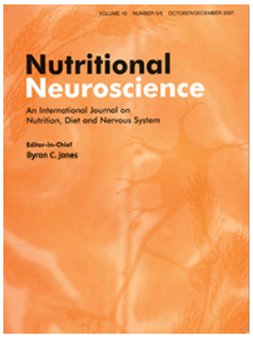

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Effects of cocoa-rich chocolate on cognitive performance in postmenopausal women. A randomised clinical trial

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ABSTRACT

Objectives: The aim of this research was to evaluate the effects of adding 10 g of cocoa-rich chocolate (99%) to the habitual diet on cognitive performance in postmenopausal women.

Methods: Following a randomised controlled parallel clinical trial, a total of 140 postmenopausal women aged 50–64 were recruited. The intervention group ($n = 73$) consumed daily 10 g of chocolate (99% cocoa) in addition to their usual food intake for 6 months, whereas the control group ($n = 67$) did not receive any intervention. Attention and executive functions, verbal memory, working memory, phonological fluency, category fluency and clinical variables were assessed at baseline and 6 months.

Results: Trail Making Test B execution time showed a decreased of −12.08 s (95% CI: −23.99, −0.18; $p = 0.047$) in the intervention group compared to the control group, after adjusting for age, educational level, time elapsed from the beginning of menopause and daily energy consumption (Cohen's $d = -0.343$). Attention, immediate or delayed verbal memory, phonological or category fluency, and working memory remained unchanged.

Conclusions: The consumption of cocoa-rich (99%) chocolate in addition to the habitual diet could be related to a slight improvement in cognitive performance regarding cognitive flexibility and processing speed in postmenopausal women, with no changes in the rest of the cognitive performance variables evaluated.

Trial registration: This clinical trial has been registered at clinicaltrials.gov as NCT03492983.

KEYWORDS
Chocolate; polyphenols; postmenopause; cognition; executive function; attention; memory

Introduction

The consumption of chocolate or cocoa-rich products has been shown to have multiple beneficial effects on health [1–4]. Cognitive function is one of the studied aspects that could improve with the intake of this type of compounds. Polyphenols are believed to act both as neuroprotectors, potentially improving cognitive performance through a signalling cascade activation mechanism in the brain, and on the vascular system, leading to beneficial changes in the cerebrovascular blood flow [5–7].

The available evidence regarding the effects of cocoa on cognitive performance is discrepant. Some studies have reported changes in cognitive performance, reflected by improvements in executive functions and verbal fluency [8], as well as working memory [9,10], and a decrease of mental fatigue [11]. Furthermore, greater chocolate consumption has been associated with better cognitive function [12]. Similarly, the intake of chocolate has been related to lower risk of dementia [13] and of cognitive decline [14]. On the other hand, the findings of other studies have shown no improvements in cognitive performance after the consumption of cocoa [15,16].

Positive effects on cognitive function associated with the intake of cocoa have been observed in different population subgroups, such as young adults [10,17] and elderly subjects, both without cognitive alterations [8,18] and with mild cognitive impairment [19]. In postmenopausal women, it appears that the change in the levels of estrogens may affect their cognitive state [20]. In this population group, improvements have been

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reported in cerebral blood flow velocity and conductance responses after the consumption of cocoa-rich chocolate [21]. However, the evidence about the effects of chocolate consumption on cognitive function in postmenopausal women is scarce.

The aim of this study was to evaluate the effects of adding 10 g of cocoa-rich chocolate (99%) to the habitual diet on cognitive performance in postmenopausal women.

Materials and methods

Design and setting

This was a randomised, controlled clinical trial with two parallel groups carried out between June 2018 and August 2019 in the Research Unit for Primary Care of Salamanca (APISAL) (Spain), which is part of the Biomedical Research Institute of Salamanca (IBSAL) and the Spanish Research Network for Preventive Activities and Health Promotion in Primary Care (REDIAPP). This clinical trial has been registered at clinicaltrials.gov as NCT03492983. This manuscript presents results on cognitive performance as a secondary outcome of the trial. Results on blood pressure, as the main outcome of the intervention study [22], as well as results on body composition, as a secondary outcome [23], have been previously published.

Study participants and recruitment

A consecutive sampling was carried out in the doctor's offices of four primary healthcare centres in the city of Salamanca (Spain) to recruit women who met the selection criteria and signed the informed consent for participation. A total of 140 women in the range of 50–64 years of age and in the postmenopausal period, defined by amenorrhea for at least 12 consecutive months, were included in the study. Thirty-two women were excluded from the trial, due to one of the following criteria: personal history of cardiovascular disease; personal history of diabetes mellitus, arterial hypertension or dyslipidaemia under pharmacological treatment; hypocaloric diets; clinically demonstrable neurological and/or neuropsychological disease; treatment with hormone replacement therapy; habitual consumption of over 210 grams per week (g/week) of cocoa; cocoa intolerance and/or allergy or similar reactions to any of the components of the supplement (Figure 1).

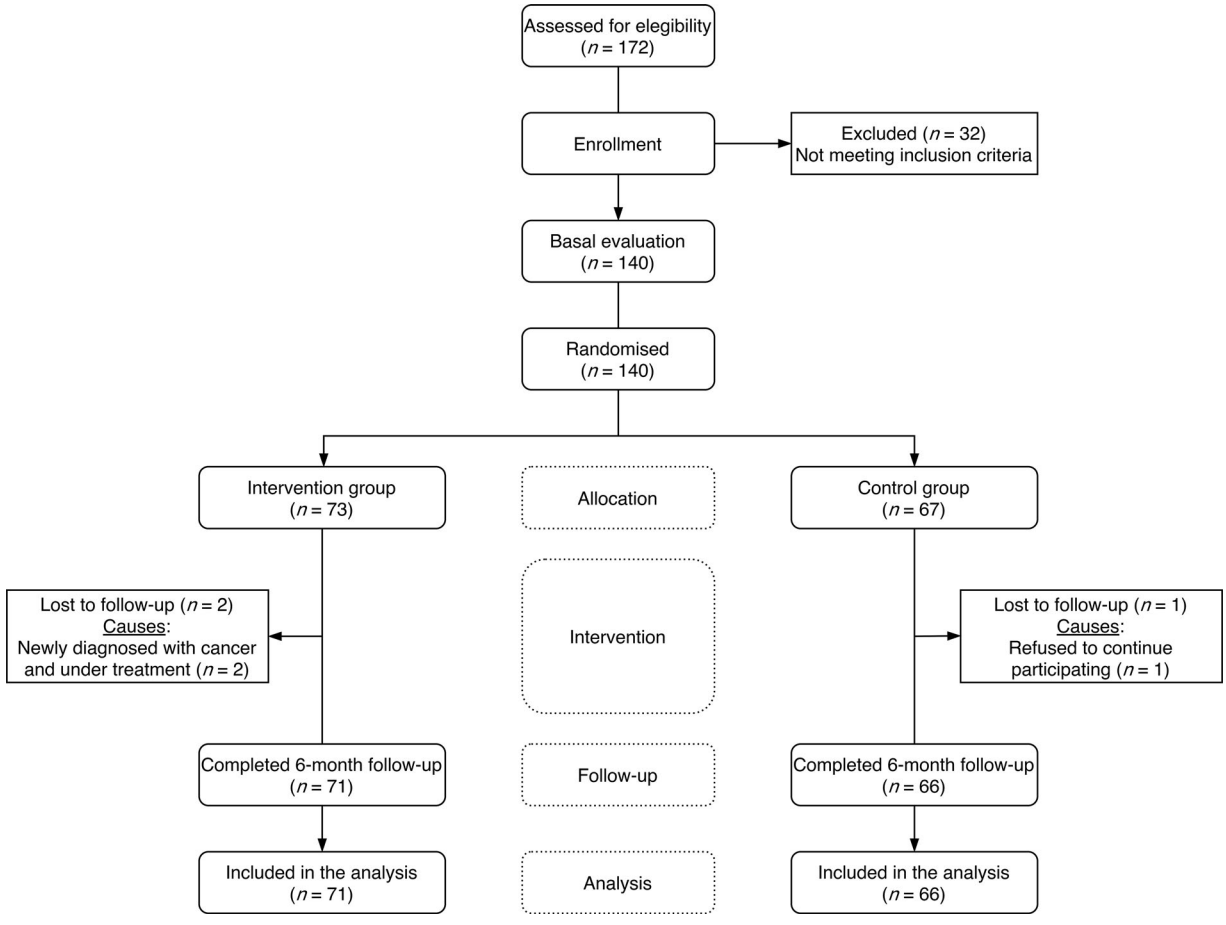

Figure 1. Flow chart of participants through the study.

Sample size

The sample size was estimated considering the change in arterial systolic pressure as the main variable of this clinical trial. To detect a minimum difference of 2.9 mm Hg in systolic arterial pressure between the two groups, 140 participants were required (70 per group), assuming an alpha risk of 0.05, a beta risk of 0.20 in a two-sided contrast, and a standard deviation of 5.8 mm Hg. In addition, a follow-up loss rate of 10% was assumed. These calculations were based on a similar study results that showed a decrease of 6.5 ± 5.8 mm Hg in systolic arterial pressure [24]. With the 140 participants included in this study, a hypothesis testing power of 80% was achieved, which allowed detecting a statistically significant difference in the mean score of the Trail Making Test B of 15 s between the intervention group and the control group, assuming an alpha risk of 0.05 in a bilateral contrast.

Procedures and randomisation

All participants paid a baseline visit and a follow-up visit 6 months after the baseline evaluation, in which the study variables were recorded (Figure 1). The intervention group paid 5 additional resupply visits 1, 2, 3, 4 and 5 months after the baseline visit, in which they were given the necessary chocolate until the next visit. Moreover, in this resupply visits, the participants of the intervention group handed a calendar with the record of the chocolate intakes done, with no other procedures involved.

The participants were randomly assigned to two parallel groups: an intervention group (IG) of 73 participants and a control group (CG) of 67 participants. The randomisation was conducted by an independent researcher using the Epidat 4.2 software [25]. The participants received the randomisation number according to the baseline visit, which was hidden until they were all assigned to a group. The information related to the treatment allocation was kept in a safe box in case of emergency unmasking.

To ensure the blinding of the study, the participants were clearly instructed not to reveal which treatment they had been assigned to in the interviews conducted by the blinded researchers. The characteristics of the intervention did not allow for the participants to be blinded. The evaluation and chocolate resupply visits in IG were conducted by different researchers in order to minimise contamination between groups.

Intervention

The CG participants did not receive any intervention. The IG participants were given chocolate with a cocoa concentration of 99% and the instructions for the consumption of 10 g of this compound as an addition to their habitual food intake every day for 6 months. After the baseline intervention, the IG participants received instructions about the consumption and storage of the chocolate supplement, recommending them to take the daily dose at the same time of the day. They were also given a calendar to record the time and intake of each day, which was handed back to the researchers in each resupply visit. The daily nutritional value of 10 g of this cocoa-rich supplement is 59 Kcal, 0.8 g carbohydrates, 1.5 g of protein and 5.1 g of fat, of which 3.1 g are saturated. The polyphenol contribution per 10 g of this product is 65.4 mg. The polyphenol profile of this supplement is shown in the previously published study protocol [26]. All participants were requested to maintain their eating and diet habits during the study period.

Main outcomes

Cognitive performance was evaluated through a brief neuropsychological test.

Attention and executive functions

Attention was measured with Trail Making Test A (TMT-A), and processing speed and cognitive flexibility (as a component of executive functions) were evaluated with Trail Making Test B (TMT-B) [27]. Furthermore, this test allows assessing visuomotor speed, visual tracking, motor function and working memory [28,29]. TMT-A consists in linking a series of numbers in ascending order, whereas TMT-B consists in linking a series of numbers and letters alternatively, with the numbers following the ascending order and the letters following the order of the alphabet. In both tests, the obtained score is based on the time (quantified in seconds) that the subject took to complete the task.

Verbal memory

Verbal memory was evaluated using the abbreviated version of the Rey Auditory Verbal Learning Test (RAVLT) [30]. Immediate verbal memory was measured based on the capacity of the participant to immediately remember a list of 15 words in 3 attempts (RAVLT-IR). The outcome variable was the number of words remembered in the third attempt. After 10 min, delayed verbal memory was measured based on the capacity of the participant to freely remember the words learned in the first part of the evaluation (RAVLT-DR).

Working memory

Working memory was assessed with the WAIS Digit Span Backward test [31]. This test consists of six categories, each with two series of numbers. The series of each category have one more number than the previous category, beginning with two numbers. The participant must immediately and inversely repeat each series. The test ends when the subject makes mistakes in the two series of one category. The score is equal to the last category of which at least one of the series of numbers was correctly followed.

Phonological fluency

Phonological fluency was evaluated with the FAS Questionnaire, which consists in naming as many words beginning with F, A and S as possible in one minute [32]. The obtained score is equal to the number of words pronounced correctly, counting out repetitions, derived words and proper nouns.

Category fluency

Category fluency measures controlled association semantic verbal fluency. This test consists in naming as many animals as possible in one minute [33]. The obtained score is equal to the sum of words pronounced correctly, counting out repetitions and derived words.

Other measurements

Sociodemographic variables

In the baseline visit, questionnaires were used to gather information about sociodemographic variables, which included questions about age, marital status and educational level. Marital status was recorded in the following categories: married/coexists, separated/divorced, single and widowed. The educational level was recorded in the following categories: primary studies, middle-high school, university studies, postgraduate studies.

Clinical variables

In the baseline visit, questionnaires were used to gather the participants' personal history of gestational diabetes, untreated hypertension and dyslipidaemia and the prescribed pharmacological treatment, as well as the time elapsed from the beginning of menopause.

Adherence to the intervention

Adherence was calculated as the percentage of days of chocolate intake with respect to the theoretical total percentage, based on the data recorded in the calendars of each IG participant.

Evaluation of chocolate consumption and habitual diet

The habitual consumption of chocolate was assessed in each of the evaluation visits through a series of questions about the amount, type and frequency of consumption in the periods between visits. To evaluate the nutritional composition of the habitual diet, which includes the distribution of macronutrients and energy consumption, a 24-hour recall was used; this reminder was recorded in 3 non-consecutive days, prior to the day of each evaluation. These data were recorded and processed using the EVIDENT app [34].

The study protocol includes the description of the method used to measure other variables that were also recorded, such as physical activity, alcohol consumption and smoking [26].

Data collection procedure, data management and monitoring

In each evaluation visit, the data were collected by a nurse, who had been previously trained for this task. Each participant of the study was identified with a unique code, which in turn identified the data collected in each of the measurements. A database was created with all the collected data, which could only be accessed by the researchers of the study.

Ethical considerations

The study was approved by the Clinical Research Ethics Committee of the Salamanca Health Area ('CREC of Health Area of Salamanca') in February 2018. The participants signed an informed consent document, in accordance with the Declaration of Helsinki. They were informed of the objectives of the project and the risks and benefits of the explorations to be carried out. The confidentiality of the participant data was guaranteed at all times in accordance with the provisions of the Organic Law 3/2018, of December 5th, on Personal Data Protection and guarantee of digital rights, and EU Regulation 2016/679 of the European Parliament and of the Council of April 27th 2016 on Data Protection (RGDP), and under the conditions established by national law 14/2007 of biomedical research.

Statistical analyses

The statistical analyses were carried out following the study protocol [26]. The data were checked for normal distribution and most data were considered normally distributed. The characteristics of the sample are presented as mean and standard deviation or median

(interquartile range) for the continuous variables, whereas the qualitative variables are expressed using frequency distribution. To evaluate the comparability between the two study groups in the baseline evaluation, the chi-square test and Student's *t*-test were used for the qualitative and quantitative variables, respectively. Fisher's exact test and Mann–Whitney *U* test were used for qualitative and quantitative variables with non-normal distribution, respectively.

The effect of the intervention on the outcome variables (cognitive performance variables) was evaluated through a covariance analysis (ANCOVA), using age, educational level, time elapsed from the beginning of menopause and daily energy consumption as covariates. The differences between groups in every case are presented as mean and 95% CI. The effect size was estimated in the change of the cognitive performance variables through the calculation of Cohen's *d*. To analyse the intragroup change in the outcome variables at 6 months with respect to the baseline evaluation, the Student's *t*-test was used for paired data, which are presented as mean and standard deviation.

Subgroup analyses were carried out based on educational level and age as baseline conditions. For the subanalysis based on age, the sample was divided taking the median of age as reference, which was 57.4 years, and the effect was evaluated through an ANCOVA adjusting for educational level, time elapsed from the beginning of menopause and daily energy consumption. To evaluate the effect in the subanalysis based on educational level, two subgroups were made (university or postgraduate studies and primary or middle-high school), and an ANCOVA was conducted using age, time elapsed from the beginning of menopause and daily energy consumption as covariates.

All analyses were performed using SPSS V.23.0 (IBM Corp, Armonk, NY) and establishing an alpha risk of 0.05 as the limit of statistical significance.

Results

Baseline characteristics of the study population

The study included 140 women: 73 in IG and 67 in CG. Three participants were lost in the follow-up: two from IG due to a newly diagnosed cancer and under treatment, and one in CG who refused to continue participating. Therefore, a total of 137 women completed the study and were included in the analysis: 71 in IG and 66 in CG (Figure 1).

The baseline characteristics of the participants showed no differences between the two groups (Table 1). Most of the women, both in IG (65.8%) and in CG (70.1%) were married or coexisted. In both groups, the educational level of most of the participants was middle-high school (30.1% in IG, 43.3% in CG). In IG, 23.3% of the women had university studies and 24.7% had postgraduate studies, whereas these percentages were 16.4% and 22.4%, respectively, in CG. The habitual consumption of chocolate, as well as the specific consumption of chocolate with over 70% of cocoa, was similar in both groups. Average adherence to the intervention (%) was 97.61 ± 3.34, with a minimum and maximum adherence of 80.56% and 100%, respectively.

Table 1. Baseline characteristics of the study population.

Variables	Intervention group (*n* = 73)	Control group (*n* = 67)	*p*[a]
Age (years)	57.1 ± 3.5	57.5 ± 3.8	0.555
Marital status (*n*, %)			0.574
Married/coexists	48 (65.8%)	47 (70.1%)	
Separated/divorced	8 (11.0%)	7 (10.4%)	
Single	15 (20.5%)	9 (13.4%)	
Widowed	2 (2.7%)	4 (6.0%)	
Educational level (*n*, %)			0.417
Primary studies	16 (21.9%)	12 (17.9%)	
Middle-high school	22 (30.1%)	29 (43.3%)	
University studies	17 (23.3%)	11 (16.4%)	
Postgraduate studies	18 (24.7%)	15 (22.4%)	
Time from menopause onset (years)	6.9 ± 4.6	6.9 ± 3.6	0.990
Untreated hypertension (*n*, %)	1 (1.4%)	0 (0.0%)	0.340
Untreated dyslipidemia (*n*, %)	8 (11.0%)	10 (14.9%)	0.484
Gestational diabetes (*n*, %)	3 (4.1%)	1 (1.5%)	0.621
Thyroid hormone treatment (*n*, %)	13 (17.8%)	10 (14.9%)	0.646
Current smoker (*n*, %)	12 (16.4%)	9 (13.4%)	0.619
Alcohol consumption (g/week)	23.1 ± 29.4	30.6 ± 48.1	0.262
Energy intake (kcal/day)	1720 ± 357	1780 ± 402	0.306
Physical activity (MET–h/week)	31.2 ± 36.8	25.7 ± 20.0	0.275
Chocolate intake (g/week)	42 (9–109)	50 (21–80)	0.925
>70% cocoa chocolate intake (g/week)	0 (0–26)	0 (0–24)	0.395

Notes**:** Values expressed as mean ± standard deviation, median (interquartile range) or frequencies (percent).
Abbreviations: MET, metabolic equivalent of task.
[a]Intergroup comparison by the Student's *t*-test or the Mann–Whitney *U* test for quantitative variables and by the chi-square test or the Fisher's exact test for the qualitative variables.

Results for baseline and 6-month evaluation of energy and nutrients intake of habitual diet are shown in Table 2.

Cognitive performance variables

TMT-A execution time decreased in both groups, with no differences between them. On the other hand, IG showed a TMT-B execution time decrease of −12.52 ± 35.40 s, whereas the change in CG was −0.97 ± 31.47 s, which implies a difference of −12.08 (−23.99, −0.18) seconds ($p = 0.047$) between the two groups after adjusting for age, educational level, time elapsed from the beginning of menopause and daily energy consumption. The Cohen's *d* value for this difference was −0.343 (Table 3).

Table 2. Energy intake and nutrients of habitual diet in postmenopausal women participants.

	IG ($n = 71$)				CG ($n = 66$)					
	Baseline	6 months	Change	p^a	Baseline	6 months	Change	p^a	Intergroup difference (IG-CG)[b]	p^b
Energy intake (kcal/day)	1712 ± 365	1722 ± 353	9.5 ± 389.1	0.840	1782 ± 402	1794 ± 339	12.2 ± 351.2	0.780	−2.7 (−129.6, 124.2)	0.967
Carbohydrates (g/day)	167.8 ± 45.6	162.3 ± 43.4	−5.5 ± 55.2	0.414	172.5 ± 50.0	177.9 ± 39.0	5.4 ± 48.1	0.367	−10.9 (−28.6, 6.9)	0.227
Proteins (g/day)	76.2 ± 16.8	81.7 ± 17.7	5.5 ± 19.2	0.020	78.3 ± 19.0	81.5 ± 15.4	3.2 ± 18.9	0.176	2.3 (−4.2, 8.8)	0.491
Fiber (g/day)	24.0 ± 7.7	22.7 ± 6.4	−1.4 ± 7.6	0.146	25.4 ± 9.5	25.4 ± 9.1	−0.2 ± 10.6	0.888	−1.2 (−4.3, 2.0)	0.463
Fats (g/day)	77.1 ± 20.7	77.7 ± 20.1	0.6 ± 23.0	0.823	80.1 ± 20.1	77.9 ± 18.1	−2.2 ± 17.8	0.315	2.9 (−4.2, 9.9)	0.425
Saturated fats (g/day)	25.1 ± 7.7	25.0 ± 7.8	−0.1 ± 8.5	0.920	25.5 ± 7.4	24.4 ± 6.4	−1.2 ± 7.5	0.219	1.0 (−1.7, 3.8)	0.451

Notes**:** Values are means ± SDs and differences are means (95% CI).
Abbreviations: CG: control group, IG: intervention group.
[a]Intragroup comparison by the Paired Student's *t*-test.
[b]Intergroup comparison by the Student's *t*-test.

Table 3. Cognitive performance variables in postmenopausal women participants.

	IG ($n = 71$)				CG ($n = 66$)								
	Baseline	6 months	Change	p^a	Baseline	6 months	Change	p^a	Intergroup difference (IG-CG)[b]	p^b	Adjusted Intergroup difference (IG-CG)[c]	p^c	Cohen's *d*
RAVLT-IR (words)	7.70 ± 1.82	8.57 ± 1.96	0.87 ± 1.82	0.000	7.47 ± 1.89	8.44 ± 2.12	0.97 ± 1.70	0.000	−0.10 (−0.70, 0.49)	0.737	−0.05 (−0.65, 0.56)	0.872	−0.057
RAVLT-DR (words)	6.79 ± 3.18	8.25 ± 2.96	1.46 ± 2.87	0.000	7.17 ± 2.78	8.35 ± 2.84	1.18 ± 2.16	0.000	0.28 (−0.58, 1.15)	0.518	0.19 (−0.69, 1.07)	0.665	0.117
Trail Making Test A (seconds)	39.32 ± 13.82	35.61 ± 11.95	−3.72 ± 12.96	0.018	40.77 ± 11.58	37.12 ± 12.31	−3.65 ± 10.74	0.007	−0.07 (−4.11, 3.97)	0.974	−0.10 (−4.34, 4.13)	0.961	0.000
Trail Making Test B (seconds)	94.00 ± 47.36	81.48 ± 31.99	−12.52 ± 35.40	0.004	91.61 ± 33.99	90.64 ± 32.64	−0.97 ± 31.47	0.803	−11.55 (−22.90, −0.20)	0.046	−12.08 (−23.99, −0.18)	0.047	−0.343
Digit Span Backwards (total score)	3.33 ± 1.26	3.20 ± 1.06	−0.13 ± 1.15	0.354	3.12 ± 1.03	3.12 ± 0.95	0.00 ± 1.16	1.000	−0.13 (−0.52, 0.26)	0.519	−0.10 (−0.51, 0.31)	0.635	−0.083
Phonological fluency (words)	13.11 ± 3.82	13.56 ± 4.22	0.45 ± 3.43	0.273	11.59 ± 3.83	12.15 ± 4.21	0.56 ± 3.54	0.203	−0.11 (−1.29, 1.07)	0.854	0.06 (−1.14, 1.25)	0.926	−0.028
Category fluency (words)	20.27 ± 5.14	22.52 ± 5.40	2.25 ± 5.43	0.001	20.12 ± 4.29	22.44 ± 4.32	2.32 ± 3.96	0.000	−0.06 (−1.68, 1.55)	0.937	0.26 (−1.40, 1.92)	0.758	0.000

Notes**:** Values are means ± SDs and differences are means (95% CI).
Abbreviations: CG: control group, IG: intervention group, RAVLT-IR: Rey Auditory Verbal Learning Test–Immediate Recall, RAVLT–DR: Rey Auditory Verbal Learning Test–Delayed Recall.
[a]Intragroup comparison by the Paired Student's *t*-test.
[b]Intergroup comparison by the Student *t* test.
[c]Intergroup comparison by ANCOVA adjusted for age, educational level, time from menopause onset and energy intake.

The results obtained in RAVLT-IR, RAVLT-DR and category fluency show a score increase in both groups, with no differences between them. With respect to the scores obtained in the WAIS Digit Span Backwards and FAS tests, no changes were found.

Subgroup analysis by age

The results of the subanalysis by age, adjusted for educational level, time elapsed from the beginning of menopause and daily energy consumption, are shown in Table 4.

The TMT-B execution time decreased in the IG participants with respect to CG both in the group of women aged ≤57.40 years and in that of women aged >57.40 years, showing no statistically significant differences.

No relevant changes were found in these subgroups in any of the cognitive performance variables measured.

Subgroup analysis by educational level

The women with university studies showed a difference of −10.39 (−24.15, 3.36) seconds in the TMT-B execution time between the two groups after adjusting for age, time elapsed from the beginning of menopause and daily energy consumption, although this difference was not statistically significant ($p = 0.136$), as was observed in the group of women without university studies (−12.11 s; −30.62, 6.41; $p = 0.196$) (Table 5).

Similarly, no relevant changes were observed in the scores of RAVLT-IR or RAVLT-DR, and no differences were found between groups based on educational level in neither TMT-A nor any of the cognitive performance variables evaluated.

Discussion

The findings of this study show a decrease of TMT-B execution time in IG after the daily consumption of 10 g of cocoa-rich (99%) chocolate, which suggests a slight improvement in the cognitive performance related to cognitive flexibility and processing speed, as components of executive functions. However, no relevant differences were found in attention, immediate or delayed verbal memory, phonological or category fluency, or working memory.

The effect of cocoa on cognitive performance has been studied by different authors, although the obtained results are heterogeneous. The findings of the present clinical trial, in which there was an improvement in the execution time of a test that explores cognitive flexibility with a moderate effect size, are in line with those reported in other studies. Nurk et al. [18] observed

Table 4. Subanalysis of cognitive performance variables by age in postmenopausal women participants.

	Age ≤57.4 years						Age >57.4 years					
	IG ($n = 38$)		CG ($n = 33$)				IG ($n = 35$)		CG ($n = 34$)			
	Baseline	6 months	Baseline	6 months	Adjusted Intergroup difference (IG-CG)[a]	p^a	Baseline	6 months	Baseline	6 months	Adjusted Intergroup difference (IG-CG)[a]	p^a
RAVLT-IR (words)	7.50 ± 1.57	8.85 ± 1.90	7.52 ± 1.75	8.66 ± 2.13	0.10 (−0.78, 0.98)	0.817	7.92 ± 2.02	8.27 ± 2.00	7.45 ± 2.02	8.23 ± 2.12	−0.33 (−1.17, 0.50)	0.425
RAVLT–DR (words)	6.42 ± 2.76	8.54 ± 2.73	7.12 ± 2.61	8.58 ± 2.88	0.26 (−1.01, 1.54)	0.681	7.26 ± 3.53	7.94 ± 3.20	7.35 ± 3.05	8.12 ± 2.83	−0.10 (−1.27, 1.07)	0.865
Trail Making Test A (seconds)	36.82 ± 9.41	34.16 ± 11.97	38.79 ± 12.64	34.70 ± 10.75	1.26 (−3.79, 6.30)	0.621	41.89 ± 16.97	37.18 ± 11.90	43.26 ± 10.49	39.55 ± 13.43	−1.66 (−8.83, 5.50)	0.644
Trail Making Test B (seconds)	90.39 ± 47.65	77.51 ± 30.89	85.24 ± 34.52	82.79 ± 27.32	−11.36 (−27.45, 4.74)	0.163	97.91 ± 46.57	85.79 ± 33.07	98.62 ± 32.44	98.48 ± 35.93	−14.30 (−32.50, 3.91)	0.122
Digit Span Backwards (total score)	3.32 ± 1.32	3.22 ± 1.18	3.12 ± 1.14	3.24 ± 0.94	−0.21 (−0.79, 0.36)	0.465	3.34 ± 1.21	3.18 ± 0.92	3.15 ± 0.93	3.00 ± 0.97	0.05 (−0.57, 0.67)	0.865
Phonological fluency (words)	13.42 ± 4.27	13.81 ± 4.34	11.67 ± 3.96	12.24 ± 4.35	−0.25 (−2.00, 1.50)	0.775	12.37 ± 3.53	13.29 ± 4.14	11.50 ± 3.70	12.06 ± 4.14	0.54 (−1.09, 2.16)	0.510
Category fluency (words)	21.11 ± 5.10	24.30 ± 5.40	21.36 ± 3.87	23.70 ± 3.96	1.38 (−1.23, 3.98)	0.296	19.14 ± 5.23	20.59 ± 4.76	18.74 ± 4.40	21.18 ± 4.35	−0.78 (−2.86, 1.30)	0.457

Notes: Subgroups were created based on median age (57.40 years). Values are means ± SDs and differences are means (95% CI).
Abbreviations: CG: control group, IG: intervention group.
[a]Intergroup comparison by ANCOVA adjusted for educational level, time from menopause onset and energy intake.

Table 5. Subgroup analysis of cognitive performance variables by educational level in postmenopausal women participants.

	University studies (Bachelor, Postgraduate)						Non-university studies (Elementary education, Middle-High school)					
	IG ($n = 35$)		CG ($n = 25$)				IG ($n = 36$)		CG ($n = 41$)			
	Baseline	6 months	Baseline	6 months	Adjusted Intergroup difference (IG-CG)[a]	p^a	Baseline	6 months	Baseline	6 months	Adjusted Intergroup difference (IG-CG)[a]	p^a
RAVLT-IR (words)	8.41 ± 1.83	9.26 ± 1.86	8.04 ± 2.21	9.24 ± 2.35	−0.43 (−1.46, 0.61)	0.413	7.05 ± 1.52	7.91 ± 1.84	7.13 ± 1.55	7.96 ± 1.83	0.12 (−0.61, 0.84)	0.749
RAVLT–DR (words)	7.54 ± 3.75	9.71 ± 2.90	8.42 ± 2.98	9.64 ± 2.66	0.82 (−0.71, 2.34)	0.287	6.16 ± 2.35	6.83 ± 2.27	6.48 ± 2.47	7.56 ± 2.68	−0.19 (−1.21, 0.82)	0.705
Trail Making Test A (seconds)	35.69 ± 11.57	33.11 ± 10.02	36.50 ± 9.94	35.68 ± 16.06	−2.68 (−9.18, 3.81)	0.411	42.53 ± 14.82	38.03 ± 13.25	43.95 ± 11.97	38.00 ± 9.46	1.17 (−4.60, 6.94)	0.687
Trail Making Test B (seconds)	75.51 ± 28.65	65.09 ± 20.32	77.57 ± 22.38	78.72 ± 26.99	−10.39 (−24.15, 3.36)	0.136	111.03 ± 54.00	97.42 ± 33.41	101.20 ± 36.90	97.90 ± 33.94	−12.11 (−30.62, 6.41)	0.196
Digit Span Backwards (total score)	3.97 ± 1.18	3.56 ± 0.96	3.54 ± 0.99	3.64 ± 0.86	−0.39 (−1.10, 0.32)	0.275	2.74 ± 1.03	2.86 ± 1.05	2.88 ± 0.98	2.80 ± 0.87	0.23 (−0.27, 0.73)	0.366
Phonological fluency (words)	14.66 ± 3.31	15.03 ± 3.94	13.38 ± 2.95	13.16 ± 4.11	0.86 (−1.15, 2.86)	0.395	11.32 ± 3.84	12.14 ± 4.04	10.44 ± 3.87	11.54 ± 4.21	−0.15 (−1.64, 1.35)	0.846
Category fluency (words)	21.97 ± 4.89	24.20 ± 5.98	22.23 ± 4.22	23.44 ± 4.44	1.93 (−1.11, 4.98)	0.208	18.50 ± 5.01	20.89 ± 4.24	18.63 ± 3.82	21.83 ± 4.18	−0.68 (−2.46, 1.11)	0.452

Notes: Values are means ± SDs and differences are means (95% CI).
Abbreviations: IG: Intervention Group, CG: Control Group, RAVLT-IR: Rey Auditory Verbal Learning Test–Immediate Recall, RAVLT–DR: Rey Auditory Verbal Learning Test–Delayed Recall.
[a]Intergroup comparison by ANCOVA adjusted for age, time from menopause onset and energy intake.

that regular consumers of chocolate performed better in the cognitive tests, with executive functions being among the most favoured functions. Similarly, other authors have reported an improvement in executive functions, shown by the reduction in the time required to complete TMT-A and TMT-B, after the 8-week consumption of two polyphenol-rich cocoa compounds against another compound with low polyphenol concentration in elderly subjects, both without cognitive deterioration [8] and with mild cognitive impairment [19]. On the other hand, the authors of another clinical trial did not observe a short-term benefit in executive functions after the consumption of chocolate [16].

The exact causes that trigger these effects on cognitive performance are still unknown, although different mechanisms have been proposed. Some studies suggest that an increase in cerebral blood flow caused by the polyphenols of cocoa can improve the results of cognitive performance tasks [10,35]. Likewise, Scholey et al. [4] state that the improvement in endothelial function and blood flow caused by polyphenols could be related to these effects. It has also been suggested that brain-derived neurotrophic factor (BDNF) could act as a mediator in the cognitive improvement after the intake of cocoa [7]. Other authors suggest that a decrease in insulin resistance may be involved in the appearance of these effects on cognitive performance [8,19]. Additionally, since the modification in the levels of estrogens may affect postmenopausal women cognitive state [20], estrogen is thought to have a key role on cognitive changes during menopause with a neuroprotective effect [36]. Evaluation of hormone levels was not carried out in the present study, however it should be considered for further research.

Regarding education, a large percentage of the women of both groups showed a high level, which was higher in IG. However, our findings do not show statistically significant differences between the two groups based on their educational level, unlike previous studies which report that people with a higher educational level show better cognitive performance [28,37].

It seems that the intervention did not affect other cognitive functions, which is in line with the results of other clinical trials, such as the one conducted in postmenopausal women by Marsh et al. [21] and the one conducted by Pase et al. [15]. On the other hand, other studies have reported beneficial effects in other cognitive aspects, such as working memory and attention [4,12]. Nurk et al. [18] observed an improvement in verbal memory related to the consumption of chocolate in an observational study. Similarly, other authors have reported an improvement in phonological fluency after the intake of polyphenol-rich cocoa

compounds in elderly people [8,19]. Furthermore, Karabay et al. [17] suggested that cocoa flavanols could improve certain aspects of attention. Likewise, Grassi et al. [9] found an increase in working memory after the consumption of flavonols with sleep deprivation.

It is important to take into account that the repetition of the tests 6 months after the baseline visit may have introduced a learning component, inducing a better performance in the tests conducted in the follow-up visit for both groups, despite the fact that this is the time period recommended to avoid the learning effect.

The clinical trial that this study is part of aimed to evaluate the effect of adding a daily amount of 10 g of chocolate high in cocoa content (99%) and polyphenols to the normal diet on blood pressure, vascular function, cognitive performance, quality of life and body composition in postmenopausal women. With a randomised clinical trial design, this intervention aimed to assess the effects of the chronic intake of cocoa-rich chocolate on a population with specific characteristics, as it is postmenopausal women. This trial had a larger number of participants compared to other studies [21,38,39] assessing the effect of cocoa rich chocolate on cognitive function, although the sample size could be insufficient for the contrast of this variable since it was not estimated based on this outcome. Moreover, the study intervention consisted on the addition of an amount of commercially available chocolate, which was not specifically designed for this purpose and has specific and unmodifiable characteristics, to the habitual diet. This provided a real clinical context and enabled the assessment of the potential benefits, as well as potential harms, of the intake of this product as a whole, as intended. Hence, this makes the results of this trial more accessible than those of other studies [8,21] using non-commercialized compounds elaborated specifically for research purposes that are not available in a real context. The amount of 10 g of cocoa-rich (99%) chocolate used in the intervention fits with the recommendations of the European Food Safety Authority [40] and coincides with the data provided by Nurk et al. [18], who reported a maximum beneficial effect on cognitive performance with the daily consumption of approximately 10 g of chocolate. Although the mean baseline chocolate intake was about 70 g/week (10 g/day) in both groups (median (interquartile range): 42 (9–109) g/week in the IG and 50 (21–80) g/week in the CG), it is important to note that this includes dark chocolate, milk chocolate and white chocolate. Nonetheless, the mean >70% cocoa chocolate (dark chocolate) intake was less than 20 g/week in both groups (median (interquartile range): 0 (0–26) g/week in the IG and 0 (0–24) g/week in the CG). The daily consumption of 10 g of cocoa-rich (99%) chocolate added to the habitual diet in the intervention group fits with the EFSA recommendations and ensures that every participant in this group complies with these recommendations. However, the chocolate used in this trial contains a lower concentration of polyphenols than the compounds used in other studies which are specifically designed for this purpose [4,8]. Moreover, dietary polyphenol intake could be very high, as observed in previous studies with an estimated mean total intake of 820 ± 323 mg polyphenols/day in the PREDIMED cohort [41], and 1193 ± 510 mg/day in the SU.VI.MAX cohort [42]. The cocoa polyphenol contribution of the amount of chocolate used in the intervention seems to be low compared with the amount of polyphenols consumed in the diet; therefore, it may be insufficient to show important changes in the effect size and should be considered for further research.

The population sample in which this study was carried out presents special characteristics. Postmenopausal women may have cognitive difficulties [20] related to hormonal changes, which are typical of this period. Therefore, it is important to develop interventions such as the one conducted in this trial, that is, with the aim of improving cognitive function in this population group without causing adverse effects.

Regarding the limitations of this study, it is worth highlighting that it was not possible to blind the participants due to the nature of the intervention, although the blinding of the researchers who recorded the measurements and conducted the statistical analyses was ensured. Lack of consideration of the amount of polyphenols consumed by individual participants as a criterion for inclusion and the lack of control of polyphenol consumption during the study could be another limitation. However, this was not possible since the tool used to assess the nutritional composition of the habitual diet does not provide data on the polyphenol content of the diet or the specific foods consumed. Dietary supplements intake was not recorded as well. Although we could assume that randomisation had balanced the groups with respect to dietary intake as well as polyphenol intake, this should be considered in future trials.

To conclude, the results of this study suggest that the consumption of cocoa-rich (99%) chocolate in addition to the habitual diet could be related to a slight improvement in cognitive performance regarding cognitive flexibility and processing speed in postmenopausal women, with no changes in the rest of the cognitive performance variables evaluated. However, it is necessary to carry out further studies that allow clarifying the possible benefits of consuming

cocoa-rich chocolate in cognitive performance for this population group.

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