Low levels of a urinary biomarker of dietary polyphenol are associated with substantial cognition decline over a three-year period in older adults: the Invecchiare in Chianti (InCHIANTI) Study

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ABSTRACT
Objectives: To investigate the association of total urinary polyphenols (TUP) and total dietary polyphenols (TDP) with cognitive decline in an older population.
Design: The Invecchiare in Chianti (InCHIANTI) study, a cohort study with a 3-year of follow-up. **Setting:** tuscany, italy. **Participants**: Non-demented adults aged 65 and older (N=652). **Measurements:** TUP and TDP concentrations were analysed at baseline using the Folin-Ciocalteu assay and a validated food frequency questionnaire, respectively. Cognition was assessed using the Mini-Mental State Examination (MMSE) and Trail Making Test (TMT) at baseline and after three years of follow-up. A substantial cognitive decline was defined as a reduction in the MMSE score of 3 or more points and as an increase of at least 29 seconds on the TMT A and 68 seconds on the TMT B (these thresholds represent the worst 10% of the distribution of decline) or as test discontinued due to multiple mistakes in TMT A and B at follow-up. **Results:** Higher TUP levels were associated with lower risk of substantial cognitive decline on the MMSE (odds ratio [OR] comparing extreme tertiles = 0.53; 95% confidence interval [CI] = 0.34–0.85; P-trend = 0.008) and on the TMT-A (OR = 0.52; 95 % CI = 0.28–0.96; P-trend = 0.03), but not on TMT-B in a logistic regression model that adjusted for baseline cognitive score and potential confounding factors. TDP did not affect the developing substantial cognitive decline in both tests. **Conclusion:** High concentrations of polyphenols, a nutritional biomarker of polyphenol intake, were associated with a lower risk of substantial cognitive decline in the older population studied over a three-year period, suggesting a protective effect against cognitive impairment.

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INTRODUCTION

Cognitive decline in older persons encompasses a number of different conditions, from a physiological age-associated reduction of cognitive functions that occurs in almost every aging individual, the cognitive impairment that often accompanies a global deterioration of health and comorbidity, up to the accelerated trajectory of cognitive decline that eventually leads to mild cognitive impairment and dementia.[1,2]

There is increasing literature suggesting that dietary factors,[3] and in particular long-chain omega-3 fatty acids, low saturated-fat intake and high vegetable intake, are related to cognitive performance in older subjects.[4] Over the last few years, there has been much scientific and public health interest in polyphenols[5] due to their potential beneficial effects against chronic diseases, particularly cardiovascular diseases, type 2 diabetes and some cancers, and overall mortality.[6-11] These secondary plant metabolites are natural bioactive compounds, which have been identified in foods and beverages.[12] Several epidemiological studies have suggested that polyphenol-rich diets are positively associated with a better cognitive function.[13,14] The neuroprotective activity of dietary polyphenols might be due to their antioxidant and anti-inflammatory properties.[15]

The protective effects of polyphenols against human diseases depend on the quantity consumed and their bioavailability, which largely differs both between compounds and between subjects (inter-individual variation), and within subjects (intra-individual variation).[16] In this context, a nutritional biomarker of total dietary polyphenols is essential to provide an accurate assessment of polyphenol exposure in order to evaluate their protective effects.[17] Total urinary polyphenol (TUP) concentration determined by the Folin-Ciocalteu assay, preferably analysed in 24-h urine samples,[18] is considered an objective biomarker of total dietary polyphenol intake (TDP),[17,18] and a proxy measure of dietary fruit and vegetable intake.[19] To the best of our knowledge, there is only one recent cross-sectional study showing that high TUP concentrations, expressed by urinary creatinine, were associated with better scores in immediate verbal memory in older subjects at high vascular risk.[20] We aimed at expanding these findings in a longitudinal perspective by studying the effect of baseline TUP concentrations, expressed by 24-h volume, and TDP, and the risk of developing cognitive decline over a three-year period in older participants free of dementia at baseline who were enrolled in the InCHIANTI (Invecchiare in Chianti, Aging in the Chianti region) study.

METHODS

Study population

The InCHIANTI (www.inchiantistudy.net) is an ongoing prospective community-based study designed to evaluate risk factors affecting the loss of mobility in the older population. It was conducted in Bagno in Ripoli and Greve in Chianti, two Italian towns adjacent to the city of Florence (Tuscany, Italy). Further details of the rationale and the study design have been described elsewhere.[21] The study protocol was approved by the Italian National Research Council of Aging Ethical Committee, and all participants provided an informed participation consent.

Data were collected at baseline and three years after enrolment. Baseline visits occurred between 1998 and 2000, and three-year assessments took place between 2001 and 2003. The study randomly sampled 1260 men and women aged 65 years and older, of whom 1155 agreed to participate (participation rate = 91.6%). Of these, we excluded 82 participants (7.1%) who were diagnosed with dementia syndrome at baseline by

geriatricians and psychologists with expertise in cognitive impairment. The final sample for the current analysis included 652 dementia-free adults aged 65 years or older who provided complete 24-h urine collection and had completed cognitive assessment after three years of follow-up. Follow-up data for Trails A and B were available for 510 and 423 participants, respectively (**Figure 1**).

Total polyphenols exposure
Twenty-four hour urine samples were collected at baseline. Urine samples were drawn, processed and stored at −80ºC until analysis. Total urinary polyphenols (TUP) were analysed by F-C assay, after solid-phase extraction as described previously.[18,19] TUP concentrations were expressed as mg gallic acid equivalents (GAE)/d.
Food intake (g/day) was assessed using the Italian version of the food frequency questionnaire developed and validated in the European Prospective Study into Cancer and Nutrition (EPIC) study.[22] Briefly, a self-administered quantitative dietary questionnaire, including 236 food items and structured by courses within a meal characteristic of Italian dietary habits, was used. Portion sizes were estimated using natural units, household measures and grammage, or with the aid of a set of photographs. Energy (kcal/d) intake was calculated using an Italian food composition database created for the EPIC study.[23] TDPs were estimated using our food composition database on polyphenols.[11] It was compiled from the three most updated U.S. Department of Agriculture databases (flavonoids, isoflavones, and proanthocyanidins)[24-26] and the Phenol-Explorer database.[27] TDP intake was calculated as the sum of flavonoids (anthocyanidins, flavonols, flavanones, flavones, flavanols [including flavan-3-ol monomers, theaflavins and proanthocyanidins] and isoflavones), phenolic acids, lignans, stilbenes and other polyphenols, and was expressed as aglycone equivalents (mg/day),[27] after the conversion of polyphenol glycoside and ester contents into aglycone contents on the basis of their respective molecular weights.

Cognitive tests
The mini-mental state examination (MMSE) is a validated method to assess global cognitive function, widely used in both clinical practice and research, and is an effective screening tool for cognitive impairment in older, community-dwelling, hospitalized and institutionalized adults.[28,29] MMSE is an 11-question test that evaluates five areas of cognitive function: orientation, registration, attention and calculation, recall, and language. Scores for the MMSE range from 0 to 30, with higher scores indicating better cognitive function. The trail making tests (TMT) is a neuropsychological test commonly used in clinical evaluation for the assessment of cognitive abilities such as visual-conceptual and visual-motor tracking, sustained attention, and task alternation abilities.[30,31] The TMT consists of two parts administered in sequence: TMT-A and TMT-B. Part A is focused particularly on attention, whereas Part B is focused on executive function. Worse performance is indicated by longer times, measured in seconds, to complete the Trails A and B.
A substantial cognitive decline was defined as a decline in the MMSE score of 3 or more points from baseline to three years later,[28,29] and as an increase of at least 29 seconds on the TMT-A and 68 seconds on the TMT-B (these thresholds represent the worst 10% of the distribution of subtracting baseline from three-year follow-up scores in seconds) or the test discontinued at follow-up, but not at baseline, due to multiple mistakes in TMT-A and TMT-B.[32]

Other baseline covariates

Trained clinicians ascertained diseases based on information from self-reported physician diagnoses, drug treatments, medical history, clinical examinations and blood tests. Smoking status (current, former, and never a smoker), age, sex, education (years of schooling) and body mass index (kg/m^2) were reported or measured. Physical activity was measured using a modified standard questionnaire[33] and was defined as: 1) sedentary (completely inactive or performing light-intensity physical activity less than <2h/wk.); 2) light physical activity (light-intensity physical activity of 2–4h/wk.); and 3) moderate to high physical activity (light-intensity physical activity of at least >4h/wk. or moderate-intensity physical activity at least 1–2 h/wk.). Specific co-morbidities considered in this analysis were congestive heart failure, stroke, cancer and diabetes. Renal function was calculated using the Cockcroft–Gault formula: (140−Age) x Weight x [0.85 *if female*] / (72 x Serum Creatinine) and was classified into a dichotomous variable: normal renal function (≥60mL/min) and impaired renal function (<60mL/min).[34] Depressive symptoms were assessed using the Center for Epidemiological Studies-Depression Scale (CES-D) and were defined as CES-D of 16 or higher and CES-D lower than 16.[35] Total blood cholesterol was measured using an automated enzymatic method.[36]

Statistical analysis

Descriptive statistics were used to describe participants' characteristics, means and standard deviations (SD) for normal continuous variables, medians and interquartile ranges (IQR) for variables with asymmetric distribution, and number of participants and percentages for categorical variables. Baseline characteristics were compared across TUP and TDP tertiles using age-adjusted generalized linear models. Spearman correlations were used to explore the relationships between TUP, TDP and the sum of fruit and vegetable intake. Linear regression models were used to evaluate associations between baseline cognitive performance (MMSE, TMT-A and TMT-B) and TDP and TUP tertiles. For TMT test performance, the inverse score was used; thus, a higher score corresponded to a better result. Logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) between TUP and TDP concentrations and substantial cognition decline. In unadjusted models, we controlled for baseline cognitive score only (Model A). In multivariable models, we adjusted for variables that have been identified as potential confounders: sex, age, education, body mass index, total energy intake, physical activity, baseline cognitive score, impaired renal function, smoking status, congestive heart failure, cancer, stroke, diabetes, depressive symptoms and total blood cholesterol (Model B). TUPs were analysed as tertiles of the whole cohort according to the following cut-off points: 126.4 and 175.5 mg GAE/d urine. TDPs were also categorized as tertiles of the whole cohort according to the following cut-off points: 514.2 and 648.4 mg/d aglycones. Tests for linear trend were performed by considering the median of each tertile as an ordinal variable. TUPs and TDPs were also analysed as a log$_2$-transformed continuous variables since they were not normally distributed. Interactions between TDP and TUP concentrations and sex, age, BMI, education and smoking status were tested by including product terms in fully adjusted logistic regression models. There was no evidence of colinearity. In sensitivity analyses, we assessed models for TDP excluding participants in the top or the bottom 1% of the distribution of the ratio of reported total energy intake to estimated energy requirement to minimize the potential effect of over- and under-reporters.

All statistical tests were two-tailed and were performed using the SPSS package program, version 20.0 (SPSS, Chicago, IL). The significance level was p<0.05.

RESULTS

The main characteristics of the study population according to tertiles of TUP and TDP, adjusted for age, are summarized in **Table 1**. The study included 361 women (55.4%) and 291 men (44.6%) with a mean age of 73 years. From the lowest to the highest TUP tertiles, participants were younger and more likely to be men. The percentage of participants who experienced substantial cognitive decline on TMT-A over the three-year follow-up, and of those who at baseline had depressive symptoms and cancer progressively decreased with increasing TUP tertiles. While the inverse associations between TUP tertiles and substantial cognitive decline and change in cognitive score on MMSE were borderline significant. Moreover, from the lowest to the highest TUP tertiles, participants had a higher energy intake. There were no significant differences across the tertiles of TUP in BMI, education, smoking status, physical activity, fruit and vegetable consumption, total cholesterol, congestive heart failure and stroke. From the lowest to the highest tertiles of TDP, participants were younger, with a lower proportion of women, less often current and former smokers, had a higher education, physical activity, energy dietary intake, dietary fruit and vegetable consumption, and had lower total cholesterol. Participants with a substantial cognitive function decline after three-years of follow-up were older and more likely to be women, had a lower educational achievement and physical activity level, and a higher prevalence of stroke and congestive heart failure than those without cognitive decline. Participants excluded from this study (n=503) were significantly older ($P<0.001$), took more medications ($P=0.04$), had lower physical activity ($P<0.001$), and had more often renal impairment ($P=0.006$) than those included, whereas there were no significant differences in sex and smoking status (results not shown). TUP were significantly correlated with the intake of fruit and vegetables (rho=0.141; $P<0.001$) and TDP (rho=0.131; $P<0.001$).

In linear regression models adjusted for potential confounding factors, TUP levels were positively associated with the MMSE ($\beta = 0.076$; SE = 0.243, $P = 0.042$) and the TMT-A ($\beta = 0.086$; SE = 5.174, $P = 0.016$), but not with the TMT-B ($\beta = 0.014$; SE = 6.835, $P = 0.73$). No associations between TDP intake and any of the cognitive tests at baseline were observed.

In logistic regression models adjusting for baseline cognitive scores and compared to participants in the lowest TUP tertile, those in the highest TUP tertile were significantly less likely to experience substantial cognitive decline both when assessed by the MMSE (OR = 0.53; 95% CI = 0.35–0.80; P-trend = 0.003) and the TMT-A (OR = 0.50; 95% CI = 0.28–0.89; P-trend = 0.02) (**Table 2, Model A**). This association remained statistically significant after full adjustment for potential confounders both for the MMSE (highest vs. lowest tertile OR = 0.53; 95% CI = 0.34–0.85; P-trend = 0.008) and the TMT-A (OR = 0.52; 95% CI = 0.28–0.96; P-trend = 0.03). No significant association between TUP concentrations and TMT-B was observed (**Table 2, Model B**). In fully adjusted models using MMSE, TMT-A and TMT-B tests, no statistically significant interactions were detected for sex, age, BMI, education and smoking status.

In multivariable logistic models adjusted for all confounders, no statistically significant associations were found between TDP and substantial cognitive decline risk using the

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MMSE (highest vs. lowest tertile OR = 1.08; 95% CI = 0.65–1.78; P-trend = 0.84), the TMT-A (OR = 0.63; 95% CI = 0.32–1.25; P-trend = 0.17) and the TMT-B (OR = 1.16; 95% CI = 0.63–2.14; P-trend = 0.77) (**Appendix Table S1, Model B**). Again, we found no significant interactions between MMSE, TMT-A or TMT-B and sex, age, BMI, education, and smoking status.

Sensitivity analyses were performed by excluding from the analysis participants in the top or the bottom 1% of the distribution of the ratio of total energy intake for TDP. The odds ratios for the sensitivity analyses were nearly identical to the results based on the whole cohort (data not shown).

DISCUSSION

In this population-based study of non-demented older people, high concentrations of TUP were associated with an approximate 47 and 48% reduction of the risk of substantial cognitive decline in global cognitive function (tested using the MMSE) and in attention (measured using the TMT-A) over a three-year period, respectively, but not with the TMT-B, which is mainly focused on measuring executive function. No significant association was found between TDP and any cognitive test, as was also observed with all-cause mortality in a previous InCHIANTI study.[11] This may be due to the difficulty in assessing TDP intake. While a part of polyphenol intake, TUP also accounts for the bioavailability of polyphenols[16] and may therefore be a more precise measure of true exposure due to the large variability between and within polyphenol absorption and metabolism.[17]

In a cross-sectional study, total polyphenols expressed by urinary creatinine normalization were linearly associated with better scores in immediate verbal memory in older adults at high cardiovascular risk.[20] In the middle-aged SU.VI.MAX cohort, high total polyphenol intake, estimated using six repeated 24h dietary recalls, was associated with better language and verbal memory after a follow-up of 13 years.[14] Similar to our results between TUP or TDP and TMT-B, no significant associations were observed between TDP and executive functioning in the SU.VI.MAX study[14]. Furthermore, in other prospective studies, the intake of flavonoids was associated with a better cognitive evolution in dementia-free older adults[13] and a lower risk of Alzheimer's disease, the most common form of dementia.[37,38] In another prospective study, the consumption of fruit and vegetables, and thus antioxidant nutrients (vitamin E and C), was associated with better verbal memory.[39]

In the present study, higher levels of TUP at baseline also had a better cognitive performance as in the PREDIMED study.[20] However, no association was observed with TDP. As far as we know, no cross-sectional studies have focused on the relationship between TDP and cognitive function. In a small subsample of post-menopausal women from the PROSPECT study, which is one of the Dutch cohorts included in the EPIC study, higher intakes of lignans, but not isoflavones, were associated with higher MMSE scores at enrolment.[40] Whereas, in the Lothian Birth Cohort 1936, flavonoid intake was not associated with any of the cognitive tests performed after adjusting for confounding factors at the age of 70 years old.[41]

The potential mechanisms of the protective effects of polyphenols on cognitive functions may be linked to their antioxidant and anti-inflammatory properties. It is known that polyphenols reduce neuronal damage and death from oxidative reactions by inhibiting the generation of reactive oxygen species, lipid peroxidation, apoptosis, protein oxidation,

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metal chelation and damage to cellular signaling.[15,42-46] Potential actions of polyphenols may be mediated by the direct interactions within ERK and PI3-kinase/Akt signalling pathways, which have been associated with the maintenance of the number and quality of synaptic connections in key brain regions.[47] Additional mechanisms are related to the inhibition of neuronal apoptosis activated by neurotoxic species or the disruption of amyloid β aggregation and effects on amyloid precursor protein processing through the inhibition of β-secretase and/or activation of α-secretase.[47]

Our study has several strengths. First of all, to the best of our knowledge, this is the first prospective study to investigate the association between total polyphenols and substantial cognitive decline in an older population. Moreover, urinary polyphenols expressed by 24-h volume are an objective biomarker of total phenolic intake[18] and a proxy biomarker of fruit and vegetable consumption.[19] The main advantage of a nutritional biomarker over a dietary biomarker is that it is a more precise and more proximal measure than dietary assessment.[48] Currently, the adapted F-C assay in urine samples is considered a valid biomarker for total polyphenol intake and is a rapid, economic and easy method to apply to large-scale epidemiological studies. TUP expressed as 24-h volume is considered the gold standard to assess urinary excretion.[18] Then cognitive functions were assessed using the MMSE – the most widely used instrument for measuring the course of cognitive change in an older adult over time[28,29] – and TMT-A and TMT-B, which are also commonly used in the assessment of psychomotor speed, visuospatial scanning, and executive ability.[30,31] Finally, our logistic regression models were adjusted for the most important confounding variables related to cognitive decline, such as: sociodemographic characteristics, health behaviours and chronic diseases.

However, our study also has some limitations. First, the InCHIANTI study was performed in community dwelling older subjects living in two sites of the Tuscany region (Italy); therefore the sample might not be representative of the general Italian population. Second, cognitive decline is a heterogeneous condition and in our study its underlying cause was not assessed. Third, our results may be influenced by measurement errors in the dietary questionnaires. Furthermore, the present study population is ≥65 years old, and therefore, they may be less reliable in recalling food intake than younger subjects. However, the food frequency dietary questionnaire was country-specific and previously validated for some polyphenol-rich foods, such as fruits, vegetables, tea, coffee and wine, in a similar population,[22] and, moreover, those participants with dementia syndrome at baseline were excluded. In addition, dietary consumption of polyphenols may be underestimated, since the food composition tables for polyphenols were not totally completed. However, for our study an extensive common database was used.[11,18]

In conclusion, we found that in non-demented older adults higher TUP concentrations were associated with a lower risk of substantial cognitive decline over a three-year period after adjusting for potential confounders. No significant association was found using TDP. Our findings suggest a protective effect of total polyphenols and, indirectly, diets rich in polyphenols, against cognitive decline risk in older adults. Further epidemiological studies and clinical trials are warranted to clarify the potential preventive role of polyphenols and their underlying mechanisms. The identification of factors that reduce or delay cognitive decline is essential to improve the autonomy and quality of life of older people.

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	Yes	No	Yes	No	Yes	No	Yes	No
Employment or Affiliation		X		X		X		X
Grants/Funds		X		X		X		X
Honoraria		X		X		X		X
Speaker Forum		X		X		X		X
Consultant		X		X		X		X
Stocks		X		X		X		X
Royalties		X		X		X		X
Expert Testimony		X		X		X		X
Board Member		X		X		X		X
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Author contributions: MR, AC and CA-L designed the research. MR, MU-S and CA-L performed the samples analyses. MR and RZ-R conducted the statistical analysis. MR drafted the manuscript and AC and RZ-R provided critical revision. SB and LF conceived of the study, participated in its design and coordination. All authors read and approved the final manuscript.

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GRAPHICS

Table 1. Characteristics of the study population according to tertiles of total urinary polyphenols (TUP) and total dietary polyphenols (TDP)

Characteristic	All (N=652)	TDP					TUP			
		Tertile 1 (n=217)	Tertile 2 (n=218)	Tertile 3 (n=217)	P-value[a]		Tertile 1 (n=217)	Tertile 2 (n=218)	Tertile 3 (n=217)	P-value[a]
Age, mean (SD)	73.4 (6.4)	75.0 (7.2)	73.1 (6.0)	72.2 (5.6)	<0.001		74.8 (6.9)	73.8 (6.5)	71.8 (5.3)	<0.001
Female, n (%)	361 (55.4)	148 (68.2)	120 (55.0)	93 (42.9)	<0.001		137 (63.1)	119 (54.6)	105 (48.4)	0.03
BMI, kg/m^2, mean (SD)	27.6 (4.0)	27.6 (4.2)	27.5 (3.9)	27.6 (3.7)	0.85		27.2 (4.0)	27.6 (4.0)	28.0 (3.8)	0.35
Education, years, mean (SD)	5.6 (3.2)	5.0 (3.0)	5.7 (3.4)	6.0 (3.2)	0.07		5.2 (3.0)	5.7 (3.8)	5.8 (2.9)	0.54
Physical activity, n (%)					0.003					0.53
Sedentary	106 (16.3)	49 (22.7)	36 (16.5)	21 (9.8)			45 (20.8)	32 (14.8)	29 (13.4)	
Light	291 (44.8)	106 (49.1)	92 (42.2)	93 (43.3)			96 (44.4)	101 (46.8)	94 (43.3)	
Moderate to high	252 (38.8)	61 (28.2)	90 (41.3)	101 (47.0)			75 (34.7)	83 (38.4)	94 (43.3)	
Smoking status, n (%)					0.04					0.33
Never smoker	387 (59.4)	147 (67.7)	130 (59.6)	110 (50.7)			138 (63.6)	137 (62.8)	112 (51.6)	
Former smoker	175 (26.8)	46 (21.2)	53 (24.3)	76 (35.0)			45 (20.7)	53 (24.3)	77 (35.5)	
Current smoker	90 (13.8)	24 (11.1)	35 (16.1)	31 (14.3)			34 (15.7)	28 (12.8)	28 (12.9)	
Energy intake, kcal/d, mean (SD)	1921 (549)	1608 (437)	1918 (456)	2237 (558)	<0.001		1830 (534)	1909 (532)	2024 (566)	0.02

Dietary fruit and vegetables, g/d, median (IQR)	436 (323-552)	309 (245-390)	445 (375-525)	576 (470-696)	<0.001		416 (296-538)	422 (319-535)	453 (365-583)	0.15
Total dietary polyphenols, mg/d, median (IQR)	574 (472-701)	430 (354-470)	574 (543-610)	766 (701-855)	<0.001		551 (452-655)	562 (457-708)	601 (518-720)	0.12
Total urinary polyphenols, mg GAE/d, median (IQR)	148 (113-196)	138 (107-173)	154 (115-197)	160 (126-208)	0.002		103 (82-113)	148 (138-163)	218 (196-254)	<0.001
Total cholesterol, mg/dL, median (IQR)	218 (195-247)	221 (195-247)	220 (197-251)	213 (192-243)	0.09		220 (195-245)	216 (196-248)	219 (194-246)	0.62
Congestive heart failure, n (%)	21 (3.2)	6 (2.8)	10 (4.6)	5 (2.3)	0.37		5 (2.3)	8 (3.7)	8 (3.7)	0.63
Stroke, n (%)	32 (4.9)	11 (5.1)	14 (6.4)	7 (3.2)	0.31		11 (5.1)	7 (3.2)	14 (6.5)	0.29
Cancer, n (%)	39 (6.0)	13 (6.0)	13 (6.0)	13 (6.0)	1.00		17 (7.8)	6 (2.8)	16 (7.4)	0.06
Diabetes, n (%)	88 (13.5)	30 (13.8)	31 (14.2)	27 (12.4)	0.85		24 (11.1)	27 (12.4)	37 (17.1)	0.15
Renal impairment, n (%)	385 (60.0)	140 (66.4)	128 (59.3)	117 (54.4)	0.76		145 (67.4)	128 (60.4)	112 (52.1)	0.44
Depressive symptoms, CES-D score $\geq$ 16, n (%)	97 (14.9)	35 (16.1)	31 (14.2)	31 (14.3)	0.97		42 (19.4)	35 (16.1)	20 (9.2)	0.06
Cognition tests score, mean (SD)										
MMSE	25.5 (3.0)	25.0 (3.3)	25.6 (2.9)	25.9 (2.5)	0.28		25.0 (3.4)	25.6 (2.9)	25.9 (2.5)	0.22
TMT-A	91.3 (57.2)	100.9 (63.5)	89.4 (5.51)	83.7 (51.4)	0.11		94.1 (57.8)	97.4 (61.9)	82.5 (50.7)	0.20
TMT-B	172.5 (75.1)	179.4 (76.0)	171.1 (72.2)	167.4 (77.2)	0.54		176.1 (76.5)	174.8 (76.0)	166.3 (72.7)	0.73
Change in cognition tests										

scores, mean (SD)										
MMSE	-0.6 (3.7)	-0.9 (4.2)	-0.5 (3.2)	-0.3 (3.5)	0.74		-0.9 (3.7)	-0.9 (4.3)	0.0 (2.8)	0.09
TMT-A	-8.5 (41.1)	-14.8 (45.1)	-5.0 (41.8)	-6.4 (35.9)	0.07		-3.7 (38.3)	-13.5 (48.5)	-8.3 (35.4)	0.12
TMT-B	-8.6 (58.3)	-14.4 (58.7)	0.4 (57.4)	-11.7 (58.6)	0.26		-8.0 (58.2)	-2.6 (50.3)	-14.5 (64.8)	0.48
Substantial cognitive decline, n (%)[b]										
MMSE	203 (31.1)	68 (31.3)	73 (33.5)	62 (28.6)	0.57		80 (36.9)	71 (32.6)	52 (24.0)	0.07
TMT-A	103 (20.2)	42 (24.7)	36 (21.2)	25 (14.7)	0.24		46 (27.1)	33 (19.4)	24 (14.1)	0.04
TMT-B	221 (52.2)	73 (51.8)	78 (55.3)	70 (49.6)	0.57		74 (52.5)	76 (53.9)	71 (50.4)	0.95

[a]Generalized linear models were adjusted for age.

[b]Substantial cognitive decline was defined as ≥ 3 points on the MMSE from baseline to three years later and as the worst 10% of the distribution of subtracting baseline from three year follow-up scores in seconds or test discontinued at follow-up for the Trails A and B.

SD = standard deviation; IQR = interquartile range; BMI = body mass index; CES-D = center for epidemiologic studies depression scale; MMSE = mini-mental state examination; TMT = trail making tests.

Table 2. Logistic regression models describing the association between total urinary polyphenols (TUP) tertiles and three-year substantial cognitive decline in older subjects

	MMSE[a]				TMT-A[a]				TMT-B[a]		
	Cut-off	No. of cases	OR (95% CI)		Cut-off	No. of cases	OR (95% CI)		Cut-off	No. of cases	OR (95% CI)
	mg GAE/d	*n*			*mg GAE/d*	*n*			*mg GAE/d*	*n*	
Model A[b]											
Tertile 1	<126.4	80	1 (reference)		<133.2	46	1 (reference)		<135.8	74	1 (reference)
Tertile 2	126.4-175.5	71	0.82 (0.55-1.21)		133.2-183.7	33	0.56 (0.32-0.97)		135.8-186.8	76	0.99 (0.60-1.64)
Tertile 3	>175.5	52	0.53 (0.35-0.80)		>183.7	24	0.50 (0.28-0.89)		>186.8	71	0.90 (0.54-1.49)
P-trend[c]			0.003				0.02				0.67
Continuous (log$_2$)		203	0.69 (0.52-0.92)			103	0.60 (0.41-0.90)			221	1.03 (0.71-1.50)
Model B[d]											
Tertile 1	<126.4	80	1 (reference)		<133.2	46	1 (reference)		<135.8	74	1 (reference)]
Tertile 2	126.4-175.5	71	0.79 (0.51-1.22)		133.2-183.7	33	0.55 (0.31-0.99)		135.8-186.8	76	1.08 (0.63-1.84)
Tertile 3	>175.5	52	0.53 (0.34-0.85)		>183.7	24	0.52 (0.28-0.96)		>186.8	71	0.95 (0.56-1.62)

P-trend[c]			0.008				0.03					0.84
Continuous (log$_2$)		203	0.71 (0.51-0.97)			103	0.65 (0.42-0.99)			221	1.11 (0.73-1.67)

[a]Substantial cognitive decline was defined as ≥ 3 points on the MMSE and as the worst 10% of the distribution of subtracting baseline from three year follow-up scores in seconds or test discontinued at follow-up for the Trails A and B.

[b]Adjusted for baseline cognitive score only.

[c]*P*-trend obtained by assigning the median of each tertile as scores.

[d]Adjusted for baseline cognitive score, sex, age, education, body mass index, total energy intake, physical activity, total cholesterol, impaired renal function, smoking status, congestive heart failure, cancer, stroke, diabetes and depressive symptoms.

TUP = total urinary polyphenols; GAE = gallic acid equivalents; OR = odds ratio; CI = confidence interval; MMSE = mini-mental state examination; TMT = trail making tests.
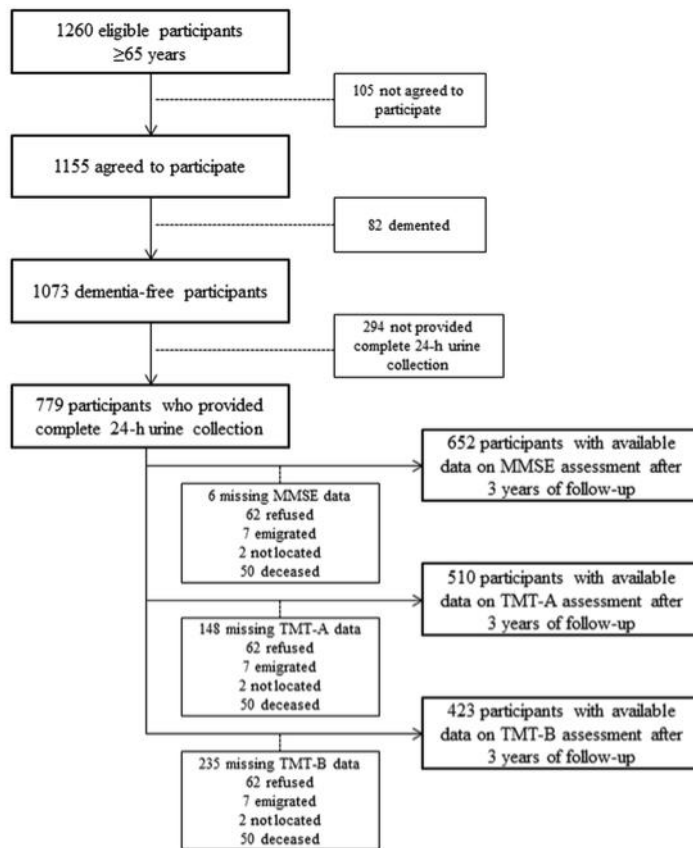
Figure 1. Flow chart of participants at each stage of the study.
MMSE = mini-mental state examination; TMT = trail making tests.

Supplementary Appendix 1
Table S1. Logistic regression models describing the association between total dietary polyphenols (TDP) tertiles and three-year substantial cognitive decline in older subjects

	MMSE[a]			TMT-A[a]			TMT-B[a]		
	Cut-off	No. of cases	OR (95% CI)	Cut-off	No. of cases	OR (95% CI)	Cut-off	No. of cases	OR (95% CI)
	mg/d	*n*		*mg/d*	*n*		*mg/d*	*n*	
Model A[b]									
Tertile 1	<514.2	68	1 (reference)	<520.6	42	1 (reference)	<525.7	73	1 (reference)
Tertile 2	514.2-648.4	73	1.09 (0.73-1.64)	520.6-659.3	36	1.04 (0.60-1.79)	525.7-676.3	78	1.61 (0.96-2.69)
Tertile 3	>648.4	62	0.86 (0.57-1.31)	>659.3	25	0.66 (0.37-1.20)	>676.3	70	1.18 (0.70-1.98)
P-trend[c]			0.45			0.16			0.70
Continuous ($\log_2$)		203	0.83 (0.59-1.18)		103	0.86 (0.53-1.38)		221	1.23 (0.80-1.89)
Model B[d]									
Tertile 1	<514.2	68	1 (reference)	<520.6	42	1 (reference)	<525.7	73	1 (reference)
Tertile 2	514.2-648.4	73	1.26 (0.80-1.97)	520.6-659.3	36	1.09 (0.61-1.96)	525.7-676.3	78	1.62 (0.94-2.80)
Tertile 3	>648.4	62	1.08 (0.65-1.78)	>659.3	25	0.63 (0.32-1.25)	>676.3	70	1.16 (0.63-2.14)
P-trend[c]			0.84			0.17			0.77
Continuous ($\log_2$)		203	0.91 (0.59-1.42)		103	0.79 (0.44-1.40)		221	1.24 (0.74-2.09)

[a]Substantial cognitive decline was defined as ≥ 3 points on the MMSE and as the worst 10% of the distribution of subtracting baseline from three year follow-up scores in seconds or test discontinued at follow-up for the Trails A and B.
[b]Adjusted for baseline cognitive score only.
[c]*P*-trend obtained by assigning the median of each tertile as scores.
[d]Adjusted for baseline cognitive score, sex, age, education, body mass index, total energy intake, physical activity, total cholesterol, impaired renal function, smoking status, congestive heart failure, cancer, stroke, diabetes and depressive symptoms.
TDP = total dietary polyphenols; OR = odds ratio; CI = confidence interval; MMSE = mini-mental state examination; TMT = trail making tests.