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The short-term effect of dark chocolate flavanols on cognition in older adults: a randomized controlled trial (FlaSeCo)

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Background: Cocoa flavanols in the diet have had positive effects on cognition, blood lipid levels, and glucose metabolism.

Methods: Cognitively healthy older adults aged 65–75 years were recruited for an eight-week randomized, double-blind controlled trial to investigate the effectiveness of cocoa flavanols on cognitive functions. At baseline, nutrient and polyphenol intakes from diet were assessed with three-day food diaries. The intervention group received 50 g dark chocolate containing 410 mg of flavanols per day, and the control group 50 g dark chocolate containing 86 mg of flavanols per day, for eight weeks. Cognition was assessed with Verbal Fluency (VF) and the Trail Making Test (TMT) A and B as the main outcome measures. Changes in blood lipids and glucose were also measured.

Results: The older adults participating numbered 100 (63% women), mean 69 y (range 65 to 74). They were highly educated with a mean 14.9 years of education (SD 3.6). No differences in changes in cognition were seen between groups. The mean change ($\pm$ SEs) in the time to complete the TMT A and B in the intervention group was -4.6 s (-7.1 to -2.1) and -16.1 s (-29.1 to -3.1), and in the controls -4.4 s (-7.0 to -1.9) and -12.5 s (-22.8 to -2.1) (TMT A $p=0.93$; TMT B $p=0.66$). No difference was apparent in the changes in blood lipids, glucose levels, or body weight between the groups.

Conclusions: The healthy older adults showed no effect from the eight-week intake of dark chocolate flavanols on cognition.

Keywords: Cocoa flavanols; Chocolate; Cognition; Aging

1. Introduction

Diets high in polyphenols have been associated with a lower risk of cognitive decline (Lamport et al. 2014). Cocoa is a rich source of polyphenols, especially the flavanols epicatechin and catechin (Willams et al. 2012, Żyżelewicz et al. 2016). Regular consumption of cocoa flavanols may reduce blood pressure, improve insulin sensitivity, reduce fat oxidation, and induce vasodilation, thus increasing blood flow in the brain, which is essential for optimal brain function (Grassi et al. 2005, Grassi et al. 2008, Sorond et al. 2013, Lamport et al. 2014, Dower et al. 2015). Similarly, epidemiological studies show that cocoa consumption has been associated with better cognition and decreased Alzheimer disease (AD) risk (Commenges et al. 2000, Crichton et al. 2016). Moreover, flavonoid and other polyphenol-rich foods have been able to improve some cognitive domains in short-time trials (Nooyens et al. 2011, Kean et al. 2015, Alharbi et al. 2016, Kent et al. 2017, Miller et al. 2018).

The antioxidant effects of flavonoids, including cocoa flavanols, were first proposed to explain their beneficial effects on cognitive functions (Rice-Evans et al. 2001). The main cocoa flavanol epicatechin can increase the bioavailability of nitric oxide, the main regulator of vascular function, leading to improvements in vascular tone and blood pressure regulation (Socci et al. 2017). Moreover, cocoa flavanols may have independent neuromodulatory and neuroprotective actions, and they apparently accumulate in the hippocampus, which is responsible for learning and memory (Sokolov et al. 2013).

In recent years, an increasing amount of evidence supports the role of cocoa-derived product and chocolate consumption in the neurocognitive and neuroprotective enhancement of executive functions, attention, and memory, particularly among subjects with cognitive decline (Socci et al. 2017). In a cross-sectional study with community-dwelling adults, high dark chocolate consumption

was associated with better cognitive function, including very old people, and the relationship was not attenuated when adjusted for cardiovascular, lifestyle, and dietary factors (Crichton et al. 2016). The few intervention studies on cocoa flavanol intake and cognitive performance have shown mixed results. Short-term studies among healthy middle-aged or older adults showed cognitive benefits from cocoa flavanol consumption in cocoa drinks or supplements (Camfield et al. 2012, Brickman et al. 2014, Mastroiacovo et al. 2015, Neshatdoust et al. 2016). Similar results have been found for older people with high blood pressure (Sorond et al. 2013) and older people with mild cognitive impairment (Desideri et al. 2012), whereas one study among healthy older participants consuming dark chocolate together with a cocoa drink showed a null result (Crews et al. 2008). The trials conducted have, however, mostly been small, and the cognitive domains were measured in very heterogenic ways (Lamport et al. 2014). It is unknown whether the cocoa flavanol in commercially available dark chocolate has cognitive benefits for healthy older people.

The aim of our study was to clarify the effect of an eight-week intake of commercially available dark chocolate with high flavanol content, especially the epicatechin content, on cognition among healthy older participants, 65–75-year-old men and women. The setting was a double-blind, randomized, controlled trial using chocolate with reduced flavanol content as a control. In addition, we assessed the changes in blood glucose, lipids, and body mass index.

2. Subjects and methods

2.1 Participants

The participants were recruited in the capital area of Finland mainly through a public release that was sent to the press and radio channels. In the release the nature of the research and the main inclusion and exclusion criteria were described. The inclusion criteria included cognitively healthy adults aged 65–75 years without diagnosed, chronic diseases, whose BMI was less than 32 (in kg/m^2) without a major weight change during the past year, who did not smoke, and who were willing to sign the informed consent. Exclusion criteria were medication for blood pressure and high cholesterol, current smokers, habitual users of antioxidant supplements, and daily consumers of chocolate or other cocoa products. Before taking any other measurements from them, the participants completed the SLUMS test (Tariq et al. 2006) to ensure that they had normal cognition. Those whose result was less than 27 points (high school education) or less than 25 (less than high school) were excluded.

Figure 1. Recruitment of the subjects.

A total of 439 subjects responded to the press release and showed interest in the study by contacting us by phone, email, or an internet-based registration form. Altogether 313 were excluded in the telephone screening, 142 due to their medical history, refusal to provide information or another reason for not fulfilling the inclusion criteria, and 171 respondents were excluded after the statistical power calculation. Based on the telephone screening, 126 persons attended the baseline visit, and their blood was sampled for the metabolic parameters (fP-Gluc, fS-Chol, fS-Chol-HDL, fS-Chol-LDL, fS-Trigly, fS-Crea, S-ALAT, S-GT, S-K, S-Na, S-Uraat). Based on the results of the laboratory analysis and the evaluation of the study geriatrician (KP), 22 subjects were excluded due

to high total cholesterol ($n=11$, >7.0 mmol/l), cognitive decline ($n=5$), high liver value ($n=4$, S-ALAT >43.0 U/l, S-GT >133.0 U/l), acute illness ($n=1$), and refusal ($n=1$). The final number in our study was 104 subjects, who were randomized into intervention and control groups. In both groups two subjects dropped out and did not give us permission to use their data. The final number of participants was 100 (Figure 1).

The Ethics Committee of the Helsinki University Central Hospital and that of the research committee of the Social Insurance Institution of Finland approved the study. This trial was registered in ACTRN 12617000748314.

2.2 Study design and outcomes

To evaluate the effect of the daily consumption of dark chocolate on cognitive performance in healthy older subjects, an eight-week randomized, double-blind, parallel-arm study was conducted from August 2017 to December 2017. The main outcome measures were Verbal Fluency (VF) from CERAD (Consortium to Establish a Registry for Alzheimer's Disease), (Morris et al. 1989) and Trail Making Test A and B (TMT; Lezak et al. 1998).

At the baseline visit the participants gave blood samples, and their cognition was evaluated with the VF and TMT tests. A nutritionist (HSS) instructed them to keep three-day food diaries, and the participants received guidance on how to include dark chocolate in their daily diet and keep their energy intake in balance. The participants were instructed to substitute their sweet snacks with the reference or intervention chocolate, and they were told to limit the intake of polyphenol-rich food items, such as wild berries and apples, which are usually consumed in autumn time in Finland, and tea, red wine, fruit and vegetable juices, and chocolate other than the research chocolate. All of the participants received leaflets on how to follow an overall healthy and balanced diet. All of the participants were also instructed to continue their usual lifestyle habits.

Before the randomization as soon as the laboratory results were ready, the participants with an abnormal metabolic and cognitive profile were excluded. At the second visit, the participants returned their food diaries, and they were randomized into intervention and control groups. The participants received chocolate pralines with a white or silver paper cover on them, representing the blinded intervention and control groups. The pralines were divided into daily portions of 50 grams (seven pralines). All of the pralines for the eight-week intervention were given at the same time.

The intervention group received 50 g dark chocolate per day containing 410 mg total of flavanols, of which 85 mg was epicatechin, and the control group received 50 g dark chocolate per day with 86 mg total of flavanols per day, of which 26 mg was epicatechin. The intervention chocolate was commercially available dark chocolate (Karl Fazer 70% Dark Chocolate Pralines). The reference chocolate with low flavanol content was produced especially for the study purposes using higher processing temperatures and a longer conching time with highly processed cocoa powder. The intervention chocolate was higher in fibre, potassium, magnesium, and zinc and lower in sucrose compared to the reference chocolate. Table 1 describes the contents of the chocolates.

Procyanidins were determined in Natural Resources Institute Finland (LUKE) according to Robbins et al. (2013). Briefly, procyanidins were extracted from defatted samples with acidified aqueous acetone. The extracts were purified with Strata SCX SPE (Phenomenex Inc., Torrance, CA, USA) cartridges and filtered into the HPLC vials. Agilent 1290 Infinity (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with fluorescence detection (FLD; λ_{ex} = 275nm, λ_{em} = 324nm) was used for HPLC analyses. Procyanidins were separated according to their degree of polymerization (DP) on a Luna Diol HILIC (Phenomenex Inc., Torrance, CA, USA) column (150 × 4.6 mm, 3 μ m) at temperature 35 °C. The mobile phase was a binary gradient (solvents A and B) consisting of acetonitrile-acetic acid (98 + 2, A) and methanol-water-acetic acid (95 + 3 + 2, B). Elution was started with 3 % of B, followed by a linear gradient to 50 % of B in 25 min, to 100 % in 28 min,

isocratically for 2 min and back to the starting point in 3 min. Flow rate was 1 mL/min and injection volume 4 μ L. The post time was 5 min before next injection. Quantification was based on an external standard of (-)-epicatechin (Sigma-Aldrich Inc., St Louis, MO, USA). Procyanidins with different DPs were quantified according to their relative response factors (in relation to (-)-epicatechin) given by Robbins et al. (2013). Each sample was analyzed in triplicate.

Table 1. Nutritional values of the intervention and reference chocolates.

Nutrients (100 g)	Reference chocolate	Intervention chocolate
Energy (kcal/kJ) ¹	558/2336	562/2353
Total fat (g) ²	35.3	40
Saturated fatty acids (g) ³	21.5	24.7
Monounsaturated fatty acids (g) ³	11.2	12.3
Polyunsaturated fatty acids (g) ³	1	1.1
Carbohydrates (g) ¹	53.7	37.2
Sucrose (g) ⁴	52.2	29.7
Non-soluble fibre (g) ⁵	3.9	7.9
Soluble fibre (g) ⁵	1	1.7
Potassium (mg) ⁶	600	840
Magnesium (mg) ⁶	84	170
Zinc (mg) ⁷	1.3	2.8
Total flavanols (mg)	172	820
Epicatechin (mg)	52	170

1 Method: (EU) No 1169/2011, Eurofins Food & Feed Testing, Linköping, Sweden

2 Method: 2009/152/EU mod., Eurofins Food & Feed Testing, Linköping, Sweden

3 Method: Internal Method – GC-FID, GC-FID, Eurofins Food & Feed Testing, Linköping, Sweden

4 Method: AOAC 982.14, mod., Eurofins Food & Feed Testing, Linköping, Sweden

5 Method: Internal Method: Enzymatic-gravimetry, Eurofins Food Testing Netherlands, Heerenveen, Netherlands

6 Method: DIN EN ISO 11885, mod., Eurofins WEJ Contaminants GmbH, Hamburg, Germany

7 Method: DIN EN ISO 17294-2-E29, mod., Eurofins WEJ Contaminants GmbH, Hamburg, Germany

The three-day food diaries and other measurements were collected prior to randomization. The average daily energy and nutrient intake were calculated based on the food diaries.

2.3 Measurements

The baseline measurements included demographic data, diagnoses, and medication. The diagnoses and medications were confirmed based on written medical records and prescriptions provided by the participants. We also assessed the participants' cognition with the Saint Louis University Mental Status (SLUMS) Exam (Tariq et al. 2006) to confirm the subjects' capability of participating in the intervention.

At baseline and follow-up, we measured the participants' weight and height and calculated their body mass index (BMI). We measured weight with a portable and calibrated scale, and height with a standardized measure against the wall. We used the equation $\text{weight (kg)}/\text{height (m)}^2$ to calculate BMI.

The Trail Making Test (TMT) A and B (Lezak et al. 1998) and Verbal Fluency (VF) (Morris et al. 1989) were used to assess the change in cognition during the intervention. The TMT is a neuropsychological test of visual attention and task switching. It consists of two parts in which the subject is instructed to connect a set of 25 dots as quickly as possible while still maintaining accuracy. A lower number of seconds indicates better performance. The test can provide information about mental flexibility, processing speed, and executive functioning. In the VF test, the participant produces as many animal words as possible in 60 seconds. A higher number of animals indicates better performance. A good performance in VF indicates mental flexibility and cognitive productivity (Sotaniemi et al. 2012).

We used the three-day food diaries to assess food and nutrient intake. During the first visit the nutritionist gave written and verbal instructions to the participants on how to keep the food diaries. She also gave the participants 100 mL, 15 mL, and 5 mL measuring cups for measuring food items such as drinks and bread spreads. The participants were instructed to complete the food diaries over a period of three days including one weekend day while maintaining their usual diet. Participants brought the completed diaries to the nutritionist, and she checked their entries. Common things

checked by the nutritionist were the types of milk and fat used, and the trademarks of the food items or the amounts of food. Energy, protein, and nutrient intake were calculated using the AIVO-diet nutrition program (<https://www.mashie.com/fi/palvelut/ravintolaskenta/>).

Blood samples were drawn from the participants after an overnight fasting period at the beginning and the end of the trial. We used a laboratory package to ensure that the participants' health condition was stable and adequate for this intervention. The participants' fasting plasma glucose, cholesterol (total, HDL, and LDL), triglycerides, haemoglobin (B-Hb, E-MCHC, E-MCH), erythrocytes (B-HKR, E-MCV), leucocytes, platelets, creatinine, alanine aminotransferase, and glutamyl transferase were determined. In addition, we wanted to explore whether the intervention had an effect on the laboratory values.

2.4 Sample size

Sample size calculation was based on the Trail Making Test (TMT A). It was assumed that a minimum group difference would be 20% in the points of the TMT A with a type I error of 5%, statistical power of 80%, and 20% of drop outs in the groups, which resulted in 50 participants per group (Mastroiacovo et al. 2015).

2.5 Randomization

The participants who met all the inclusion criteria and had sufficient cognitive performance ($n = 104$) were randomly allocated to the intervention ($n = 52$) and control ($n = 52$) groups according to a computer-generated randomization list. Four participants dropped out and refused permission to use their data, resulting in 50 in the intervention and 50 in the control group.

2.6 Blinding

The intervention was double-blinded. Neither the researchers nor participants were aware of the treatment allocation. The chocolate pralines were wrapped with a white or silver paper cover representing the blinded intervention and control groups. The code was revealed after the intervention was finished.

2.7 Statistical methods

Data are presented as means with standard deviation (SD) or as counts (n) with percentages (%). Statistical comparison between the groups was performed by t-test. The method (achieved significance level) was used when the theoretical distribution of the test statistics was unknown or in the case of a violation of the assumptions (e.g. non-normality). Effect size (d) for the intervention was calculated by using Cohen's method where an effect size of 0.20 is considered small, 0.50 moderate, and 0.80 large. CIs for the effect sizes were obtained by bias-corrected bootstrapping (5000 replications). The normality of the variables was tested by using the Shapiro-Wilk W test. The Stata 15.1, StataCorp LP (College Station, TX, USA) statistical package was used for the analysis.

3. Results

3.1 Baseline

The number of participants was 100 (63% women), and their mean age was 69 years (SD 2.5). The mean BMI of the participants in the whole group was 25.0 kg/m² (SD 3.6). They had a mean education of 14.9 years (SD 3.6), and their HRQoL was good. The mean level of blood glucose was 5.0 mmol/l (SD 0.4) and total cholesterol 5.6 mmol/l (SD 0.7).

The groups were similar at baseline. The baseline characteristics in the intervention and reference groups appear in Table 2.

Table 2. Background characteristics, lifestyle factors, and metabolic and cognitive parameters at baseline.

	Reference group Low-flavanol chocolate N=50	Intervention group High-flavanol chocolate N=50
Women, n (%)	30 (60)	31 (62)
Age, mean (SD)	68 (3)	69 (2)
Years of education, mean (SD)	14.9 (3.8)	14.4 (3.0)
Cohabiting, n (%)	27 (54)	29 (58)
SLUMS, mean (SD)	27.8 (1.4)	28.0 (1.2)
Dietary intake, mean (SD)		
Energy (kJ)	7564 (1650)	8228 (2109)
Energy (kcal)	1807 (394)	1965 (504)
Total fat (g)	79.9 (26.2)	89.0 (33.3)
Saturated fat (g)	28.2 (10.7)	31.9 (14.3)
Protein (g)	72.9 (20.9)	81.5 (25.3)
Carbohydrates (g)	221 (49)	232 (61)
Sucrose (g)	84.9 (28.2)	85.2 (30.0)
Fibre (g)	23.4 (8.1)	24.7 (10.4)
Salt (mg)	7310 (2914)	7418 (2343)
Vitamin D (µg)	8.89 (4.95)	9.76 (6.80)
Folate (µg)	276 (138)	287 (100)
Potassium (mg)	3741 (875)	3787 (1105)
Calcium (mg)	978 (332)	1024 (386)
Clinical parameters, mean (SD)		
BMI (kg/m ²)	24.3 (3.6)	25.4 (3.5)
Waist circumference (cm)		
Women	83 (11)	86 (11)
Men	94 (8)	96 (9)
GDS	1.8 (3.0)	2.3 (3.6)
Laboratory parameters, mean (SD)		
Fasting plasma glucose (mmol/L)	4.93 (0.29)	5.00 (0.45)
Total cholesterol (mmol/L)	5.60 (0.74)	5.66 (0.63)
LDL cholesterol (mmol/L)	3.42 (0.56)	3.37 (0.55)
HDL cholesterol (mmol/L)	1.73 (0.44)	1.84 (0.47)
Total triglycerides (mmol/L)	1.02 (0.29)	1.00 (0.39)

3.2 Intervention

In the intervention group the participants ate 94% of the offered chocolate, whereas in the control group they ate 98% of their chocolate.

No differences were seen in changes in cognition between groups. The mean change (95% CI) in the time to complete TMT A and B in the intervention group was -4.6 s (-7.1 to -2.1) and -16.1 s (-29.1 to -3.1), and in the controls -4.4 s (-7.0 to -1.9) and -12.5 s (-22.8 to -2.1)(Table 3).

Table 3. Changes in cognitive parameters during the intervention.

	Baseline		Change from baseline		P-value	Effect Size* (95% CI)
	Reference N=50 Mean (SD)	Intervention N=50 Mean (SD)	Reference N=50 Mean (95% CI)	Intervention N=50 Mean (95% CI)		
Trail Making Test, s						
A	41.0 (10.4)	41.1 (10.3)	-4.4 (-7.0 to -1.9)	-4.6 (-7.1 to -2.1)	0.93	0.02 (-0.37 to 0.41)
B	91.1 (41.8)	95.3 (44.4)	-12.5 (-22.8 to -2.1)	-16.1 (-29.1 to -3.1)	0.66	0.09 (-0.31 to 0.48)
Verbal Fluency test, words/60 s	23.0 (4.9)	23.6 (5.5)	2.8 (1.4 to 4.3)	3.0 (1.6 to 4.3)	0.90	0.02 (-0.37 to 0.42)
SLUMS	27.8 (1.4)	28.0 (1.2)	1.1 (0.6 to 1.6)	1.3 (0.8 to 1.6)	0.59	0.11 (-0.29 to 0.50)
Cerad	14.2 (1.1)	13.8 (1.0)	0.3 (0.1 to 0.6)	0.5 (0.2 to 0.8)	0.35	0.19 (0.21 to -0.58)

*Effect size (d) was calculated by using the Cohen's method where an effect size of 0.20 is considered small, 0.50 moderate, and 0.80 large. CIs for the effect sizes were obtained by bias-corrected bootstrapping (5000 replications).

No differences were seen in the changes of metabolic parameters during the intervention between the intervention and control groups (Table 4.).

Table 4. Changes in metabolic parameters during the intervention.

	Baseline		Change from baseline		P-value
	Control N=50 Mean (SD)	Intervention N=50 Mean (SD)	Control N=50 Mean (95% CI)	Intervention N=50 Mean (95% CI)	
Weigh, Kg	70.0 (12.9)	73.4 (12.3)	0.6 (0.3 to 0.9)	0.3 (0.0 to 0.7)	0.20
Fasting plasma glucose (mmol/L)	4.93 (0.29)	5.00 (0.45)	-0.08 (-0.16 to -0.00)	-0.06 (-0.16 to 0.04)	0.70
Total cholesterol (mmol/L)	5.60 (0.74)	5.66 (0.63)	-0.01 (-0.14 to 0.11)	-0.01 (-0.14 to 0.12)	0.93

LDL cholesterol (mmol/L)	3.42 (0.56)	3.37 (0.55)		-0.07 (-0.20 to 0.05)	-0.05 (-0.15 to 0.06)	0.75
HDL cholesterol (mmol/L)	1.73 (0.44)	1.84 (0.47)		0.01 (-0.04 to 0.06)	0.00 (-0.05 to 0.06)	0.88
Total triglycerides (mmol/L)	1.02 (0.29)	1.00 (0.39)		0.07 (-0.02 to 0.16)	0.08 (-0.02 to 0.18)	0.87

4. Discussion

Daily consumption of 50 grams of dark chocolate with high flavanol content for eight weeks did not influence the cognitive parameters that were studied among healthy participants aged 65–75 years in this double-blind, placebo-controlled, randomized trial. In addition, no differences were seen in the changes in the intervention and control participants' weight or blood glucose and lipid levels. The compliance with the chocolate intake was excellent in both groups. This is one of the few randomized double-blind controlled trials studying the effects of dark chocolate that is naturally high in cocoa flavanols on the cognitive functions of cognitively healthy older people.

The heterogeneity of a study design that studies the effects of cocoa flavanols on cognitive functions is a major challenge. Yet only a few trials have examined the effects of cocoa flavanol intake on cognitive functions in middle-aged or older adults (Crews et al. 2008, Camfield et al. 2012, Desideri et al. 2012, Sorond et al. 2013, Brickman et al. 2014, Mastroiacovo et al. 2015, Neshatdoust et al. 2016). In addition to our study, Crews et al. (2008) is the only trial investigating the effects of dark chocolate naturally high in cocoa flavanols on cognitive functions. Crews et al. (2008) studied the effects of 60% dark chocolate bars (37 g, 397 mg flavanols) and cocoa beverage (237 ml, 357 mg flavanols) on neuropsychologic functions and cardiovascular health among healthy subjects over 60 years of age in a six-week intervention. Our results are in line with their findings, which did not support the predicted beneficial effects of short-term dark chocolate consumption on

cognitive functions or cardiovascular health. The amount of cocoa flavanols in the study by Crews et al. (2008) was almost double compared to the dose in our trial (410 mg/50 g daily dose of dark chocolate).

In most trials, cocoa flavanols have been consumed as drinks (Desideri et al. 2012, Sorond et al. 2013, Mastroiacovo et al. 2015, Neshatdoust et al. 2016) or as part of a diet that includes supplements (Brickman et al. 2014), and only one previous study included a dark chocolate bar in addition to a cocoa drink (Crews et al. 2008). The intervention chocolate in this trial was commercially available dark chocolate naturally high in cocoa flavanols (410 mg/daily portion), which is close to the daily dose (494–520 mg) that has been proven effective in previous trials (Desideri et al. 2012, Mastroiacovo et al. 2015, Neshatdoust et al. 2016). The type and bioavailability of cocoa flavanols may differ in enriched products such as drinks (Desideri et al. 2012, Sorond et al. 2013, Mastroiacovo et al. 2015, Neshatdoust et al. 2016) and supplements (Brickman et al. 2014) compared to dark chocolate naturally high in cocoa flavanols (Crews et al. 2008), which may have affected the results. Further studies should compare the neurocognitive effects of different types of products containing cocoa flavanols.

In previous trials, the participants' age, health and cognitive status, and educational level have been heterogeneous. A few trials have included healthy middle-aged and older adults (Camfield et al. 2012, Brickman et al. 2014, Mastroiacovo et al. 2015, Neshatdoust et al. 2016), another studied subjects with mild cognitive impairment (Desideri et al. 2012), and one study examined participants with vascular risk factors (Sorond et al. 2013). Our participants were 65–75 years of age, and at this age the decline in cognitive performance begins to show in tests. However, the participants in this study had a healthy cognitive status and metabolic profile compared to the population of the same age group (Koponen et al. 2018, FinHealth 2017 Study). Furthermore, their dietary intake at

baseline met the recommendations well (Valsta et al. 2018, FinDiet 2017 Study). In addition, the education level of the subjects was high with a mean over 14 years of education, and at baseline they performed cognitive tests with excellent results. Higher educational levels compared with lower levels have been associated with a greater cognitive reserve and result in better performance in cognitive tests (Lezak et al. 2004). These facts may have caused a ceiling effect on the cognition outcome parameters.

Some previous studies in different age groups have shown favourable effects of cocoa flavanols on the parameters of cardiovascular health such as LDL- and HDL-cholesterol levels, flow-mediated dilatation, and insulin resistance; however, the results are conflicting (Hooper et al. 2012). The latest trials with a higher cocoa flavanol dose in drinks compared to the dose in dark chocolate in our trial have shown positive effects on metabolic profile (Desideri et al. 2012, Mastroiacovo et al. 2015). In our trial, we did not find any differences in weight, lipid profile, or fasting glucose levels between the baseline and end of the intervention, suggesting that dark chocolate is a safe source of cocoa flavanols for healthy older adults.

We chose the Trail Making Test (TMT) and Verbal Flow test as the main outcome measures based on the latest scientific knowledge (Morris et al. 1989, Lezak et al. 1998) because the combination of these tests is feasible for this kind of trial and the most sensitive for measuring changes in intervention among older adults (Lezak et al. 2004). The same cognitive measurements have also been used in previous trials (Desideri et al. 2012, Sorond et al. 2013, Mastroiacovo et al. 2015). The cognitive tests have evaluated the indirect association between cocoa flavanol intake and cognitive performance but do not provide information on the direct mechanisms of action of cocoa flavanols. Two potential explanations are improvements in the cerebral blood volume or an increase in serum BDNF (brain-derived neurotrophic factor) concentration (Brickman et al. 2014, Neshatdoust et al.

2016). A recent meta-analysis found that patients with Alzheimer's disease, but not mild cognitive impairment, have significantly lower serum BDNF levels compared to healthy controls (Ng et al. 2019). Future research should include modern neuroimaging techniques that enable measuring the changes in neuromodulatory properties such as brain signalling activity, neurogenesis, and blood flow (Sokolov et al. 2013). Functional near infrared spectroscopy (fNIRS) is a novel method which measures the cortical blood flow or hemodynamics (Ho CS et al 2016). fNIRS can be used to assess cognitive impairment and neuropsychiatric disorder (Ho CS et al 2018). As depression was found to be associated with cardiovascular risk factors (Ho RC et al 2018) and regular consumption of cocoa flavanols may reduce cardiovascular risk factors. Further research is required to assess the potential benefits on hemodynamics and depression in older adults (Husain et al 2019).

The strength of our study is the study design of a randomized, double-blind, and controlled trial using commercially available dark chocolate naturally high in cocoa flavanols and dark chocolate with low flavanol content as a reference. The recruitment process was very fluent due to the high interest in an intervention concerning chocolate. The compliance with consuming the daily dark chocolate portion was high, and the drop-out level was very low. The limitations of our study are the small sample size and high educational level and cognitive status compared to the average in the same age group. Thus, the cognitive outcomes may have had a ceiling effect. The participants might have been a selected group of people that are especially interested in their health and nutrition.

5. Conclusions

In conclusion, the short-term use of dark chocolate naturally high in flavanols showed no benefit in the studied cognitive parameters in cognitively healthy older adults.

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Author Statement

Dr Suominen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Highlights:

- Only few trials have studied how dark chocolate affects cognition in older adults.
- Short-term dark chocolate consumption did not show beneficial effects on cognition.
- Dark chocolate is a safe source of cocoa flavanols for healthy older adults.