

# 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  $\approx 990$  mg (high flavanols),  $\approx 520$  mg (intermediate flavanols), or  $\approx 45$  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 ( $38.10\pm 10.94$  and  $104.10\pm 28.73$  seconds, respectively) and intermediate flavanols ( $40.20\pm 11.35$  and  $115.97\pm 28.35$  seconds, respectively) in comparison with those assigned to low flavanols ( $52.60\pm 17.97$  and  $139.23\pm 43.02$  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 ( $27.50\pm 6.75$  versus  $22.30\pm 8.09$  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  $\approx 40\%$  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.<sup>1,2</sup> 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.<sup>3</sup> 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.<sup>4</sup> 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.<sup>4</sup>

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<sup>5</sup> and with a lower prevalence of cognitive impairment,<sup>6</sup> a better cognitive evolution over a 10-year period,<sup>7</sup> and better dose-dependent performance of several cognitive abilities in elderly subjects.<sup>8</sup> 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

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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.<sup>9</sup> 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.<sup>9</sup>

Substantial evidence exists supporting the link between cognition and cardiovascular risk factors.<sup>10</sup> In particular, high blood pressure has been linked with the pathophysiology of MCI other than with Alzheimer disease and vascular dementia.<sup>10,11</sup> This relationship may be mediated by endothelial dysfunction and microvascular diseases, leading to an impairment of vascular reserve.<sup>10,11</sup> 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.<sup>12–14</sup> Peripheral insulin resistance could promote cognitive dysfunction by reducing brain insulin uptake, raising brain levels of amyloid  $\beta$  and  $\tau$  phosphorylation and promoting vascular damage through oxidative stress, proinflammatory cytokines, advanced glycation end products, and dyslipidemia,<sup>12,13</sup> with chronic dysglycemia thought to contribute to cognitive dysfunction by promoting the development of cerebral microvascular disease and inflammation.<sup>15</sup> 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.<sup>12,16</sup> In this regard, dietary flavanol consumption has been shown to reduce blood pressure and improve insulin sensitivity,<sup>17,18</sup> as well as to induce vasodilation of the peripheral<sup>17,18</sup> and cerebral vascular system,<sup>19</sup> increasing brain blood flow and perfusion.<sup>19,20</sup> 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.<sup>21</sup> 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,<sup>22</sup> 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;  $\approx$ 990 mg of flavanols per serving), intermediate (IF;  $\approx$ 520 mg of flavanols per serving), or low level (LF;  $\approx$ 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 (Cocopro 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 ( $\pm$ 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  $\alpha$  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.  $\chi^2$  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 $\pm$ 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<sup>2</sup>), 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 $\pm$ 8.39 kg; IF, 68.59 $\pm$ 8.19 kg; LF, 71.17 $\pm$ 8.65 kg;  $P=0.486$ ) or body mass index (HF, 27.20 $\pm$ 2.7 kg/m<sup>2</sup>; IF, 26.27 $\pm$ 2.50 kg/m<sup>2</sup>; LF, 26.90 $\pm$ 2.75 kg/m<sup>2</sup>;  $P=0.427$ ) 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 ( $-14.3\pm 4.2$  seconds;  $P<0.0001$ ) and IF ( $-8.8\pm 3.4$  seconds;  $P<0.0001$ ) interventions but not in those assigned to the LF intervention ( $+1.1\pm 13.0$  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 ( $-29.2\pm 8.0$  seconds;  $P<0.0001$ ) and IF ( $-22.8\pm 5.1$  second;  $P<0.0001$ ) subjects but not in those assigned to the LF intervention ( $+3.8\pm 16.3$

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 ( $+8.0\pm 5.3$  words per 60 seconds;  $P<0.0001$ ) and IF ( $+5.1\pm 3.1$  words per 60 seconds;  $P<0.0001$ ) groups and, to a lesser extent, in those assigned to the LF group ( $+1.2\pm 2.7$  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 ( $+0.693\pm 0.223$ ;  $P<0.0001$ ) and IF ( $+0.404\pm 0.141$ ;  $P<0.0001$ ) groups demonstrating significant improvement; no change was observed in the LF group ( $-0.072\pm 0.383$ ;  $P=0.31$ ; 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:  $-10.0\pm 3.1$  mm Hg,  $P<0.0001$ ; diastolic:  $-4.8\pm 1.8$  mm Hg,  $P<0.0001$ ) and IF (systolic:  $-8.2\pm 3.5$  mm Hg,  $P<0.0001$ ; diastolic:  $-3.4\pm 2.0$  mm Hg,  $P<0.0001$ ) subjects but not in those assigned to the LF group (systolic:  $-1.4\pm 5.4$  mm Hg,  $P=0.16$ ; diastolic:  $-0.9\pm 3.4$  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 ( $-0.6\pm 0.3$  mmol/L;  $P<0.0001$ ) and IF ( $-0.5\pm 0.1$  mmol/L;  $P<0.0001$ ) but not in those assigned to LF ( $-0.1\pm 0.5$  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 ( $-1.6\pm 1.0$ ;  $P<0.0001$ ) and IF ( $-0.9\pm 0.2$ ;  $P<0.0001$ ) but not in those assigned to LF ( $-0.1\pm 0.5$ ;  $P=0.29$ ; 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	$F=0.53$ $P=0.59$	NS
Week 8	28.07±1.01	27.43±1.94	27.93±1.20	$F=1.61$ $P=0.21$	NS
ANOVA	$F=18.4$ $P=0.0002$	$F=6.27$ $P=0.02$	$F=5.18$ $P=0.03$		
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	$F=0.36$ $P=0.70$	NS
Week 8	38.10±10.94	40.20±11.35	52.60±17.97	$F=9.67$ $P=0.0002$	<0.05*
ANOVA	$F=341.94$ $P<0.0001$	$F=206.16$ $P<0.0001$	$F=0.22$ $P=0.65$		
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	$F=0.16$ $P=0.86$	NS
Week 8	104.10±28.73	115.97±28.35	139.23±43.02	$F=8.26$ $P=0.0005$	<0.05*
ANOVA	$F=399.01$ $P<0.0001$	$F=607.89$ $P<0.0001$	$F=1.67$ $P=0.21$		
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	$F=0.69$ $P=0.51$	NS
Week 8	27.50±6.75	24.20±6.10	22.30±8.09	$F=4.20$ $P=0.018$	<0.05†
ANOVA	$F=67.75$ $P<0.0001$	$F=74.22$ $P<0.0001$	$F=6.84$ $P=0.014$		
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	$F=0.26$ $P=0.77$	NS
Week 8	0.687±0.482	0.340±0.663	0.000±0.803	$F=8.07$ $P=0.0006$	<0.05†
ANOVA	$F=290.49$ $P<0.0001$	$F=246.89$ $P<0.0001$	$F=1.06$ $P=0.31$		

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<sub>2α</sub> 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<sub>2α</sub> 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 <sub>2α</sub> , 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<sub>2α</sub>, 8-iso-prostaglandin F<sub>2α</sub>. 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  $\approx 2\%$  and  $\approx 7\%$  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.<sup>6–8,23</sup> 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.<sup>24</sup> 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,<sup>19</sup> vascular function, and blood pressure were observed.<sup>25</sup> 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<sup>25</sup> 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.<sup>26</sup> 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  $\approx 40\%$  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.<sup>27</sup> 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.<sup>19</sup> 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,<sup>17,18,25</sup> which, in turn, plays a pivotal role in modulating cerebrovascular tone.<sup>28</sup>

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.<sup>17,18,29,30</sup> 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  $\leq 2\%$  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.<sup>31</sup> 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.<sup>3,32</sup>

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.<sup>33</sup> These effects probably represent the consequence of the global effects of these nutrients on the cardiovascular system rather than 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.<sup>34</sup> 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  $\approx 7\%$  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,<sup>22</sup> 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.<sup>1,2</sup> 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.<sup>35</sup>

### 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.

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### Disclosures

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## 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.