A double-blind, placebo-controlled, randomized trial of the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health: clinical findings from a sample of healthy, cognitively intact older adults

W David Crews Jr, David W Harrison, and James W Wright

ABSTRACT

Background: In recent years, there has been increased interest in the potential health-related benefits of antioxidant- and phytochemical-rich dark chocolate and cocoa.

Objective: The objective of the study was to examine the short-term (6 wk) effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health in healthy older adults.

Design: A double-blind, placebo-controlled, fixed-dose, parallel-group clinical trial was used. Participants (n = 101) were randomly assigned to receive a 37-g dark chocolate bar and 8 ounces (237 mL) of an artificially sweetened cocoa beverage or similar placebo products each day for 6 wk.

Results: No significant group (dark chocolate and cocoa or placebo)-by-trial (baseline, midpoint, and end-of-treatment assessments) interactions were found for the neuropsychological, hematological, or blood pressure variables examined. In contrast, the midpoint and end-of-treatment mean pulse rate assessments in the dark chocolate and cocoa group were significantly higher than those at baseline and significantly higher than the midpoint and end-of-treatment rates in the control group. Results of a follow-up questionnaire item on the treatment products that participants believed they had consumed during the trial showed that more than half of the participants in both groups correctly identified the products that they had ingested during the experiment.

Conclusions: This investigation failed to support the predicted beneficial effects of short-term dark chocolate and cocoa consumption on any of the neuropsychological or cardiovascular health-related variables included in this research. Consumption of dark chocolate and cocoa was, however, associated with significantly higher pulse rates at 3- and 6-wk treatment assessments.


INTRODUCTION

In the past 10 y, there has been increased interest in the potential health-related benefits of antioxidant- and phytochemical-rich dark chocolate and cocoa (1–3). Research has identified an array of potential mechanisms through which chocolate and cocoa products may promote cardiovascular health and provide cardioprotective effects (4–29). Specifically, chocolate and cocoa-related products have been shown to decrease or inhibit both LDL oxidation (5–10) and platelet activation or function (11–15), to enhance serum lipid profiles (9, 16, 17), to favorably modify eicosanoid synthesis (18), to lower blood pressure [BP (16, 19, 20)], to promote endothelium-dependent relaxation or dilation (21, 22), and to inhibit free radical–induced erythrocyte hemolysis (23). Furthermore, preliminary in vitro investigations have suggested that cocoa flavonols or procyanidins may possess immunoregulatory effects and may help to modulate immune responses (24–26).

Similarly, the beneficial neuronal, neurocognitive, and neuroprotective effects of dietary and herbal compounds (eg, blueberry, cranberry, and Ginkgo biloba extracts) that are high in antioxidant activity and phytochemicals have been shown in numerous previous studies involving laboratory animals and humans (27–32; also: CC Neto et al, unpublished observations, 2003). Moreover, preliminary or pilot evidence has shown that flavanol-rich cocoa can increase cerebral blood flow in healthy elderly subjects, as measured by transcranial Doppler ultrasound (33), and in healthy young participants, as measured by functional magnetic resonance imaging (fMRI) in response to a cognitive task (ie, the task-switching paradigm) (34). It should be noted, however, that, in the fMRI study (34), no significant effects were observed in participants’ behavioral reaction times, the costs of switching between 2 sets of rules, or heart rates after the ingestion of the flavanol-rich cocoa. Francis et al (34) hypothesized that the fMRI changes may have been related to cognitive changes that were not manifest in the behavioral measures used in their project, especially in young, healthy participants who likely were functioning at a high level of cognitive ability.

There appears to be a relative lack of clinical trials that examined the effects of dark chocolate and cocoa on variables associated with neuropsychological functioning and cardiovascular health.
health in healthy, older, cognitively intact (CI) adults. This dearth has occurred despite research indicating that dark chocolate and cocoa are rich in antioxidants and phytochemicals, which may have beneficial effects on the cardiovascular and immune systems and on the brain and nervous system. In light of this past research, the purposes of the present investigation were to conduct the first-known clinical trial of the short-term (ie, 6 wk) effects of dark chocolate and cocoa on the neuropsychological functioning of healthy CI adults ≥60 y old and to extend to that cohort the findings of previous studies involving primarily young to middle-aged participants showing the potential beneficial effects of dark chocolate and cocoa on variables associated with cardiovascular health.

SUBJECTS AND METHODS

Subjects

Healthy, volunteer men (n = 41) and women (n = 60) ≥60 y old who reported no history of dementia or significant neurocognitive impairment were initially enrolled and randomly assigned in this study. To be considered CI and included in the trial, participants were required to obtain a total score on the Mini-Mental State Examination [MMSE (36)] of ≥24 out of a possible 30. Participants’ histories were unremarkable for active or clinically significant cardiovascular, neurological, pulmonary, endocrine, renal or urological, hepatic, gastrointestinal, or hematological disorders; uncontrolled hypertension; significant head injuries (ie, loss of consciousness for ≥5 min); episodes of anoxia or hypoxia; learning disabilities; color blindness; or psychiatric or substance abuse disorders. Persons being treated with antihypertensive, hypolipidemic, nonsteroidal antiinflammatory, anticoagulant, or psychotropic medications also were excluded from the present study. Medications for other preexisting conditions were not discontinued, although changes or additions to medication regimens during the study resulted in the removal of one participant from the trial.

Persons using any chocolate- or cocoa-related products before entering the study were requested to terminate such usage ≥7 d before their initial, pretreatment baseline assessments. Participants were also requested to avoid or, to the extent possible, limit their consumption of flavonoid-rich products such as blueberries, cranberries or cranberry juice, grapes or grape juice, red wine, soy (eg, soy milk or tofu), tea, pomegranate, acai, and isoflavone supplements for the duration of the study. Furthermore, participants’ histories were unremarkable for uncorrected conditions (eg, vision or hearing difficulties) that could have precluded their participation in all trial procedures.

Written informed consent was obtained from each participant before any investigational procedures were initiated. The study was approved by the Institutional Review Board and Human Subjects Committee at Virginia Polytechnic Institute and State University.

Experimental design and procedures

This study used a 6-wk, randomized, double-blind, fixed-dose, placebo-controlled, parallel-group experimental design. Persons meeting inclusionary criteria were randomly assigned to either the dark chocolate and cocoa group (n = 51) or the placebo group (n = 50). The study used flavanoid- and procyanidin-rich dark chocolate bars and artificially sweetened (with aspartame) cocoa beverage mix products, as well as low-polyphenol placebo bars that were matched for appearance (eg, color and quantity), smell, taste, and caloric content. The dark chocolate, cocoa, and placebo products were produced, analyzed (ie, regarding their polyphenol and nutritional contents), and furnished by the manufacturer (The Hershey Company, Hershey, PA). Computerized randomization of the products was conducted by an independent researcher. The boxes and containers containing the products (and their randomization numbers, 1–101) were subsequently issued to participants in an ascending and sequential order as they entered the study (at the time of their pretreatment baseline assessments). One dark chocolate bar (37.0 g; containing 60% cacao, =11 g natural cocoa, and 397.30 mg total proanthocyanins/g) and one 8-ounce (237 mL) cup of the cocoa beverage (dry weight 12 g, of which 11 g was natural cocoa; also containing 357.41 mg total proanthocyanins/g) or similarly matched placebo products were taken orally on a daily basis; these products originated from the same batches and lot numbers. Total proanthocyanin concentrations for each placebo bar and beverage were 0.20 mg/g and 40.87 mg/g, respectively. The products’ total proanthocyanin concentrations were measured by the manufacturer with the use of methods described previously by Gu et al (35).

Before the initiation of the treatment phase, participants completed a self-report history questionnaire devised by 2 of us (WDC and DWH) that assessed their medical and psychiatric histories. Participants meeting the trial’s medical, psychiatric, and medication inclusion criteria (as defined earlier) subsequently were individually administered the MMSE (36). Three screening BP and pulse rate measurements, taken at 3-min intervals via an automated BP device (Advantage 6014 Blood Pressure Monitor; American Diagnostic Corporation, Hauppauge, NY), also were obtained from each participant’s left upper arm after he or she sat comfortably in a quiet room for 5 min. Participants who exhibited mean (averaged across the 3 readings) systolic BPs > 160 mm Hg or diastolic BPs > 95 mm Hg were referred to their primary care physician for follow-up assessment and were excused from trial participation.

Participants who met the preliminary inclusion criteria were scheduled for 12-h fasting hematologic assessments consisting of a coronary risk panel (including direct measurements of total serum cholesterol, HDL cholesterol, and triacylglycerols and calculated measurements of LDL and VLDL cholesterol) and an ultrasensitive C-reactive protein (CRP) test. Blood (6 mL/participant) was drawn via venipuncture by trained laboratory phlebotomists. Participants’ serum cholesterol and triacylglycerol concentrations were subsequently analyzed by using a Dimension RxL Max with HM Module Integrated Chemistry System (Dade Behring Inc, Deerfield, IL), and their ultrasensitive CRP concentrations were analyzed with the use of a latex particle-enhanced immunoturbidimetric assay on a Roche/Hitachi 912 automated analyzer (Roche Diagnostics, F Hoffmann-La Roche Ltd, Basel, Switzerland). Participants found to have fasting total cholesterol concentrations > 300 mg/dL, HDL-cholesterol concentrations < 30 mg/dL, total triacylglycerol concentrations > 400 mg/dL, or ultrasensitive CRP concentrations > 10.0 mg/L were referred to their primary care physician for follow-up assessment and were excused from trial participation. Height and weight measurements were also obtained during this session, and
each participant’s body mass index (BMI; in kg/m²) was subsequently calculated by using the adult BMI calculator of the Centers for Disease Control and Prevention (37).

Participants who also met the study’s hematologic inclusion criteria were scheduled for a pretreatment baseline assessment. At this session, 3 BP and pulse measurements, conducted at 3-min intervals, were obtained from each participant’s left upper arm by using procedures identical to those described previously. Neuropsychological testing was then initiated. All participants were individually administered the following series of neuropsychological tests: the Selective Reminding Test (38), the Wechsler Memory Scale-III Faces I and Faces II subtests (39), the Trail Making Test (40, 41), the Stroop Color-Word Test (42, 43), the Wechsler Adult Intelligence Scale-III Digit Symbol-Coding subtest (44), and the Activation-Deactivation Adjective Check List, General Activation subscale [A-DACL (45, 46)]. We strictly adhered to each measure’s standardized administration and scoring procedures. After all testing procedures, participants were assigned the next ascending participant number and provided with a supply of randomly assigned boxes or containers containing the dark chocolate and cocoa or placebo products, as well as detailed instructions and forms onto which product consumption could be recorded daily.

At the midpoint of the treatment phase (after 3 wk), 3 BP and pulse measurements, conducted at 3-min intervals, were obtained from each participant’s left upper arm by using procedures identical to those described previously. Participants were also asked to complete the A-DACL (45, 46) for an assessment of mental energy. In addition, in an effort to ensure sustained dosing, participants were asked to consume either one 37-g dark chocolate bar, an 8-ounce (237 mL) cocoa beverage, or a similar placebo product 2 h before their planned assessments.

After 6 wk of dark chocolate and cocoa or placebo treatment, and just before the termination of the regimen, identical hematologic, BMI, BP and pulse, and neuropsychological assessments, with 2 exceptions, were conducted for each participant. Because the MMSE (36) was used as an inclusion or exclusion criterion measure, it was administered only during the initial screening assessment. In addition, the Follow-up Self-report Questionnaire (31) was administered only at the end of the second neuropsychological assessment to subjectively assess participants’ perceptions of changes over the 6-wk span in their overall ability to remember, thinking abilities and processes, mood, energy levels, and overall health and to ascertain the types of products (ie, dark chocolate and cocoa, placebo, or unknown) they believed they had consumed during the study.

It should be noted that the 12-h fasting hematologic and BMI assessments and the BP-neuropsychological assessments were conducted on alternate days to control for any potential confounding that the required fasts may have had on participants’ neuropsychological test performances. Furthermore, in an effort to ensure sustained dosing, participants were asked to consume either one 37-g dark chocolate bar, an 8-ounce (237 mL) cocoa beverage, or a similar placebo product 2 h before their end-of-treatment BP/neuropsychological assessments.

Treatment adherence was assessed via tabulations of the total amounts of dark chocolate and cocoa beverage (or similar placebo products) consumed by the endpoint of the project; a deviation of ≥20% from the optimum treatment regimen was defined as nonadherence. Adverse events were assessed at the end of treatment and on an as-needed basis.

**Statistical analysis**

All statistical analyses were conducted by using SPSS software (version 11.5; SPSS Inc, Chicago, IL).

**RESULTS**

A trial flow diagram for this experiment is provided in Figure 1. For purposes of the present trial, the per-protocol data set was used in the statistical analyses. Of the 101 randomly assigned participants, 38 men and 52 women completed the trial protocol and were available for the efficacy analyses. Of the 11 participants who were excluded, 6 from the dark chocolate and cocoa group withdrew or were withdrawn prematurely secondary to the following events: gastrointestinal upset and headaches (n = 1);
group participant was excluded because of nonadherence to the treatment regimen.

To examine any differences between the treatment groups, separate analyses of variance (ANOVARs) were conducted on the descriptive and criterion measures cited in Table 1. For the BMI data, a 2-factor mixed ANOVA was conducted by using the fixed factor of group (dark chocolate and cocoa or placebo) and repeated measures of trial (baseline or end-of-treatment assessments). No significant differences were observed among any of these variables. A Pearson chi-square analysis also was conducted by using the variables of sex (men or women) and treatment group (dark chocolate and cocoa or placebo). No significant relation was observed between the numbers of men and women who constituted the 2 groups.

For the neuropsychological measures and test scores, 2-factor mixed ANOVAs were conducted to examine possible interaction effects by using the fixed factor of group (dark chocolate and cocoa or placebo) and repeated measures of trial (baseline and end-of-treatment assessments). Because of the absence of previous clinical trials that have examined the effects of dark chocolate and cocoa on the neuropsychological test performances of CI older adults, primary and secondary endpoints were not defined in advance. Rather, a series of endpoints was chosen that could be more rigorously defined and tested in future trials. No significant group-by-trial interactions were found for any of the endpoints. To conduct these analyses, a series of separate analyses of variance (ANOVAs) were conducted on the descriptive and criterion measures cited in Table 1. For the BMI data, a 2-factor mixed ANOVA was conducted by using the fixed factor of group (dark chocolate and cocoa or placebo) and repeated measures of trial (baseline or end-of-treatment assessments). No significant differences were observed among any of these variables. A Pearson chi-square analysis also was conducted by using the variables of sex (men or women) and treatment group (dark chocolate and cocoa or placebo). No significant relation was observed between the numbers of men and women who constituted the 2 groups.

No significant group by trial interactions were observed among the variables.

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chocolate and cocoa group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y) 1</td>
<td>68.76 ± 8.62</td>
<td>68.73 ± 8.01</td>
</tr>
<tr>
<td>Education level (y) 1</td>
<td>15.52 ± 2.66</td>
<td>15.18 ± 2.75</td>
</tr>
<tr>
<td>Mini-Mental State Examination</td>
<td>28.51 ± 1.42</td>
<td>28.62 ± 1.39</td>
</tr>
<tr>
<td>Treatment regimen adherence (%)</td>
<td>97.79 ± 3.91</td>
<td>98.56 ± 2.58</td>
</tr>
<tr>
<td>BMI 2</td>
<td>25.16 ± 3.40</td>
<td>25.51 ± 3.56</td>
</tr>
<tr>
<td>End-of-treatment</td>
<td>25.09 ± 3.47</td>
<td>25.56 ± 3.55</td>
</tr>
</tbody>
</table>

1 All values are $\bar{x} \pm$ SD. n = 45 and 45 for the dark chocolate and cocoa group and the placebo group, respectively. No significant differences were observed among the variables.

2 Analyzed with ANOVA.

3 Analyzed with a 2-factor mixed ANOVA.

### Table 2

<table>
<thead>
<tr>
<th>Test or variable</th>
<th>Baseline</th>
<th>End-of-treatment</th>
<th>Change in score (end-of-treatment − baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chocolate and cocoa group</td>
<td>Placebo group</td>
<td>Chocolate and cocoa group</td>
</tr>
<tr>
<td>Selective Reminding Test (raw score)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate free recall</td>
<td>101.78 ± 21.90 1</td>
<td>99.36 ± 21.70 1</td>
<td>107.00 ± 22.22</td>
</tr>
<tr>
<td>Long-term storage</td>
<td>93.64 ± 32.16</td>
<td>91.04 ± 28.45</td>
<td>99.01 ± 30.07</td>
</tr>
<tr>
<td>Short-term recall</td>
<td>17.80 ± 11.57</td>
<td>18.36 ± 11.15</td>
<td>15.91 ± 10.28</td>
</tr>
<tr>
<td>Long-term retrieval</td>
<td>83.98 ± 32.68</td>
<td>81.04 ± 31.24</td>
<td>91.09 ± 31.46</td>
</tr>
<tr>
<td>Consistent long-term retrieval</td>
<td>58.00 ± 34.98</td>
<td>55.60 ± 36.12</td>
<td>70.82 ± 36.15</td>
</tr>
<tr>
<td>Random long-term retrieval</td>
<td>25.98 ± 14.49</td>
<td>25.29 ± 13.50</td>
<td>20.27 ± 13.06</td>
</tr>
<tr>
<td>Cued recall</td>
<td>9.16 ± 2.02</td>
<td>9.07 ± 2.30</td>
<td>9.40 ± 2.17</td>
</tr>
<tr>
<td>Delayed free recall</td>
<td>8.87 ± 3.22</td>
<td>8.64 ± 2.89</td>
<td>8.91 ± 3.36</td>
</tr>
<tr>
<td>Delayed recognition</td>
<td>11.73 ± 0.81</td>
<td>11.58 ± 0.89</td>
<td>11.82 ± 0.68</td>
</tr>
<tr>
<td>Wechsler Memory Scale-III (raw score)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faces I</td>
<td>33.62 ± 4.49</td>
<td>34.62 ± 4.34</td>
<td>36.73 ± 5.03</td>
</tr>
<tr>
<td>Faces II</td>
<td>35.27 ± 4.56</td>
<td>35.73 ± 4.40</td>
<td>38.62 ± 4.09</td>
</tr>
<tr>
<td>Wechsler Adult Intelligence Scale-III (raw score)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit symbol</td>
<td>65.51 ± 12.18</td>
<td>66.89 ± 13.60</td>
<td>68.56 ± 12.86</td>
</tr>
<tr>
<td>Trail Making Test (total time)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part A</td>
<td>31.53 ± 10.50</td>
<td>33.73 ± 8.90</td>
<td>28.62 ± 9.28</td>
</tr>
<tr>
<td>Part B</td>
<td>87.71 ± 42.12</td>
<td>84.16 ± 31.37</td>
<td>76.91 ± 38.46</td>
</tr>
<tr>
<td>Stroop Color-Word Test (raw score)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Word Page</td>
<td>98.04 ± 16.56</td>
<td>96.49 ± 15.18</td>
<td>99.31 ± 16.49</td>
</tr>
<tr>
<td>Color Page</td>
<td>64.62 ± 11.66</td>
<td>66.13 ± 12.37</td>
<td>66.49 ± 12.49</td>
</tr>
<tr>
<td>Color-Word Page</td>
<td>32.18 ± 9.72</td>
<td>32.84 ± 9.42</td>
<td>35.24 ± 9.23</td>
</tr>
</tbody>
</table>

1 $n = 45$ and 45 for the dark chocolate and cocoa group and the placebo group, respectively. All variables were analyzed with a 2-factor mixed ANOVA.

2 Group × trial interaction.

3 $\bar{x} \pm$ SD (all such values).
variables. A summary of the groups’ neuropsychological test scores and the group-by-trial interaction values is provided in Table 2.

Similarly, for the A-DACL scores (45, 46), a 2-factor mixed ANOVA was conducted by using the fixed factor of group (dark chocolate and cocoa or placebo) and repeated measures of trial (baseline, midpoint, or end-of-treatment assessments). The group-by-trial interaction was nonsignificant. An overview of the group scores at each trial visit and the group-by-trial interaction values is provided in Table 3.

For the follow-up self-report questionnaire items concerning participants’ subjective perceptions of changes from pretreatment baseline to the end-of-treatment phase in their overall abilities to remember, thinking abilities and processes, mood, energy levels, and health, separate Pearson chi-square analyses were conducted with the use of the treatment groups’ self-report data. Frequency data for each question were grouped into the following categories: much worse, somewhat worse, no change, somewhat improved, or much improved. As noted in Table 4, no significant relations were found across any of these 5 questions.

In contrast, for the follow-up self-report questionnaire item regarding the products that participants believed they had consumed during the clinical trial (ie, dark chocolate and cocoa, placebo, or unknown), a Pearson chi-square analysis showed a significant \( P = 0.004 \) relation between the treatment group to which participants had been assigned (ie, dark chocolate and cocoa or placebo) and the products that participants believed they had consumed. In particular, more than half (55.6\%; \( n = 25 \)) of the participants who received the dark chocolate and cocoa products correctly believed that they had consumed these products; only 22.2\% (\( n = 10 \)) of that group believed that they had consumed the placebo products, and another 22.2\% (\( n = 10 \)) were uncertain as to which products they had ingested. Similarly, more than half (55.6\%; \( n = 25 \)) of the participants who received placebo products correctly believed that they had consumed those products; 26.7\% (\( n = 12 \)) of that group believed that they had received the dark chocolate and cocoa products, and 17.8\% (\( n = 8 \)) were unsure as to which products they had ingested.

For the hematologic test results, 2-factor mixed ANOVAs were conducted to examine possible interaction effects by using the fixed factor of group (dark chocolate and cocoa or placebo) and repeated measures of trial (baseline or end-of-treatment assessments). As noted in Table 5, which provides a summary of the groups’ scores and group-by-trial interaction values for each hematologic test, none of the interactions were significant.

The physiologic variables of systolic and diastolic BPs and pulse rate also were examined for interaction effects via 2-factor mixed ANOVAs by using the fixed factor of group (dark chocolate and cocoa or placebo) and repeated measures of trial (baseline, midpoint, or end-of-treatment assessments). An overview of the groups’ BP and pulse rate scores and group-by-trial interaction values is shown in Table 6.

On comparison of the groups’ mean pulse rates, a significant \( P = 0.007 \) group-by-trial interaction was found. Specifically, post hoc analyses using Tukey’s test showed that the mean pulse rate at the 3-wk (midpoint) assessment was significantly higher in the dark chocolate and cocoa group than at baseline (\( P < 0.01 \)) and than in the placebo group at midpoint (\( P < 0.01 \)). Post hoc

### Table 3

Scores on the Activation-Deactivation Adjective Check List, General Activation subscale\(^7\)

<table>
<thead>
<tr>
<th>Trial visit</th>
<th>Chocolate and cocoa group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment baseline</td>
<td>16.76 ± 3.38</td>
<td>15.72 ± 3.69</td>
</tr>
<tr>
<td>Midpoint</td>
<td>16.67 ± 3.33</td>
<td>16.63 ± 3.36</td>
</tr>
<tr>
<td>End-of-treatment</td>
<td>17.18 ± 3.71</td>
<td>16.44 ± 3.48</td>
</tr>
</tbody>
</table>

\( ^7 \) All values are \( \bar{x} \pm SD. \( n = 45 \) and 43 for the dark chocolate and cocoa group and the placebo group, respectively. Analyzed with a 2-factor mixed ANOVA. No significant group × trial interaction was observed, \( P = 0.475 \).

### Table 4

Pearson chi-square tests for the summarized follow-up self-report questionnaire frequency data\(^7\)

<table>
<thead>
<tr>
<th>Question</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>5.355</td>
<td>0.148</td>
</tr>
<tr>
<td>Thinking processes</td>
<td>4.047</td>
<td>0.256</td>
</tr>
<tr>
<td>Mood</td>
<td>2.995</td>
<td>0.392</td>
</tr>
<tr>
<td>Energy</td>
<td>1.984</td>
<td>0.576</td>
</tr>
<tr>
<td>Overall health</td>
<td>3.352</td>
<td>0.340</td>
</tr>
<tr>
<td>Product group</td>
<td>11.218</td>
<td>0.004</td>
</tr>
</tbody>
</table>

\( ^7 \) \( n = 45 \) and 45 for the dark chocolate and cocoa group and the placebo group, respectively.

### Table 5

Hematologic test results\(^7\)

<table>
<thead>
<tr>
<th>Test or variable</th>
<th>Baseline</th>
<th>End-of-treatment</th>
<th>Change in score (end-of-treatment — baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chocolate and cocoa group</td>
<td>Placebo group</td>
<td>Chocolate and cocoa group</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>209.33 ± 33.26(^3)</td>
<td>208.91 ± 31.62</td>
<td>208.36 ± 37.51</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>130.07 ± 25.97</td>
<td>132.87 ± 24.13</td>
<td>129.93 ± 27.86</td>
</tr>
<tr>
<td>VLDL cholesterol (mg/dL)</td>
<td>15.27 ± 7.92</td>
<td>15.84 ± 6.95</td>
<td>15.44 ± 7.63</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>63.36 ± 16.50</td>
<td>58.91 ± 13.79</td>
<td>62.18 ± 14.63</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>95.20 ± 49.39</td>
<td>99.20 ± 43.76</td>
<td>96.80 ± 47.32</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>1.59 ± 1.56</td>
<td>1.52 ± 1.26</td>
<td>1.86 ± 2.60</td>
</tr>
</tbody>
</table>

\( ^7 \) \( n = 45 \) and 45 for the dark chocolate and cocoa group and the placebo group, respectively. All variables were analyzed with a 2-factor mixed ANOVA. No significant group × trial interactions were observed among the variables.

\( ^3 \) \( \bar{x} \pm SD \) (all such values).
## TABLE 6
Blood pressure (BP) and pulse rate results

<table>
<thead>
<tr>
<th>Test and variable</th>
<th>Baseline</th>
<th>Midpoint</th>
<th>End-of-treatment</th>
<th>Change in score (midpoint — baseline)</th>
<th>Change in score (end-of-treatment — midpoint)</th>
<th>Change in score (end-of-treatment — baseline)</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>126.83 ± 14.31</td>
<td>128.57 ± 14.25</td>
<td>125.52 ± 12.71</td>
<td>−2.33 ± 11.27</td>
<td>−2.05 ± 11.99</td>
<td>−3.58 ± 10.10</td>
<td>0.970</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74.22 ± 8.20</td>
<td>73.50 ± 8.80</td>
<td>73.72 ± 7.49</td>
<td>−0.72 ± 6.04</td>
<td>−0.95 ± 6.78</td>
<td>0.22 ± 5.93</td>
<td>0.015</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>71.34 ± 9.19</td>
<td>69.88 ± 10.06</td>
<td>70.22 ± 9.80</td>
<td>−2.66 ± 10.39</td>
<td>−0.58 ± 6.30</td>
<td>4.72 ± 7.93</td>
<td>0.007</td>
</tr>
</tbody>
</table>

1 n = 45 and 43 for the dark chocolate and cocoa group and the placebo group, respectively. All variables were analyzed with a 2-factor mixed ANOVA. Post hoc analyses were conducted with Tukey’s test.

2 Group × trial interaction.

3 Significantly different from the midpoint mean for the chocolate and cocoa treatment, P < 0.01.

4 Significantly different from the end-of-treatment mean for the chocolate and cocoa treatment, P < 0.01.

5 Significantly different from the respective placebo treatment, P < 0.01.

6 Significantly different from the respective placebo treatment, P < 0.01.

## DISCUSSION
The findings from this double-blind, placebo-controlled, randomized clinical trial failed to support the predicted beneficial effects of short-term (6 wk) consumption of dark chocolate and cocoa on any of the neuropsychological, hematologic, or physiologic variables included in the investigation. The null neuropsychologic effects of the dark chocolate and cocoa group, whereas the placebo group acknowledged 10 such adverse events. An overview of these adverse events classified by body system and treatment group is provided in Table 7.

Only one serious adverse event was reported in the dark chocolate and cocoa group. One participant in the dark chocolate and cocoa group experienced an episode of atrial arrhythmia (type unknown) that required medical attention and outpatient hospitalization; however, this event was not believed to be related to the consumption of the trial’s products. All of the remaining reported adverse events were categorized as mild to moderate in intensity, and no causal relations between the treatments and any of these events were identified.
The null hematologic results also were in contrast to the results of past studies, which showed the favorable effects of dark chocolate and cocoa on LDL oxidation susceptibility (5–10) and on serum total and LDL-cholesterol concentrations (16). Two additional studies involving the ingestion of either cocoa or dark chocolate for up to 3 wk by healthy, younger adults also have provided evidence of treatment-related greater concentrations of HDL cholesterol (9, 17).

Alternatively, the present trial’s null hematologic findings appear consistent with several studies in healthy, young to middle-aged participants that have failed to show the effects of dark chocolate and cocoa on serum total (7, 9, 12, 17, 19, 49), LDL- (7, 9, 17, 19, 49), VLDL (9), and HDL- (7, 12, 17, 19, 49) cholesterol or triglyceride or triacylglycerol (7, 9, 12, 17, 19, 49) concentrations. Furthermore, the trial’s nonsignificant ultrasensitive CRP results were consistent with past research that has found no effects of cocoa extract supplementation (7) or dark chocolate (16) on markers of inflammation, including high-sensitivity CRP concentrations, in healthy participants (7) and persons with essential hypertension (16), respectively.

Although it remains possible that dark chocolate and cocoa have only limited or no effect on individual persons’ lipid profiles and CRP concentrations, it should be noted that the present trial used a sample of healthy participants whose mean baseline serum HDL- and VLDL cholesterol, triacylglycerol, and ultrasensitive CRP concentrations fell within the “normal” reference ranges, whereas their mean serum LDL- and total cholesterol concentrations were slightly to moderately above the reference ranges’ cutoffs. Thus, it is possible that participants’ baseline hematologic values were not elevated (or abnormal) to the degree necessary for the effects of short-term dark chocolate and cocoa to be observed, but this possibility is only speculative at this time.

Whereas the present study’s nonsignificant BP results appear contrary to previous short-term (≤15 d of treatment) research that found dark chocolate and cocoa consumption to be associated with significantly lower systolic (16, 19, 20) and diastolic (16, 20) BPs, it should be noted that 2 of those studies involved participants with either never-treated grade I essential hypertension (16) or untreated stage 1, mild, isolated systolic hypertension (20), whereas the third study used healthy, nonhypertensive participants whose mean age was <34 y (19). The present experiment’s null BP findings appear consistent, however, with past short-term (≤28 d of treatment) studies investigating the effectiveness of dark chocolate and cocoa in healthy adults; those studies also did not find significant treatment-related decreases in systolic (12, 48, 50) or diastolic (12, 19, 48, 50) BPs. These results, along with the current trial’s findings, suggest that short-term (≤6 wk) consumption of dark chocolate and cocoa may be of limited efficacy in lowering BP in normotensive, healthy adults.

In contrast, the dark chocolate and cocoa group had significantly higher mean pulse rates at both the 3-wk (midpoint) and 6-wk (end-of-treatment) assessments, as compared with their baseline mean pulse rate and with the placebo group’s midpoint and end-of-treatment mean pulse rates. Although the precise mechanism or mechanisms responsible for these findings remain unknown, the higher pulse rates were likely related to the well-known stimulant effects of methylxanthines (eg, theobromine and caffeine) that are found in dark chocolate and cocoa and to the fact that participants were requested to consume either a dark chocolate bar or cup of the cocoa beverage (or similar placebo

<table>
<thead>
<tr>
<th>Body system and adverse event</th>
<th>Total (n = 101)</th>
<th>Chocolate and cocoa group (n = 51)</th>
<th>Placebo group (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial arrhythmia</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dental or oral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denture staining</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dermatological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itching (upper body)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea or gastrointestinal disturbance</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Nausea or vomiting</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Rectal bleeding upon defecation</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Soft stools or fecal incontinence</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Stool color change</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoulder injury or pain</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Nervous system</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jitteriness or increased energy</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory or allergic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchitis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sinus or cold symptomatology</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold sweats</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>
products) 2 h before their midpoint and end-of-treatment BP or neuropsychological assessments.

In addition, several other factors may have contributed to the overall absence of positive findings in the present experiment. For one, the sample size of the current trial may not have been of sufficient magnitude, which could have decreased the power of the statistical measures to find true, significant differences that may actually have existed between the treatment groups. The relatively short duration (6 wk) of the treatment phase and the quantity of dark chocolate and cocoa products consumed by participants in the present trial also may have contributed to the overall null findings. Whereas the possibility is speculative, a concomitant of greater product consumption and a relatively longer time span may have resulted in more potent antioxidant and phytochemical effects, which, in turn, could have been more readily measured or observed in such healthy, CI, older adults.

Furthermore, despite the investigators’ efforts to educate participants to limit their consumption of certain antioxidant- or phytochemical-rich products during this trial, control of every aspect of their diets was not attempted. Thus, it is likely that participants from both treatment groups continued to consume a diversity of other fruit and vegetables containing antioxidants or phytochemicals (or both), which may have confounded the current results to some extent. Whereas it remains unknown whether or how these various factors may have contributed to the trial’s generally null findings, it seems probable that several of these factors interacted synergistically to produce the current results.

For the follow-up self-report questionnaire, more than half of the participants in the dark chocolate and cocoa (55.6%; n = 25) and placebo (55.6%; n = 25) groups correctly identified the types of products that they had ingested during the experiment. Whereas this finding suggests that more than half of each treatment group was not fully blinded to the trial’s products, in light of the predominately null findings from this study, no evidence was found for any type of participant expectancy effects or bias that may have confounded the results.

Future research is required to address some of the potential limitations of the current study. It may prove especially enlightening to examine large, heterogeneous samples consisting of persons of diverse backgrounds, health statuses, educational levels, and ages, so that any differential effects of such factors can be more readily shown. Also needed are longitudinal trials that involve the consumption of dark chocolate and cocoa over extended periods (i.e., several months to ≥1 y) and that result in the consumption of larger quantities of such products, which will likely promote more potent antioxidant and phytochemical effects.

We are indebted to PL Kelly Harrison for expert assistance with the randomization procedures and statistical analyses and Leland E. Bertrand Jr for invaluable help with the participant screening process. We also thank Laura Campbell, Gladys R Crews, Christen Short, and Sara Valentino for assistance with participant recruitment and scheduling, neuropsychological and physiologic testing, and data collection. We especially thank Susan E Short, Don Pizzullo, and Anne Aker at the Virginia Tech Roanoke Center (Roanoke, VA) for their gracious hospitality and support and the use of their excellent facilities and Glen J McIver, Donna Rice, Sherry McPherson, and Tiffany Walter at Centra Health Laboratory (Lynchburg, VA) for their expert help with the coordination, collection, and analysis of the hematologic data. We are also indebted to Debra Miller and Dave Stuart at the Hershey Company (Hershey, PA) for the invaluable support and advice they provided throughout the study. Finally, we deeply appreciate the generosity of the Reverend Douglas Pillow and Court Street United Methodist Church (Lynchburg, VA) for opening the doors of the church to this research project.

The authors’ responsibilities were as follows—WDC and DWH (co-principal investigators): contributed to the design and coordination of the study, data collection, data analysis, and manuscript preparation; and JWW (medical director): contributed to the study design, data collection, data analysis, and manuscript preparation. None of the authors had a personal or financial conflict of interest.

REFERENCES


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