumers[1]

Food group intake	Nonconsumers	Dark chocolate consumers	*P*-value[3]	
n	1317	824	—	—
Cereals, g/d	259.9 ± 2.0	242.9 ± 2.5	<0.0001	0.004
Potatoes, *g/d*	30.9 ± 0.6	31.0 ± 0.8	0.940	—
Legumes, *g/d*	31.2 ± 0.6	32.6 ± 0.8	0.166	—
Vegetables, *g/d*	185.0 ± 2.1	189.3 ± 2.7	0.213	—
Fruits, *g/d*	378.5 ± 5.9	390.3 ± 7.5	0.219	—
Nuts and seeds, *g/d*	0.7 ± 0.1	1.1 ± 0.1	<0.001	0.002
Dairy products, *g/d*	187.7 ± 3.7	215.4 ± 4.6	<0.0001	0.050
Meat, *g/d*	111.9 ± 1.1	104.5 ± 1.4	<0.0001	0.109
Fish, *g/d*	41.8 ± 0.7	46.6 ± 0.9	<0.0001	0.018
Vegetables oils, *mL/d*	24.8 ± 0.2	25.0 ± 0.3	0.597	—
Fat, *g/d*	2.7 ± 0.1	2.8 ± 0.1	0.477	—
Sweets (no chocolate), *g/d*	48.1 ± 1.1	65.1 ± 1.4	<0.0001	<0.001
Sweet beverages, *g/d*	47.9 ± 2.7	58.6 ± 3.5	0.016	0.823
Coffee, *mL/d*	94.1 ± 1.8	103.2 ± 2.3	0.002	0.563
Tea, *mL/d*	16.1 ± 1.3	22.7 ± 1.7	0.002	0.036
Alcoholic beverages, *mL/d*	304.4 ± 9.7	216.4 ± 12.3	<0.0001	0.002
Total energy intake,[2] *kcal/d*	2191 ± 18	2363 ± 23	<0.00001	<0.001

[1] Values are means ± SEM.
[2] 1 kcal = 4.184 kJ.
[3] *P*-values are from ANOVA adjusted for total energy intake in the first column. *P*-values from multivariate analysis adjusted for all the variables associated with chocolate consumption at the level $P < 0.10$ in the univariate analysis in the 2nd column.

TABLE 3 Estimated daily macro- and micronutrient intakes according to nonconsumers and dark chocolate consumers[1]

Nutrient intake	Nonconsumers	Dark chocolate consumers	*P*-value[2]	
n	1317	824	—	—
Macronutrient intake, *units/d*				
Total protein, *g*	87.5 ± 0.3	88.2 ± 0.4	0.134	—
Total lipid, *g*	77.2 ± 0.4	81.9 ± 0.5	<0.0001	0.183
SFA, *g*	27.3 ± 0.2	29.3 ± 0.2	<0.0001	0.166
Monounsaturated fatty acids, *g*	37.4 ± 0.2	39.2 ± 0.2	<0.0001	0.143
Polyunsaturated fatty acids, *g*	8.29 ± 0.04	8.83 ± 0.06	<0.0001	0.769
Dietary cholesterol, *mg*	327.0 ± 2.3	350.8 ± 2.9	<0.0001	0.269
Carbohydrates, *g*	278.2 ± 1.1	278.8 ± 1.4	0.751	—
Dietary fiber, *g*	22.1 ± 0.1	22.4 ± 0.2	0.168	—
Alcohol, *g*	23.7 ± 0.6	17.0 ± 0.8	<0.0001	0.123
Mineral intake, *units/d*				
Iron, *mg*	14.2 ± 0.1	13.9 ± 0.1	0.001	0.267
Calcium, *mg*	958.5 ± 7.5	1016.5 ± 9.4	<0.0001	<0.001
Sodium, *mg*	2582.4 ± 14.8	2534.3 ± 18.7	0.045	0.034
Potassium, *mg*	3265.7 ± 14.5	3325.7 ± 18.4	0.011	0.976
Phosphorus, *mg*	1374.2 ± 4.9	1401.6 ± 6.1	<0.001	<0.001
Zinc, *mg*	12.1 ± 0.1	12.0 ± 0.1	0.152	—
Vitamin intake, *units/d*				
Thiamine, *mg*	0.97 ± 0.01	1.02 ± 0.01	<0.0001	0.151
Riboflavine, *mg*	1.44 ± 0.01	1.53 ± 0.01	<0.0001	0.586
Niacin, *mg*	17.9 ± 0.1	18.1 ± 0.1	0.052	0.642
Vitamin C, *mg*	133.3 ± 1.5	142.7 ± 1.9	<0.001	0.354
Vitamin B-6, *mg*	1.83 ± 0.01	1.87 ± 0.01	0.008	0.141
Folic acid, *μg*	272.3 ± 1.5	280.0 ± 1.9	0.002	0.779
Retinol equivalent, *μg*	880.4 ± 11.9	921.7 ± 15.1	0.032	0.475
β-Carotene, *μg*	2779 ± 39	2933 ± 50	0.016	0.653
Vitamin E, *μg*	7.3 ± 0.1	7.8 ± 0.1	<0.0001	0.976
Vitamin D, *μg*	2.31 ± 0.02	2.58 ± 0.03	<0.0001	0.003

[1] Values are means ± SEM.
[2] *P*-values are from ANOVA adjusted for total energy intake. *P*-values from multivariate analysis adjusted for all the variables associated with chocolate consumption at the level $P < 0.10$ in the univariate analysis.

and sweet consumption (interaction terms $P > 0.05$ for all). Similar results were found after stratification for indicators of healthy status such as BMI, physical activity, social status, or energy intake.

Dose-response curve. The fully adjusted regression model 5 (see note in Table 4 for definition), including a linear and a quadratic term for the intake of dark chocolate, was used to construct the average dose-response curve. The relationship found was interpreted as a J-shaped curve, because after an initial decrease in the serum CRP concentrations by increasing consumption of dark chocolate, the curve reaches a plateau at higher intake [up to 6.7 g/d, corresponding to a consumption of 1 serving (20 g) of dark chocolate every 3 d] and tends to be reversed at the highest consumption explored (linear term $\beta_1 = -0.051 \pm 0.021$; $P = 0.014$; quadratic term $\beta_2 = 0.0025 \pm 0.0010$; $P = 0.015$) (**Fig. 1**).

Discussion

Flavonoids contained in cocoa and dark chocolate might protect against risk factors for CVD (24,25).

We report here that regular intake of dark chocolate is inversely related to serum CRP concentrations in a cross-sectional analysis. This is, to our knowledge, the first study showing such an effect of dark chocolate on an inflammatory marker in a large healthy population of men and women randomly recruited from city hall registries in a region of southern Italy.

Dark chocolate consumption was associated with young age, high social status, and lower total physical activity. However, adjustment for these variables did not modify the association between CRP and dark chocolate. In the univariate analysis, dark chocolate consumption was also associated with lower systolic BP; however, the association disappeared after adjustment for possible covariates. Differences from the results of a previous study (9) may be due to differences in sample population and study design, because we did not restrict our analysis to elderly subjects.

Dark chocolate consumers had more healthy dietary habits than nonconsumers; indeed, they consumed less meat, refined cereal, and alcoholic beverages but more fish, nuts and seeds, sweets other than chocolate, coffee, and tea. To take into account the possibility that the relationship between dark chocolate consumption and serum CRP was mediated by the healthier lifestyle of chocolate consumers, 2 levels of adjustment were used in addition to environmental confounders: either food groups or macro- and micronutrient intake. In both cases, the association between serum CRP and dark chocolate consumption was still significantly present, although decreased.

Among the variables associated with chocolate consumption, only age, sex, and sweets were also independently associated with serum CRP concentrations. To further clarify whether dark chocolate was associated with serum CRP concentrations independently from such variables, we tested the association in their absence or presence in a stratified analysis for such factors.

TABLE 4 Serum concentrations of CRP in dark chocolate consumers and nonconsumers[1]

Serum CRP and model	Non consumers	Dark chocolate consumers	*P*-value[2]	*P*-value[3]
$n = 2141$	1317	824	—	—
Age adjusted	1.30 (1.24−1.36)	1.13 (1.06−1.20)	0.0005	0.0005
Model 1[4]	1.29 (1.23−1.36)	1.14 (1.07−1.21)	0.002	0.001
Model 2[5]	1.29 (1.23−1.36)	1.14 (1.07−1.21)	0.002	0.002
Model 3[6]	1.28 (1.22−1.35)	1.16 (1.09−1.23)	0.017	0.012
Model 4[7]	1.27 (1.22−1.33)	1.18 (1.11−1.25)	0.038	0.034
Model 5[8]	1.28 (1.22−1.34)	1.17 (1.10−1.24)	0.016	0.019

[1] Values are geometric means (95% CI).
[2] *P*-values are from multivariate ANOVA.
[3] *P*-values are from multivariate binomial Poisson regression.
[4] Adjusted for age, sex, social status (as a continuous variable) and physical activity (as a continuous variable).
[5] Adjusted for variables given for model 1 and intake of total lipid, alcohol, iron, calcium, thiamine, riboflavin, vitamin C, folic acid, vitamin E, and vitamin D.
[6] Adjusted for variables given for model 1 and intake of fish, meat, sweets (no chocolate), coffee, alcoholic beverages.
[7] Adjusted for variables given for model 3 and BMI, waist:hip ratio, systolic BP.
[8] Adjusted for variables given for model 2 and BMI, waist:hip ratio, systolic BP.

The association between dark chocolate consumption and serum CRP concentrations did not significantly vary in the presence or absence of such factors.

As previously shown by others (9), energy intake was higher in dark chocolate consumers than in nonconsumers, although BMI, waist:hip ratio, and systolic BP values were all lower. After further adjustment for anthropometric variables, we still found a strong relationship between dark chocolate consumption and serum CRP. In contrast, the association of anthropometric variables with serum CRP disappeared. The apparent contradictory association between dark chocolate consumption and low BMI in the presence of high energy intake and low physical activity was probably due to differences in age and social status between consumers and nonconsumers, because it disappears after adjustment for such variables. As an alternative explanation, the association between dark chocolate consumption and low BMI could be attributed to the physiologic activity of polyphenols, as it has been described for tea polyphenols in humans and rats (26,27). However, this hypothesis remains to be demonstrated.

Consumption of nuts and seeds, coffee, and tea, which were all strongly related to dark chocolate consumption and are excellent sources of antioxidants, was not associated with any reduction in serum CRP, suggesting a specific effect of dark chocolate on this inflammatory marker.

To eliminate the possibility that the associations found were dependent on either changes in lifestyle (particularly in dietary habits) as a consequence of a disease or the presence of more chocolate consumers in healthy people, we had preliminarily excluded from our analyses all subjects with previous CVD, those undergoing therapy for hypertension, diabetes, or dyslipidemia, and those following any type of dietary regimen. The possibility of reverse causation was also excluded by stratification analysis, showing that dark chocolate consumption did not differ between strata of BMI, smoking habits, social status, physical activity, and energy intake, all parameters considered as indicators of healthy status.

Two small, short-term clinical trials (10,28) previously investigated the effect of cocoa on markers of inflammation and did not find any association. Besides the small size of such studies that might have generated false negative results, they used relatively high doses of chocolate that could have masked the effect. Our dose-response curve indeed shows that the effect of dark chocolate on serum CRP was present up to the intake of 1 serving every 3 d and tended to disappear at higher doses. We can only speculate on the mechanism(s) for that narrow range of protection. Such a low dose contributed to a low amount of total daily energy and nutrient intake (29). Increasing the dose of chocolate would also lead to increased total energy and SFA intake that could oppose the beneficial effects of polyphenols on inflammation (30). Moreover, polyphenols could behave both as antioxidants and pro-oxidants and therefore act as either antiinflammatory or proinflammatory compounds, depending on their concentration and free radical source (31).

Our data are in agreement with those of the Zutphen Elderly Study (9) in which men with a usual consumption of 4.2 g/d of cocoa (corresponding to ~10 g of dark chocolate) had a lower systolic BP compared with men with no or very low intake. In contrast, intervention studies showed that a short-term consumption of 10 times more chocolate was needed to decrease BP. More recently, Taubert et al. (29) have shown that a longer supplementation with low doses (6.3 g/d) of dark chocolate efficiently reduced BP and improved formation of vasodilatory nitric oxide. These 3 studies agree to suggest that long-term regular intake of small amounts of dark chocolate can positively affect health parameters. Due to its small contribution to total calorie intake [because 100 g of dark chocolate amounts to ~515 kcal (2154.8 kJ), 2–6 g/d will provide ~10–30 kcal (41.8–125.5 kJ)], dark chocolate consumption should have no harmful effect on anthropometric variables such as BMI and waist:hip ratio and can be viewed as a promising behavioral approach to lower, in a quite pleasant way, cardiovascular risk factors at a general population level.

According to data reported in apparently healthy American men and women (11), ranges of serum CRP measured in our nonchocolate consumer population would belong to a "moderate" risk estimate quintile, whereas the ranges found in dark chocolate consumers would be classified as a "mild" risk estimate. For the decrease in serum CRP values from moderate to mild quintile, the relative risk of suffering a future cardiovascular event would apparently decrease by 26% in men (95% CI, 11–44%) and 33% in women (95% CI, 13–56%). It is reasonable therefore to suggest that in an apparently healthy population, even a small reduction of a low-grade inflammation such as that associated in this study with regular consumption of dark chocolate might have clinically relevant benefits in the primary prevention of CVD.

Chocolate products generally contain a higher total flavan-3-ol concentration on a per weight basis than is found in most plant foods and beverages containing such flavonoids (3). Chocolate components have been shown in vitro to decrease inflammation by several mechanisms (7,8,31,32). Furthermore, consumption of chocolate products with a high content in procyanidin lowered proinflammatory leukotrienes levels in a feeding clinical trial (33).

We have now shown that dark chocolate consumption decreases serum CRP, a marker of inflammation in a cross-sectional study, independently of possible covariates such as sex, age, and sweets consumption that can, in turn, also independently influence serum CRP concentrations.

Limitations of this study. There are some limitations of our study. First, the cross-sectional nature of our study does not enable determination of causality. Second, self-report of chocolate

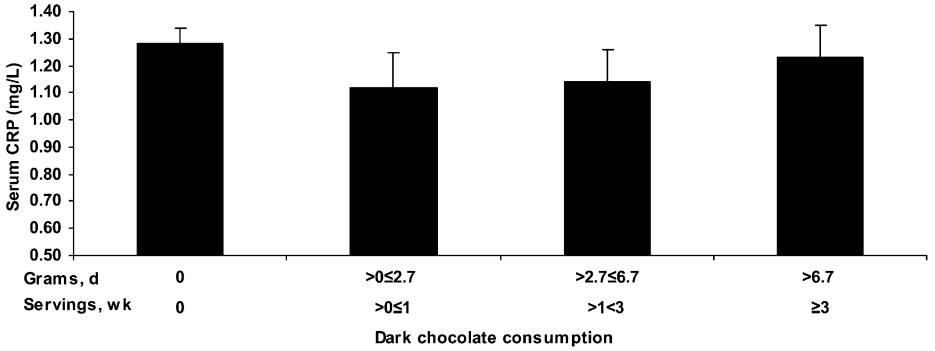

FIGURE 1 J-shaped relationship of serum CRP concentrations according to tertiles of dark chocolate intake among all subjects. Values are geometric means (95% CI), n = 1317, 211, 301, and 312 for nonconsumers, T1, T2, and T3, respectively. Consumption of dark chocolate was expressed both as g/d and as servings/wk (one serving = 20 g of dark chocolate).

intake by participants may underestimate the true consumption rate. Third, a reliable distinction between serum CRP concentrations according to the chocolate intake was difficult, because of the small sample size of persons consuming chocolate more than once a day. Finally, we cannot exclude that healthy lifestyles, associated with chocolate consumption, can at least partially contribute to the decrease in serum CRP concentrations, because adjustment for such factors slightly reduced the association of serum CRP with chocolate without affecting its significance.

In conclusion, the present study, by showing a significant inverse association between dark chocolate and serum CRP, adds new insight into the relationship between flavonoid-rich foods, inflammation, and cardiovascular protection. Additional studies are necessary to explain the mechanisms linking dark chocolate consumption and regulation of serum CRP concentrations.

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