Benefits in Cognitive Function, Blood Pressure, and Insulin Resistance Through Cocoa Flavanol Consumption in Elderly Subjects With Mild Cognitive Impairment

The Cocoa, Cognition, and Aging (CoCoA) Study

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Abstract—Flavanol consumption is favorably associated with cognitive function. We tested the hypothesis that dietary flavanols might improve cognitive function in subjects with mild cognitive impairment. We conducted a double-blind, parallel arm study in 90 elderly individuals with mild cognitive impairment randomized to consume once daily for 8 weeks a drink containing ≈990 mg (high flavanols), ≈520 mg (intermediate flavanols), or ≈45 mg (low flavanols) of cocoa flavanols per day. Cognitive function was assessed by Mini Mental State Examination, Trail Making Test A and B, and verbal fluency test. At the end of the follow-up period, Mini Mental State Examination was similar in the 3 treatment groups ($P=0.13$). The time required to complete Trail Making Test A and Trail Making Test B was significantly ($P<0.05$) lower in subjects assigned to high flavanols (38.10±10.94 and 104.10±28.73 seconds, respectively) and intermediate flavanols (40.20±11.35 and 115.97±28.35 seconds, respectively) in comparison with those assigned to low flavanols (52.60±17.97 and 139.23±43.02 seconds, respectively). Similarly, verbal fluency test score was significantly ($P<0.05$) better in subjects assigned to high flavanols in comparison with those assigned to low flavanols (27.50±6.75 versus 22.30±8.09 words per 60 seconds). Insulin resistance, blood pressure, and lipid peroxidation also decreased among subjects in the high-flavanol and intermediate-flavanol groups. Changes of insulin resistance explained ≈40% of composite z score variability through the study period (partial $r^2=0.4013$; $P<0.0001$). To the best of our knowledge, this is the first dietary intervention study demonstrating that the regular consumption of cocoa flavanols might be effective in improving cognitive function in elderly subjects with mild cognitive impairment. This effect appears mediated in part by an improvement in insulin sensitivity. (***Hypertension***. **2012;60:794-801.)** ● **Online Data Supplement**

Key Words: mild cognitive impairment ■ cognitive function ■ cocoa flavanols ■ blood pressure ■ insulin

Mild cognitive impairment (MCI) is a clinical state of individuals who are memory impaired but otherwise well functioning and without clinical demonstration of dementia.[1,2] MCI represents an interesting field of research, because MCI increases the risk of later developing dementia, and, in this phase, an interventional therapy could have the greatest potential to improve cognitive performance and to slow down disease progression.[3] A growing body of evidence suggests that specific dietary components may impact brain function through the regulation of neurotransmitter pathways, signal-transduction pathways, and synaptic transmission.[4] Several dietary components, including omega-3 fatty acids, B vitamins, vitamins D and E, and choline, have been identified as having favorable effects on cognitive abilities.[4]

More recently, evidence suggests that the consumption of flavonoids, a diverse group of polyphenolic compounds widely present in plant-based foods, may be associated with a decreased risk of incident dementia[5] and with a lower prevalence of cognitive impairment,[6] a better cognitive evolution over a 10-year period,[7] and better dose-dependent performance of several cognitive abilities in elderly subjects.[8] Among the flavonoids, flavanols, a subclass abundant in tea, grapes, red wine, apples, and cocoa products, including chocolate, have been proposed to be highly effective in

Received February 11, 2012; first decision March 6, 2012; revision accepted July 11, 2012.

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The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.112.193060/-/DC1.

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Hypertension **is available at http://hyper.ahajournals.org** **DOI: 10.1161/HYPERTENSIONAHA.112.193060**

reversing age-related declines in neurocognitive performance by increasing the number of and strength of connections between neurons, reducing neuronal loss attributed to neurodegenerative processes, and through their ability to interact with the cellular and molecular architecture of the brain responsible for memory.[9] These cellular effects of flavanols are proposed to be attributed to selective actions on different pathways, including binding to ATP sites on enzymes and modulation of proteins central to intracellular signaling cascades, such as the mitogen-activated protein kinase signaling pathway and the phosphoinositide 3-kinase/AKT signaling cascade, thus working both directly and indirectly by modulating transcription factor activation and binding to promoter sequences.[9]

Substantial evidence exists supporting the link between cognition and cardiovascular risk factors.[10] In particular, high blood pressure has been linked with the pathophysiology of MCI other than with Alzheimer disease and vascular dementia.[10,11] This relationship may be mediated by endothelial dysfunction and microvascular diseases, leading to an impairment of vascular reserve.[10,11] Alterations in the insulin signaling pathway have also been suggested as potential contributors to cognitive dysfunction because of the pivotal role of this hormone in modulating brain structure and function.[12–14] Peripheral insulin resistance could promote cognitive dysfunction by reducing brain insulin uptake, raising brain levels of amyloid β and τ phosphorylation and promoting vascular damage through oxidative stress, proinflammatory cytokines, advanced glycation end products, and dyslipidemia,[12,13] with chronic dysglycemia thought to contribute to cognitive dysfunction by promoting the development of cerebral microvascular disease and inflammation.[15] Interestingly, it has been also proposed that the brain itself could become insulin resistant and that this promotes or even triggers key pathophysiological events leading to cognitive dysfunction.[12,16] In this regard, dietary flavanol consumption has been shown to reduce blood pressure and improve insulin sensitivity,[17,18] as well as to induce vasodilation of the peripheral[17,18] and cerebral vascular system,[19] increasing brain blood flow and perfusion.[19,20] Taken together, these previously reported effects provide a strong foundation for the idea that the regular consumption of flavanols may have implications for cognitive function, particular among those at risk.

To the best of our knowledge, no dietary intervention study has yet specifically investigated the topic of neurocognitive performance in response to dietary flavanols in subjects with MCI. In the current report, we tested the hypothesis that the regular dietary inclusion of a beverage containing cocoa flavanols would be effective in improving cognitive performance in subjects with MCI. Furthermore, the impact of this daily dietary modification on blood pressure, glucose metabolism, and oxidative stress was also studied in this population because of their potential influential role on cognitive function.

Methods

Participants

Participants were recruited among those referred to the Alzheimer unit of the University of L'Aquila Geriatric Division for MCI, with MCI diagnosed according to the revised Petersen criteria.[21] These criteria included a decline in memory, objectively verified by neuropsychological testing in combination with a precise history from the participant, proxy, or both, as suggested by Petersen,[22] and adjusted for age and education, or a decline in other cognitive domains, with normal functional of activities. Among 184 subjects screened, 61 individuals did not meet eligibility criteria, and 33 individuals refused to participate. The remaining 90 individuals were enrolled in the study (Figure S1, available in the online-only Data Supplement). The study was approved by the local ethics committee, and written informed consent to participate was obtained by each participant. Please see the online-only Data Supplement for a detailed Methods section.

Study Design and Outcomes

To investigate the impact of regular cocoa flavanol consumption on cognitive function in individuals with MCI, an 8-week double-blind, randomized, parallel arm study was conducted. After a 1-week run-in period, participants were randomly assigned to consume once daily a dairy-based cocoa drink containing cocoa flavanols either at a high (HF; ≈990 mg of flavanols per serving), intermediate (IF; ≈520 mg of flavanols per serving), or low level (LF; ≈45 mg of flavanols per serving) for 8 weeks. The dairy-based cocoa drinks used in this study were specially designed so that they were indistinguishable in taste and appearance, calorically balanced, and contained similar macronutrient, mineral, theobromine, and caffeine content, varying significantly only in the content of cocoa flavanols (Table S1, available in the online-only Data Supplement). The HF and IF drink mixes were made with a flavanol-rich cocoa powder (Cocoapro processed cocoa powder; Mars Inc), whereas the LF drink was made with a highly processed, alkalized cocoa powder.

Main outcome measures examined were changes in cognitive function after 8 weeks of regular cocoa flavanol consumption. Secondary outcome measures examined included changes in blood pressure, metabolic parameters, and plasma markers of lipid peroxidation.

Cognitive Function Assessment

Cognitive testing was performed at baseline and after 8 weeks (±2 days) using a combination of 4 well-validated standardized tests: the Mini Mental State Examination, Trail Making Test (TMT) A, TMT B, and a verbal fluency test. As a predefined procedure, an integrated measure of overall cognitive function, composite cognitive z score, was also constructed for each participant by converting the log-transformed raw scores from the individual tests to standardized scores (z score) that were based on the means and pooled SDs of the whole population at baseline.

Statistical Analysis

The statistical analysis was conducted according to the intention-to-treat principle. Accurate prestudy sample-size calculation was hindered by the lack of available data on the effect of cocoa flavanol administration on cognitive function in subjects with MCI. Thus, the study was based on an estimated sample size of 90 subjects, with a ratio of 1:1:1 for the 3 treatment groups, which has been calculated to be adequate to achieve 90% power to detect a moderate effect size (Cohen d=0.40) with 2 degrees of freedom and an α of 0.05 on the global cognitive z score between HF and LF. SAS version 9.1 was used to perform a 2-way mixed-design ANOVA using the repeated statement with the general linear model procedure. Post hoc analysis between treatment groups was performed by Tukey studentized range − honestly significant difference. χ^2 was used to compare categorical variables. Spearman nonparametric correlation was used to evaluate correlations between variables. Change of composite cognitive z score was entered as the dependent variable in a hierarchical multiple regression analysis in which homeostasis model assessment–insulin resistance (HOMA-IR), isoprostanes, and blood pressure were entered sequentially. Analysis was performed on variables logarithmically transformed to enhance symmetry of

measures. If it is not otherwise specified, data are presented as mean±SD.

Results

Study Population

General characteristics of the study population are shown in Table S2. According to the selection criteria, none of participants was obese (body mass index >30 kg/m^2), a current smoker, or under statin treatment. Hypertension was the most prevalent cardiovascular risk factor. The 3 study groups were comparable with regard to sex, age, anthropometric characteristics, cardiovascular risk factor prevalence, and pharmacological treatments (Table S2). Dietary total flavanol intake was also similar in the 3 study groups at enrollment and slightly decreased during the run-in period to a similar degree in all of the groups, a consequence of the dietary prescriptions (Table S3).

Adherence to the Study Protocol

Two participants assigned to IF discontinued beverage consumption after 4 weeks because of personal reasons, whereas 1 participant assigned to LF treatment discontinued beverage consumption after 2 weeks because of reported gastric discomfort. All 3 of these participants were followed up during the entire study period, and the data were included in the database for statistical analysis according to an intention-to-treat procedure. The overall compliance was 99.6% at week 4 and 99.4% at week 8, without difference among the LF, IF, and HF interventions. Adherence to the study protocol was evaluated at each visit by a checklist questionnaire of specified food items and by monitoring body weight. Compliance with the dietary restrictions was good, because none of the subjects reported regular consumption of the restricted flavanol/procyanidin-containing foods and beverages. Furthermore, caloric intake was controlled throughout the study, because no significant differences in body weight (HF, 70.67±8.39 kg; IF, 68.59±8.19 kg; LF, 71.17±8.65 kg; P=0.486) or body mass index (HF, 27.20±2.7 kg/m^2; IF, 26.27±2.50 kg/m^2; LF, 26.90±2.75 kg/m^2; P=0.427) among the 3 study groups were observed at the end of follow-up.

Cognitive Function Measures

Baseline performance on the cognitive function tests was similar for the 3 treatment groups, indicating an adequate randomization procedure (Table 1). Mini Mental State Examination score did not significantly change in relation to the 3 different treatments during the study period (P=0.13; Table 1).

The time required to complete TMT A significantly changed throughout the study period (P<0.0001), with significant reductions observed in subjects assigned to HF (−14.3±4.2 seconds; P<0.0001) and IF (−8.8±3.4 seconds; P<0.0001) interventions but not in those assigned to the LF intervention (+1.1±13.0 seconds; P=0.65; Table 1). Similarly, the time required to complete TMT B significantly changed during the study period (P<0.0001), with significant reductions observed among HF (−29.2±8.0 seconds; P<0.0001) and IF (−22.8±5.1 second; P<0.0001) subjects but not in those assigned to the LF intervention (+3.8±16.3 seconds; P=0.21; Table 1). Thus, TMT A and TMT B scores at the end of follow-up were significantly (P<0.05) better in subjects assigned to HF and IF treatments in comparison with those assigned to the LF group (Table 1).

Verbal fluency test scores significantly improved (P<0.0001) in the study, again with improvements demonstrated in the HF (+8.0±5.3 words per 60 seconds; P<0.0001) and IF (+5.1±3.1 words per 60 seconds; P<0.0001) groups and, to a lesser extent, in those assigned to the LF group (+1.2±2.7 words per 60 seconds; P=0.014). The improvement of verbal fluency test score was significantly (P<0.05) greater in HF subjects in comparison with those assigned to the LF group (Table 1).

According to the above results, the composite cognitive z score significantly changed during the study period (P<0.0001), with HF (+0.693±0.223; P<0.0001) and IF (+0.404±0.141; P<0.0001) groups demonstrating significant improvement; no change was observed in the LF group (−0.072±0.383; P=0.31; Table 1 and Figure S2). Composite cognitive z score at the end of follow-up was significantly (P<0.05) better in subjects assigned to HF in comparison with those assigned to LF (Table 1 and Figure S2).

Blood Pressure and Metabolic Parameters

Baseline blood pressure levels and metabolic parameters were similar for the 3 treatment groups (Table 2).

Blood Pressure

Systolic and diastolic blood pressures significantly (P<0.0001) changed through the study period with significant pressure reductions among HF (systolic: −10.0±3.1 mm Hg, P<0.0001; diastolic: −4.8±1.8 mm Hg, P<0.0001) and IF (systolic: −8.2±3.5 mm Hg, P<0.0001; diastolic: −3.4±2.0 mm Hg, P<0.0001) subjects but not in those assigned to the LF group (systolic: −1.4±5.4 mm Hg, P=0.16; diastolic: −0.9±3.4 mm Hg, P=0.14; Table 2). Thus, systolic and diastolic blood pressure levels at the end of follow-up were significantly (P<0.05) lower in subjects assigned to HF and IF in comparison with those assigned to LF (Table 2).

Metabolic Parameters

Plasma glucose levels significantly changed during the study period (P<0.0001), with significant reductions observed in subjects assigned to HF (−0.6±0.3 mmol/L; P<0.0001) and IF (−0.5±0.1 mmol/L; P<0.0001) but not in those assigned to LF (−0.1±0.5 mmol/L; P=0.19; Table 2). Thus, plasma glucose levels at the end of follow-up were significantly (P<0.05) lower in subjects assigned to HF and IF in comparison with those assigned to LF (Table 2). With regard to plasma insulin levels, no significant differences were found among the 3 treatment groups at the end of follow-up (Table 2). HOMA-IR significantly improved through the study period (P<0.0001), with significant reductions in subjects assigned to HF (−1.6±1.0; P<0.0001) and IF (−0.9±0.2; P<0.0001) but not in those assigned to LF (−0.1±0.5; P=0.29; Table 2). HOMA-IR at the end of follow-up was significantly (P<0.05) better in subjects assigned to HF in comparison with those assigned to LF (Table 2). Spearman nonparametric correlation revealed significant relationships between changes of plasma glucose and HOMA-IR during

Table 1. Changes in Neuropsychological Test Score During the Study Period in the 3 Treatment Groups

Neuropsychological Tests	High CF	Intermediate CF	Low CF	ANOVA	Tukey HSD
MMSE		Time×treatment interaction: F=2.10; *P*=0.13			
Week 0	27.43±1.28	27.17±2.09	27.60±1.45	F=0.53	NS
				P=0.59	
Week 8	28.07±1.01	27.43±1.94	27.93±1.20	F=1.61	NS
				P=0.21	
ANOVA	F=18.4	F=6.27	F=5.18		
	P=0.0002	*P*=0.02	*P*=0.03		
TMT A, s		Time×treatment interaction: F=27.62; *P*<0.0001			
Week 0	52.37±14.87	49.00±14.35	51.50±18.16	F=0.36	NS
				P=0.70	
Week 8	38.10±10.94	40.20±11.35	52.60±17.97	F=9.67	<0.05*
				P=0.0002	
ANOVA	F=341.94	F=206.16	F=0.22		
	P<0.0001	*P*<0.0001	*P*=0.65		
TMT B, s		Time×treatment interaction: F=78.19; *P*<0.0001			
Week 0	133.33±36.52	138.80±33.41	135.40±44.04	F=0.16	NS
				P=0.86	
Week 8	104.10±28.73	115.97±28.35	139.23±43.02	F=8.26	<0.05*
				P=0.0005	
ANOVA	F=399.01	F=607.89	F=1.67		
	P<0.0001	*P*<0.0001	*P*=0.21		
VFT, words per 60 s		Time×treatment interaction: F=22.79; *P*<0.0001			
Week 0	19.53±6.07	19.04±5.42	21.01±8.06	F=0.69	NS
				P=0.51	
Week 8	27.50±6.75	24.20±6.10	22.30±8.09	F=4.20	<0.05†
				P=0.018	
ANOVA	F=67.75	F=74.22	F=6.84		
	P<0.0001	*P*<0.0001	*P*=0.014		
Z score		Time×treatment interaction: F=62.13; *P*<0.0001			
Week 0	−0.007±0.523	−0.064±0.704	0.072±0.912	F=0.26	NS
				P=0.77	
Week 8	0.687±0.482	0.340±0.663	0.000±0.803	F=8.07	<0.05†
				P=0.0006	
ANOVA	F=290.49	F=246.89	F=1.06		
	P<0.0001	*P*<0.0001	*P*=0.31		

CF indicates cocoa flavanol; MMSE, Mini Mental State Examination; TMT, trail making test; VFT, verbal fluency test; NS, not significant. $P<0.05$ is considered a statistically significant difference.

*Data are low vs high and intermediate CF.

†Data are low vs high CF.

the study period and their respective values at baseline (r=−0.325, $P=0.0018$ and r=−0.421 and $P<0.0001$, respectively) in the study population considered as a whole.

With regard to total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides concentrations, no significant differences were found among the 3 treatment groups at the end of follow-up (Table 2). Plasma total 8-iso-$PGF_{2\alpha}$ levels significantly decreased during the study period ($P<0.0001$), with significant reductions observed among subjects assigned to HF (−99.8±60.3 pg/L; $P<0.0001$) and IF (−65.2±87.2 pg/L; $P=0.0003$), but not in those assigned to LF (−3.6±51.4 pg/L; $P=0.71$) drinks (Table 2). Thus, plasma total 8-iso-$PGF_{2\alpha}$ levels at the end of follow-up were significantly ($P<0.05$) lower in subjects assigned to HF in comparison with those assigned to LF (Table 2).

Determinant of Cognitive Changes After Flavanol Consumption

Because of the collinearity between systolic and diastolic blood pressures, only changes in systolic blood pressure were entered in the hierarchical regression analysis, because they

Table 2. Changes of Blood Pressure and Metabolic Parameters During the Study Period in the 3 Treatment Groups

Variables	High CF	Intermediate CF	Low CF	ANOVA
SBP, mm Hg		Time×treatment interaction: $P<0.0001$		
Week 0	141.1±9.9	142.4±9.8	141.4±8.6	$P=0.85$
Week 8	131.0±9.2	134.2±8.5	140.0±10.9	$P=0.0018$*
ANOVA	$P<0.0001$	$P<0.0001$	$P=0.16$	
DBP, mm Hg		Time×treatment interaction: $P<0.0001$		
Week 0	84.5±5.6	86.4±7.3	86.0±6.4	$P=0.51$
Week 8	79.7±5.3	83.0±6.9	85.1±7.1	$P=0.007$*
ANOVA	$P<0.0001$	$P<0.0001$	$P=0.14$	
Glucose, mmol/L		Time×treatment interaction: $P<0.0001$		
Week 0	5.45±0.60	5.41±0.75	5.45±0.80	$P=0.98$
Week 8	4.81±0.48	4.95±0.68	5.33±0.74	$P=0.0065$*
ANOVA	$P<0.0001$	$P<0.0001$	$P=0.19$	
Insulin, mU/L		Time×treatment interaction: $P<0.0001$		
Week 0	15.63±8.28	12.52±5.66	13.80±9.22	$P=0.31$
Week 8	10.54±6.78	9.65±5.37	13.67±9.12	$P=0.08$
ANOVA	$P<0.0001$	$P<0.0001$	$P=0.72$	
HOMA-IR		Time×treatment interaction: $P<0.0001$		
Week 0	3.90±2.37	3.07±1.55	3.44±2.49	$P=0.35$
Week 8	2.33±1.61	2.19±1.33	3.28±2.35	$P=0.023$†
ANOVA	$P<0.0001$	$P<0.0001$	$P=0.29$	
TC, mmol/L		Time×treatment interaction: $P<0.0001$		
Week 0	5.31±1.05	5.37±0.99	5.12±0.89	$P=0.60$
Week 8	4.85±0.98	4.94±0.95	5.06±0.86	$P=0.70$
ANOVA	$P<0.0001$	$P<0.0001$	$P=0.09$	
LDL-C, mmol/L		Time×treatment interaction: $P<0.0001$		
Week 0	3.38±0.80	3.25±0.81	3.22±0.74	$P=0.70$
Week 8	2.92±0.74	2.85±0.74	3.16±0.71	$P=0.25$
ANOVA	$P<0.0001$	$P<0.0001$	$P=0.10$	
HDL-C, mmol/L		Time×treatment interaction: $P=0.29$		
Week 0	1.25±0.38	1.41±0.45	1.25±0.35	$P=0.20$
Week 8	1.24±0.38	1.43±0.46	1.25±0.36	$P=0.14$
ANOVA	$P=0.75$	$P=0.08$	$P=0.77$	
TG, mmol/L		Time×treatment interaction: $P<0.0001$		
Week 0	1.53±0.32	1.55±0.33	1.42±0.37	$P=0.32$
Week 8	1.49±0.32	1.45±0.31	1.42±0.38	$P=0.74$
ANOVA	$P<0.0001$	$P<0.0001$	$P=0.83$	
8-iso-$PGF_{2\alpha}$, pg/L		Time×treatment interaction: $P<0.0001$		
Week 0	377.3±112.8	402.5±125.01	412.2±147.5	$P=0.56$
Week 8	277.4±112.1	337.3±114.9	408.7±140.6	$P=0.0004$†
ANOVA	$P<0.0001$	$P=0.0003$	$P=0.71$	

CF indicates cocoa flavanol; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol: LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$. Plus-minus values are mean±SD. Differences within groups were analyzed by ANOVA. Differences between groups were analyzed by ANOVA followed by Tukey HSD.

*$P<0.05$ low vs high and intermediate CF.

†$P<0.05$ low vs high CF.

were found to be more strictly correlated with changes of composite cognitive *z* score than those of diastolic blood pressure ($r=-0.4967$, $P<0.0001$ versus $r=-0.4036$, $P<0.0001$). Changes of HOMA-IR were found to be the main determinants of changes in cognitive function, explaining ≈40% of composite *z* score variability through the study period ($r^2=0.4013$, $\beta=-0.2910$; $P<0.0001$; Table S4). Changes in systolic blood pressure levels and plasma isopros-

tane concentrations explained ≈2% and ≈7% of cognitive improvement throughout the study period, respectively (Table S4).

Discussion

In this randomized, double-blind, parallel arm study, we sought to evaluate the effect of daily consumption of flavanol-rich cocoa drinks for 8 weeks on cognitive function in older adults with early memory decline. We found that cognitive performance was improved with regular cocoa flavanol consumption without evidence of any relevant adverse effects. In addition to these cognitive improvements, the regular dietary inclusion of cocoa flavanols was associated with significant reductions in blood pressure, as well as in improvements in some metabolic markers. Interestingly, the improvement of cognitive performance was associated with a reduction in insulin resistance, suggesting a possible influential role of glucose metabolism in modulating cognitive function in these subjects.

Epidemiological studies have previously reported a favorable association between increased flavonoid consumption and cognitive function in elderly subjects.[6–8,23] Moreover, it has been demonstrated recently that 12-week supplementation with polyphenol-rich concord grape juice may enhance cognitive function in a small group (n=12) of older adults with early memory decline.[24] The current study sheds new light on this relevant topic, providing the first evidence that regular cocoa flavanol consumption can positively affect cognitive function in older adults with early memory decline. To the best of our knowledge, this is the first well-controlled dietary intervention study of this size and duration examining neurocognitive performance in response to multiple levels of dietary flavanols. We found significant improvements in performance on a battery of neuropsychological tests mainly exploring executive function but also aspects of working memory, short-term memory, long-term episodic memory, processing speed, and global cognition, after 8 weeks of regular cocoa flavanol consumption. In particular, the benefits were remarkably evident for processing speed, executive function, and working memory, as indicated by the improvement in TMT A and TMT B scores. Interestingly, these improvements were already evident at an intermediate cocoa flavanol content, suggesting a particular sensitivity of this subset of neuropsychological functions to the benefits of cocoa flavanols. According to the above findings, a significant improvement in performance in the verbal fluency test, a test commonly used as a measure of executive function and language, was also observed in subjects assigned to HF consumption. On the other hand, the lack of an effect of flavanols on the Mini Mental State Examination score is likely a reflection of the low sensitivity of this test to detect small changes at the upper end of cognitive performance over time. Taken together, these data are suggestive of a possible clinical benefit derived from the regular dietary inclusion of cocoa flavanol-containing foods in subjects with MCI. In this regard, the level of flavanols used in this current study is comparable to the level of cocoa flavanols fed in previous studies where improvements in brain blood flow,[19] vascular function, and blood pressure were observed.[25] However, our study is the first well-controlled trial that addresses the relationship between cocoa flavanols and cognitive function, and from this study alone, we cannot say what is the effective flavanol amount that is required to improve or maintain cognition.

Our data do not provide definite information as to possible mechanisms underlying the observed beneficial effects, although some putative pathways can be considered. In this regard, we found a significant improvement of insulin resistance after cocoa flavanol consumption. This evidence fully fits with evidence from the recent meta-analysis by Hooper et al[25] indicating consistent acute and chronic benefits of chocolate- and cocoa-derived flavanols on insulin resistance. Interestingly, flavonoids have been reported recently to exert additional benefits to established hypoglycemic therapy in postmenopausal type 2 diabetic patients.[26] Although it is unclear the mechanism underlying this effect, these previously published studies provide consistent evidence that the consumption of flavanols, either directly or indirectly, can mediate important improvements in insulin sensitivity. We found an independent negative relationship between changes in composite cognitive z score and change in HOMA-IR, which explained ≈40% of composite cognitive z score variation throughout the study period. These data suggest a possible influential role of insulin-resistance in modulating cognitive function in subjects with early memory decline. Supportive of this concept, it has been demonstrated recently that the insulin sensitizer rosiglitazone could protect against cognitive decline in diabetic subjects with MCI.[27] An improvement in brain perfusion could have also played a contributory role in the ameliorating cognitive performance in our study population. This hypothesis is supported by the findings of another study, which demonstrated that 2 weeks of regular flavanol-rich cocoa consumption, providing an intake of cocoa flavanols close to that of HF drinks used in our study, was associated with a significant increase of cerebral blood in healthy elderly.[19] In this regard, although we did not specifically evaluate the effect of cocoa flavanols on endothelial function, a number of studies, some also from our group, clearly demonstrated the ability of flavonoids to improve NO bioavailability in vasculature,[17,18,25] which, in turn, plays a pivotal role in modulating cerebrovascular tone.[28]

The current demonstration of significant reductions in both systolic and diastolic blood pressure levels after regular cocoa flavanol consumption is consistent with previous findings obtained both in normotensives and in hypertensive subjects, as well as medicated individuals.[17,18,29,30] Our findings seem to exclude any relevant contribution of blood pressure reduction to the variations of cognitive performance after 8 weeks of cocoa flavanol consumption, because blood pressure changes explained ≤2% of cognitive improvement throughout the study period. However, we cannot exclude that these reductions in blood pressure, in addition to improving cardiovascular health, may contribute to supporting improvements in cognitive performance and even help in the prevention of blood pressure-related cognitive declines across a longer time course.[31] Indeed, evidence is mounting that the management of high blood pressure and other conventional

cardiovascular risk factors could favorably affect the clinical course of MCI.[3,32]

The last interesting finding of our study was the significant reduction of circulating levels of plasma F2-isoprostanes after flavanol-rich cocoa consumption. F2-isoprostanes are generated from arachidonic acid through a process of nonenzymatic free radical-catalyzed lipid peroxidation, thus representing an established marker of oxidative stress.[33] These effects probably represent the consequence of the global effects of these nutrients on the cardiovascular system rather then the expression of a direct effect of cocoa flavanol. Indeed, a direct antioxidant effect of polyphenols in vivo has been questioned recently, because concentrations in blood are low compared with other antioxidants, and extensive metabolism after ingestion lowers their antioxidant activity.[34] However, the contribution of oxidative stress, at least as assessed throughout lipid peroxidation products, to the variations of cognitive performance observed after cocoa flavanol consumption was quite modest, because changes of plasma isoprostane level concentrations explained ≈7% of cognitive improvement throughout the study period.

In summary, the results of the current study provide encouraging evidence that the regular inclusion of flavanol-containing foods may be an effective dietary approach for improving some aspects of cognitive dysfunction in adults with MCI. Other than the possible direct effects of flavanols on cognitive function, general improvements in cardiovascular function and specific metabolic parameters could have, alone or in combination, played a role in improving cognitive performance in this study population.

Limitations

Although results of our study are remarkably good, their potential clinical relevance requires some considerations. First, because standard MCI criteria do not define optimal tests to establish cognitive performance in subjects with MCI,[22] cognitive testing was performed using a battery of well-validated, standardized, and widely used neuropsychological tests exploring only some aspects of the complex cognitive dysfunction in subjects with MCI.[1,2] Second, because our study consisted of a 2-month intervention, the extent of the cognitive benefits and their duration, as well as their impact on a clinical course of MCI, remain to be established. Third, from pathophysiological perspective, it is yet unclear whether the observed benefits in neurocognition are a direct consequence of cocoa flavanol themselves or a secondary effect related to general improvements in cardiovascular function or health. Finally, subjects enrolled in our study were generally in good health and without known cardiovascular disease. Thus, our study population could be not completely representative of all subjects with MCI. On the other hand, the exclusion of habitual consumers of dietary supplements with antioxidant properties likely did not affect the representativeness of our study population, because the consumption of these supplements among our elderly population is quite low, generally <10%, similar to that observed in other cohorts from our country.[35]

Perspectives

Our data seem to support the hypothesis that the regular consumption of cocoa flavanols may be able to improve cognitive performance in MCI adults in a relatively short period of time. Although additional confirmatory studies are warranted, the findings reported herein suggest that the regular dietary inclusion of flavanols could be one element of a dietary approach to the maintaining and improving not only cardiovascular health but also specifically brain health.

Sources of Funding

This study was supported by a grant from Mars Inc, which also supplied the standardized powdered cocoa drinks used in this investigation.

Disclosures

C.K.-U. is employed by Mars Inc.

References

1. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol*. 1999;56:303–308.
2. Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. Practice parameter: early detection of dementia–mild cognitive impairment (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*. 2001;56:1133–1142.
3. Li J, Wang YJ, Zhang M, Xu ZQ, Gao CY, Fang CQ, Yan JC, Zhou HD. Vascular risk factors promote conversion from mild cognitive impairment to Alzheimer disease. *Neurology*. 2011;76:1485–1491.
4. Gómez-Pinilla F. Brain foods: the effects of nutrients on brain function. *Nat Rev Neurosci*. 2008;9:568–578.
5. Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues JF. Intake of flavonoids and risk of dementia. *Eur J Epidemiol*. 2000;16:357–363.
6. Kuriyama S, Hozaka A, Ohmori K, Shimazu T, Matsui T, Ebihara S, Awata S, Nagatomi R, Arai H, Tsuji I. Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project. *Am J Clin Nutr*. 2006;83:355–361.
7. Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P. Flavonoid intake and cognitive decline over a 10-year period. *Am J Epidemiol*. 2007;165:1364–1371.
8. Nurk E, Refsum H, Drevon CA, Tell GS, Nygaard HA, Engedal K, Smith AD. Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. *J Nutr*. 2009;139:120–127.
9. Williams RJ, Spencer JPE. Flavonoids, cognition, and dementia: actions, mechanisms, and potential therapeutic utility for Alzheimer disease. *Free Radic Biol Med*. 2012;52:35–45.
10. Gorelick PB, Scuteri A, Black SE, DeCarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Petersen RC, Schneider JA, Tzourio C, Arnett DK, Bennett D, Chui HC, Higashida RT, Lindquist R, Nilsson PR, Roman GC, Selke FW, Seshadri S. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:2672–2713.
11. Novak V, Hajjar J. The relationship between blood pressure and cognitive function. *Nat Rev Cardiol*. 2010;7:686–698.
12. De la Monte S. Insulin resistance and Alzheimer's disease. *BMB Reports*. 2009;42:475–481.
13. Neumann KF, Rojo L, Navarrete LP, Navarrete LP.as G, Reyes P, Maccioni RB. Insulin resistance and Alzheimer's disease: molecular links & clinical implications. *Curr Alzheimer Res*. 2008;5:438–447.
14. Li L, Holscher C. Common pathological processes in Alzheimer disease and type 2 diabetes: a review. *Brain Res Rev*. 2007;56:384–402.
15. Strachan MW, Reynolds RM, Marioni RE, Price JF. Cognitive function, dementia and type 2 diabetes mellitus in the elderly. *Nat Rev Endocrinol*. 2011;7:108–114.
16. Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE. Demonstrated

brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest*. 2012;122:1316–1338.
17. Grassi D, Desideri G, Necozione S, Lippi C, Casale R, Properzi G, Blumberg JB, Ferri C. Blood pressure is reduced and insulin sensitivity increased in glucose-intolerant, hypertensive subjects after 15 days of consuming high-polyphenol dark chocolate. *J Nutr*. 2008;138:1671–1676.
18. Grassi D, Necozione S, Lippi C, Croce G, Valeri L, Pasqualetti P, Desideri G, Blumberg JB, Ferri C. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension*. 2005;46:398–405.
19. Sorond FA, Lipsitz LA, Hollenberg NK, Fisher NDL. Cerebral blood flow response to flavanol-rich cocoa in healthy elderly humans. *Neuropsychiatr Dis Treat*. 2008;4:433–440.
20. Francis ST, Head K, Morris PG, MAcDonald IA. The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *J Cardiovasc Pharmacol*. 2006;47:S215–S220.
21. Winblad B, Palmer K, Palmer K, Jelic V, Fratiglioni L, Wahlund LO, Nordberg A, Bäckman L, Albert M, Almkvist O, Arai H, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack C, Jorm A, Ritchie K, van Duijn C, Visser P, Petersen RC. Mild cognitive impairment–beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J Intern Med*. 2004;256:240–246.
22. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med*. 2004;256:183–194.
23. Macready AL, Kennedy OB, Ellis JA, Williams CM, Spencer JPE, Butler LT. Flavonoids and cognitive function: a review of human randomized controlled trial studies and recommendations for future studies. *Genes Nutr*. 2009;4:227–2242.
24. Krikorian R, Nash TA, Shidler MD, Shukitt-Hale B, Joseph JA. Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. *Br J Nutr*. 2010;103:730–734.
25. Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, Cassidy A. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. *Am J Clin Nutr*. 2012;95:740–751.
26. Curtis PJ, Sampson M, Potter J, Dhatariya K, Kroon PA, Cassidy A. Chronic ingestion of flavan-3-ols and isoflavones improves insulin sensitivity and lipoprotein status and attenuates estimated 10-year CVD risk in medicated postmenopausal women with type 2 diabetes: a 1-year, double-blind, randomized, controlled trial. *Diabetes Care*. 2012;35:226–232.
27. Abbatecola AM, Lattanzio F, Molinari AM, Cioffi M, Mansi L, Rambaldi P, DiCioccio L, Cacciapuoti F, Canonico R, Paolisso R. Rosiglitazone and cognitive stability in older persons with type 2 diabetes and mild cognitive impairment. *Diabetes Care*. 2010;33:8:1706–1711.
28. Andresen J, Shafi NI, Bryan RM Jr. Endothelial influences on cerebrovascular tone. *J Appl Physiol*. 2006;100:318–327.
29. Egan BM, Laken MA, Donovan JL, Woolson RF. Does dark chocolate have a role in the prevention and management of hypertension?: commentary on the evidence. *Hypertension*. 2010;55:1289–1295.
30. Heiss C, Jahn S, Taylor M, Real WM, Angeli FS, Wong ML, Amabile N, Prasad M, Rassaf T, Ottaviani JI, Mihardja S, Keen CL, Springer ML, Boyle A, Grossman W, Glantz SA, Schroeter H, Yeghiazarians Y. Improvement of endothelial function with dietary flavonoids is associated with mobilization of circulating angiogenic cells in patients with coronary artery disease. *J Am Coll Cardiol*. 2010;56:218–224.
31. Kilander L, Nyman H, Boberg M, Hansson L, Lithell H. Hypertension is related to cognitive impairment: a 20-year follow-up of 999 men. *Hypertension*. 1998;31:780–786.
32. Solfrizzi V, Panza F, Colacicco AM, D'Introno A, Capurso C, Torres F, Grigoletto F, Maggi S, Del Parigi A, Reiman EM, Caselli RJ, Scafato E, Farchi G, Capurso A, for the Italian Longitudinal Study on Aging Working Group. Vascular risk factors, incidence of MCI, and rates of progression to dementia. *Neurology*. 2004;63:1882–1891.
33. Patrono C, Fitzgerald GA. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. *Arterioscler Thromb Vasc Biol*. 1997;17:2309–2315.
34. Hollman PC, Cassidy A, Comte B, Heinonen M, Richelle M, Richling E, Serafini M, Scalbert A, Sies H, Vidry S. The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. *J Nutr*. 2011;141:989S–1009S.
35. Skeie G, Braaten T, Hjartåker A, Lentjes M, Amiano P, Jakszyn P, Pala V, Palanca A, Niekerk EM, Verhagen H, Avloniti K, Psaltopoulou T, Niravong M, Touvier M, Nimptsch K, Haubrock J, Walker L, Spencer EA, Roswall N, Olsen A, Wallström P, Nilsson S, Casagrande C, Deharveng G, Hellström V, Boutron-Ruault MC, Tjønneland A, Joensen AM, Clavel-Chapelon F, Trichopoulou A, Martinez C, Rodríguez L, Frasca G, Sacerdote C, Peeters PH, Linseisen J, Schienkiewitz A, Welch AA, Manjer J, Ferrari P, Riboli E, Bingham S, Engeset D, Lund E, Slimani N. Use of dietary supplements in the European Prospective Investigation into Cancer and Nutrition calibration study. *Eur J Clin Nutr*. 2009;63:S226–S238.

Novelty and Significance

What Is New?

- For the first time, regular cocoa flavanol consumption has been shown to positively affect cognitive function in older adults with early memory decline.

What Is Relevant?

- The cognitive improvements were demonstrated along with significant improvements in blood pressure and insulin sensitivity.

Summary

Regular consumption of cocoa flavanols might be effective in improving cognitive function in elderly subjects with MCI. This effect appears mediated in part by an improvement in insulin sensitivity.

Benefits in Cognitive Function, Blood Pressure, and Insulin Resistance Through Cocoa Flavanol Consumption in Elderly Subjects With Mild Cognitive Impairment: The Cocoa, Cognition, and Aging (CoCoA) Study

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Hypertension. 2012;60:794-801; originally published online August 14, 2012;
doi: 10.1161/HYPERTENSIONAHA.112.193060
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0194-911X. Online ISSN: 1524-4563

Title: Benefits in Cognitive Function, Blood Pressure and Insulin Resistance Through Cocoa Flavanol Consumption in Elderly Subjects with Mild Cognitive Impairment: Cocoa, Cognition and Aging (CoCoA) Study

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EXPANDED METHODS SECTION

Participants

Participants were recruited among those referred to the Alzheimer's Unit of the Geriatric Division of the University of L'Aquila for Mild Cognitive Impairment (MCI) diagnosed according to the revised Petersen criteria (1). These criteria included a decline in memory, objectively verified by neuro-psychological testing in combination with a precise history from the participant, proxy, or both, as suggested by Petersen (2), and adjusted for age and education, or a decline in other cognitive domains, with normal functional of activities. Subjects with cerebrovascular lesions, overt dementia, or clinically significant neurologic disease were excluded. To avoid confounding due to the influence of concomitant depression on the performance on cognitive tests (3) subjects with a score of Geriatric Depression Scale >11 were also excluded (4). All subjects were requested to have undergone magnetic resonance or computed tomography in order to exclude clinically significant central nervous system disorders. Medical records of all participants were reviewed in order to assess that participants were free of clinically significant coexisting medical conditions, including cardiovascular disease, cerebrovascular events and inflammatory diseases. Current smokers, users of antioxidant supplements (including vitamin C and E), habitual consumers of chocolate or other cocoa products (daily consumption of any amount), or individuals to whom medications known for having antioxidant properties (including statins and glitazones) were prescribed were all excluded from participation in the current study. Other concomitant treatments were kept constant throughout the study period. Individuals with a body mass index (BMI) of more than 30 and subjects reporting weight change $\pm$10% body weight within the last 6 months before entering the study were also excluded. One hundred eighty four subjects were screened by evaluation of medical history, physical examination, laboratory parameters, and assessment of the individuals' habitual diet (Figure S1). Sixty one individuals did not meet eligibility criteria (20 had vascular disease, 4 used vitamins, 8 were current smokers, 3 consumed cocoa, 21 had BMI >30 kg/m^2, 5 used statins) and 33 individuals refused to participate. The remaining 90 individuals were enrolled in the study. The study was approved by the local ethics committee and written informed consent to participate was obtained by each participants.

Study design and outcomes

To investigate the impact of regular cocoa flavanol consumption on cognitive function in individual with MCI, an 8-week double-blind, randomized, parallel arms study was conducted between February 2007 and July 2008. After the enrollment, study participants met with a dietician in order to evaluate current diet habits and correct any nutritional insufficiencies. Dietary total flavanol intake was estimated by a validated semi-quantitative dietary questionnaire (5). Participants were then instructed to maintain their usual lifestyle and intake of fruits and vegetables and to avoid or, to the maximum extent possible, limit the intake of specific flavanol- and procyanidin-rich foods and beverages including tea, red wine, fruit and vegetable juices and chocolate. A detailed list of foods was given to each participant allowing participants appropriate dietary choices without imposing restrictions on the total consumption of fruits, vegetables, and beverages (i.e., total consumption of fruit and vegetables was not restricted, merely the recommendation was made to modify the types of these foods consumed). All participants were encouraged to continue with their usual physical activity throughout the study period. After a 1 week run-in period, participants were randomly assigned to consume once daily a dairy-based cocoa drink containing cocoa flavanols either at a high (HF: ≈990 mg flavanols/serving), intermediate (IF: ≈520 mg flavanols/serving) or low level (LF: ≈48 mg flavanols/serving) for 8 weeks. Frequent participant monitoring was used to maximize compliance with the intervention and to minimize changes in overall diet and lifestyle. All the participants were invited to return excess sachets and empty sachets to check compliance. This latter was calculated by taking the amount of servings ingested divided by the amount the participants should have ingested and multiplied by 100. Although the measurements of plasma

flavanols would have been a better measure of compliance, we did not assess this parameter as blood samples were taken after a 12 hour fast, well past the expected clearance time of flavanols in plasma (6).
Randomization ensured that the groups at baseline were approximately equal, averaging out between-subject differences on potential confounding due to baseline nutritional habits. Computerized randomization of the products was conducted by an independent researcher. Personnel not involved in the trial attached a label with the number 1 to 90 to the identical boxes containing individual anonymized sachets. The boxes were subsequently issued to participants in an ascending and sequential order as they entered the study (at the time of their pre-treatment baseline assessments). Neither the treating physicians, nor the patients were aware of treatment allocation. Main outcome measures examined were changes in cognitive function following 8 weeks of regular cocoa flavanol consumption. Secondary outcome measures examined included changes in blood pressure, metabolic parameters and plasma markers of lipid peroxidation.

Cocoa drinks
The dairy-based cocoa drinks were supplied as a dry beverage mix in individual anonymized sachets. The food products used in this study were specially designed as there were indistinguishable in taste and appearance, calorically balanced and carefully nutrient matched. The three beverage mixes contained similar macronutrient, mineral , theobromine, and caffeine content, varying significantly only in the content of cocoa flavanols (Table S1). The HF and IF drink mixes were made with a flavanol-rich cocoa powder (Cocoapro® processed cocoa powder; Mars Inc, USA), while the LF drink was made with a highly processed, alkalised cocoa powder. Flavanol and procyanidin compositions of the cocoa drinks are also shown in Table S1. In this study, the term "cocoa flavanols" is used to define the sum of all monomeric flavanols and their oligomeric derivatives (procyanidins) up to and including decamers (10 monomeric subunits). To prepare the drinks, the contents of the packets were mixed by a hand-held mixer with 250 mL warm water and immediately consumed. The assigned drink was consumed once a day, in the morning, during the entire length of the study. Participants were instructed to promptly inform researchers if any adverse events occurred.

Cognitive function assessment
Study participants arrived on the morning of evaluation after a small breakfast had been consumed at home, but before taking the daily cocoa drink. The participants were tested during a morning visit in a quiet room by neuro-psychologically trained research assistants who were unaware of the aim of the study. The same research assistant administered the neuro-psychologically tests on the same subject during the study to minimize examiner bias. Cognitive testing was performed at baseline and after 8 weeks (± 2 days) using a combination of four well-validated standardized tests: Mini Mental State Examination (MMSE), Trail Making Test (TMT) A, TMT B and verbal fluency test (VFT). The MMSE is a widely used screening tool for cognitive impairment and covers five areas of cognitive function including orientation, attention, calculus, recall and language with scores ranging from 0 to 30 (7). The TMT, which explores visual-conceptual and visual-motor tracking, is a frequently used neuropsychological test because of its sensitivity to brain damage that consists of two parts: TMT-A and TMT-B. TMT-A, a visual scanning test, requires one to draw a line connecting consecutive numbers. TMT-B adds cognitive flexibility to TMT-A and requires one to draw a line connecting numbers and letters in alternating sequence. The score is given by the amount of time in seconds to complete the task (8). To test verbal fluency, participants were asked to list as many nouns as possible beginning with given letters (9). As predefined procedure an integrated measure of overall cognitive function - composite cognitive z-score - was also constructed for each participant by converting the log-transformed raw scores from the individual tests to standardised scores (z score) that were based on the means and pooled standard deviations of the whole population at baseline.

Blood pressure measurement
Before neuro-psychological testing , clinic systolic and diastolic blood pressure levels were recorded in the morning, using of a validated oscillometric device with appropriately sized cuffs (Omron 705 CP; Omron Matsusaka Co. Ltd., Matsusaka-City, Japan) on the non-dominant upper arm. These evaluations were performed by staff blinded to the study protocol. At each visit, participants rested 15 min in a seated position, the first blood pressure measurement was taken but discarded and the subsequent three consecutive blood pressure readings, taken at 3-min intervals, were recorded. The average of these latter measures was considered for statistical analysis.

Laboratory analysis
Twenty-four h before or after neuro-psychological testing blood samples were drawn from each participant after an overnight fasting period for determinations of lipids profile, fasting plasma glucose and insulin. The homeostasis model assessment of insulin resistance (HOMA-IR) index [fasting serum insulin (mU/L) x fasting plasma glucose (mmol/L)/22.5] was calculated from fasting glucose and insulin level as a marker of insulin resistance. Plasma total 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) levels, as index of oxidative stress-related lipid peroxidation (10), were assessed by enzyme immunoassay (Assay Design Inc, Ann Arbor, MI) according to the manufacturer's instructions.

References

1) Winblad B, Palmer K, KivipeltoM, Jelic V, Fratiglioni L, Wahlund LO, Nordberg A, Bäckman L, Albert M, Almkvist O, Arai H, Basun H, Blennow K, de Leon M, DeCarli C, Erkinjuntti T, Giacobini E, Graff C, Hardy J, Jack C, Jorm A, Ritchie K, van Duijn C, Visser P, Petersen RC. Mild cognitive impairment–beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med. 2004;256:240-246.
2) Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004;256:183-194.
3) Burt DB, Zembar MJ, Niederehe G. Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. Psychol Bull. 1995;117:285-305.
4) Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, Leirer VO. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res. 1982;17:37-49.
5) Gaddi A, Cicero AF, Wani FO, Dormi A, Pasquarelli V, D'Addato S. The realization of a project aimed at reducing the plasmatic lipid level in a large Italian population improves the mean calcium daily intake: the Brisighella Study. Eur J Clin Nutr. 2001;55:97-106.
6) Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-Uribe C, Schmitz HH, Kelm M. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci U S A. 2006;103:1024-9.
7) Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189-198
8) Reitan, RM. Validity of the trail making test as an indicator of organic brain damage. Percept Mot Skills 1958;8:271–276.
9) Borkowski J, Benton A, Spreen O. Word fluency and brain damage. Neuropsychologia. 1967;5:135–140.
10) Patrono C, Fitzgerald GA. Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. Arterioscler Thromb Vasc Biol. 1997;17:2309–2315.

TABLE S1: **NUTRIENT CONTENT OF THE COCOA DRINKS**

Nutrient content per dose (58 g)	Low CF	Intermediate CF	High CF
Calories	215	216	218
Total fat (g)	3	3	3
Satured fat (g)	1	1	1
Cholesterol (mg)	10	10	9
Total carbohydrates (g)	31	31	32
Dietary fiber (g)	7	6	6
Sugars (g)	17	17	18
Protein (g)	17	17	17
Caffeine (g)	46	44	41
Theobromine (mg)	400	429	458
Sodium (mg)	450	415	380
Potassium (mg)	1195	1088	980
Calcium (mg)	451	455	458
Iron (mg)	9	7	5
Phosphorus (mg)	496	509	521
Magnesium (mg)	141	149	158
Zinc (mg)	3	3	3
Copper (mg)	0.8	0.8	0.7
Manganese (mg)	1.0	1.1	1.1
Flavanol and procyanidin composition			
Epicathechin (mg)	5	95	185
Catechin (mg)	8	35	62
Dimers (mg)	10	96	182
Trimers (mg)	4	72	141
Tetramers (mg)	2	64	126
Pentamers-decamers (mg)	17	158	297
Total flavanols (mg)	48	520	993

TABLE S2: BASELINE GENERAL CHARACTERISTICS OF THE STUDY PARTICIPANTS DISTINGUISHED ACCORDING TO THE TREATMENT ASSIGNED.

	High CF	Intermediate CF	Low CF	p value
General characteristics				
Gender – m/f	14/16	13/17	16/14	0.732
Age (years)	71.2±4.9	71.3±4.5	71.0±4.5	0.967
range	65 – 80	65 - 82	64 – 81	
Weight (kg)	71.0±8.0	69.2±8.2	71.7±8.1	0.486
BMI - kg/m^2 - mean ± SD	27.4±2.5	26.6±2.3	27.1±2.5	0.427
range	23 - 30	22 - 30	22 - 30	
Hypertension – n (%)	21 (70)	22 (73)	23 (77)	0.843
treated – n (%)	12 (57)	12 (54)	14 (61)	0.833
ACE-I – n (%)	6 (50)	6 (50)	8 (57)	0.773
ARB – n (%)	1 (8)	2 (17)	2 (14)	0.809
CCB – n (%)	6 (50)	5 (42)	8 (57)	0.627
Diuretics – n (%)	4 (33)	3 (25)	3 (21)	0.894
Diabetes – n (%)	5 (16)	6 (20)	7 (23)	0.812
oral agents – n (%)	4 (80)	4 (67)	5 (71)	0.914
insulin – n (%)	none	none	none	
Hypercholesterolemia – n (%)	6 (20)	2 (7)	2 (7)	0.165
Former smokers – n (%)	6 (20)	8 (27)	5 (17)	0.627

CF: cocoa flavanol; ACE-I: angiotensin converting enzyme inhibitor; ARB: angiotensin II Type 1 receptor blocker; CCB: calcium channel blocker. Plus–minus values are means ±SD. ANOVA was used for continuous variables; Chi-square test was used for categorical variables. $p<0.05$ is considered a statistically significant difference.

TABLE S3: MEAN DIETARY TOTAL FLAVANOL INTAKE AT ENROLLMENT AND AFTER THE 1 WEEK RUN-IN PERIOD IN THE THREE TREATMENT GROUPS.

Variables	High CF	Intermediate CF	Low CF	ANOVA
DF (mg/dL)	*time x treatment interaction: P=0.873*			
Enrollment	209.9±47.2	196.8±48.5	207.3±56.9	P=0.577
End run-in	194.9±38.9	182.2±31.1	194.7±39.2	P=0.312
ANOVA	P<0.0001	P=0.0008	P=0.0043	

CF: cocoa flavanol; DF: dietary flavonoids
Plus–minus values are means ±SD. Differences within groups were analyzed by ANOVA. Differences between groups were analyzed by ANOVA.

TABLE S4: DETERMINANT OF COMPOSITE COGNITIVE Z SCORE CHANGES FROM BASELINE IN THE STUDY POPULATION.

Variables	R	R^2	R^2 change	F	F change	β	t	p
ΔHOMA-IR	- 0.6335 p<0.0001	0.4013	-	58.99	-	- 0.2910	- 7.681	<0.0001
ΔHOMA-IR		0.4734	0.0721	39.11	19.88	- 0.2373	- 6.09	<0.0001
Δ8-iso-$PGF_{2\alpha}$	- 0.4991 p<0.0001					- 0.0014	- 3.45	0.0009
ΔHOMA-IR		0.4957	0.0223	28.18	10.93	- 0.2013	- 4.73	<0.0001
Δ8-iso-$PGF_{2\alpha}$						- 0.0013	- 3.06	0.0030
ΔSBP	-0.4967 p<0.0001					- 0.0133	- 1.95	0.0547

ΔHOMA-IR: changes of homeostasis model assessment of insulin resistance; Δ8-iso-$PGF_{2\alpha}$: changes of 8-iso-prostaglandin $F_{2\alpha}$; ΔSBP: changes of systolic blood pressure.

FIGURE S1: FLOW OF PARTICIPANTS THROUGH THE PHASES OF THE STUDY

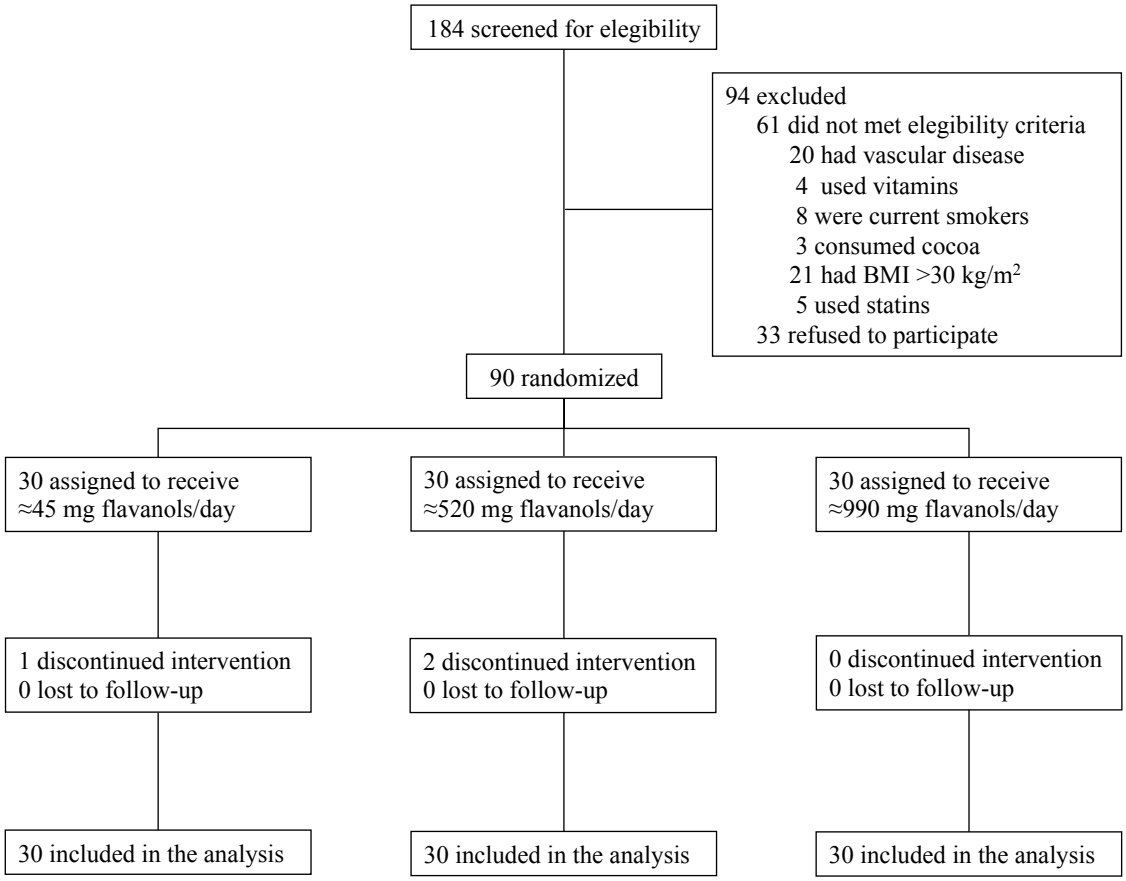
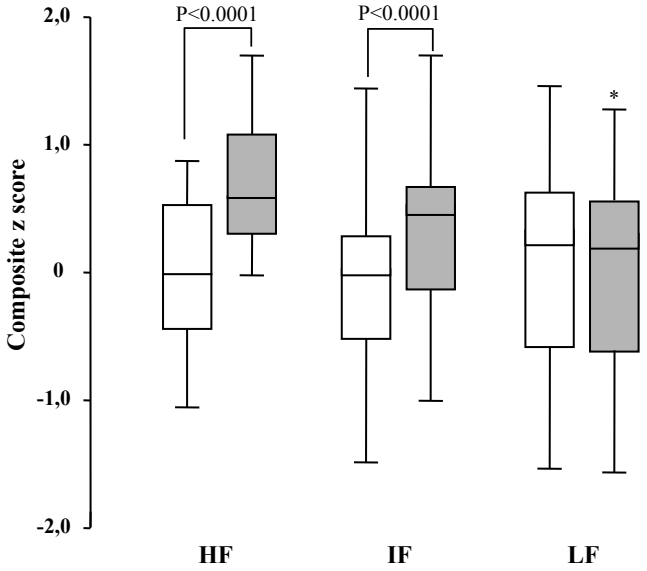


FIGURE S2: CHANGES IN COMPOSITE COGNITIVE Z SCORE AFTER 8 WEEKS UNDER EITHER HIGH (HF) OR INTERMEDIATE (IF) OR LOW COCOA FLAVANOL (LF) CONTENT BEVERAGES.



Boxes represent interquartile ranges with the median value shown as a horizontal bar within each box. Bars outside each box show minimum and maximum values. White boxes indicate baseline values, grey boxes indicate 8-week values. Differences within group were analyzed by ANOVA. Differences between groups were analyzed by ANOVA followed byTukey's Studentized Range – Honestly Significant Difference * $p<0.05$ LF vs HF.