Safety and efficacy of cocoa flavanol intake in healthy adults: a randomized, controlled, double-masked trial

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Abstract

Background: Evidence from dietary intervention studies shows that the intake of flavanols and procyanidins can be beneficial for cardiovascular health. Nevertheless, there is a clear need for advancing our understanding with regard to safe amounts of intake for these bioactives.

Objective: The aim was to investigate in healthy adults the effects of cocoa flavanol (CF) intake amount and intake duration on blood pressure, platelet function, metabolic variables, and potential adverse events (AEs).

Design: This investigation consisted of 2 parts. Part 1 was an open-label, intake-amount escalation study, in which 34 healthy adults (aged 35–55 y) consumed escalating amounts of CFs, ranging from 1000 to 2000 mg/d over 6 wk. Primary outcomes were blood pressure and platelet function, select metabolic variables, and the occurrence and severity of AEs. Secondary outcomes included plasma concentrations of CF-derived metabolites and methylxanthines. On the basis of the outcomes of study part 1, and assessing the same outcome measures, part 2 of this investigation was a controlled, randomized, double-masked, 2–parallel–arm dietary intervention study in which healthy participants (aged 35–55 y) were asked to consume for 12 consecutive weeks up to 2000 mg CFs/d (n = 46) or a CF-free control (n = 28).

Results: Daily intake of up to 2000 mg CFs/d for 12 wk was not associated with significant changes in blood pressure or platelet function compared with CF-free controls in normotensive, healthy individuals who exhibited a very low risk of...
cardiovascular disease. There were no clinically relevant changes in the metabolic variables assessed in either of the groups. AEs reported were classified as mild in severity and did not significantly differ between study arms.

**Conclusion:** The consumption of CFs in amounts up to 2000 mg/d for 12 wk was well tolerated in healthy men and women. This trial was registered at clinicaltrials.gov as NCT02447770 (part 1) and NCT02447783 (part 2).

**Keywords:**

cocoa  epicatechin  flavanol  procyanidin  safety

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**INTRODUCTION**

Flavanols, including catechin and epicatechin, as well as their related oligomers, the procyanidins, are plant-derived bioactives (1). Evidence from dietary intervention trials supports the concept that the intake of these bioactives can provide beneficial vascular effects in humans (2–4). However, the intake amount of flavanols and procyanidins studied in many intervention trials significantly exceeds the average, habitual dietary intake of these bioactives by most populations (5). Although this is not unusual per se when compared with a variety of other nutrients and bioactives, including vitamin D, folate, and fiber, for example, and regardless of the potential positive health effects, it is important to investigate whether the chronic intake of dietary flavanols and procyanidins poses risks for human health and well-being (2, 6). Although increasing the consumption of flavanol- and procyanidin-containing foods and beverages would intuitively seem to be safe, because such foods are present in the typical human diet and, to date, neither epidemiologic nor medical anthropological data indicate otherwise (5, 7), to our knowledge the potential risks of a high-flavanol/procyanidin diet have not been specifically investigated in a prospective study. To underscore the need for such studies, although many investigators have suggested that increasing the intake of flavanols/procyanidins via dietary guidance, food fortification, or dietary supplementation may result in a number of health benefits, others have raised concerns with regard to the potential risks that might be associated with the chronic intake of higher amounts of these bioactives. In particular, there has been concern with regard to the potential hepatotoxic effects of galloylated flavanols, which are, among other sources, particularly abundant in green tea–based dietary supplements (8–10). Such concerns highlight the importance of the question as to whether all flavanols and procyanidins can be treated as equals when assessing the potential risks that might be associated with this class of bioactives. Finally, although flavanol and procyanidin consumption has been associated with improvements in vascular function in populations at higher cardiovascular disease (CVD) risk (11–17), the efficacy and safety of the dietary intake of these bioactives in “healthy adults” have not been studied in depth.

Given the above, in the current study we investigated in healthy adults who were classified as being at low risk of CVD the effects of cocoa flavanols (CF) intake amount and intake duration on blood pressure (BP), platelet function, metabolic variables, and potential adverse events (AEs). We hypothesized that a daily intake of up to 2000 mg CFs is well tolerated in healthy humans and would exert beneficial changes in BP and platelet function.
METHODS

Subjects

Healthy men and women between 30 and 55 y of age were recruited by public advertisement in the city of Davis, California, and surrounding areas. Exclusion criteria included the following: BMI (in kg/m^2) >30; BP >140/90 mm Hg; allergies to peanut or cocoa; avoidance of caffeinated food products and beverages; a history of CVD, stroke, gastrointestinal tract disorders, or renal, hepatic, or thyroid disease; previous gastrointestinal surgery (except for appendectomy); the current intake of herb-, plant-, or botanicals-containing dietary supplements; following a vegan/vegetarian diet; or adhering to an uncommon diet or a weight-loss program. To determine eligibility, participants were asked to complete health and lifestyle questionnaires; have their height, weight, and in-office BP determined; and to provide a blood sample for a complete blood count (CBC), liver panel, lipid panel, and metabolic panel assessments. Enrolled participants commenced the study protocol between 1 and 3 wk after eligibility was determined. While participating in the study, volunteers were asked to maintain their typical daily activities and diet. Volunteers were asked to fast for 12 h before each study day when blood samples were collected (water was allowed ad libitum) and to refrain from taking nonsteroidal anti-inflammatory drugs (including aspirin and ibuprofen) 7 d before the study day.

Test materials

All test materials investigated were delivered in cellulose-based capsules intended for oral consumption. The macro-/micronutrient composition of the test materials is detailed in Table 1. The CF-containing test material was made by using cocoa extract (CE) manufactured by the CocoaPro process (Mars Inc.), which represents a food-grade extract derived from seeds of *Theobroma cacao* L. by means of a flavanol-/procyanidin-preserving manufacturing process. The CE capsules were standardized with regard to their total CF content, namely 500 mg/capsule (see Table 1). The term "cocoa flavanols," and consequently the CF intake amount, is defined here as the sum of all flavanols and procyanidins ranging from monomers to decamers (i.e., degrees of polymerization from 1 to 10). The control capsules were cellulose-based capsules that contained a highly alkalized cocoa powder (Jet Black; Blommer Chocolate Co.) with no detectable CF content [limit of detection: 0.3 mg epicatechin/g product (18, 19)]. CE and control capsules were matched for their macro-/micronutrients as well as for their theobromine and caffeine content, their size, and overall appearance. All capsules were produced under established good manufacturing practices.

| TABLE 1 |
| Composition of the test materials¹ |

Study design

Study parts 1 and 2 were conducted at the Ragle Human Nutrition Center at the
University of California, Davis (UC Davis). Recruitment for part 1 started in April 2012, and the study was completed within 3 mo. Part 1 consisted of an open-label, intake-amount escalation study in the course of which participants were asked to consume an increasing number of CE capsules in successive 2-wk intervals, followed by a 2-wk washout period during which participants did not consume CE capsules (Figure 1). The corresponding CF intake amounts were as follows: 1000 mg/d (2 CE capsules/d) for 2 wk, 1500 mg/d (3 CE capsules/d) for 2 wk, and 2000 mg/d (4 CE capsules/d) for 2 wk, followed by a 2-wk washout period. Because previous investigations focused on studying the effects of CF intake at amounts of up to 1000 mg/d (11–17), we aimed here to investigate in healthy individuals the longer–term effects on both efficacy and safety of CF intake amounts of up to 2000 mg/d (corresponding to the respective intakes of 248 mgflavanol monomers and 1752 mg procyanidins/d). In addition to supporting data from preceding CF–based studies, this approach was also deemed tenable by earlier investigations that provided monomeric flavanols from tea in amounts of up to 850 mg/d (20, 21) as well as by data on the very limited absorption and systemic availability of procyanidins (22) and the habitual intake of procyanidins in certain populations (23).

Participants were asked to consume their CE capsules at 2 separate intake occasions per day (with breakfast and lunch or with lunch and dinner). On each study visit (scheduled for study days 1, 15, 29, 43, and 57; Figure 1), participants underwent an in–office BP measurement and a blood draw to assess platelet function, metabolic variables, and plasma concentration of CFs and methylxanthine metabolites. In addition, participants were scheduled to have 1 of their 2 daily intake occasions at the study site (corresponding to CF intakes of 500 mg at visit 1; 1000 mg at visits 2, 3, and 4; and no capsule/CF intake at visit 5; Figure 1), where the capsules were consumed with 250 mL water instead of a meal to allow for assessing absorption and metabolism.

The recruitment for study part 2 started in June 2012, and the study was completed within 7 mo. Part 2 consisted of a randomized, parallel–arm, double–masked, controlled dietary intervention study. The minimum sample size required to determine whether in–office BP would be affected by CF intake (n = 30; α = 0.05, power = 0.8) was determined by ANOVA testing via imputing an SD of 8.6
and a mean difference of 6.4 for systolic BP on the basis of a previous study (24). Participants were randomly assigned to receive CE or control capsules by using computer-generated lists of random numbers via the randomly permuted block method and stratified by sex. The allocation ratio to receive CE or control capsules was 2:1 (CE:control). The allocation list was generated by one of the authors (JIO), recruitment of participants was conducted by another author (JK), and allocation of participants was conducted by a third author (JLE). Participants, the physician on record, and researchers assessing outcomes as well as researchers involved with the statistical analysis of data were masked to the nature of the intervention. Participants were asked to consume CE capsules (CF group) or CF–free control capsules (control group) daily for 12 consecutive weeks in the following manner. Participants followed a run-in period consuming 2 capsules/d (corresponding to 1000 mg CFs/d in the CF group) during week 1 and 3 capsules/d (corresponding to 1500 mg CFs/d in the CF group) during week 2; after the run-in period, participants consumed 4 capsules/d (corresponding to 2000 mg CFs/d in the CF group) from week 3 to week 12, followed by a 2-wk washout period during which they did not consume any control or CE capsules (Figure 1). Participants were asked to divide the amount of capsules consumed per day into 2 intake occasions and to consume capsules with breakfast and lunch. Before each scheduled study visit, participants were asked to fast for 12 h and to refrain from taking aspirin and ibuprofen for the previous week. At each study visit (study days 1, 43, 85, and 99; Figure 1), participants underwent an in–office BP measurement and a blood draw to assess platelet function, metabolic variables, and plasma concentrations of CFs and methylxanthine metabolites. At visit 1 (study day 1) and visit 3 (study day 85), subjects had their 24-h ambulatory BP (ABP) measured. During visits to the research facility and scheduled interviews, volunteers were reminded to maintain their normal daily routine, activities, and diet.

Research ethics

The study protocol was approved by the Institutional Review Board of UC Davis. All subjects gave their written informed consent to participate. This trial was registered at clinicaltrials.gov as NCT02447770 (part 1) and NCT02447783 (part 2). This manuscript is compliant with CONSORT (Consolidated Standards for Reporting Trials).

Quantification of CF-derived metabolites and methylxanthines in plasma

Blood samples were collected with the use of EDTA as an anticoagulant. Plasma was obtained by whole–blood centrifugation at 1800 × g for 15 min at 4°C, separated into aliquots, and combined with ascorbic acid (final concentration: 1 mg/mL). Plasma samples were stored at 80°C until analyzed. CF–derived metabolites determined in plasma corresponded to the structurally related (−)-epicatechin metabolites (SREMs) and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone metabolites (γ-VLMs) and were measured by using validated methods as previously described (22, 25). Methylxanthines, including theobromine, caffeine, paraxanthine, and theophylline, were quantified to assess compliance as previously described (26).

AE monitoring and analysis
All participants were actively monitored throughout the study for the occurrence of AEs. This monitoring consisted of completing a questionnaire with open-ended questions that inquired about the general health and well-being of the participant and about the potential occurrence of any health–related events. The questionnaire was completed during interviews scheduled weekly (in study part 1) and biweekly (in study part 2). In addition, self-reporting of any AE was encouraged by providing participants with a telephone number and an e-mail address to report any AE occurring at any time throughout the study. The terminology used to describe AEs, as well as the degree of severity of AEs reported, is that outlined in the Common Terminology Criteria for Adverse Events (CTCAE), NIH (version 4.0, 2009). Grade 1 or mild AEs consisted of asymptomatic or mild symptoms, clinical or diagnostic observations only, without required medical intervention. Grade 2 or moderate AEs consisted of moderate symptoms with minimal, local, or noninvasive intervention indicated and limiting age–appropriate instrumental activities of the daily life (e.g., preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.). Grade 3 (severe AEs) and grades 4 and 5 (serious AEs including life-threatening AEs and death, respectively) were not reported in this study. Participants were informed through the consent form of potential types of AEs that may occur during the study, which included gastrointestinal–related AEs (including nausea, gastroesophageal reflux, abdominal/stomach pain, constipation, bloating, flatulence, vomiting, and dyspepsia), headaches, and blood draw–related AEs (including arm soreness and lightheadedness). Other type of AEs recorded during the study were considered unexpected. The relation of the AE to the subject’s participation in the study was determined by the physician on record (Alan Shindel, UC Davis Medical Center), while remaining masked to the nature of the intervention that participants received. This procedure was conducted by using a 4-category system (not related, possibly related, probably related, and related) according to the following guidelines:

1) Not related: an AE was considered not related to the study when the AE could be reasonably explained by other factors, including underlying disorders or conditions of the participants or other circumstances unrelated to either the study or any underlying disorder or condition of the participants. AEs that were not related to the study were not reported in this study.

2) Possibly related: an AE was considered possibly related to the study when it was not possible to exclude that the AE was possibly caused by the procedures of the study, although other factors, such as underlying disorders or conditions of the participants, were presumable.

3) Probably related: an AE was considered probably related to the study when there was a reasonable possibility that the AE may have been caused by the procedures involved in the study and that other factors, such as underlying disorders or conditions of the participants, could be excluded.

4) Related: an AE was considered to be related to the study when there
was a reasonable hypothesized cause–effect relation between the subject’s participation in the study with the occurrence of the AE.

**Metabolic variables**

Blood samples were collected by venipuncture with the use of EDTA and heparin as an anticoagulant. Collected samples were used for the assessment of a comprehensive metabolic panel and CBC at the Department of Pathology and Laboratory Medicine, UC Davis Medical Center. The resultant data were independently reviewed by 2 physicians at the UC Davis Medical Center (Alan Shindel and Jeanna Welborn, both of UC Davis Medical Center).

**Assessment of BP**

After a 15-min rest in a seated position, in–office BP was measured by using an automated oscillometric Welch Allyn Spot Vital Signs LXi BP monitor (Welch Allyn). Three consecutive readings were taken by a single observer in 5-min intervals. The first measurement was discarded; and BPs, including systolic BP, diastolic BP, mean BP, and heart rate, were determined as the average of the last 2 measurements. For the assessment of 24-h ABP, participants were fitted with a Spacelabs ABP model 90217–1Q monitor (Spacelabs Health Care). The ABP cuff was placed on the nondominant arm over the brachial artery, and ABPs were recorded every 30 min from 0700 to 2200 h, and every 60 min during 2200–0700 h. Participants were asked to keep the monitor in place at all times. An activity diary was recorded by each participant to determine daytime and nighttime averages for systolic BP, diastolic BP, mean BP, and heart rate. Recordings of 24-h ABP were used to determine 24-h averages for systolic BP, diastolic BP, mean BP, and heart rate. All BP measurements for the same participants were conducted by using the same monitor, cuff size, and designated arm.

**Assessment of platelet function**

Blood samples were collected by using citrate as an anticoagulant. Platelet function was then assessed by using the PFA-100 analyzer (Dade Behring International) with collagen–epinephrine and collagen–ADP stimulation as described elsewhere (27).

**Assessment of background diet**

A 3-d food record (3-DFR) for 1 weekend day and 2 adjoining weekdays was collected the week before study visits 2 (study day 15) and 4 (study day 43) during study part 1 and before study visits 2 (study day 43) and 3 (study day 85) during study part 2. On these days, the subjects were asked to record everything they ate and drank, including approximate amounts, on provided data sheets. 3–DFRs were analyzed with Food Processor SQL (version 10.12.0; ESHA Research) for the content of macro- and micronutrients, caffeine, as well as (−)–epicatechin, (+)–catechin, procyanidins (dimers, trimers, tetramers to hexamers, and heptamers to decamers), and galloylated flavanols [(−)–epicatechin gallate, (−)–epigallocatechin, and (−)–epigallocatechin gallate] with the use of USDA tables (28, 29). Data obtained from the two 3–DFRs from the same participant were averaged, and results were standardized to a total energy intake of 8 MJ.
Materials

Authentic de novo chemically synthesized (−)-epicatechin metabolites and 5-((3′,4′-dihydroxyphenyl)-γ-valerolactone (γ-VL) were provided by Mars Inc. The synthesis of (−)-epicatechin metabolites and γ-VL was performed according to Zhang et al. (30) and Hamada et al. (31), respectively. These standards were chemically synthesized and characterized by using mass spectrometry and nuclear magnetic resonance (data not shown). Sulfatase and β-glucuronidase enzymes from Helix pomatia (G0751) were purchased from Sigma. HPLC-grade water, methanol, and acetonitrile were purchased from Fisher.

Statistical analysis

Data are presented as means ± SDs. The primary test used to analyze results from study part 1 was a 1-factor repeated-measures ANOVA (factor: amount of CFs consumed). The primary test used to analyze results from study part 2 was a 2-factor repeated-measures ANOVA (factor 1: intervention; factor 2: time). The primary test used to analyze the AEs was Fisher’s exact test. ANOVA, Tukey P values, and Fisher’s exact test were computed with SigmaStat 3.5 (Systat Software). P values ≤0.05 were considered significant.

RESULTS

Study population

We screened 49 and 83 volunteers for study parts 1 and 2, respectively. The number of volunteers enrolled in each study part and the number of volunteers available for intention-to-treat analysis are depicted in Figure 2. Twenty volunteers participated in both study parts. These volunteers had a minimum of 2 wk between the completion of study part 1 and the beginning of study part 2. Characteristics of the volunteers who were randomly assigned to study parts 1 and 2 are given in Table 2. The calculation of 10-y CVD risk shows that the volunteers recruited for study parts 1 and 2 represented a population at low risk of CVD [i.e., <10% 10-y CVD risk (32)]. No significant differences existed between the participants of study part 1 and the 2 groups enrolled in study part 2. On the basis of the 3-DFRs collected, the average habitual daily intake of (−)-epicatechin and procyanidins was equal to 7% and 5% of the amount of (−)-epicatechin and procyanidins provided by the CE capsules, respectively. The average habitual daily intake of caffeine derived from participants’ background diet was 260% higher than the amount of caffeine provided by the intake of CE and control capsules.

TABLE 2

| Table 2 | Baseline characteristics of the study population and background dietary intake of flavanols and caffeine assessed during the study1 |

FIGURE 2
Metabolic variables and AEs reported with the intake of increasing amounts of CFs

A total of 48 AEs were reported by 27 participants during study part 1. All of the reported AEs were NIH CTCAE grade 1 (mild). The data did not show an association between intake amount and number of AEs reported, that is, there was no difference in the number or type of AEs reported whether volunteers consumed 2 CE capsules/d (1000 mg CFs/d), 3 CE capsules/d (1500 mg CFs/d), or 4 CE capsules/d (2000 mg CFs/d) (Table 3). However, 94% of all AEs reported occurred during the 6-wk interval in which participants consumed CE capsules, whereas only 6% occurred during the 2-wk washout period (P < 0.001, Fisher’s exact test) (Table 3). Gastrointestinal–related AEs represented the majority of AEs reported (75%; P < 0.005, Fisher’s exact test). Nausea was the most predominant gastrointestinal–related AE (n = 16 reports; 44% of all gastrointestinal–related AEs reported; P < 0.05, Fisher’s exact test), and it was predominantly reported during the study visits (Supplemental Figure 1) when participants were asked to consume the CE capsules on an empty stomach after an overnight fast. Other reports of nausea occurred when participants consumed CE capsules with only a light snack. Vomiting (n = 3) was reported by 2 participants when consuming CE capsules after 12 h of fasting (n = 2) or after only a light snack (n = 1). In addition to gastrointestinal–related AEs, we recorded headaches (n = 6), blood draw–related AEs (n = 3), and other AEs, including insomnia (n = 1), agitation (n = 1), and hot flashes (n = 1). No clinically relevant changes in metabolic variables were observed during study part 1 (Supplemental Table 1).

| TABLE 3 |
| Number of AEs reported during study part 1 per type of AE and amount of CFs consumed¹ |

BP and platelet function after the intake of increasing amounts of CFs

Results for in–office BP assessments and platelet function measurements after ex vivo stimulation with collagen–epinephrine and collagen–ADP are presented in Table 4. No significant changes in systolic or diastolic BP or platelet function were...
observed after the daily intake of increasing numbers of CE capsules over 2–wk intervals. No acute changes in BP and platelet function were observed 2 h after the consumption of CE capsules (Table 4).

### TABLE 4

| In-office BP and platelet function determination during study part 1 |

| Concentration of CF-derived metabolites in plasma after the intake of increasing amounts of CFs |

On visit 1 before CE capsule intake (12-h fasting plasma), the concentration of SREMs in plasma was below the limit of detection (<25 nM) in 27 of 33 volunteers. The consumption of 1 CE capsule [500 mg CFs/55 mg (−)-epicatechin] and 2 CE capsules [1000 mg CFs/110 mg (−)-epicatechin] resulted in a significant increase in the concentration of SREMs in plasma within 2 h after ingestion, reaching concentrations of 855 ± 458 and 1832 ± 968 nM, respectively (Supplemental Figure 2). The main SREMs determined in plasma corresponded to (−)-epicatechin-3′-β-D-glucuronide, (−)-epicatechin-3′-sulfate, and 3′-O-methyl(−)-epicatechin sulfated in positions 5 and 7. No differences in the profile of SREMs were observed 2 h after the intake of 1 CE capsule [55 mg (−)-epicatechin] or 2 CE capsules [110 mg (−)-epicatechin; Supplemental Figure 2]. The concentration of SREMs in 12-h fasting plasma (0 h) significantly increased after 2 wk of daily consumption of 3 CE capsules/d [165 mg (−)-epicatechin/d; 164 ± 138 nM] and 4 CE capsules/d [220 mg (−)-epicatechin/d; 293 ± 399 nM; P < 0.05, repeated-measures ANOVA). On visit 5 (2 wk after the cessation of CE capsule intake), the concentration of SREMs in 12-h fasting plasma was not significantly different from that quantified on visit 1 (at the beginning of the study).

Unconjugated (−)-epicatechin was detected in the plasma of 28 of 33 volunteers 2 h after CF intake, although it accounted for only 1.2% ± 1.5% of the amount of all SREMs in plasma. No statistical differences were observed in the concentration of unconjugated (−)-epicatechin 2 h after the ingestion of 2 CE capsules [110 mg (−)-epicatechin] on 3 different occasions (corresponding to study visits 2, 3, and 4). Unconjugated (−)-epicatechin was not detected in any of the 12-h fasting plasma (0 h) samples.

The concentration of γ-VLM was assessed before and after the daily intake of increasing amounts of CE capsules in 12-h fasting plasma (Supplemental Figure 3). There was an increase in the concentration of γ-VLM in 12-h fasting plasma that was dependent on the amount of CE capsules consumed (P < 0.01, r = 0.969), reaching concentrations of up to 568 ± 784 nM after a daily intake of 2000 mg CFs/d.

The concentration of methylxanthines was determined in 12-h fasting plasma after the intake of CE capsules that, in addition to CF, contained theobromine and caffeine (Table 1). Daily CE capsule intake resulted in a significant increase in the...
plasma concentration of theobromine, from 5 ± 4 μM up to 18 ± 9 μM, that was dependent on the amount of CE capsules ingested (P < 0.001, r = 0.999; Supplemental Figure 3). Significant increases in the plasma concentration of caffeine and its main metabolite in humans, paraxanthine, were observed after the consumption of 4 CE capsules (38 mg caffeine/d) for 2 wk (Supplemental Figure 3). No changes in the concentration of theophylline were detected. On visit 5 (2 wk after the cessation of CE capsule intake), the concentrations of theobromine, caffeine, and paraxanthine were not significantly different from those quantified on visit 1 (beginning of the study).

Metabolic variables and AEs reported after a 12-wk dietary intervention with CFs

A total of 46 AEs were reported by 31 participants, with 28 AEs reported by 19 participants in the CF group (n = 46 participants) and 18 AEs reported by 12 participants in the control group (n = 28 participants). There was no significant difference in the number of AEs reported between the study groups (P = 1.000, Fisher’s exact test; Table 5). The majority of AEs reported were NIH CTCAE grade 1 (mild; 98%), except for one AE classified as NIH CTCAE grade 2 (moderate; nature of the AE was headaches, which receded after cessation of intake) in the CF group. Gastrointestinal–related AEs represented 62% of all AEs reported, with no significant differences between groups (Table 5; P = 0.119, Fisher’s exact test). Regardless of the test material consumed, 93% of the gastrointestinal–related AEs reported during study part 2 took place within the first 6 wk of the study (Supplemental Figure 4). The number of reports of headaches in the CF group (n = 46 participants) was 11, including 8 reports of recurring headaches, whereas in the control group (n = 28 participants) 2 events were reported, including 1 of recurring headaches; these numbers were not significantly different (P = 0.190, Fisher’s exact test). In the CF group, 9 of 11 reports of headaches occurred in the first 6 wk of the intervention, with 4 being reported during the first week of the study (Supplemental Figure 4). We recorded 1 blood draw–related AE and other AEs that included 1 report of acne (CF group), 1 report of prodromal acne (control group), and minor petechiae due to the use of the ABP cuff. No clinically relevant changes in liver panels (Table 6), metabolic panels, or CBC results were observed during the study or between groups (Supplemental Table 2).

<table>
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<th>TABLE 5</th>
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<td>Number of AEs reported during study part 2 per type of AE and study group</td>
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<th>TABLE 6</th>
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<td>Liver panel results during study part 2</td>
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BP and platelet function after a 12-wk dietary intervention with CFs
Results for both in office-BP and ABP are given in Table 7. There were no significant changes in systolic or diastolic BP over time compared with the beginning of the study or between study groups (Table 7). Results for platelet function, as assessed by closure time after ex vivo stimulation with epinephrine and ADP, are shown in Table 8. No significant differences in closure time were observed over time compared with the beginning of the study or between study groups (Table 8).

### TABLE 7

| In-office BP and 24-h ABP during study part 2
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### TABLE 8

| Platelet function assessed during study part 2
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Concentration of CF-derived metabolites and methylxanthines in plasma after a 12-wk dietary intervention

The concentration of γ-VLM in 12-h fasting plasma was measured before (baseline) and 43, 85, and 99 d after the initiation of the intervention with the control and CE capsules (Supplemental Figure 5). Compared with baseline, in the CF group there was a significant increase in the concentration of γ-VLM on day 43 (47 ± 30 vs. 648 ± 604 nM; P < 0.05, repeated-measures ANOVA); values on day 85 (498 ± 511 nM) were similar to the day 43 data. Two weeks after the cessation of CE capsule ingestion (study day 99), concentrations of γ-VLM in 12-h fasting plasma were similar to those at baseline (92 ± 124 nM). No changes in the concentration of γ-VLM were detected in the control group throughout the study compared with the baseline values for this group (42 ± 21 nM).

Methylxanthines were quantified in 12-h fasting plasma before (baseline) and 43, 85, and 99 d after the initiation of the intervention with the control and CE capsules. Both groups showed a significant increase in the concentration of theobromine on day 43 (17 ± 10 μM for the CF group and 17 ± 7 μM for the control group; P < 0.05, repeated-measures ANOVA) and day 85 (16 ± 8 μM for the CF group and 16 ± 9 μM for the control group; P < 0.05, repeated-measures ANOVA) compared with baseline concentrations (7 ± 8 μM for the CF group and 4 ± 3 μM for the control group; Supplemental Figure 5). No significant differences were observed in the concentration of theobromine between study groups. Two weeks after the cessation of test product ingestion (study day 99), the concentration of theobromine returned to concentrations similar to those detected at baseline (6 ± 6 μM for the CF group and 5 ± 4 μM for the control group). Intakes of CE and control capsules resulted in similar increases in caffeine and paraxanthine concentrations in plasma (Supplemental Figure 5). No changes in plasma concentrations of theophylline were detected throughout the study.
DISCUSSION

We showed here that the daily consumption for 12 wk of up to 2000 mg CFs did not affect BP, platelet function, liver panel, or metabolic markers in a cohort of healthy middle-aged men and women (n = 46). We further report that all but one of the AEs recorded during this study were mild (NIH CTCAE grade 1) and the occurrence of AEs was not associated with the amount of CFs consumed. We did not observe an association between the type or number of AEs and increased periods of daily CF intake (up to the maximal duration of 12 wk studied here). Collectively, these findings suggest that the consumption of up to 2000 mg CFs daily is well tolerated by healthy men and women.

Contrary to our initial assumption, we did not observe any significant changes in BP or platelet function in our study cohort after either an acute intake of CFs in amounts of up to 1000 mg or after the consumption of up to 2000 mg CFs/d for 12 wk. Although the current results may seem to be at odds with findings from previous studies, in which changes in platelet function and BP after CF intake were observed (11–17), the participants in those studies were often individuals who had either been diagnosed with CVD or who exhibited a high risk of CVD. In marked contrast to the above, the population recruited here consisted of a healthy cohort who exhibited a very low CVD risk (Table 2). In this context, similar outcomes were observed in studies with quercetin supplements, in which volunteers with hypertension showed improvements after the intake of a quercetin supplement, whereas healthy individuals showed no detectable change in BP (33–35) over the course of the investigation.

It may also be meaningful to consider that a general limitation of studies in healthy individuals is based on how investigators assess the health status of study participants. Although it is a common practical approach to define as healthy those individuals for whom absence of disease can be demonstrated, such assessments do not allow for a more granular and differentiated assessment of the health status of any given individual. This may be one of the reasons for interstudy inconsistencies, especially in short-term studies, as can perhaps be exemplified by differences in the outcomes of this and other recently published flavanol–based dietary interventions in apparently healthy individuals (36, 37).

With regard to AEs, the majority of AEs recorded here corresponded to mild gastrointestinal–related AEs (all NIH CTCAE grade 1), although no causal association between gastrointestinal–related AEs and the intake of CFs could be established. Note that, compared with study part 1, a lower number of gastrointestinal–related AEs were reported in study part 2. This may be attributed to differences in the study protocol. Although in part 1 we asked volunteers to consume the test capsules on an empty stomach after fasting overnight, because we intended to collect data on the absorption and metabolism of CFs, in part 2 we advised participants to consume the test capsules with food. This notion is supported by the fact that the majority of gastrointestinal–related AEs in study part 1 (64%) were reported during the study visits. From these observations, it is tenable to derive recommendations for the consumption of CFs, suggesting that the ingestion of CF–containing capsules with a meal may be a preferred practice to lessen the potential occurrence of gastrointestinal–related AEs. In study part 1, we
collected one report of insomnia. Considering that both CE and control capsules contained caffeine and theobromine (Table 1), we suggested that during study part 2 volunteers should avoid taking capsules at night; we instead asked that the capsules be taken at breakfast and lunch only. We did not receive reports of insomnia during study part 2. Headaches were also recorded during the study, but there were no significant differences in occurrence between the CF and control groups, which suggested no causal association between CF intake and headaches. Thus, considering that 1) the vast majority (99%) of the AEs collected during study parts 1 and 2 were classified as mild (NIH CTCAE grade 1), 2) none of the AEs recorded was causally linked to the consumption of CFs, and 3) there were no clinically relevant changes in metabolic variables determined in blood, we suggest that CF intake is well tolerated in healthy adults when ingested in amounts up to 2000 mg/d. The above findings confirm the primary hypothesis tested in the current work.

During study part 1, we observed a CE-capsule-intake amount-dependent increase in the concentrations of theobromine in 12-h fasting plasma (Supplemental Figure 3). There was an ∼3-fold increase in the concentration of theobromine in 12-h fasting plasma after daily intake of CE and control capsules for 12 wk in study part 2 (Supplemental Figure 5). These changes in theobromine concentration were consistent with the consumption of CE and control capsules as required by the study design, thus suggesting that participants were compliant with the study regimen. In addition, we observed that the acute and daily intakes of CE capsules resulted in the respective presence of up to 1832 ± 968 nM SREMs and up to 568 ± 784 nM γ-VLMs in plasma in study part 1 (Supplemental Figures 2 and 3), showing that CFs are indeed available for absorption when consumed in the CE capsule format tested in this study.

Limitations of this study lie in its duration and the number of volunteers studied. However, considering that remarkably little has been published on the safety in healthy humans of dietary flavanol and procyanidin intake, and that many previous investigators made no comment on potential AEs, we propose that this study provides relevant and needed information for current safety assessments as well as for the design of future studies in this context.

Because the current work specifically addressed the safety of CF consumption, it is relevant to ask whether the data presented here can be extrapolated to other flavanols and procyanidins. A significant difference between CFs and flavanols and procyanidins derived from other sources, especially tea and some grape seed extracts, for example, is that the contribution to the total flavanol content in CFs of galloylated flavanol derivatives is negligible. This may be of importance because the absorption, distribution, metabolism, and excretion, and thus the biological activities of galloylated flavanols/procyanidins, have been shown to exhibit significant differences (38). Previous case report studies showed detrimental changes in liver function after the consumption of high amounts of (−)-epicagallocatechin gallate–containing tea extracts for periods that ranged from 1.2 to 260 wk (median of 8 wk) (8). In contrast, CF intake in this study did not result in clinically relevant changes in liver panels. Consequently, the extrapolation of information on the overall safety of flavanols and procyanidins based on the data provided here is limited and should only be undertaken with caution.
In conclusion, the intake of CFs in the form of CF-containing CE that provided up to 2000 mg CFs/d for 12 wk was well tolerated in healthy adults with a low risk of CVD. Importantly, the amount of CFs consumed in the current study, as well as the duration of CF intake studied, significantly exceeded those in previous dietary intervention studies (11–17). Taken together, the data presented here support the notion that the positive health effects previously reported to be associated with the intake of CFs can be achieved at intake amounts that are well below those that might result in an increased risk of CF-related AEs. The outcomes of this study also highlight that future research into the broader role of bioactives (nonessential nutrients) in health maintenance and healthy aging will require a renewed focus on advancing our concept/definition and biomarker-assisted assessment of health and the health status of individuals. The mere absence of disease or the identification of a low disease risk status on the basis of biomarkers primarily developed for diagnosing/assessing discrete disease states, however important, can only provide a limited toolkit when aiming at understanding health maintenance and healthy aging more holistically. With regard to CFs specifically, the long-term and large-scale COSMOS (COcoa Supplement and Multivitamin Outcomes Study; NCT02422745) represents a unique opportunity to comprehensively study the health benefits of CFs.

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The authors’ responsibilities were as follows—JIO, HS, and CLK: designed the research, wrote the manuscript, and had primary responsibility for final content; JIO, JK, JLE, RF, and TYM: conducted research, analyzed data, and performed statistical analyses; CK–U: provided test products used in this study; MB and CK–U: critically reviewed the manuscript; and all authors: read and approved the final manuscript. JIO, MB, CK–U, and HS are employed by Mars Inc., a company with long-term research and commercial interests in flavanols and procyanidins. CLK has received an unrestricted research grant from Mars Inc. and is the current holder of the Mars Chair in Developmental Nutrition. In addition, CLK has consulted for other food companies and government agencies with an interest in health and nutrition, as well as in phyt nutrients, including flavanols and procyanidins. JK, JLE, RF, and TYM declared no conflicts of interest.

Footnotes

6 Abbreviations used: ABP, ambulatory blood pressure; AE, adverse event; BP, blood pressure; CBC, complete blood count; CF, cocoa flavanol; CTCAE, Common Terminology Criteria for Adverse Events; CVD, cardiovascular disease; CE, cocoa extract; SREM, structurally related (−)–epicatechin metabolite; UC Davis, University of California, Davis; γ-VLM, 5-(3’,4’-dihydroxyphenyl)–γ–valerolactone metabolite; 3–DFR, 3–d food record.
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