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High Concentrations of a Urinary Biomarker of Polyphenol Intake Are Associated with Decreased Mortality in Older Adults[1,2]

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Abstract

Polyphenols might have a role in the prevention of several chronic diseases, but evaluating total dietary polyphenol (TDP) intake from self-reported questionnaires is inaccurate and unreliable. A promising alternative is to use total urinary polyphenol (TUP) concentration as a proxy measure of intake. The current study evaluated the relationship between TUPs and TDPs and all-cause mortality during a 12-y period among older adult participants. The study population included 807 men and women aged 65 y and older from the Invecchiare in Chianti study, a population-based cohort study of older adults living in the Chianti region of Tuscany, Italy. TUP concentrations were measured at enrolment (1998–2000) using the Folin-Ciocalteau assay after a solid-phase extraction. TDPs were also estimated at baseline throughout a validated food frequency questionnaire and using our database based on USDA and Phenol-Explorer databases. We modeled associations using Kaplan-Meier survival and Cox proportional hazards models, with adjustment for potential confounders. During the 12-y follow-up, 274 participants (34%) died. At enrollment, TUP excretion adjusted for age and sex tended to be greater in participants who survived [163 ± 62 mg gallic acid equivalents (GAE)/d)] than in those who died (143 ± 63 mg GAE/d) (P = 0.07). However, no significant differences were observed for TDPs. In the multivariable Cox model, participants in the highest tertile of TUP at enrolment had a lower mortality rate than those in the lowest tertile [HR = 0.70 (95% CI: 0.49–0.99); P-trend = 0.045], whereas no significant associations were found between TDP and overall mortality. TUP is an independent risk factor for mortality among community-dwelling older adults, suggesting that high dietary intake of polyphenols may be associated with longevity. J. Nutr. 143: 1445–1450, 2013.

Introduction

Epidemiological data suggest that people consuming diets rich in fruit and vegetables are at a lower risk of several chronic diseases and overall mortality (1). Plants are abundant food sources of micronutrients and phytochemicals such as polyphenols, carotenoids, and vitamin C. Polyphenols are products naturally occurring from the secondary metabolism of plants. More than 8000 different phenolic compounds have been identified in plants, although edible plants contain only several hundred phenolic structures (2).

Beyond their antioxidants and free radical scavenger effects, polyphenols also exert other potentially beneficial biological activities, such as antiinflammatory, anticarcinogenic, antidiabetic, antiobesity, antiallergic, and hepato- and gastroprotective effects (3–6). Accordingly, epidemiological studies suggest that intake of flavonoids may protect against cardiovascular disease (CVD)[9] (7–10), neurodegenerative diseases (11,12), and some cancers (9,13), particularly gastrointestinal cancers (14).

The health effects of polyphenols depend on their quantity consumed and bioavailability, which varies greatly from one molecule to another (15) and among individuals (16). For this

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[9] Abbreviations used: CVD, cardiovascular disease; GAE, gallic acid equivalent; InCHIANTI, Invecchiare in Chianti; TDP, total dietary polyphenol; TUP, total urinary polyphenol.

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reason, a nutritional biomarker of total dietary polyphenols (TDPs) is needed to accurately assess the relationship between total polyphenols and chronic diseases. Total urinary polyphenol (TUP) estimated by the Folin-Ciocalteu urine assay is considered a valid nutritional biomarker for TDP (17,18) and a proxy biomarker of dietary fruit and vegetable intake (17,19). In a previous study, TUP was negatively associated with blood pressure levels and prevalence of hypertension in the PREDIMED study of an elderly Mediterranean population at high risk of CVD (19). However, the association between TUP as a measure of TDP and all-cause mortality has not been evaluated. Therefore, we examined the relationship between total polyphenol intake, measured by a dietary questionnaire (TDP) or a nutritional biomarker (TUP), and all-cause mortality during a 12-y follow-up period among older adults in the Invecchiare in Chianti (InCHIANTI, "Aging in the Chianti Area") study.

Methods

Population. The InCHIANTI study is a prospective cohort investigation aimed at assessing factors affecting loss of mobility in later life (20). Of the 1256 eligible participants aged 65 y and older who were randomly selected and recruited from Greve in Chianti and Bagno in Ripoli, 1155 (90.1%) agreed to participate. Of these, 807 (69.9%) participants had 24-h urine measures and complete data for all covariates to be included in this study. A total of 274 participants died during the 12-y follow-up period. The study protocol complied with the Declaration of Helsinki and was approved by the Italian National Institute of Research and Care on Aging Ethical Committee. All participants received a full description of the study (20). At the end of the field data collection, we gathered data on the mortality of the original InCHIANTI cohort, with data from the General Mortality Registry of the Tuscany Region and death certificates, which are immediately deposited after death at the registry office of the municipality of residence.

Dietary, lifestyle, and medical data collection. At baseline, usual food intakes were estimated by personal interview using the Italian version of the FFQ developed and validated in the European Prospective Study into Cancer and Nutrition study (21). Energy and nutrients were calculated using an Italian food composition database (22). TDPs were calculated using our food composition database on polyphenols (23) based on USDA databases (24,25) and Phenol-Explorer (26). Total polyphenol data were calculated as the sum of phenolic acids, flavonoids (anthocyanidins, flavonols, flavanones, flavones, flavanols, and isoflavones), lignans, stilbenes, and other polyphenols, which were analyzed by HPLC with or without a previous hydrolysis and expressed as mg aglycones/100 g (26).

The disease status of participants was ascertained by self-reported physician diagnoses, current pharmacological treatments, medical records, clinical examinations, and blood tests. Diseases included in this analysis were CVD (including angina, myocardial infarction, congestive heart failure, stroke, and hypertension), diabetes mellitus, cancer, dementia, Parkinson's, and chronic obstructive pulmonary diseases. BMI was calculated as weight in kilograms divided by height in meters squared. Smoking history was determined from self-report and participants were classified as former smoker, current smoker, or never smoked. Educational level was recorded as the number of years of schooling. Physical activity in the year prior to the interview was classified on an ordinal scale based on responses to a modified standard questionnaire: *1*) sedentary (completely inactive or light-intensity activity <2 h/wk); *2*) light physical activity (light-intensity activity 2–4 h/wk); and *3*) moderate to high physical activity (light-intensity activity ≥4 h/wk or moderate-intensity activity 1–2 h/wk) (27). Renal function was classified using the Cockcroft-Gault equation: [140−age (y)] · weight (kg) · 0.85 (if female)/[serum creatinine (mg/dL) · 72] and participants were classified as having normal renal function (≥60 mL/min), impaired renal function (≥30 to <60 mL/min), or profoundly impaired renal function or renal failure (<30 mL/min) (28).

Laboratory analyses. Twenty-four-hour urine samples were collected from all participants at baseline. Urine samples were aliquoted and stored at –80°C until analysis. Clinicians and laboratory technicians were unaware of the data. TUP was analyzed by Folin-Ciocalteu assay after a solid-phase clean-up as described elsewhere (17). TUP equivalents were expressed as mg gallic acid equivalent (GAE)/24-h urine collection.

Statistical analysis. Variables are reported as means ± SDs or percentages. Characteristics of participants, according to their vital status, were compared using age- and sex-adjusted generalized linear models with differences expressed as means ± SEMs. The relationships between both TUP and TDP and age were explored using Spearman correlation coefficients, and by sex using the Mann-Whitney U test. TUPs were analyzed as tertiles, defined as <123, 123–173, and >173 mg GAE/d. TDPs were also analyzed as tertiles, defined as <509, 509–645, and >645 mg/d aglycones. Tests for linear trend were performed by assigning the median of each tertile as scores. Kaplan-Meier survival curves assessing the relationships between TUP or TDP tertiles and mortality were compared by using the log-rank test. Cox proportional hazards models unadjusted (model 1), adjusted for age and sex (model 2), and additionally adjusted for education, BMI, total energy intake, alcohol intake, smoking history, physical activity, CVD, cancer, diabetes mellitus, dementia, Parkinson's disease, and chronic obstructive pulmonary disease (model 3), were used to examine the relationship between both TDPs and TUPs and mortality. Tests and graphs based on Schoenfeld residuals were used to assess the proportional hazards assumption. All analyses were performed using SPSS software v19.0 with significance set at $P < 0.05$.

Results

During 12 y of follow-up, 274 (34%) of 807 participants died, of which 66 (24%) deaths were due to CVD, 112 (41%) to cancer, and 74 (27%) to other causes. Moreover, 22 (8%) participants had missing information on cause of death. TUP and TDP decreased with aging (rho = −0.22, $P < 0.001$; and rho = −0.15, $P < 0.001$, respectively), and sex (men vs. women) 17.9 ± 4.4 mg GAE/d ($P < 0.001$) and 82.4 ± 13.5 mg/d ($P < 0.001$), respectively. In models adjusted for age and sex, baseline TUP excretion tended to be higher among survivors than those who died (20.0 ± 10.3 mg GAE/d; $P = 0.07$). TDP excretion did not differ between surviving participants and those who died ($P = 0.64$). The characteristics of participants at baseline, adjusted for age and sex, are shown in **Table 1**. Participants who died were significantly older, more likely to be men and current smokers, to be sedentary, and to have chronic obstructive pulmonary disease, dementia, or CVD compared with those who survived. Participants aged 65 y and older who were excluded from this study ($n = 348$) had a higher rate of mortality (56.6%; $P < 0.001$), were older ($P < 0.001$), and were more likely to be sedentary ($P < 0.001$) compared with those who were not excluded. Sex, educational level, and smoking status did not significantly differ.

The overall survival curves of participants by TUP or TDP tertiles are shown in **Figure 1**. Participants in the highest TUP tertile experienced lower all-cause mortality than those in the lowest TUP tertile (log-rank = 29.44; $P < 0.001$). No significant association was observed between TDP tertiles and mortality (log-rank = 3.30; $P = 0.19$).

In Cox proportional hazards models adjusted for age and sex only, participants with the highest TUP tertile at baseline had a lower mortality rate than those in the lowest tertile [HR = 0.63 (95% CI: 0.46–0.87); P-trend = 0.004] (**Table 2**). In contrast, participants across TDP tertiles had similar mortality risk, even when comparing the highest with the lowest tertile [HR = 1.08 (95% CI: 0.77–1.52); P-trend = 0.66]. After adjustment for

TABLE 1 Characteristics of study population at baseline (InCHIANTI study)[1]

Characteristic	All (n = 807)	Survived (n = 533)	Died (n = 274)	P^2
Age, *y*	74.3 ± 6.9	71.8 (5.3)	79.2 (7.2)	<0.001
Sex, *% female*	55.4	58.7	48.9	<0.001
BMI, *kg/m²*	27.5 ± 4.0	27.8 ± 3.9	27.0 ± 4.2	0.59
Education, y	5.4 ± 3.3	5.7 ± 3.3	4.6 ± 3.0	0.09
Energy intake, *kcal/d*	1910 ± 557	1930 ± 585	1880 ± 496	0.89
Alcohol, *g/d*	14.4 ± 20.2	15.1 ± 21.8	13.2 ± 16.5	0.11
Fruit and vegetable intake, *g/d*	450 ± 182	460 ± 182	430 ± 181	0.56
TUP, *mg GAE/d*	156 ± 63	163 ± 62	143 ± 63	0.07
TDP, *mg/d*	594 ± 196	600 ± 201	584 ± 185	0.64
Renal function, *%*				0.44
Normal	37.8	43.7	25.8	
Impaired	60.5	56.0	69.9	
Failure	1.7	0.4	4.3	
Smoking status, *%*				0.003
Never smoker	59.4	61.0	56.2	
Former smoker	26.9	26.8	27.0	
Current smoker	13.8	12.2	16.8	
Physical activity, *%*				<0.001
Sedentary	18.7	10.5	34.6	
Light	44.0	45.0	41.9	
Moderate to high	37.4	44.4	23.5	
CVDs, *%*	9.2	6.4	14.8	0.007
Diabetes mellitus, *%*	10.8	10.9	10.6	0.77
Cancer, *%*	6.4	6.6	6.2	0.23
Chronic obstructive pulmonary disease, *%*	2.8	1.7	4.9	0.030
Dementia, *%*	3.7	0.9	9.1	0.002
Parkinson disease, *%*	0.8	0.2	1.9	0.019

[1] Values are means ± SDs or percentages. CVD, cardiovascular disease; GAE, gallic acid equivalent; InCHIANTI, Invecchiare in Chianti; TDP, total dietary polyphenol; TUP, total urinary polyphenol.
[2] Generalized linear models were adjusted for age and sex.

multiple confounders including age, sex, education, BMI, smoking status, renal function, physical activity, and chronic diseases, participants in the highest TUP tertile had a lower mortality rate than those in the lowest tertile [HR = 0.70 (95% CI: 0.49–0.99); P-trend = 0.045]. In the multivariable model, no signification associations were found between TDP and overall mortality [highest vs. lowest tertile HR = 1.22 (95% CI: 0.85–1.76); P-trend = 0.31].

Discussion

To our knowledge, this is the first study to examine the association between TUP and TDP on all-cause mortality in a large, community–dwelling, older adult population. Our results suggest that older participants with low TUP concentrations are at higher risk of death, whereas no association was found for TDP.

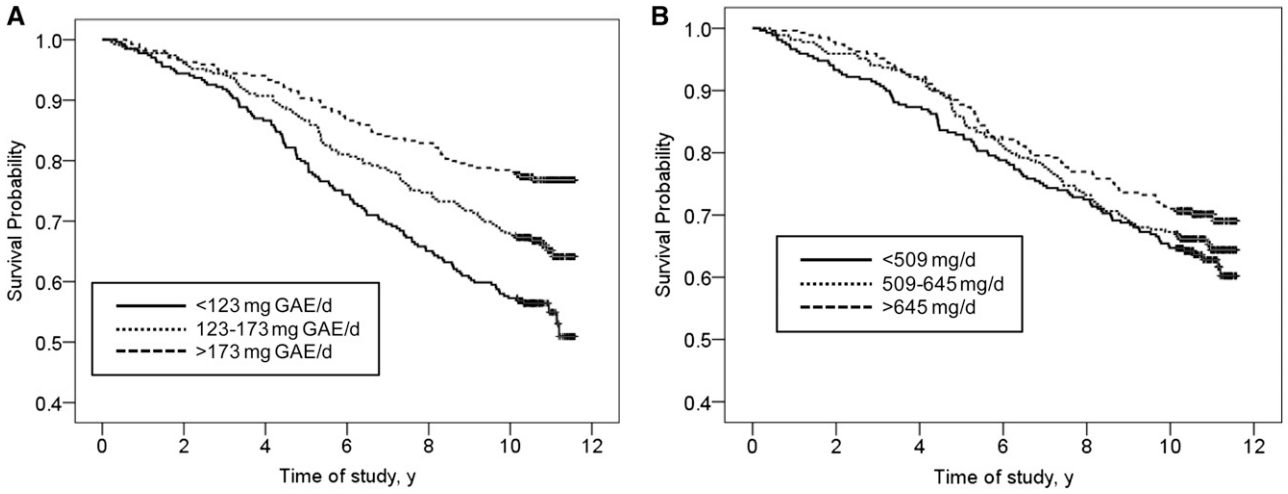

FIGURE 1 Kaplan-Meier plots of all-cause mortality for 12 y of follow-up in the InCHIANTI study by tertiles of TUPs (log-rank = 29.44; $P < 0.001$ by log-rank test between extreme tertiles) (*A*) and TDPs (log-rank = 3.30; $P = 0.19$) (*B*). GAE, gallic acid equivalent; InCHIANTI, Invecchiare in Chianti; TDP, total dietary polyphenol; TUP, total urinary polyphenol.

TABLE 2 Relationship between TUPs or TDPs and all-cause mortality in older participants (InCHIANTI study)[1]

	TUPs				TDPs			
	Cutoff	Survived/died	HR (95% CI)	*P* value	Cutoff	Survived/died	HR (95% CI)	*P* value
	mg GAE/d	*n*			*mg/d*	*n*		
Model 1[2]								
Tertile 1	<123	149/120	1 (ref)		<509	169/100	1 (ref)	
Tertile 2	123–173	177/92	0.70 (0.53–0.91)	0.009	509–645	176/93	0.90 (0.68–1.20)	0.47
Tertile 3	>173	207/62	0.44 (0.32–0.60)	<0.001	>645	188/91	0.76 (0.57–1.02)	0.07
P-trend[3]				<0.001				0.07
Model 2[4]								
Tertile 1	<123	149/120	1 (ref)		<509	169/100	1 (ref)	
Tertile 2	123–173	177/92	0.75 (0.57–0.98)	0.037	509–645	176/93	1.11 (0.82–1.49)	0.51
Tertile 3	>173	207/62	0.63 (0.46–0.87)	0.005	>645	188/91	1.08 (0.77–1.52)	0.65
P-trend[3]				0.004				0.66
Model 3[5]								
Tertile 1	<123	149/120	1 (ref)		<509	169/100	1 (ref)	
Tertile 2	123–173	177/92	0.89 (0.65–1.20)	0.43	509–645	176/93	1.18 (0.85–1.63)	0.33
Tertile 3	>173	207/62	0.70 (0.49–0.99)	0.045	>645	188/91	1.22 (0.85–1.76)	0.29
P-trend[3]				0.045				0.31

[1] CVD, cardiovascular disease; GAE, gallic acid equivalent; InCHIANTI, Invecchiare in Chianti; TDP, total dietary polyphenol; TUP, total urinary polyphenol.
[2] Model 1: unadjusted model.
[3] *P*-trend obtained by assigning the median of each tertile as scores.
[4] Model 2: adjusted for age (y) and sex, and energy intake (kcal/d) only for TDPs.
[5] Model 3: adjusted for age (y), sex, education (y of education), BMI (kg/m^2), alcohol intake (g/d), smoking status (never, former, current), renal function (normal, impaired, failure), physical activity (sedentary, light, moderate to high), CVD, diabetes, cancer, chronic obstructive pulmonary disease, dementia, Parkinson's disease, and energy intake (kcal/d) only for TDPs.

Polyphenol biomarkers have several advantages over dietary data collected using self-reported questionnaires (29). The main advantage of dietary biomarkers is that they provide an objective measure of exposure that is independent of many of the biases and errors associated with self-report methods (30). TUP has been validated as a biomarker of TDP in 2 different epidemiological studies (17,18). Moreover, the adapted Folin-Ciocalteu assay is an inexpensive, fast, and easy method for analyzing TUP concentrations, making it especially suitable for large epidemiological studies. In this study, high TUP concentrations were associated with a 30% reduction in all-cause mortality. To date, TUP concentrations analyzed by the adapted Folin-Ciocalteu assay have only been associated with a reduction in both systolic and diastolic blood pressure and with the prevalence of hypertension (19), which are well-known risk factors for CVD and therefore of mortality. Some epidemiological studies using biomarkers of individual polyphenol compounds, particularly phytoestrogens (isoflavones and lignans), have reported significant negative associations with breast, prostate, and colorectal cancer (31,32).

Several epidemiological studies have suggested that both polyphenol-rich foods and dietary polyphenol intake, particularly flavonoids, are inversely associated with chronic diseases, such as CVD (8–10), neurodegenerative diseases (11,12), and some cancers (9,13), although the evidence thus far remains inconclusive. Flavonoids have also been inversely associated with total (33) and CVD mortality (34,35); however, to our knowledge, no studies assessing the relationship between total polyphenols (measured by either questionnaire or biomarker) and all-cause mortality have been conducted to date.

TUP concentrations have also been positively associated with the intake of fruits and vegetables, as measured by both Folin-Ciocalteu assay (17) and liquid chromatography-MS (36,37). Therefore, the potential effects of high fruit and vegetable consumption could be partially explained by polyphenols. Epidemiological data have shown that people with a high consumption of fruit and vegetables are at a lower risk of several types of cancers (38), CVD (39), and overall mortality (1) compared with those with a low consumption. Indeed, comparable results have also been reported when the link between mortality and a greater adherence to a Mediterranean diet, which is a dietary pattern rich in fruits, vegetables, and nuts, was evaluated (40,41).

The underlying mechanisms by which high TUP concentrations can contribute to a reduction of all-cause mortality are still unknown but may be due to their cardiovascular-protective and anticarcinogenic effects, because CVDs and cancer are the 2 main causes of mortality in this section of the population (6,9). Several reviews have summarized the potential chemopreventive mechanisms of certain polyphenols, including their ability to modulate carcinogen metabolism (e.g., phase I and II metabolic enzymes), regulate inflammatory pathways (e.g., nuclear transcription factor $\kappa\beta$, cyclooxygenase-1, and cyclooxygenase-2), and inhibit cell proliferation and induce apoptosis (e.g., intracellular protein β-catenin) (4,14,42). Based on cell culture studies, polyphenols may positively affect critical steps in atherogenesis [for review, see (43,44)], including LDL oxidation, NO release, inflammation, oxidative stress, chemotaxis, cell adhesion, foam cell formation, smooth muscle cell proliferation, and platelet aggregation.

Our study has several limitations. TUP concentrations were measured only once, so we do not have information on intra- or inter-individual variability. Information on cause-specific mortality (CVD and cancer) was also not available. Although the specific causes of death were known in the InCHIANTI study, the ability to detect a relationship between TUP concentrations and specific causes of mortality may currently be limited because of the small sample size. Finally, our population cannot be considered to be representative of the general Italian population,

because our cohort is predominantly made up of elderly people and so may not be generalizable to the younger population. Nevertheless, our study has several strengths. This study is, to the best of our knowledge, the first population-based prospective study to assess the association between TUP concentrations and mortality in older participants. Moreover, we used TUP concentrations as a biomarker of TDP, which is a more reliable and accurate measure of intake (17,18). Moreover, food composition tables for polyphenols were not totally completed, so an underestimation of their intake is inevitable (18). Finally, our Cox proportional hazards models were adjusted for potentially important confounders, including age, sex, lifestyle factors, physical activity, energy intake, BMI, renal function, and chronic diseases.

In conclusion, the findings from our study suggest that high TUP concentrations are associated with reduced all-cause mortality in an elderly, free-living population, whereas no significant association was found using TDP. Further investigations are needed to confirm this protective association in other populations, especially younger people and different countries with higher dietary variability.

Acknowledgments

R.Z.-R., A.C., and C.A.-L. designed the research; M.R. and M. U.-S. conducted the laboratory analyses; R.Z.-R. performed the statistical analyses; A.C., S.B., L.F., and C.A.-L. contributed to the recruitment and data collection; and R.Z.-R. drafted the manuscript. All authors read and approved the final manuscript.

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