Association of habitual dietary resveratrol exposure with the development of frailty in older age: the InCHIANTI Study[1-4]

Montserrat Rabassa, Raul Zamora-Ros, Mireia Urpi-Sarda, Stefania Bandinelli, Luigi Ferrucci, Cristina Andres-Lacueva, Antonio Cherubini

[1]From the Biomarkers and Nutrimetabolomics Laboratory, Nutrition and Food Science Department, XaRTA, INSA, Campus Torribera, Pharmacy Faculty, University of Barcelona, Spain (MR, CA-L, MU-S); Biomarkers group (BMA), Nutrition and Metabolism Section (NME), International Agency for Research on Cancer (IARC), Lyon, France (RZ-R); Geriatric Rehabilitation Unit, Azienda Sanitaria Firenze, Florence, Italy (SB); Clinical Research Branch, National Institute on Aging, NIH, Baltimore, Maryland, United States (LF); Geriatrics and Geriatric Emergency Care, Italian National Research Centre on Aging (INRCA), Ancona, Italy (AC).
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[3]Reprints not available. Address correspondence to C Andres-Lacueva, Biomarkers and Nutrimetabolomic Laboratory, Nutrition and Food Science Department, Campus Torribera, Pharmacy Faculty, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain. E-mail: candres@ub.edu. Phone: +34.934034840.
[4]Abbreviations used: ADL, Activities of Daily Living; BMI, body mass index; CES-D, Center for Epidemiologic Studies Depression scale; EPIC, European prospective study into cancer and nutrition study; FFQ, food frequency questionnaire; FS, frailty syndrome; hsCRP, high-sensitivity C-reactive protein; IADL, Instrumental Activities of Daily Living; IL-1ra, Interleukin-1 receptor antagonist; IL-6, Interleukin-6; InCHIANTI, *Invecchiare in Chianti*; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MMSE, Mini-Mental State Examination; SPE, solid-phase extraction; TDR, total dietary resveratrol; TNF-α, Tumor necrosis factor-α; TUR, total urinary resveratrol; SIRT1, sirtuin 1.
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Abstract

Background

Resveratrol may play a protective role against the frailty syndrome (FS) due to its antioxidant and anti-inflammatory properties.

Objective

Our objective was to prospectively evaluate the association between habitual dietary resveratrol exposure and the development of FS after three-, six- and nine-year follow-up periods in a community-dwelling older population.

Design

We conducted a longitudinal analysis using data from 769 participants aged ≥65 years from the InCHIANTI study. Total dietary resveratrol (TDR) intake was estimated at baseline with a validated food frequency questionnaire, which was developed to assess participants' usual food intake over the previous year, and an ad hoc resveratrol database. Total urinary resveratrol (TUR) was analysed by liquid chromatography-tandem mass spectrometry with a previous solid phase extraction at baseline. The combination of both measures (TDR+TUR) was computed using the Howe's method. FS was assessed at baseline and at three-, six-, and nine-year follow-up and was defined as the presence of at least three of the following five criteria: shrinking, exhaustion, sedentariness, slowness and weakness.

Results

TDR+TUR levels were inversely associated with FS risk over three-year follow-up [odds ratio comparing extreme tertiles = 0.11; 95% confidence interval = 0.03–0.45; *P*-trend = 0.002], but not after six- and nine-years of follow-up, in multinomial logistic regression models adjusted for baseline frailty status and potential confounders. These results did not differ when analyses were additionally adjusted for inflammatory markers.

Conclusion

A higher habitual dietary resveratrol exposure was associated with a lower risk of older community-dwellers developing FS during the first three years of follow-up, but not after longer follow-up periods.

INTRODUCTION

Frailty is a geriatric syndrome characterized by a decrease in both physiological reserve and resistance to stressors that embodies an elevated risk of illness, disability, hospitalization, institutionalization, and mortality (1-3). The clinical phenotype of frailty is characterized by an unintentional weight loss, global muscle weakness, exhaustion or poor endurance, slowed performance, and low physical activity (4). Studies have suggested that oxidative stress and chronic inflammation are key factors in the causal link between aging and the development of frailty syndrome (FS) (5, 6).

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) and its glycoside piceid (3,5,4'-trihydroxystilbene-3-*O*-β-D-glucopyranoside) are bioactive compounds mainly present in grapes and red wine, and in very low concentrations in peanuts, pistachios, berries, tomatoes, chocolate, apple and beer (7-11). Several studies have shown the potential effects of resveratrol against aging-related diseases through the activation of sirtuins, mimicking the benefits of energy restriction (12), as well as its antioxidant and anti-inflammatory activities (13). To the best of our knowledge, there is no epidemiological evidence linking resveratrol with FS, although two recent randomized clinical trials have shown protective effects of resveratrol on markers of inflammation and oxidative

stress, such as reactive oxygen species, total antioxidant capacity, C-reactive protein (CRP) and tumour necrosis factor-alpha (TNF-α) in healthy subjects (14-15).
Although cognitive impairment is not included in the phenotypic definition of FS, its prevalence in frail subjects varies between 20% and 50% in the literature (6). For that reason, the use of self-reported dietary questionnaires in cognitively impaired participants may be a limitation, thus the use of nutritional biomarkers is essential to confirm and reinforce the results obtained using questionnaires. Recently, a mass-spectrometric methodology has been improved to fully characterize the resveratrol metabolome in humans through the measurement of total urinary resveratrol (TUR) (16). In epidemiologic studies, TUR has been validated as a biomarker of habitual dietary resveratrol and wine intake (17-18). In the studies of diet-disease associations, the combination of both measures provides higher statistical power of the true exposure than each method used separately (19).
In the current prospective study, we examined the association between the risk of FS and each of its five criteria at the three-, six- and nine-year follow-up, and the habitual dietary resveratrol exposure measured at baseline, using a validated food frequency questionnaire (FFQ), an objective dietary biomarker, and a combination of both measures, in older adults from the InCHIANTI (*Invecchiare in Chianti*, Aging in Chianti) study.

SUBJECTS AND METHODS

Study population

The InCHIANTI study is a cohort study of risk factors for late-life disability conducted in Bagno a Ripoli and Greve in Chianti, two municipalities adjacent to the city of Florence (Italy). A detailed description of the study rationale, design, and methods has been provided elsewhere (20). Briefly, 1299 participants aged ≥65 years were randomly selected from the population registries, using a multistage stratified sampling method. Because 39 subjects had died or emigrated, 1260 subjects were eligible, of whom 1155 participants agreed to take part in the study. The participation was 91.7%. The present study is based on data collected at baseline (1998–2000) and after the three- (2001–2003), six- (2004–2006) and nine-year (2007–2009) follow-up periods. Finally, 769 participants had both measures of resveratrol exposure (biomarker and FFQ data) and frailty data available at baseline, of whom 529 (68.8%), 442 (57.5%), and 322 (41.9%) completed the frailty assessment at three-, six-, and nine-year follow-up visits, respectively. The main reasons for unavailability of data at three-year follow-up were participation refusal (8.1%) and death (7.2%), whereas at six- and nine-year follow-up it was death, accounting for 19.8% and 30.8% of the total initial cohort, respectively (**Figure 1**). The National Institute of Research and Care of Aging ethical committee approved the study protocol, and all participants signed an informed consent.

Assessment of resveratrol exposure

Twenty-four-hour urine samples were collected from participants at baseline. Urine samples were aliquoted, coded and stored at –80 ºC until analysis. TUR was extracted by solid-phase extraction (SPE) and analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described (16, 21). Briefly, 1 mL of urine with the internal standard was loaded into a preconditioned Waters Oasis® HLB 96-well plate for SPE (Milford, Massachusetts, USA). TUR was eluted with acidified methanol solution and ethyl acetate after washing the plate. After evaporation to dryness, the samples were reconstituted with 100 μL of the mobile phase and then analysed in the LC-MS/MS system. TUR was calculated as the sum of both total individual metabolites

from phase-II enzymes (17) and total dihydro-resveratrol metabolites, which are produced by intestinal microbiota (16, 22) and was expressed as 24-hour volume (nmol/24h).
Total dietary resveratrol (TDR) intake was assessed at baseline using the Italian version of the FFQ developed and validated in the European Prospective Study into Cancer and Nutrition (EPIC) study (23) and an ad hoc food composition database on resveratrol (7, 8). TDR intake was computed as the sum of *trans*- and *cis*-resveratrol, and *trans*- and *cis*-piceid (7), and was reported as mg/d.

Assessment of frailty syndrome
FS was defined according to five-component criteria proposed by Fried and colleagues (4): shrinking or unintended weight loss, self-reported exhaustion, muscle weakness or poor grip strength, slowness or slow walking speed, and low physical activity or sedentariness. Each criterion was operationalized using previously published methods (24). Shrinking was defined as self-reported weight loss of more than 4.5 kg within the past year for reasons other than dieting. Exhaustion was defined as a response of 'occasionally (3–4 days in the last week)' or 'often or always (5–7 days in the last week)' to the statement 'I felt that everything was an effort'. Muscle weakness at baseline was defined as grip strength in the lowest quintile, stratified by sex and body mass index (BMI) quartiles, and at follow-up visit was determined using the baseline cut-off points. Grip strength was measured using a handheld dynamometer (Nicholas Muscle Tester, Sammon Preston, Inc., Chicago, IL) using a standard method. Slowness at baseline was defined as the time to walk 4.57 m or 15 feet (the mean of two repetitions) in the slowest quintile, stratified by sex and standing height. Slow walking speed at follow-up visit was determined using the baseline cut-off points. Sedentariness was defined as either complete inactivity or spending less than 1 h/wk performing low-intensity activities. Frailty, pre-frailty and robustness were defined as the presence of at least three, one or two, and zero of the five-component criteria, respectively (25).

Baseline covariates assessment
Trained geriatricians conducted a comprehensive assessment of health, functional status and anthropometrical measures using standardized methods. BMI was calculated as weight/height^2 (kg/m^2). Smoking habit, based on self-report, was classified as 'current smokers' and 'non-smokers' (including former and never smoker). Level of education was recorded as years of education. Wine (g/d) consumption was assessed using the validated FFQ (23). Total energy (kcal/d) and alcohol (g/d) intakes were calculated using an Italian food composition database (26). Global cognitive performance was assessed by the Mini-Mental State Examination (MMSE). Participants with an MMSE score <24 were considered cognitively impaired (27). Depressive symptoms were evaluated using the Center for Epidemiologic Studies Depression Scale (CES-D). A CES-D score ≥16 was defined as depressed mood (28). Functional status was assessed using the Activities of Daily Living scale (ADL) (29) and the Instrumental Activities of Daily Living scale (IADL) (30). Comorbidities were ascertained combining information from self-reported, medical records, and clinical examinations (31). Specific comorbidities considered in this analysis were angina pectoris, myocardial infarction, hypertension, congestive heart failure, stroke, peripheral arterial disease, diabetes, osteoarthritis, cancer, renal disease, and chronic obstructive pulmonary disease. Comorbidities were defined as the presence of at least one chronic disease.
Inflammatory markers were measured in serum samples. Interleukin (IL)-6 and IL-1 receptor antagonist (IL-1ra) were measured by high-sensitivity enzyme-linked

immunoabsorbent assays (ELISAs) using commercial kits (BIOSOURCE International Inc., Camarillo, CA). TNF-α was measured using multiplex technology (Human Serum Adipokine Panel B LINCOplex kit; Linco Research, Inc., St. Charles, MO). The high-sensitivity C-reactive protein (hsCRP) was measured by an ELISA colorimetric competitive immunoassay that used purified protein and polyclonal anti-CRP antibodies (20, 32).

Statistical analysis

Habitual dietary resveratrol exposure was assessed using: TDR, TUR, and the combination of both measures TDR+TUR in a score according to Howe's method (19). Briefly, participants were ranked from lowest to highest value for TDR intake and for TUR concentrations, and the two ranks were then summed.

Descriptive analyses of the baseline characteristics across habitual dietary resveratrol exposure tertiles were assessed by using age- and sex-adjusted generalized linear models. Spearman's rank correlation analyses were performed to examine the relationship between dietary resveratrol exposure and alcohol and wine consumption.

Multinomial logistic regression models were used to estimate ORs and 95% CIs between FS (using robust as the reference category) and habitual dietary resveratrol exposure tertiles. A parsimonious approach was used, and therefore only confounding variables associated ($P < 0.10$) with FS and habitual dietary resveratrol exposure in univariate analyses were considered as covariates for adjusting in the subsequent multivariate models. Three separate models were presented: the unadjusted one (model 1), the model 2 adjusted for status of frailty (or its components) at baseline, age, sex, study municipality, education, BMI, total energy intake (except in TUR), smoking status, comorbidities, depression mood and cognitive impairment, and the model 3 additionally adjusted for inflammatory markers (IL–6, TNF–α, hsCRP and IL–1RA). The last model was used to evaluate the mediation effect of inflammation in the associations between dietary resveratrol exposure and FS. Moreover, we estimated the percentage of excess risk mediated by inflammatory markers for FS using inverse ORs of the dietary resveratrol exposure as a continuous variable (33).

Logistic regression models were used to evaluate the association between each of the five criteria of FS and TDR, TUR, and TDR+TUR. These risk estimates were similar; thus only data from TDR+TUR models are presented. TDR and TDR+TUR were continuously analysed as log-transformed variables and TUR after a Box–Cox transformation (α=0.00001; λ=0.25) since they were not normally distributed. TDR, TUR and TDR+TUR were also analysed as tertiles. Tests for linear trend were performed by considering the median of each tertile as an ordinal variable for TDR and TUR. For TDR+TUR score, the linear trend was performed by treating the tertiles as a continuous variable in the model. Interactions between TDR+TUR and sex, age, BMI and cognitive impairment in relation to FS were tested by including product terms in fully adjusted multinomial logistic regression models. In the sensitivity analysis, we excluded heavy drinkers (>56g/d of alcohol) (34) in multinomial logistic regression models. There was no evidence of collinearity. Repeated measures general linear models were used to assess changes in log-transformed TDR intakes over time. All statistical tests were two-tailed, and the significance levels were $P < 0.05$, and were conducted with the SPSS software (version 18.0; SPSS Inc, Chicago, IL).

RESULTS

The main characteristics of the study population according to TDR+TUR tertiles at baseline, adjusted for age and sex, are reported in **Table 1**. The mean ± SD age of the

cohort at baseline was 72.7 ± 5.8 years, with 55.4% of the population being women. Participants in the highest TDR+TUR tertile were more likely to be younger and men than those in the lowest tertile. Participants in the top TDR+TUR tertile were more likely to be located in the wine-growing Chianti municipality, and to have a higher total energy intake than those in the bottom tertile. The proportion of participants with IADL disability tended to decrease progressively with increasing TDR+TUR tertiles. There were no significant differences across the dietary resveratrol exposure by BMI, current smokers, comorbidities, cognitive impairment and ADL disabilities, whereas there were significant differences across the TDR and TUR tertiles by sex, total energy intake, depressed mood and IADL disabilities (**Supplemental tables 1 and 2**).

Frail participants at the three-year follow-up were older, had a higher BMI, and had a lower habitual dietary resveratrol exposure (TDR), and had a higher frequency of depressed mood and ADL and IADL disabilities than non-frail participants (data not shown). The most common frailty criterion for frail participants at the three-year follow-up period was slowness and at the six- and nine-year follow-up visits it was sedentariness. TDR+TUR was significantly correlated with TDR (rho=0.925; $P<0.0001$), TUR (rho=0.924; $P<0.0001$), wine (rho=0.908; $P<0.0001$) and alcohol (rho=0.907; $P<0.0001$) consumption at the three-year follow-up visit. TDR and TUR were statistically correlated with each other (rho=0.713; $P<0.0001$).

In comparison to subjects excluded from this study due to incomplete data (n=626) at three-year follow-up, those included were younger (72.7 ± 5.7 vs 77.8 ± 8.2, $P<0.001$), had higher education (5.6 ±3.3 vs 5.0 ±3.3, $P=0.001$), lower rates of ADL and IADL disability (1.5% vs 17.3%, 11.9% vs 41.9%, respectively, $P<0.001$) and had lower prevalence of depressed mood (28.0% vs 38.5% , $P<0.001$) and cognitive impairment (21.2% vs 43.0%, $P<0.001$). Subjects excluded at six- and nine-year follow-up (n=713 and n=833) were older (77.7 ± 8.0 and 77.3 ± 7.8, $P<0.001$, respectively). ADL and IADL disability rates were significantly much lower among those included at the six- and nine-years follow-up (15.7% vs 0.9% and 13.7% vs 0.6%, and 39.6% vs 9.7% and 37.0% vs 5.3%), respectively. The prevalence of depressed mood and cognitive impairment was significantly lower in subjects included at six- and nine-years of follow-up (25.6% vs 38.8% and 23.6% vs 37.5%, and 18.6% vs 41.9%and 11.8% vs 41.2%, respectively).

The associations between habitual dietary resveratrol exposure measures at baseline and FS over the three-year follow-up are shown in **Table 2**. In unadjusted multinomial logistic regression models (model 1), participants in the highest tertile of all measures of habitual dietary resveratrol exposure (TDR, TUR, and TDR+TUR) had a lower risk of developing FS than those in the lowest tertile. The strength of these associations remained after adjustment for FS at baseline and potential confounders (model 2) for TDR (OR = 0.17; 95% CI = 0.05, 0.63; P trend = 0.02), TUR (OR = 0.32; 95% CI = 0.09, 1.11; P trend = 0.03), and TDR+TUR (OR = 0.11; 95% CI = 0.03, 0.45; P trend = 0.002). After the adjustment for all inflammatory markers (model 3), inverse associations between resveratrol exposure and FS risk were almost identical and remained statistically significant (**Table 2**). The percentages of excess risk mediated by inflammatory markers for TDR, TUR and TDR+TUR after three-year follow-up were only 0, 4.5 and 2.9%, respectively. These results mean that the effect of dietary resveratrol exposure on FS risk was not mediated by inflammatory markers. After six- and nine-year follow-up, inverse associations between measures of dietary resveratrol exposure and FS risk were also observed, although the results were not statistically significant in the fully adjusted models (**Supplemental tables 3 and 4, respectively**).

No statistically significant interactions were detected for sex, age, BMI and cognitive impairment in relation to the association between TDR+TUR and FS after any of the follow-up periods in the fully adjusted models. Sensitivity analyses were performed by repeating the models after the exclusion of 31, 30 and 24 participants at the three-, six- and nine- year follow-up, respectively, because they consumed more than 56 g/d of alcohol. The associations between TDR, TUR, and TDR+TUR and FS at the three-, six- and nine-year follow-up were almost identical to results based on the whole population (data not shown).

The associations of TDR+TUR with individual FS criteria during the three-year follow-up are shown in **Table 3**. After adjustment for FS at baseline and potential covariates, participants in the highest tertile of TDR+TUR had lower risk of feeling exhaustion than those in the lowest tertile (OR = 0.20; 95 % CI = 0.09, 0.43; *P*-trend < 0.001). No associations were observed for other FS criteria. Low-level of physical activity showed a significant inverse association with total dietary resveratrol exposure between participants in raw models at three-, six- and nine-year follow-up periods, but not in the adjusted models (**Table 3, and supplemental tables 5 and 6**).

Since TDR intake could change over time, we assessed TDR over the nine-year follow-up visits in the 247 participants with both dietary and frailty data in all three follow-ups. The median (IQR) of TDR intakes at three-, six-, and nine-year follow-up visits were 0.44 (0.04-1.68), 0.46 (0.04-1.12), and 0.60 (0.03-1.15), respectively. The observed change in TDR intake over the nine-year follow-up visits was not statistically significant (*P*=0.18). The intra-class correlation of TDR intake over the nine-year of follow-up period was 0.65. The Spearman´s rank correlations between TDR intake at the three-, six-, and nine-year follow-up visits and urinary resveratrol concentrations at baseline were 0.62 (*P* < 0.001), 0.63 (*P* < 0.001), and 0.61 (P < 0.001), respectively (data not shown).

DISCUSSION

Our study shows that high levels of habitual dietary resveratrol exposure were associated with a lower risk of developing FS in older adults followed over a three-year period. No significant associations were observed over six- and nine-year follow-ups, although the results were in the same direction.

First of all, the loss of statistical significance at six and nine-year follow up could be partially due to the reduction in the sample size (42.5% and 58.1% of loss at six- and nine-year follow-up, respectively, mainly due to death) and consequently a decrease in the statistical power. Furthermore, changes in other dietary and environmental factors, and changes of health status of the participants during the follow-up may be the cause of the attenuated association with long-term FS risk.

Frailty is a multifactorial and complex syndrome. The potential underlying mechanisms of FS are many, including oxidative stress, inflammation, decreased immune function, and endocrine system and musculoskeletal alterations (6). Energy restriction has been shown to increase longevity and decrease FS (6). These effects are suggested to be mediated by sirtuins, particularly sirtuin 1 (SIRT1), which is an important molecular target for regulating cellular energy metabolism and mitochondrial homeostasis (35). There is a vast interest in resveratrol regarding its role in the prevention of common clinical conditions of aging through the activation of SIRT1 in the liver, skeletal muscle, heart, neocortex, and adipose tissue (36-38). Resveratrol could attenuate oxidative stress-mediated modification of SIRT 1 by increasing the SIRT1 deacetylase activity/level leading to the inhibition of inflammatory/apoptotic signaling and thereby improving skeletal muscle after disuse in aging (39). Randomized clinical trials have

shown that the potential effects of resveratrol may be involved in the interaction with markers of inflammatory status and oxidative stress (14-15). However, in our study, as previously described (34), the correlation of resveratrol exposure and inflammatory markers was not statistically significant. Furthermore, our data suggest that the association between habitual resveratrol exposure and FS was not mediated by inflammation, since the ORs did not change after adjusting for inflammatory markers, and the percentage of excess risk mediated by inflammation was less than 5%.

Frailty is related to an array of diseases such as obesity, hypertension, cardiovascular disease, diabetes, osteoporosis, cancer, cognitive impairment, and depression (6). Although there is limited epidemiological evidence of the protective effects of resveratrol on these chronic diseases; a cross-sectional study showed that dietary resveratrol via wine consumption, evaluated as TUR, was associated with beneficial effects on fasting blood glucose, triglycerides and heart rate in high-cardiovascular risk subjects (40). However, several clinical trials have assessed the health benefits of using high resveratrol doses in these chronic diseases and their associated risk factors (13). Daily ingestion of 250 mg of resveratrol for three months improved systolic and diastolic blood pressures, and glycated haemoglobin A_{1c} (HA_{1c}) and total cholesterol in type 2 diabetic subjects on oral hypoglycaemic treatment (41), whereas, daily ingestion of 10 mg of resveratrol for four weeks improved insulin resistance, decreased blood glucose levels and delayed the appearance of glucose peaks after a test meal (42). A recent pilot trial also described an improvement in insulin sensitivity and post-meal plasma glucose in overweight/obese and moderately insulin-resistant older adults upon intake of 1, 1.5 or 2 g resveratrol for one month (43). In healthy obese men, supplementation with resveratrol at 150 mg/d for one month improved the metabolic profile that mimics energy restriction, reduced the sleeping and resting metabolic rate, blood pressure, insulin levels and hepatic lipid content, and improvement in skeletal muscle intrinsic mitochondrial function, among others (38). Resveratrol has also been postulated as a neuroprotective and antidepressant molecule (44-45). As recently shown in an animal study, *trans*-resveratrol at doses of 40 and 80 mg/kg increased serotonin and noradrenaline levels in brain regions (45). The effects of resveratrol on cognition were postulated in a recent double-blind placebo-controlled interventional study which found that 26 wk of supplementation with resveratrol (200 mg/d) improved memory performance in healthy older adults (46). Interestingly, we found that habitual dietary intake of resveratrol was correlated to depressed mood (Spearman correlation coefficient between TDR+TUR and CES-D was -0.25, $P<0.001$) and cognitive function (Spearman correlation coefficient between TDR+TUR and MMSE was 0.17, $P<0.001$) at baseline.

Major strengths of our study were its longitudinal design, its long follow-up, and its relative large sample size of old subjects that is the target population for studying FS. Moreover, this is an Italian cohort, a population with a high heterogeneity in wine consumption, the main food source of resveratrol (7). Another important strength is the assessment of dietary resveratrol exposure with a combinative approach using TUR concentrations in 24-h urine samples as a nutritional biomarker (17, 40), and FFQ-reported TDR intake (7, 23). This approach provides a more accurate estimation than only one measure and may attenuate the misclassification of habitual exposure (19). A further advantage was the standardized assessment of FS by trained geriatricians and, consequently, the identification of frail and pre-frail cases using a validated methodology (25).

However, this study has some limitations. First of all, moderate wine drinking habit has been associated with optimal social, cognitive, and personality factors (47). Moreover,

participants excluded from the study, due to data unavailability, were older and less healthy (more disabilities and cognition impairment). Though we have adjusted our models for several important indicators of psychological functioning and social status, the presence of residual confounding cannot be excluded. Second, TUR were analysed only a single time at baseline; however, TDR intake in the diets of our whole population tends to be conservative, as was also observed in a previous InCHIANTI study (34). Third, our results could also be affected by measurement error in TDR intake, although the FFQ was previously validated (23). Finally, shrinking was self-reported, and therefore it may be influenced by memory of older participants. In order to minimize recall bias, the data was collected by trained personnel in a home interview using a standardized protocol.

In conclusion, our study showed that a higher habitual exposure to resveratrol was associated with a lower risk of developing frailty in a cohort of older population studied during a three-year period, but not during a six- and nine-year follow-up. Our data did not suggest that inflammation mediated the protective effects of resveratrol against FS. Future clinical trials on the relationships between dietary resveratrol exposure and FS are needed to clarify this potential association and the underlying mechanisms avoiding the limitations of observational studies.

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The authors' contributions to the manuscript were as follows: LF, SB, AC and CA-L designed the research; MR, AC, RZ-R and CA-L conducted the research; MR, MU-S and CA-L performed the samples analyses; MR and RZ-R conducted the statistical analysis; MR wrote the paper; RZ-R, AC, MU-S, LF, SB and CA-L provided critical revision, and AC and CA-L have the primary responsibility for final content. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

None of the authors had any financial or personal conflicts of interest. All founders were governmental entities, except the International Nut and Dried Fruit Council Foundation. It did not exert any influence on the design, implementation, analysis and interpretation of the data from this project.

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Table 1. Characteristics of the study population according to TDR+TUR tertiles at baseline[1]

		TDR+TUR tertiles			
	Total	**Lowest tertile**	**Intermediate tertile**	**Highest tertile**	***P***
Participants, *n* (%)	529	176 (33.3)	177 (33.4)	176 (33.3)	—
Age, y	72.7 ± 5.8[2]	72.6 ± 6.0	73.5 ± 6.2	71.9 ± 4.9	0.07
Female gender, *n* (%)	293 (55.4)	138 (47.1)	115 (39.2)	40 (13.7)	<0.001
Study municipality, *n* (%)					0.04
Greve in Chianti (rural)	245 (46.3)	78 (44.3)	75 (42.4)	92 (52.3)	
Bagno a Ripoli (urban)	284 (53.7)	98 (55.7)	102 (57.6)	84 (47.7)	
BMI, kg/m^2	27.7 ± 4.0	28.0 ± 4.3	27.5 ± 3.7	27.7 ± 3.9	0.51
Education, y	5.7 ± 3.3	5.0 ± 2.4	5.8 ± 3.6	6.2 ± 3.7	0.10
Current smokers, *n* (%)	312 (59.0)	128 (72.7)	107 (60.5)	77 (43.8)	0.28
Total energy intake, kcal/d	1957 ± 573	1742 ± 508	1885 ± 548	2245 ± 543	<0.001
Alcohol intake, g/d	15.2 ± 21.1	0.6 ± 2.3	10.0 ± 13.7	35.0 ± 22.6	<0.001
Wine consumption, g/d	53.6 (0.0–250.0)[3]	0.0 (0.0–0.0)	53.6 (17.9–125.0)	250.0 (134.0–375.0)	<0.001
TDR, mg/d	0.5 (0.0–1.7)	0.0 (0.0–0.0)	0.5 (0.1–1.0)	2.1 (1.1–3.2)	<0.001
TUR, nmol/24h	4596.4 (1596.0–14263.6)	876.6 (207.0–1877.4)	4742.5 (2855.7–8360.1)	24211.0 (12771.4–49766.6)	<0.001
Inflammatory markers					
IL-6, pg/ml	2.8 (2.0–3.8)	2.6 (1.8–3.6)	2.8 (2.0–3.9)	2.9 (2.1–3.9)	0.98
TNF-α, pg/ml	4.4 (3.0–5.7)	4.5 (3.0–5.7)	4.3 (3.0–5.7)	4.3 (3.2–5.6)	0.56
hsCRP, µg/ml	2.7 (1.3–5.1)	2.4 (1.5–5.4)	2.8 (1.3–5.9)	2.7 (1.2–4.6)	0.38
IL-1RA, pg/ml	128.9 (95.0–177.0)	129.6 (93.9–170.9)	126.1 (94.6–180.8)	132.9 (97.3–188.4)	0.92
Comorbidities, *n* (%)	473 (90.4)	158 (90.8)	163 (93.1)	152 (87.4)	0.57
Cognitive impairment, MMSE score ≤ 24, *n* (%)	112 (21.2)	47 (26.7)	41 (23.2)	24 (13.6)	0.40
Depressed mood, CES-D score ≥16, *n* (%)	148 (28.0)	65 (36.9)	52 (29.4)	31 (17.6)	0.14

Frailty, *n* (%)	23 (4.4)	8 (4.6)	10 (5.7)	5 (2.9)	0.14
Pre-frailty, *n* (%)	196 (37.4)	74 (42.3)	70 (40.0)	52 (29.9)	0.76
ADL disabilities, *n* (%)	8 (1.5)	3 (1.7)	4 (2.3)	1 (0.6)	0.60
IADL disabilities, *n* (%)	63 (11.9)	31 (17.6)	26 (14.7)	6 (3.4)	0.06

[1]Descriptive analyses were compared between habitual dietary resveratrol exposure tertiles with the use of age- and sex- generalized linear models. ADL, activities of daily living; BMI, body mass index; CES-D, Center for Epidemiologic Studies Depression Scale; hsCRP, high-sensitivity C-reactive protein; IADL, instrumental activities of daily living; IL-1ra, Interleukin-1 receptor antagonist; IL-6, Interleukin-6; MMSE, Mini-Mental State Examination; TDR, total dietary resveratrol; TNF-α, Tumor necrosis factor-α; TUR, total urinary resveratrol.
[2]Mean ± SD (all values).
[3]Median; IQR in parentheses (all such values).

Table 2. Association of dietary resveratrol exposure measures at baseline with the probability of developing frailty during a follow-up of 3 years (follow-up 1)[1]

	TDR (FFQ)					TUR (biomarker)					TDR+TUR (FFQ+ biomarker)[2]			
		Pre-Frailty		Frailty			Pre-Frailty		Frailty		Pre-Frailty		Frailty	
	Cut-off (mg/d)	*n*	OR (95% CI)	*n*	OR (95% CI)	Cut-off (nmol/d)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
Model 1														
Lowest tertile	<0.1	66	1 (referent)	26	1 (referent)	<2273.0	74	1 (referent)	18	1 (referent)	70	1 (referent)	23	1 (referent)
Intermediate tertile	0.1–1.1	74	1.04 (0.66, 1.62)	12	0.43 (0.20, 0.90)	2273.0–10342.7	63	0.76 (0.49, 1.19)	20	0.99 (0.49, 2.00)	70	0.92 (0.59, 1.44)	17	0.68 (0.34, 1.37)
Highest tertile	>1.1	64	0.78 (0.50, 1.21)	7	0.22 (0.09, 0.52)	>10342.7	67	0.75 (0.48, 1.16)	7	0.32 (0.13, 0.80)	64	0.71 (0.46, 1.11)	5	0.17 (0.06, 0.46)
P-trend[3]			0.19		0.001			0.33		0.009		0.12		<0.001
Continuous		204	0.96 (0.88, 1.04)	45	0.75 (0.65, 0.86)		204	0.99 (0.98, 1.00)	45	0.98 (0.96, 0.99)	204	0.84 (0.65, 1.08)	45	0.50 (0.34, 0.73)
Model 2														
Lowest tertile	<0.1	66	1 (referent)	26	1 (referent)	<2273.0	74	1 (referent)	18	1 (referent)	70	1 (referent)	23	1 (referent)
Intermediate tertile	0.1–1.1	74	1.02 (0.62, 1.66)	12	0.33 (0.11, 0.98)	2273.0–10342.7	63	0.90 (0.55, 1.46)	20	1.34 (0.49, 3.65)	70	0.86 (0.52, 1.40)	17	0.48 (0.17, 1.35)
Highest tertile	>1.1	64	0.94 (0.53, 1.66)	7	0.17 (0.05, 0.63)	>10342.7	67	1.05 (0.61, 1.80)	7	0.32 (0.09, 1.11)	64	0.96 (0.55, 1.69)	5	0.11 (0.03, 0.45)
P-trend[3]			0.78		0.02			0.71		0.03		0.85		0.002
Continuous		204	1.01(0.91, 1.12)	45	0.67 (0.54, 0.84)		204	0.99 (0.98, 1.01)	45	0.98 (0.95, 1.00)	204	1.01 (0.74, 1.38)	45	0.47 (0.26, 0.85)

Model 3														
Lowest tertile	<0.1	66	1 (referent)	26	1 (referent)	<2273.0	74	1 (referent)	18	1 (referent)	70	1 (referent)	23	1 (referent)
Intermediate tertile	0.1–1.1	74	1.01 (0.61, 1.66)	12	0.37 (0.12, 1.11)	2273.0–10342.7	63	0.90 (0.55, 1.49)	20	1.85 (0.64, 5.35)	70	0.84 (0.51, 1.39)	17	0.55 (0.19, 1.57)
Highest tertile	>1.1	64	0.84 (0.47, 1.50)	7	0.17 (0.04, 0.64)	>10342.7	67	1.02 (0.59, 1.77)	7	0.34 (0.10, 1.24)	64	0.89 (0.50, 1.58)	5	0.11 (0.02, 0.46)
P-trend[3]			0.50		0.02			0.81		0.03		0.65		0.003
Continuous		204	1.00 (0.90, 1.12)	45	0.67 (0.53, 0.84)		204	1.00 (0.98, 1.01)	45	0.98 (0.95, 1.01)	204	0.97 (0.71, 1.34)	45	0.47 (0.26, 0.85)

[1]Multinomial logistic regression models were used and three separate models were presented: the unadjusted one (model 1), the model 2 adjusted for status of frailty at baseline, age, sex, study municipality, education, BMI, total energy intake (except in TUR), smoking status, comorbidities, depression mood and cognitive impairment, and the model 3 additionally adjusted for inflammatory markers (IL–6, TNF–α, hsCRP and IL–1RA). FFQ, food frequency questionnaire; TDR, total dietary resveratrol; TUR, total urinary resveratrol.

[2]TDR+TUR: Howe's method based on ranks.

[3]*P*-trend obtained by assigning the median of each tertile as scores or entered as ordinal variable as appropriate.

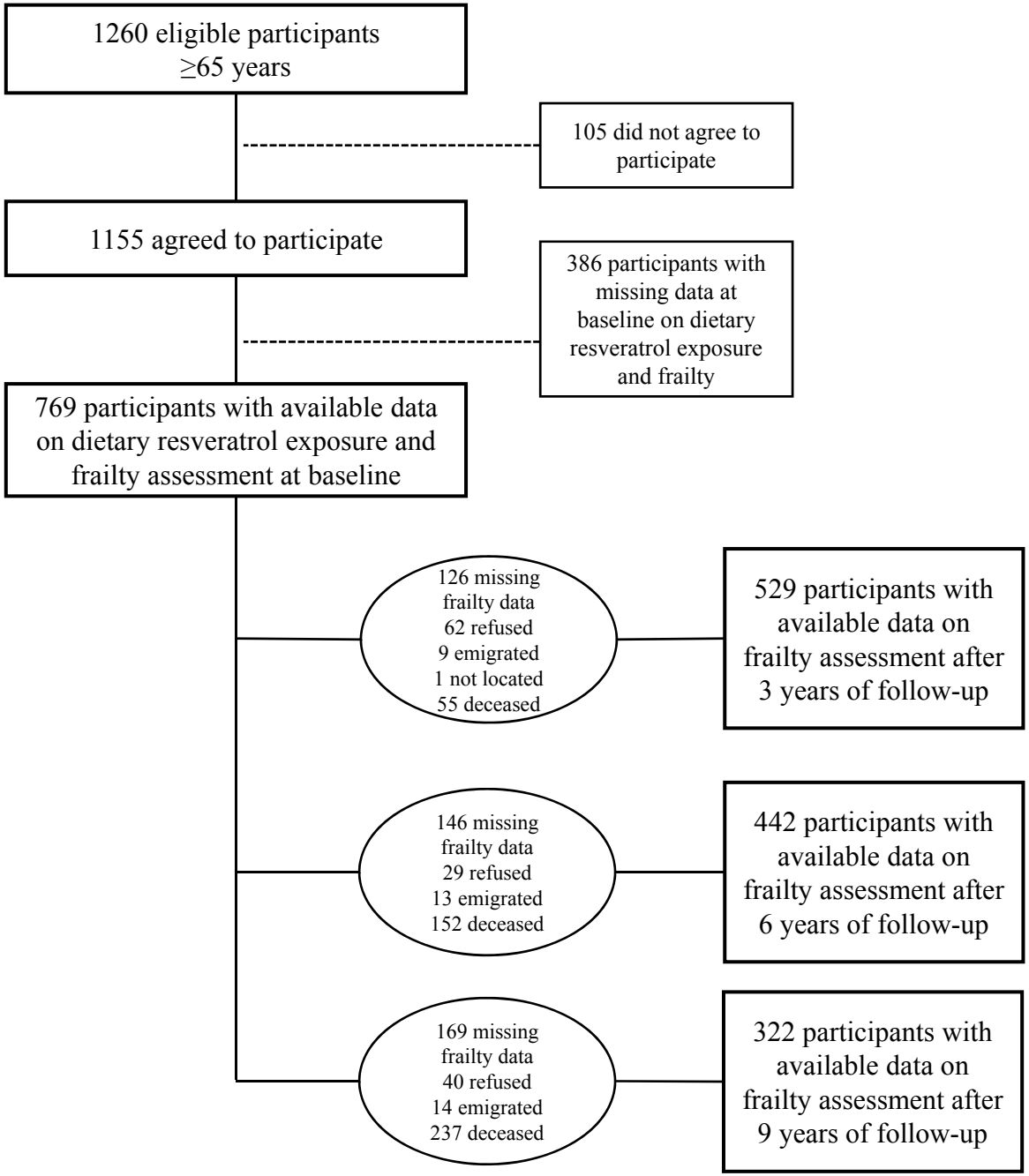
Table 3. Association of TDR+TUR tertiles at baseline with the probability of frailty components during a follow-up of 3 years (follow-up 1)[1]

	Unintentional weight loss		Feeling of exhaustion		Low-level physical activity		Low walking speed		Poor muscle strength	
	n	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
Model 1										
Lowest tertile	16	1 (referent)	58	1 (referent)	43	1 (referent)	46	1 (referent)	11	1 (referent)
Intermediate tertile	15	0.93 (0.44, 1.94)	32	0.45 (0.27, 0.74)	37	0.82 (0.50, 1.35)	46	0.99 (0.62, 1.60)	15	1.39 (0.62, 3.12)
Highest tertile	11	0.67 (0.30, 1.48)	12	0.15 (0.07, 0.29)	20	0.40 (0.22, 0.71)	35	0.70 (0.43, 1.16)	13	1.20 (0.52, 2.75)
P-trend[2]		0.33		<0.001		0.002		0.17		0.68
Continuous	42	0.87 (0.58, 1.32)	102	0.47 (0.35, 0.62)	100	0.63 (0.48, 0.84)	127	0.83 (0.64, 1.08)	39	1.19 (0.74, 1.91)
Model 2										
Lowest tertile	16	1 (referent)	58	1 (referent)	43	1 (referent)	46	1 (referent)	11	1 (referent)
Intermediate tertile	15	0.77 (0.34, 1.72)	32	0.40 (0.23, 0.70)	37	0.81 (0.44, 1.46)	46	0.85 (0.48, 1.49)	15	1.27 (0.49, 3.27)
Highest tertile	11	0.64 (0.24, 1.70)	12	0.20 (0.09, 0.43)	20	0.68 (0.32, 1.46)	35	0.74 (0.38, 1.46)	13	0.57 (0.19, 1.68)
P-trend[2]		0.36		<0.001		0.30		0.38		0.30
Continuous	42	0.88 (0.54, 1.43)	102	0.56 (0.40, 0.78)	100	0.81 (0.56, 1.18)	127	0.89 (0.63, 1.25)	39	0.90 (0.51, 1.58)
Model 3										
Lowest tertile	16	1 (referent)	58	1 (referent)	43	1 (referent)	46	1 (referent)	11	1 (referent)
Intermediate tertile	15	0.65 (0.27, 1.54)	32	0.41 (0.23, 0.73)	37	0.76 (0.41, 1.39)	46	1.01 (0.54, 1.88)	15	1.39 (0.51, 3.84)
Highest tertile	11	0.55 (0.19, 1.60)	12	0.17 (0.07, 0.39)	20	0.66 (0.30, 1.45)	35	0.79 (0.37, 1.67)	13	0.42 (0.13, 1.40)
P-trend[2]		0.24		<0.001		0.27		0.57		0.15
Continuous	42	0.84 (0.50, 1.42)	102	0.55 (0.39, 0.79)	100	0.80 (0.54, 1.16)	127	0.97 (0.67, 1.43)	39	0.83 (0.45, 1.51)

[1]Logistic regression models were used and three separate models were presented: the unadjusted one (model 1), the model 2 adjusted for its components at baseline, age, sex, study municipality, education, BMI, total energy intake, smoking status, comorbidities, depression mood and cognitive impairment, and the model 3 additionally adjusted for inflammatory markers (IL–6, TNF–α, hsCRP and IL–1RA).TDR, total dietary resveratrol; TUR, total urinary resveratrol.

[2]*P*-trend entered as ordinal variable.

Figure legends:
Figure 1. Flow chart of participants at each stage of the study.


1260 eligible participants
≥65 years
105 did not agree to participate
1155 agreed to participate
386 participants with missing data at baseline on dietary resveratrol exposure and frailty
769 participants with available data on dietary resveratrol exposure and frailty assessment at baseline
126 missing frailty data
62 refused
9 emigrated
1 not located
55 deceased
529 participants with available data on frailty assessment after 3 years of follow-up
146 missing frailty data
29 refused
13 emigrated
152 deceased
442 participants with available data on frailty assessment after 6 years of follow-up
169 missing frailty data
40 refused
14 emigrated
237 deceased
322 participants with available data on frailty assessment after 9 years of follow-up