Original Investigation

Resveratrol Levels and All-Cause Mortality in Older Community-Dwelling Adults

Richard D. Semba, MD, MPH; Luigi Ferrucci, MD, PhD; Benedetta Bartali, PhD; Mireia Urpí-Sarda, PhD; Raul Zamora-Ros, PhD; Kai Sun, MS; Antonio Cherubini, MD, PhD; Stefania Bandinelli, MD; Cristina Andres-Lacueva, PhD

IMPORTANCE Resveratrol, a polyphenol found in grapes, red wine, chocolate, and certain berries and roots, is considered to have antioxidant, anti-inflammatory, and anticancer effects in humans and is related to longevity in some lower organisms.

OBJECTIVE To determine whether resveratrol levels achieved with diet are associated with inflammation, cancer, cardiovascular disease, and mortality in humans.

DESIGN Prospective cohort study, the Invecchiare in Chianti (InCHIANTI) Study ("Aging in the Chianti Region"), 1998 to 2009 conducted in 2 villages in the Chianti area in a population-based sample of 783 community-dwelling men and women 65 years or older.

EXPOSURES Twenty-four–hour urinary resveratrol metabolites.

MAIN OUTCOMES AND MEASURES Primary outcome measure was all-cause mortality. Secondary outcomes were markers of inflammation (serum C-reactive protein [CRP], interleukin [IL]-6, IL-1β, and tumor necrosis factor [TNF]) and prevalent and incident cancer and cardiovascular disease.

RESULTS Mean (95% CI) log total urinary resveratrol metabolite concentrations were 7.08 (6.69-7.48) nmol/g of creatinine. During 9 years of follow-up, 268 (34.3%) of the participants died. From the lowest to the highest quartile of baseline total urinary resveratrol metabolites, the proportion of participants who died from all causes was 34.4%, 31.6%, 33.5%, and 37.4%, respectively (P = .67). Participants in the lowest quartile had a hazards ratio for mortality of 0.80 (95% CI, 0.54-1.17) compared with those in the highest quartile of total urinary resveratrol in a multivariable Cox proportional hazards model that adjusted for potential confounders. Resveratrol levels were not significantly associated with serum CRP, IL-6, IL-1β, TNF, prevalent or incident cardiovascular disease, or cancer.

CONCLUSIONS AND RELEVANCE In older community-dwelling adults, total urinary resveratrol metabolite concentration was not associated with inflammatory markers, cardiovascular disease, or cancer or predictive of all-cause mortality. Resveratrol levels achieved with a Western diet did not have a substantial influence on health status and mortality risk of the population in this study.

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Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Richard D. Semba, MD, MPH, Department of Ophthalmology, The Johns Hopkins University School of Medicine, Smith Bldg, M015, 400 N Broadway, Baltimore, MD 21287 (rdsemba@jhmi.edu).

Resveratrol (3,5,4′-trihydroxystilbene), a polyphenol found in grapes, red wine, peanuts, chocolate, and certain berries and Asiatic plant roots, has been shown to exert anti-inflammatory effects in vitro and following supplementation in animal models[1,2] and to increase lifespan and health in mice fed a high-calorie diet.[3-6] Studies performed in animal models have shown that resveratrol and other chemically related compounds inhibit sirtuin 1 (SIRT1) and mimic the effects of caloric restriction.[6,7] In 1992, Siemann and Creasy[8] postulated that the cardioprotective effects of red wine could be attributed to resveratrol. The "French paradox," in which a low incidence of coronary heart disease occurs in the presence of a high dietary intake of cholesterol and saturated fat in France, has been attributed to the regular intake of red wine[9] and in particular, to resveratrol and other polyphenols contained in wine.[10]

Some preliminary evidence also suggests that resveratrol in humans may have anti-inflammatory effects, prevent cancer, diminish arterial stiffness, and improve endothelial reactivity in older women.[4-6] In a randomized trial of 20 healthy adults, plasma concentrations of C-reactive protein (CRP) and tumor necrosis factor (TNF) decreased by about one-third during 6 weeks of supplementation with a plant extract containing resveratrol.[11] In addition, peripheral blood mononuclear cell messenger RNA (mRNA) expression of interleukin (IL)-6 and TNF decreased in the group receiving resveratrol over the same intervention period. In a small crossover trial, a supplement containing resveratrol and polyphenols from muscadine grape suppressed the increase of IL-1β following a high-fat, high-carbohydrate meal.[12] A recent phase 2 study of SRT501, a micronized oral formulation of resveratrol that activates SIRT1, in multiple myeloma patients was halted early owing to a high level of adverse effects and renal failure.[13]

Although resveratrol has attracted a great deal of attention owing to its effects on inflammation, carcinogenesis, and longevity in vitro or in lower organisms, and in trials involving supraphysiologic doses of resveratrol in humans, there is little epidemiologic data to support a link between physiologic levels of resveratrol achieved with the diet alone and health in humans. Some of the challenges in studying resveratrol in humans are the rapid uptake, metabolism, and excretion of resveratrol and the low concentrations found in plasma.[4] Recently, mass spectrometric methods have been developed that allow insights into resveratrol metabolism in humans through the measurement of resveratrol metabolites in urine.[14] We hypothesized that higher urinary concentrations of resveratrol metabolites were associated with reduced risk of all-cause mortality and associated with lower inflammation and lower prevalence and incidence of cardiovascular disease and cancer. To test these hypotheses, we measured urinary metabolites of resveratrol in a population-based cohort study.

Methods

Study Population

The study participants were men and women, 65 years or older, who participated in the Invecchiare in Chianti, "Aging in the Chianti Area" (InCHIANTI) Study, conducted in 2 small towns in Tuscany, Italy. The rationale, design, and data collection methods have been described elsewhere,[15] and the main outcome of this longitudinal study is mobility disability. Briefly, in August 1998, 1270 people 65 years or older were randomly selected from the population registry of Greve in Chianti (population, 11 709) and Bagno a Ripoli (population, 4704), and of 1256 eligible subjects, 1155 (90.1%) agreed to participate. Participants received an extensive description of the study and participated after written, informed consent was obtained. 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 and by the Institutional Review Board of the Johns Hopkins University School of Medicine. InCHIANTI Study participants were evaluated for a 3-year follow-up visit from 2001 to 2003 (n = 926), a 6-year follow-up visit from 2004 to 2006 (n = 844), and 9-year follow-up visit from 2007 to 2009 (n = 768).

Data Collection and Definition

Data on demographic characteristics, lifestyle factors, and medication use were collected using standardized questionnaires. Smoking history was determined from self-report. Daily alcohol intake, expressed in grams per day, and resveratrol intake, expressed in milligrams per day, were determined at each study visit from participants answers to the European Prospective Investigation into Cancer and Nutrition (EPIC) food frequency questionnaire, which has been validated in the Italian population.[16] Education was recorded as number of years of school.

All participants were examined in a standardized manner by a study geriatrician. Diseases were ascertained according to standard, preestablished criteria and algorithms similar to those used in the Women's Health and Aging Study for diabetes mellitus, coronary heart disease, chronic heart failure, stroke, and cancer.[17] The algorithm for the diagnosis of diabetes was based on the use of insulin and oral hypoglycemic agents and on responses to a questionnaire administered to the primary care physician of the study participant.[17] Systolic and diastolic blood pressures were calculated as the mean of 3 measures taken with a standard mercury sphygmomanometer during the physical examination. Weight and height were measured using a high-precision mechanical scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The Mini-Mental State Examination (MMSE) was administered at enrollment, and an MMSE score lower than 24 was considered consistent with *cognitive impairment*.[18] *Chronic kidney disease* was defined as estimated glomerular filtration rate of less than 60 mL/min/1.73 m^2 using the 4-variable Chronic Kidney Disease-Epidemiology Collaboration equation of Levey and colleagues.[19]

Mortality data were collected using data from the Mortality General Registry maintained by the Tuscany Region. Analyses include those who refused to participate in the follow-up after baseline and those who moved away but were known to be alive at the time of censoring of this analysis. Causes of death were not available for all participants who died because cause-specific data have not yet been released by the Tuscany re-

gional authorities. Therefore, the analysis in the present study uses all-cause mortality.

Laboratory Studies

Twenty-four-hour urine samples were collected from participants at baseline. Urine samples were then aliquoted and immediately stored at −80°C. Of the 1155 adults 65 years or older who enrolled in the study, 783 had 24-hour urine samples available for measurements of resveratrol. Resveratrol conjugates derived from gut and microbial metabolism were measured in 24-hour urine samples using liquid chromatography–tandem mass spectrometry (LC-MS/MS).[14] Briefly 1 mL of urine with the internal standard was loaded into a previously equilibrated Oasis (Waters) HLB (hydrophilic-lipophilic-balanced) solid-phase extraction 96-well plate (30 mg). Urinary resveratrol metabolites were eluted with acidified methanol solution and ethyl acetate. After evaporation, the samples were reconstituted with 100 µL of the mobile phase and then analyzed by liquid chromatography (PerkinElmer S200) coupled to a triple-quadrupole mass spectrometer (API3000; Applied Biosystems) as described elsewhere.[14] The overall time taken per sample was about 14-minutes, including the cleanup by solid-phase extraction, optimized runtime by liquid chromatography, and mass spectrometry detection.[14] Intra-batch and inter-batch coefficients of variation were less than 10.5% and less than 10.7%, respectively. Because we were uncertain whether all the participants collected urine for a full 24-hour period, all results for urinary resveratrol metabolites were corrected for creatinine. Urinary creatinine was measured by the modified Jaffe method,[20] and results for 24-hour urinary resveratrol metabolites are reported as nanomoles per gram of creatinine.

Serum CRP, IL-6, IL-1β, and TNF were measured in duplicate by high-sensitivity enzyme-linked immunosorbent assays (ELISA) using commercial kits (BioSource International), as described in detail elsewhere.[21] Commercial enzymatic tests (Roche Diagnostics) were used for measuring serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol concentrations. Low-density lipoprotein (LDL) cholesterol level was calculated using the Friedewald formula.[22] Plasma glucose concentration was measured by the glucose oxidase method (Beckman Instruments Inc). Normal, impaired, and diabetic fasting glucose levels were set at fasting plasma glucose levels of 99 mg/dL or lower, 100 to 125 mg/dL, and higher than 125 mg/dL, respectively.[23]

Statistical Analysis

Variables are reported as means (SDs) or as percentages. Variables that were highly skewed (ie, markers of inflammation) were log-transformed to achieve a normal distribution. Characteristics of subjects were compared across quartiles of urinary resveratrol metabolites using Kruskal-Wallis tests for continuous variables and χ^2 tests for categorical variables. Spearman correlation was used to examine the relationship between alcohol intake and urinary resveratrol metabolites. Cox proportional hazards models were used to examine the relationship between quartile of total urinary resveratrol metabolites and all-cause mortality, incident cardiovascular disease, and incident cancer over 9 years of follow-up. Multivariable Cox proportional hazards models were adjusted for age, sex, BMI, and then other variables that were significant in the univariate analyses. All analyses were performed using SAS software, version 9.1.3 (SAS Institute) with a type I error of 0.05.

Results

Overall, mean (95% CI) log total urinary resveratrol metabolite concentrations were 7.08 (6.69-7.48) nmol/g of creatinine. Less than 1% of the study population reported using any type of nutritional supplement. The characteristics of the participants across quartiles of total urinary resveratrol metabolite concentrations are listed in **Table 1**. There were significantly more men in the highest quartiles of total urinary resveratrol metabolites. Alcohol consumption, current smoking, and physical activity were higher among participants in the highest quartile of total urinary resveratrol metabolites compared with the lower quartiles. The proportion of participants with abnormal fasting plasma glucose levels was significantly different across quartiles, with the highest proportion of subjects with diabetic fasting glucose levels in the lowest and highest quartiles. The proportion of participants with cognitive impairment (MMSE score <24) was significantly lower in the participants in the highest quartile of total urinary resveratrol metabolites. There were no significant differences across the quartiles of total urinary resveratrol metabolite concentrations by age, education, BMI, CRP, IL-6, IL-1β, TNF, mean arterial blood pressure, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, or by prevalence of hypertension, heart failure, peripheral artery disease, stroke, cancer, and chronic kidney disease. The prevalence of coronary artery disease and diabetes were higher among those in the lowest quartile of total urinary resveratrol metabolites. The Spearman correlation between alcohol consumption in grams per day and total urinary resveratrol metabolite concentrations was 0.67 ($P < .001$).

We compared the characteristics of the 782 participants with resveratrol measurements with the 273 participants who had no resveratrol measurements at baseline. The participants with no resveratrol measurements had a significantly higher proportion with cognitive impairment (MMSE score <24), stroke, lower physical activity, and mortality compared with participants who had resveratrol measurements. They also had lower levels of total cholesterol and higher levels of IL-1β and TNF compared with the participants who had resveratrol measurements. There were no significant differences in age, education, BMI, smoking, chronic disease, or other variables as, detailed in Table 1 between those with and without resveratrol measurements.

During 9 years of follow-up, 268 (34.2%) of the participants died. There were no significant differences in the proportion of participants who died across quartiles of total urinary resveratrol metabolite concentrations. The baseline characteristics of the participants by vital status during follow-up are listed in **Table 2**. Participants who died were older, more likely to be male, with lower education, lower BMI, physi-

Table 1. Baseline Characteristics of Study Participants by Quartiles of Total Urinary Resveratrol Metabolites[a]

Characteristic	Quartiles of Total Urinary Resveratrol Metabolites, nmol/g of Creatinine				*P* Value[b]
	<1554 (n = 195)	1554-4996 (n = 196)	>4996-15 010 (n = 196)	>15 010 (n = 196)	
Age, mean (SD), y	75.2 (7.5)	74.3 (7.0)	74.5 (7.0)	73.8 (6.2)	.52
Sex					
Male	28.7	33.2	48.2	68.7	<.001
Female	71.3	66.8	51.8	31.3	
Education, mean (SD), y	5.0 (2.6)	5.1 (3.1)	5.6 (3.8)	5.8 (3.4)	.14
Alcohol intake, mean (SD), g/d	2.4 (5.8)	8.8 (15.9)	16.3 (16.0)	31.1 (24.7)	<.001
Consumes alcohol	37.4	66.8	87.8	99.0	<.001
Current smoker	7.7	12.8	8.6	25.1	<.001
BMI, mean (SD)	27.5 (4.2)	27.7 (4.1)	27.5 (3.8)	27.3 (4.0)	.48
Physical activity					
Inactive	24.6	17.9	17.9	15.0	.002
Low	44.6	51.3	41.8	36.8	
Moderate-high	30.8	30.8	40.3	48.2	
Fasting plasma glucose					
Normal	72.8	80.1	75.1	69.7	.05
Impaired	15.9	12.8	18.8	15.9	
Diabetic	11.3	7.1	6.1	14.4	
Log, mean (SD)					
CRP, µg/mL	1.09 (1.07)	1.08 (0.98)	0.97 (1.04)	0.99 (1.01)	.52
IL-1β, pg/mL	−2.15 (1.30)	−2.19 (1.14)	−2.24 (1.22)	−2.25 (0.92)	.24
IL-6, pg/mL	1.08 (0.55)	1.06 (0.50)	1.16 (0.58)	1.17 (0.57)	.18
TNF, pg/mL	1.47 (0.59)	1.43 (0.52)	1.45 (0.54)	1.54 (0.78)	.59
Mean arterial BP, mm Hg	105 (11)	106 (11)	105 (10)	106 (12)	.78
Cholesterol, mean (SD), mg/dL					
Total	215 (37)	223 (41)	219 (42)	220 (38)	.28
HDL	56 (14)	56 (15)	55 (16)	58 (15)	.45
LDL	133 (31)	142 (36)	138 (37)	138 (34)	.16
Triglycerides	130 (79)	132 (63)	128 (73)	123 (57)	.20
MMSE score <24	32.8	31.1	31.0	16.4	<.001
Hypertension	48.2	50.5	44.2	47.7	.65
Coronary artery disease	6.7	1.5	7.1	3.6	.03
Heart failure	6.7	3.1	4.6	3.6	.32
Peripheral artery disease	6.2	4.6	4.1	7.7	.40
Stroke	4.1	3.1	5.6	3.1	.53
Diabetes mellitus	15.9	10.2	9.1	18.5	.02
Cancer	7.7	6.1	7.6	3.4	.30
Chronic kidney disease	27.2	23.0	20.3	16.4	.07
Died during follow-up, overall	34.4	31.6	33.5	37.4	.67

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); BP, blood pressure; CRP, C-reactive protein; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; MMSE, Mini-Mental State Examination[18]; TNF, tumor necrosis factor.

SI conversion factors: To convert ethanol to moles, multiply by 0.0217; total cholesterol, HDL cholesterol, and LDL cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113; CRP to micromoles per liter, multiply by 9.524; IL-6 to micromoles per liter, multiply by 0.131.

[a] Unless otherwise indicated, data are reported as percentage of participants.

[b] Kruskal-Wallis test for continuous variables and χ^2 test for categorical variables.

cally inactive, with diabetic fasting plasma glucose and higher CRP, IL-6, and TNF concentrations, higher mean arterial blood pressure, and lower total, HDL, and LDL cholesterol levels. A higher proportion of those who died had MMSE scores lower than 24, heart failure, peripheral artery disease, stroke, diabetes, and chronic kidney disease. There were no significant differences between participants who died during the study period and those who did not in alcohol intake, current smoking, gut resveratrol metabolites, microbial resveratrol metabolites, total urinary resveratrol metabolites, IL-1β, triglycerides, coronary artery disease, or cancer.

The relationship between total urinary resveratrol metabolites and all-cause mortality was examined using multivariable Cox proportional hazards models (**Table 3**). Total urinary resveratrol metabolites concentration was not significantly associated with mortality in models adjusting for age, sex, BMI, serum levels of lipids, chronic diseases, and other variables. The relationship between total urinary resveratrol metabolites and mortality did not change in additional models that included markers of inflammation in addition to the covariates used in the final models (data not shown). Sensitivity analyses were conducted to take into consideration mor-

Table 2. Baseline Characteristics of Study Participants by Vital Status During 9 Years of Follow-up[a]

Characteristic	Died (n = 268)	Alive (n = 515)	*P* Value[b]
Age, mean (SD), y	79.3 (7.2)	71.9 (5.3)	<.001
Sex			
Male	51.1	41.4	.01
Female	48.9	58.6	
Education, mean (SD), y	4.6 (2.9)	5.8 (3.4)	<.001
Alcohol intake, mean (SD), g/d	13.4 (16.7)	15.3 (21.6)	.91
Current smoker	16.0	12.2	.14
BMI, mean (SD)	26.9 (4.1)	27.8 (4.0)	.002
Physical activity			
Inactive	34.6	1.7	<.001
Low	41.0	45.0	
Moderate-high	24.4	44.3	
Fasting plasma glucose			
Normal	72.0	75.7	.04
Impaired	14.6	16.5	
Diabetic	13.4	7.8	
Log, mean (SD)			
Gut RMs, nmol/g of creatinine	2.29 (8.93)	1.51 (9.06)	.08
Microbial RMs, nmol/g of creatinine	5.72 (7.16)	6.59 (5.90)	.91
Total urinary RMs, nmol/g of creatinine	6.69 (6.40)	7.29 (5.21)	.51
CRP, µg/mL	1.23 (1.10)	0.92 (0.97)	<.001
IL-1β, pg/mL	−0.28 (1.21)	−2.17 (1.12)	.13
IL-6, pg/mL	1.30 (0.59)	1.02 (0.51)	<.001
TNF, pg/mL	1.57 (0.58)	1.43 (0.63)	<.001
Arterial BP, mean (SD), mm Hg	107 (11)	105 (11)	.01
Cholesterol, mean (SD), mg/dL			
Total	211 (41)	224 (38)	<.001
HDL	55 (17)	56 (14)	.07
LDL	130 (35)	141 (34)	<.001
Triglycerides	129 (70)	128 (68)	.64
MMSE score <24	41.8	20.6	<.001
Hypertension	50.8	46.0	.21
Coronary artery disease	4.9	4.7	.91
Heart failure	9.0	2.1	<.001
Peripheral artery disease	12.7	1.9	<.001
Stroke	7.8	1.9	<.001
Diabetes mellitus	16.8	11.7	.05
Cancer	7.8	5.4	.19
Chronic kidney disease	31.3	16.7	<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); BP, blood pressure; CRP, C-reactive protein; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein; MMSE, Mini-Mental State Examination[18]; RMs, resveratrol metabolites; TNF, tumor necrosis factor.

SI conversion factors: To convert ethanol to moles, multiply by 0.0217; total cholesterol, HDL cholesterol, and LDL cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113; CRP to micromoles per liter, multiply by 9.524; IL-6 to micromoles per liter, multiply by 0.131.

[a] Unless otherwise indicated, data are reported as percentage of participants.

[b] Kruskal-Wallis test for continuous variables and χ^2 test for categorical variables.

tality in the first year following resveratrol measurements, because those who died within the first year may have been ill at the time of resveratrol measurements, and potential effects of excessive alcohol consumption, since resveratrol was strongly associated with alcohol intake, as shown previously. In an alternative analysis, 12 participants who died within 1 year of enrollment were excluded. Total urinary resveratrol metabolites were not significantly related to all-cause mortality in multivariable Cox proportional hazards models adjusting for the same covariates as the models in Table 3 (data not shown). In another analysis, 40 participants were exclude because they consumed more than 4 drinks (>56 g or ethanol) per day (1 drink = 14 g of ethanol). Total urinary resveratrol metabolites were not significantly related to all-cause mortality in multivariable Cox proportional hazards models adjusting for the same covariates as the models in Table 3 (data not shown).

To corroborate the relationship between urinary resveratrol metabolites and all-cause mortality further, we also examined the relationship of dietary intake of resveratrol with all-cause mortality. In the 783 participants, the mean (95% CI) of log dietary intake of resveratrol in was −2.42 (−2.55 to −2.28) mg/d. The Spearman correlation between dietary intake of resveratrol and total resveratrol metabolites was 0.67 ($P < .001$). The relationship between dietary intake of resveratrol and all-cause mortality was examined using a multivariable Cox proportional hazards model with the same covariates as in the fi-

Table 3. Relationship Between Total Urinary Resveratrol Metabolites and All-Cause Mortality in Separate Multivariable Cox Proportional Hazards Models

Covariates in Models	Quartiles of Total Urinary Resveratrol Metabolites, nmol/g creatinine[a]				*P* Value[b]
	<1554	1554-4996	>4996-15 010	>15 010	
Age, sex	0.83 (0.58-1.17)	0.95 (0.67-1.36)	0.75 (0.53-1.05)	1 [Referent]	.55
Age, sex, education, BMI, physical activity, total cholesterol, HDL cholesterol, MMSE score	0.74 (0.51-1.08)	0.90 (0.62-1.30)	0.71 (0.49-1.02)	1 [Referent]	.30
Age, sex, education, BMI, physical activity, total cholesterol, HDL cholesterol, MMSE score, mean arterial BP, and chronic diseases[c]	0.80 (0.54-1.17)	1.03 (0.70-1.51)	0.84 (0.58-1.22)	1 [Referent]	.43

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); BP, blood pressure; HDL, high-density lipoprotein; MMSE, Mini-Mental State Examination.[18]

SI conversion factors: To convert ethanol to moles, multiply by 0.0217; total cholesterol, HDL cholesterol, and LDL cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113; CRP to micromoles per liter, multiply by 9.524; IL-6 to micromoles per liter, multiply by 0.131.

[a] Unless otherwise indicated, data are reported as hazards ratios (95% CIs) for each quartile of urinary resveratrol metabolites relative to the highest quartile (Referent).

[b] For trend across quartiles.

[c] Chronic diseases include coronary heart disease, heart failure, stroke, peripheral artery disease, diabetes, cancer, and chronic kidney disease.

nal model in Table 3. Compared with the highest quartile of resveratrol intake, the hazard ratios (95% CIs) for all-cause mortality in the lowest (first), second, and third quartiles of resveratrol were 1.17 (0.75-1.81), 1.29 (0.84-1.99), and 1.42 (0.97-2.09), respectively, after adjusting for age, sex, education, BMI, physical activity, total energy intake, total cholesterol, HDL cholesterol, MMSE score, mean arterial blood pressure, and chronic diseases.

Since dietary intake of resveratrol could change over time, we assessed dietary resveratrol intake in 608, 517, and 434 participants seen at the 3-, 6-, and 9-year follow-up visits, respectively. The mean (95% CI) log findings of dietary intake of resveratrol at the 3-, 6-, and 9-year follow-up visits were −2.71 (−2.87 to −2.55), −2.82 (−2.99 to −2.64), and −2.66 (−2.81 to −2.50) mg/d, respectively. The intra-class correlation of dietary intake of resveratrol over the 9-year follow-up period was 0.52. The Spearman correlations between dietary resveratrol intake at the 3-, 6-, and 9-year follow-up visits and urinary resveratrol concentrations at baseline were 0.59 ($P < .001$), 0.56 ($P < .001$), and 0.45 ($P < .001$), respectively.

Of 639 participants who were free of cardiovascular disease at enrollment, 174 (27.2%) developed cardiovascular disease during follow-up. The proportions of participants with incident cardiovascular disease from the lowest to the highest quartiles of resveratrol were 22.3%, 29.6%, 28.4%, and 28.0%, respectively ($P = .44$). Of 734 participants who were free of cancer at enrollment, 34 (4.6%) developed cancer during follow-up. The proportions of participants with incident cancer from the lowest to the highest quartiles of resveratrol were 4.4%, 4.9%, 5.0%, and 4.3%, respectively ($P = .98$).

Discussion

The present study shows, contrary to all our hypotheses, that urinary resveratrol metabolites are not predictive of longevity in older community-dwelling adults and, in addition, are not significantly associated with markers of inflammation, prevalent or incident cardiovascular disease, or cancer. Resveratrol levels achieved with the diet do not show any apparent protective association with disease and markers of disease in humans and are not associated with lifespan. To our knowledge, this is the first large, observational epidemiologic study to examine the relationship between urinary resveratrol from normal dietary intake and health outcomes in humans. To our knowledge, no urinary resveratrol concentration has been established as an effective threshold in humans. Therefore, we analyzed urinary resveratrol concentrations as quartiles, as has been done in a previous study of resveratrol and health outcomes.[24]

The strengths of the study are the population-based sampling, the strict criteria for assessment of chronic diseases in the cohort, low use of nutritional supplements (<1%), the measurement of multiple biomarkers for inflammation, the high follow-up rates, and the availability of 9 years of data from monitoring of vital events. In addition, urinary resveratrol metabolites were measured in urine samples that had been collected over a 24-hour period. Urinary resveratrol levels in the present study are similar to those reported elsewhere.[25] The lack of an association between resveratrol, health, and longevity might be owing to variability in resveratrol intake in a population that has a large variability in exposure to resveratrol, interindividual variation, and variability of host-gut microbiota,[26,27] which might imply that a much larger sample size would be needed to detect the association.

Although annual sales of resveratrol supplements have reached $30 million in the United States alone,[28] there is limited and conflicting human clinical data demonstrating any metabolic benefits of resveratrol, and there are no data concerning its safety in high doses or for long-term supplementation in older people, who often have multiple comorbidities for which they are taking multiple medications. Supraphysiologic doses of resveratrol (100 mg) from Asiatic roots have been shown to decrease circulating levels or expression of CRP, IL-6, TNF, and IL-1β in human trials.[11,12] In addition, recent trials show that supplementation for 1 year with lower doses of resveratrol (8 mg) decreased levels of CRP, TNF-α, IL-6/IL-10 ratio and increased levels of the anti-inflammatory cytokine IL-10 in 75 subjects with cardiovascular risk factors.[29] Another study of resveratrol, 150 mg/d ad-

ministered for 30 days to healthy obese men, did not find significant changes to circulating CRP, IL-6, IL-1β, and IL-18, but there was a reduction in TNF concentrations.[30]

In the present study of community-dwelling older adults, there were no significant associations between urinary resveratrol metabolites and serum CRP, IL-6, IL-1β, or TNF. A previous study showed that resveratrol supplementation decreased fasting plasma glucose levels in adults with type 2 diabetes[31] and in healthy obese men.[30] Resveratrol supplementation decreased LDL cholesterol levels in patients recovering from myocardial infarction.[32] In 75 patients with cardiovascular disease, supplementation with grape extract for 6 months decreased oxidized LDL and ApoB levels.[33] In the present study, there was no significant relationship between urinary resveratrol metabolites and total cholesterol, HDL cholesterol, LDL cholesterol, or triglycerides.

On the other hand, there are trials with resveratrol that reported negative results. A trial of resveratrol-enriched grape extract supplementation for 1 year in hypertensive men with type 2 diabetes mellitus showed no impact of resveratrol on blood pressure, glucose, and lipids, but there was a significant reduction in serum IL-6 and alkaline phosphatase levels and reduction of the expression of proinflammatory cytokines CCL3, IL-1β, and TNF.[34] Resveratrol supplementation did not change body composition, resting metabolic rate, plasma lipids, or inflammatory markers in a randomized, double-blind, placebo-controlled trial in nonobese women with normal glucose tolerance.[33] In addition, resveratrol did not affect its putative molecular targets, including SIRT1, in either skeletal muscle or adipose tissue. In a randomized, double-blind, placebo-controlled trial, high-dose resveratrol supplementation had no effect on glucose metabolism, insulin sensitivity, resting energy expenditure, or inflammatory markers in obese men.[35]

Resveratrol is only one of many polyphenols that are found in red wine and grapes. In the present study, urinary resveratrol levels were significantly associated with alcohol intake. The study population is located in the wine-growing Chianti region of Tuscany. The moderately high correlation between alcohol intake and urinary resveratrol is most likely attributed to a correlation between wine intake and resveratrol. A previous study has shown that urinary resveratrol levels are a valid biomarker of wine consumption.[25] Human studies of the oral absorption of ^{14}C-resveratrol show that the elimination half-life of total resveratrol metabolites is about 6 to 15 hours after oral doses.[36] Resveratrol metabolites can be detected in the urine of humans who consume 1 glass of wine per week if the last drink was consumed 3 days previously, or in those who consume 3 glasses of wine per week if the last drink was consumed 5 days previously.[25]

Conclusions

In conclusion, this prospective study of nearly 800 older community-dwelling adults shows no association between urinary resveratrol metabolites and longevity. This study suggests that dietary resveratrol from Western diets in community-dwelling older adults does not have a substantial influence on inflammation, cardiovascular disease, cancer, or longevity.

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Author Affiliations: Department of Ophthalmology, The Johns Hopkins University School of Medicine, Baltimore, Maryland (Semba, Sun); Longitudinal Studies Section, National Institute on Aging, Baltimore, Maryland (Ferrucci); New England Research Institute, Boston, Massachusetts (Bartali); Nutrition and Food Science Department, Biomarkers and Nutrimetabolomics Laboratory, Food Technology Reference Net and Nutrition and Food Safety Research Institute (XaRTA and INSA), Pharmacy School, University of Barcelona, Barcelona, Spain (Urpí-Sarda, Zamora-Ros, Andres-Lacueva); Unit of Nutrition, Environment and Cancer, Cancer Epidemiology and Research Program, Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain (Urpí-Sarda, Zamora-Ros, Andres-Lacueva); Geriatrics and Geriatric Emergency Department, Istituto Nazionale di Riposo e Cura per Anziani V.E.II.–Istituto di Ricovero e Cura a Carattere Scientifico (INRCA-IRCCS), Ancona, Italy (Cherubini); Azienda Sanitaria, Florence, Italy (Bandinelli).

Author Contributions: Dr Semba had full access to all of the data in the study and takes responsibility for the integrity and accuracy of the data analyses.
Study concept and design: Semba, Ferrucci, Cherubini, Andres-Lacueva.
Acquisition, analysis, or interpretation of data: Semba, Ferrucci, Bartali, Urpí-Sarda, Zamora-Ros, Sun, Cherubini, Bandinelli, Andres-Lacueva.
Drafting of the manuscript: Semba, Cherubini, Andres-Lacueva.
Critical revision of the manuscript for important intellectual content: Ferrucci, Bartali, Urpí-Sarda, Zamora-Ros, Sun, Cherubini, Bandinelli, Andres-Lacueva.
Statistical analysis: Sun.
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Study supervision: Ferrucci, Andres-Lacueva.

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