The Relationship between Urinary Total Polyphenols and the Frailty Phenotype in a Community-Dwelling Older Population: the InCHIANTI Study

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Abbreviations: ADL, activities of daily living; CI, confidence interval; DTP, dietary total polyphenols; GAE, gallic acid equivalents; IADL, instrumental activities of daily living; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; UTP, urinary total polyphenols.

ABSTRACT

Background. Frailty, an age-related state of increased vulnerability, is associated with a higher risk of multiple adverse events. Studies have suggested that the quality of dietary intake may affect the development of frailty. We hypothesized that frailty in older subjects would be associated with dietary total polyphenols intake (DTP) and its biomarker, urinary total polyphenols (UTP).

Methods. The Invecchiare in Chianti (InCHIANTI) Study is a prospective cohort study set in the Chianti area (Italy). We used data at baseline from 811 participants aged ≥65 years. UTP was determined using the Folin-Ciocalteu assay after solid-phase extraction. DTP was estimated using a validated FFQ and our own polyphenol database. The frailty, prefrailty and nonfrailty states were defined according to the Fried and colleagues' criteria. Multinomial logistic regressions adjusted for potential confounders were used to assess the relationship between polyphenols and frailty.

Results. Both DTP and UTP concentrations progressively decrease from nonfrail to frail participants. Participants in the highest UTP tertile compared to those in the lowest tertile were significantly less likely to be both frail [OR=0.36(0.14-0.88), *P*=0.025] and prefrail [OR=0.64(0.42-0.98), *P*=0.038]. Exhaustion and slowness were the only individual frailty criteria significantly associated with UTP tertiles. No significant association was observed between frailty and DTP, after adjustment for covariates.

Conclusions. High concentrations of UTP were associated with lower prevalence of frailty and prefrailty in an older community-dwelling population. A polyphenol-rich diet may protect against frailty in older persons. Our findings should be confirmed in longitudinal studies.

INTRODUCTION

Polyphenols are phytochemical compounds present in plant-based foods, such as fruits and vegetables and derived foods (1). Epidemiological studies reported an inverse association between diets rich in polyphenols and risk of cardiovascular diseases, some cancers, diabetes, premature death (2-4), functional limitations and disabilities (5). Many scientists believe that the effects of polyphenols on health depend on the amount of polyphenols consumed, but also on their bioavailability (including inter- and intra-variability) and bioactivity, which cannot be assessed by simply evaluating dietary intake of total polyphenols (DTP) (6, 7). In this respect, urinary total polyphenols (UTP) take into account all the above-mentioned factors, and might be a better measure of exposure to polyphenols than dietary assessment (6).

Frailty is a condition characterized by an extreme vulnerability due to a decline across multiple physiological systems that increases the risk of adverse health events, such as disability, falls, dependency, need for long-term care, and death (8). Several instruments exist for measuring the level of frailty as reviewed by de Vries et al. (9). The frailty phenotype of Fried et al. is probably the most widely used definition of frailty. According to Fried et al., frailty is defined as the presence of at least three of the following criteria: reduced physical activity, muscle weakness, slow walking speed, fatigue or poor endurance and unintentional weight loss (8). Epidemiological studies have found that high plasma concentrations of antioxidants, such as carotenoids, vitamin E and D are associated with lower prevalence of frailty and related conditions (10-13). Indeed, low serum carotenoid concentrations have been considered a risk factor for accelerated muscle strength decline, and high levels of carotenoids were associated with reduced risk of walking disabilities and sarcopenia (12, 14).

The relationship between dietary intake of polyphenols and frailty has been studied very little. A recent cross-sectional study in the National Health and Nutrition Examination Survey (NHANES) showed that urinary concentrations of an isoflavone metabolite were inversely associated with frailty in women (15). In addition, in the InCHIANTI study, a greater adherence to a Mediterranean diet, which is a polyphenol-rich diet, was associated with a lower risk of developing frailty (16).

In the current study, we aimed at investigating whether polyphenol exposure, measured either as UTP or as DTP, was associated with the frailty syndrome and its individual criteria in an older community-dwelling population.

METHODS

Study Population

The InCHIANTI is a prospective population-based study conducted in two towns (Greve in Chianti and Bagno a Ripoli) in the Chianti geographic area (Tuscany, Italy) aimed at evaluating factors affecting mobility in an older community-dwelling population. A detailed description of the study design, sampling procedure and data collection method has been previously published elsewhere (17). Baseline data collection started in September 1998 and was completed in March 2000. The Italian National Research Council of Aging ethical committee examined and approved the study protocol, and all participants gave informed consent. The study randomly sampled 1,260 men and women, of whom 1,155 (91.6%) were enrolled. Of these, 811 (70.2%) participants had 24-hour urine measures and complete data for the evaluation of frailty. At baseline, data collection included: a home interview followed by a full medical and functional evaluation performed by trained personnel, and 24-hour urine collection and blood drawing that were coded and stored at -80 ºC until analysis.

Determination of Total Polyphenols in Urine Samples

The analysis of total polyphenols in baseline urine samples was performed using the Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) after solid-phase extraction using Oasis® MAX 96-well plates (Waters, Milford, Massachusetts) according to our previous methodology (6). Results were expressed as UTP concentrations in mg gallic acid equivalents (GAE) (Sigma-Aldrich, St. Louis, MO) in 24-hour urine.

Frailty Syndrome

Frailty was defined according to the following criteria proposed by Fried and colleagues (8): (a) *Shrinking or weight loss*; (b) *Exhaustion or lack of energy*; (c) *Low physical*

activity or sedentariness; (d) *Weakness or poor muscle strength*; (e) *Slowness or low walking speed* (see Supplementary Data). Participants who had one or two of these criteria were considered prefrail, those with three to five criteria were considered frail, and participants with no criteria were considered not frail and were called nonfrail.

Covariates

Data on socio-demographics (age and gender) and lifestyle variables were collected by standardized questionnaires. Food intake (g/day) at baseline was estimated using the Italian version of the food frequency questionnaire developed and validated in the European Prospective Study into Cancer and Nutrition (EPIC) study (18). DTP intake was estimated using a custom-made food composition database on polyphenols (7), based on the USDA and Phenol-Explorer databases. Individual polyphenols, expressed as aglycones, were summed to calculate DTP (7). A description of other covariates used in the study is included in the Supplementary Data.

Statistical Analysis

Variables with symmetric distribution were reported as means and standard deviations (SD). Variables with asymmetric distribution were summarized as medians and interquartile ranges. Categorical variables were summarized as number of participants and percentages. UTP and DTP concentrations were not normally distributed, and were normalized using a Box–Cox transformation (α=0.00001; λ=0.25) (6) and as a log2-transformation, respectively. Spearman's correlation test was used to evaluate the associations between UTP and fruits and vegetables consumption and DTP.

UTP and DTP were analyzed as tertiles according to the following cut-off points: 123.6 and 173.4 mg GAE/24-hour and 509.2 and 645.2 mg/d, respectively. Differences between frail, prefrail and nonfrail participants, as well as differences across UTP and

DTP tertiles, were estimated using analysis of variance, Kruskal–Wallis or chi-square tests, as appropriate. The association between UTP and DTP as tertiles and as continuous variables and both prefrailty and frailty (using nonfrail as the reference group) was tested using a multivariate multinomial logistic regression in three additive models. The interaction between gender and UTP and DTP as tertiles and as continuous variables was evaluated by using the models with and without an interaction term. Additionally, logistic regression was used to associate each frailty criteria with UTP and DTP as tertiles and as continuous variables. *P*-values < 0.05 (two-tailed) were considered to be significant. The statistical analyses were performed using the SPSS package program version 18.0 (SPSS Inc., Chicago, IL).

RESULTS

The study included a total of 811 participants, 44.9% of whom were men, with a mean age of 74.3 ± 6.9 years. In the overall sample, the prevalence of prefrailty and frailty was 39.6% and 8.9%, respectively. In comparison to those excluded due to incomplete data (n=344), those included in the study were significantly younger (mean±SD, 74.3 ± 6.9 vs 78.0 ± 8.5 years, $P<0.001$), had lower rates of activities of daily living (ADL) (5.4% vs 20.9%) and instrumental activities of daily living (IADL) (21.9% vs 42.7%) disabilities (both, $P<0.001$) and had a lower prevalence of frailty (8.9% vs 12.2%) and dementia (3.8% vs 14.8%) ($P<0.05$). From the nonfrail to the frail group, participants were less physically active, and had a higher prevalence of disability in more than one ADL and IADL as well as of physical performance impairment. In addition, from the nonfrail to the frail group, participants had lower DTP, lower plasma antioxidants such as α-tocopherol and lower urinary UTP (see Supplementary Table 1).

The characteristics of study participants across UTP and DTP tertiles are displayed in Table 1. From the lowest to the highest UTP tertiles, participants had a higher Mediterranean Diet score and higher energy intake. The prevalence of participants with disability in more than one ADL and IADL, and with physical performance impairment, decreased with increasing UTP or DTP tertiles. While the prevalence of participants with frailty progressively decreased from the lowest to the highest UTP and DTP tertiles, prefrailty prevalence only decreased significantly through the UTP tertiles.

The association between frailty and UTP or DTP concentrations through the tertiles and as continuous variables is shown in Table 2. Frailty was significantly associated with UTP levels, independent of age, gender, creatinine clearance and other factors such as body mass index, total energy intake, alcohol consumption, smoking habit and activity level (Table 2, Model 1). This association was unchanged after further adjustment for

inflammatory markers, i.e. IL-6 and CRP (Table 2, Model 2), and for chronic diseases (Table 2, Model 3). Participants in the highest UTP tertile were 64 % (P = 0.025) and 36 % (P = 0.038) less likely to be frail and prefrail, respectively, than those in the lowest UTP tertile. In addition, there were significant inverse associations between frailty and prefrailty and UTP (OR=0.69 (0.54-0.88), P=0.003; OR=0.85 (0.76-0.96), P=0.011, respectively). Frailty and prefrailty status were not associated with DTP tertiles or continuous DTP variable (Table 2). Moreover, when the interaction between gender and UTP and DTP as tertiles or as continuous variables was evaluated, no statistically significant interactions were observed (data not shown).

The association of UTP with individual frailty criteria is shown in Table 3. After adjustment for covariates, the participants in the highest UTP tertile were less likely to report exhaustion [OR=0.42 (0.24–0.72), P=0.002] and to walk slowly [OR=0.55 (0.33–0.93), P=0.026] than those in the lowest UTP tertile. Furthermore, those in the middle UTP tertile were also less likely to walk slowly [OR=0.58 (0.35–0.95) P=0.029] than those in the lowest UTP tertile. In addition, there were significant inverse associations between UTP and exhaustion and slowness criteria (OR=0.81 (0.70-0.94), P=0.005; OR=0.86 (0.74-0.99), P= 0.036, respectively). Although two of the five criteria (weight loss and low physical activity) showed no significant association with UTP tertiles, weakness showed a borderline significant association (P=0.059) between participants in the highest and in the lowest UTP tertiles in the fully adjusted model (Table 3, Model 3). This association became significant when the lineal variable was considered (OR=0.84 (0.73-0.96), P=0.01).

DISCUSSION

This cross-sectional study shows that high concentrations of urinary total polyphenols (UTP) were associated with lower prevalence of prefrailty and frailty in a community-dwelling older population. Indeed, participants in the highest UTP tertile were 36% and 64% less likely to be prefrail and frail, respectively, than those in the lowest UTP tertile. On the contrary, dietary total polyphenols (DTP) was not significantly associated with frailty status. In a previous study, a significant association was observed between UTP and total mortality, while no significant association was found with DTP (7).

To our knowledge, only one epidemiological study, the NHANES study, has investigated the association between polyphenol biomarkers and frailty. It showed an inverse association between the urinary concentration of O-desmethylangolensin, an isoflavone metabolite, and frailty in women (15). Moreover, two recent epidemiological studies showed inverse associations between a greater adherence to the Mediterranean diet and frailty risk (16, 19). In addition, not consuming fruits and vegetables daily was associated with frailty and prefrailty in the participants of the Whitehall II prospective cohort study (20). Finally, several observational studies, which evaluated other antioxidant micronutrients, reported that frailty was associated with low intakes of vitamins D, E, C and folate, low concentrations of plasma carotenoids and α-tocopherol, and a low diet quality index (12, 21-23).

In the present study, a statistically significant inverse association was observed between UTP concentrations and some frailty criteria, particularly exhaustion and slowness, and, although with borderline significance, with the weakness criterion. To our knowledge, no epidemiological studies have evaluated these associations so far, although similar results were reported for other antioxidant micronutrients. Thus, both high consumption of carotenoid-rich foods and high plasma levels of carotenoids were associated with a

decline in muscle strength, impaired lower extremity performance and walking disability in older adults (12, 14, 24, 25). In addition, low intake and low serum concentrations of vitamin E were associated with reduced physical performance, frailty and disability (21, 24, 26). Moreover, a poor nutritional score determined as the sum of several vitamins, minerals and proteins was associated with weakness and exhaustion, although this last association lost its significance after adjustment for energy intake (21). Taking into account the weight loss criterion, the number of older subjects reporting weight loss is low, due to the good health of participants at baseline, and therefore there could be insufficient power to test the association between this variable and UTP. Moreover, in a previous InCHIANTI study, authors suggested that weight loss may not be a sensitive proxy measure of inadequate diet or undernutrition (21). The protective effect of polyphenols towards frailty might be accounted for by their anti-inflammatory and antioxidant activity (27-29). Greater adherence to the Mediterranean diet was associated with lower levels of inflammatory biomarkers, e.g. IL-6 and CRP, and particularly those related to endothelial function (ICAM-1 and VCAM-1) (29, 30). Moreover, it has been hypothesized that diets rich in antioxidants (polyphenols and some vitamins) could prevent sarcopenia, a major component of frailty (27). Antioxidant containing diets resulted in less oxidative stress, elevated activities of glutathione and a reductive shift in the glutathione redox state in muscle homogenates and mitochondria in mice (31), as well as protected skeletal muscle from oxidative damage (32).

The different results obtained from the dietary measure (DTP) and the urinary biomarkers (UTP) are probably mainly due to the fact that the latter measure also takes into account the bioavailability of food polyphenols. After absorption and metabolism, compounds reach target tissues where they will deliver the biological activity. UTP is

an unspecific polyphenol biomarker that is associated with the consumption of fruits and vegetables in our population (rho=0.15; $P<0.001$) and in other populations such as the Spanish Predimed study (33). In addition, UTP is also associated with DTP (rho=0.134; $P<0.001$) which was evaluated in depth in a previous study in the whole InCHIANTI cohort (r=0.211, $P<0.001$) (6). Although the use of FFQ by itself has limitations in the dietary assessment measure and in capturing data of food consumed, the FFQ used in this study has been validated for this specific population and in the EPIC Italian population (6). This FFQ has been used to correlate plasma carotenoids with the consumption of fruit and vegetables (6). Therefore, the main strength of this study was the use of a validated biomarker, UTP concentrations in 24-hour urine samples, to measure polyphenol exposure which is a more reliable and accurate measure of intake than DTP and takes into account the bioavailability of each type of polyphenol (6). A further advantage was that two of the criteria (slowness and weakness) for evaluating frailty were objectively measured. These criteria were those inversely associated with higher UTP concentrations. Finally, our models were built considering potential confounders of the relationship between frailty and polyphenol exposure.

The main limitation of this study is the cross-sectional design, which did not allow a causal role of low UTP in increasing frailty risk to be established. We could not assess whether the lower excretion of total polyphenols, related to their consumption, represented the initial point of the pathway causing the frailty syndrome or whether the frailty syndrome led to less consumption of polyphenol-rich foods. In addition to this point, it is impossible to differentiate which polyphenols are associated with frailty using this measure. Second, three of the criteria (exhaustion, shrinking and sedentariness) for evaluating frailty were self-reported measures, and these may have been influenced by the memory of participants, especially in older subjects. Therefore, a

potential misclassification in frailty status cannot be excluded. Despite this, assessment of frailty is standardized in geriatrics and the information was collected by trained personnel in a home interview in order to reduce memory biases. Third, the DTP intake may be underestimated, although the dietary intake was optimized by using a validated Italian FFQ and a comprehensive database on polyphenols (6, 7).

In conclusion, the present study showed that UTP concentrations, a marker of polyphenol intake, were inversely associated with frailty and prefrailty status in a community-dwelling older population in Italy. Participants in the highest UTP tertile had lower risks of frailty and prefrailty by 64 and 36%, respectively, when compared with those in the lowest UTP tertile. Furthermore, when frailty was explored by individual criteria, participants in the highest UTP tertile had significantly lower risk of suffering exhaustion and slowness and a borderline significantly lower risk of weakness. Our data provide new evidence suggesting that polyphenol-rich diets may protect against the occurrence of frailty in older subjects.

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No conflicts of interest are declared.

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Table 1. Baseline Characteristics of InCHIANTI Participants Belonging to the Tertiles of Urinary Total Polyphenols (UTP) and to the Tertiles of Dietary Total polyphenols (DTP).

Characteristic		UTP (Tertiles)			DTP (Tertiles)		
		1 (n=270) (< 123.6 mg GAE/24h)	2 (n=271) (123.6-173.4 mg GAE/24h)	3 (n=270) (> 173.4 mg GAE/24h)	1 (n=268) (< 509.2 mg/day)	2 (n=267) (509.2-645.2 mg/day)	3 (n=268) (> 645.2 mg/day)
Demographics							
	Age, yr, mean ± SD	76.2 ± 7.4	74.3 ± 6.8	72.5 ± 5.9 ‡	75.9 ± 7.4 74.3	74.3 ± 6.7	72.8 ± 6.2 ‡
	Men, n (%)	99 (36.7)	124 (45.8)	141 (52.2) ‡	83 (31.0)	124 (46.4)	154 (57.5) ‡
Behavior-related variables							
	Body mass index, kg/m^2, mean ± SD	27.0 ± 4.2	27.5 ± 3.9	28.0 ± 3.8 *	27.4 ± 4.3	27.5 ± 3.8	27.7 ± 3.9
	Smoking, packs/year, median (IQR)	0.0 (0.0-14.6)	0.0 (0.0-16.8)	0.0 (0.0-28.0) *	0.0 (0.0-7.4)	0.0 (0.0-20.4)	0.0 (0.0-19.3) ‡
	Energy intake, kcal/d, mean ± SD	1826.5 ± 542.8	1911.0 ± 536.5	2013.0 ± 572.1 ‡	1601.1 ± 441.9	1902.0 ± 439.5	2247.7 ± 573.2 ‡
	Mediterranean diet score, mean ± SD	4.0 ± 1.6	4.0 ± 1.6	4.4 ± 1.7 *	3.3 ± 1.5	4.3 ± 1.5	4.9 ± 1.6 ‡
	Alcohol intake, g/d, median (IQR)	5.7 (0.0-17.4)	6.6 (0.0-17.5)	7.9 (0.0-26.8)	1.9 (0.0-13.4)	7.6 (0.2-19.3)	13.4 (0.5-28.3) ‡
	Activity level in the last year, h/wk, mean ± SD	3.2 ± 1.1	3.3 ± 1.0	3.3 ± 0.9 *	3.1 ± 1.1	3.3 ± 0.9	3.4 ± 1.0 ‡
	Disability in ≥ 1 ADL, n (%)	25 (9.3)	13 (4.8)	6 (2.2) ‡	19 (7.1)	14 (5.2)	7 (2.6) §
	Disability in ≥ 1 IADL, n (%)	86 (31.9)	51 (18.8)	41 (15.2) ‡	80 (29.9)	60 (22.5)	33 (12.3) ‡
	Physical performance impairment, SPPBS ≤ 9, n (%)	93 (36.0)	59 (23.1)	43 (16.5) ‡	82 (32.5)	64 (24.5)	49 (18.8) †
	Dietary total polyphenols (DTP), mg/day, mean ± SD	570.1 ± 202.1	595.0 ± 200.7	620.4 ± 180.9 *	407.4 ± 77.5	574.9 ± 38.9	803.2 ± 167.9 ‡
Plasma parameters							
	IL-6, pg/ml, median (IQR)	1.6 (0.8-2.7)	1.3 (0.9-2.1)	1.5 (0.9-2.0)	1.6 (1.0-2.7)	1.5 (0.9-2.2)	1.4 (0.8-1.9)*
	CRP, µg/ml, median (IQR)	2.6 (1.3-5.8)	2.5 (1.3-5.6)	2.8 (1.4-5.5)	2.8 (1.5-5.9)	2.6 (1.4-5.8)	2.5 (1.2-5.1)
	Total cholesterol, mg/dL, mean ± SD	219.7 ± 38.0	220.4 ± 40.3	217.4 ± 39.2	220.1 ± 39.9	221.7 ± 39.7	215.9 ± 37.6
	Uric acid, mg/dL, mean ± SD	5.1 ± 1.3	5.2 ± 1.4	5.2 ± 1.3	5.1 ± 1.4	5.1 ± 1.2	5.3 ± 1.4 *
	α-tocopherol, µmol/L, median (IQR)	29.0 (24.3-33.8)	29.7 (24.8-35.3)	30.5 (25.3-36.2) *	28.7 (24.8-34.7)	30.4 (25.1-36.1)	29.5 (24.4-34.8)
	Creatinine clearance, ml/min, mean ± SD	59.1 ± 20.6	63.8 ± 18.0	68.6 ± 18.1 ‡	59.0 ± 19.0	64.7 ± 18.6	68.1 ± 19.3 ‡
Urinary parameters							
	UTP, mg GAE/24h, mean ± SD	94.0 ± 21.7	147.9 ± 14.7	229.5 ± 48.7 ‡	145.6 ± 61.2	158.5 ± 65.5	167.4 ± 64.6 ‡
	Normalized UTP, mg GAE/24h, mean ± SD	8.4 ± 0.8	9.9 ± 0.3	11.5 ± 0.8 ‡	9.7 ± 1.4	10.0 ± 1.5	10.2 ± 1.4 ‡
Frailty phenotype							
	Frailty, n (%)	37 (24.5)	22 (13.6)	13 (7.3) ‡	30 (19.9)	24 (14.0)	24 (8.7) *
	Prefrailty, n (%)	119 (51.1)	109 (43.8)	93 (36.2) †	117 (49.2)	95 (39.1)	107 (42.1) §
Diseases and conditions							
	Dementia, n (%)	18 (6.7)	8 (3.0)	5 (1.9) †	16 (6.0)	9 (3.4)	5 (1.9) *

Diabetes, n (%)	27 (10.0)	32 (11.8)	45 (16.7) *	37 (13.8)	35 (13.1)	31 (11.6)
Hypertension, n (%)	131 (49.6)	134 (50.2)	117 (43.8)	137 (52.7)	118 (44.5)	123 (46.2)
Renal failure, n (%)	188 (71.5)	159 (60.9)	142 (54.0) ‡	175 (68.6)	166 (63.4)	144 (54.3) †

Notes: GAE: Acid gallic equivalents. Statistical comparisons are from ANOVA analysis of variance, Kruskal–Wallis or chi-square, as appropriate. * $P< 0.05$, † $P< 0.01$, ‡ $P<0.001$, § $P=0.06$-0.07.

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Table 2. Multinomial Logistic Model Describing the Association Between Tertiles of Urinary Total Polyphenols (UTP) or Dietary Total Polyphenols (DTP) and Prefrailty and Frailty Status in the InCHIANTI Study.

URINARY TOTAL POLYPHENOLS (UTP)						
		Prefrail (1-2 characteristics)			**Frail (3-5 characteristics)**	
Models	**n**	**OR (95 % CI)**	***P***	**n**	**OR (95 % CI)**	***P***
Model 1						
Tertiles						
Lowest	119	Reference		37	Reference	
Middle	109	0.82 (0.54-1.22)	0.326	22	0.64 (0.30-1.37)	0.248
Highest	93	0.65 (0.43-0.99)	0.042	13	0.33 (0.13-0.80)	0.014
Continuous	321	0.86 (0.76-0.97)	0.013	72	0.68 (0.54-0.86)	0.001
Model 2						
Tertiles						
Lowest	119	Reference		37	Reference	
Middle	109	0.83 (0.55-1.25)	0.365	22	0.66 (0.31-1.43)	0.291
Highest	93	0.64 (0.42-0.97)	0.034	13	0.35 (0.14-0.86)	0.022
Continuous	321	0.85 (0.76-0.96)	0.010	72	0.69 (0.54-0.87)	0.002
Model 3						
Tertiles						
Lowest	119	Reference		37	Reference	
Middle	109	0.83 (0.55-1.26)	0.382	22	0.68 (0.31-1.50)	0.340
Highest	93	0.64 (0.42-0.98)	0.038	13	0.36 (0.14-0.88)	0.025
Continuous	321	0.85 (0.76-0.96)	0.011	72	0.69 (0.54-0.88)	0.003
DIETARY TOTAL POLYPHENOLS (DTP)						
		Prefrail (1-2 characteristics)			**Frail (3-5 characteristics)**	
Models	**n**	**OR (95 % CI)**	***P***	**n**	**OR (95 % CI)**	***P***
Model 1						
Tertiles						
Lowest	117	Reference		30	Reference	
Middle	95	0.74 (0.49-1.13)	0.164	24	1.13 (0.51-2.48)	0.769
Highest	107	1.03 (0.66-1.62)	0.885	14	1.16 (0.45-2.99)	0.764
Continuous	319	0.97 (0.65-1.44)	0.877	68	1.48 (0.63-3.48)	0.365
Model 2						
Tertiles						
Lowest	117	Reference		30	Reference	
Middle	95	0.80 (0.52-1.22)	0.298	24	1.11 (0.50-2.46)	0.796
Highest	107	1.16 (0.73-1.83)	0.536	14	1.24 (0.47-3.28)	0.662
Continuous	319	1.03 (0.69-1.55)	0.875	68	1.59 (0.68-3.76)	0.287
Model 3						
Tertiles						
Lowest	117	Reference		30	Reference	
Middle	95	0.81 (0.53-1.24)	0.331	24	1.10 (0.49-2.47)	0.811
Highest	107	1.19 (0.75-1.88)	0.466	14	1.26 (0.47-3.34)	0.645
Continuous	319	1.05 (0.70-1.58)	0.811	68	1.60 (0.67-3.81)	0.288

Notes: Tertiles and continuos variable of urinary total polyphenols (UTP) are expressed as mg GAE/24h. Tertiles and continuos variable of dietary total polyphenols (DTP) are expressed as mg/day. Number of cases (n), odds ratio (OR), confidence interval (CI), and *P*-test are estimated in Models 1 to 3. Model 1 adjusting for age, gender, body mass index, total energy intake, alcohol consumption, smoking habit, activity level, creatinine clearance. Model 2 adjusting for all variables present in Model 1 plus IL6 and CRP. Model 3 adjusting for all variables present in Model 2 plus diabetes, hypertension, dementia and renal failure.

Table 3. Covariate-Adjusted Associations of UTP Tertiles with Individual Frailty Criteria in the InCHIANTI Study.

	Weight loss		Exhaustion		Low physical activity *		Weakness		Slowness	
Models	**n**	**OR (95 % CI)** ***P***	**n**	**OR (95 % CI)** ***P***	**n**	**OR (95 % CI)** ***P***	**n**	**OR (95 % CI)** ***P***	**n**	**OR (95 % CI)** ***P***
Model 1										
Tertiles										
Lowest	12	Reference	58	Reference	70	Reference	75	Reference	81	Reference
Middle	18	1.59 (0.71-3.61) 0.263	53	0.94 (0.60-1.47) 0.769	44	0.69 (0.42-1.11) 0.127	55	0.76 (0.49-1.18) 0.225	48	0.55 (0.34-0.90) 0.016
Highest	10	1.01 (0.40-2.55) 0.983	29	0.43 (0.26-0.73) 0.002	38	0.78 (0.47-1.30) 0.344	39	0.60 (0.37-0.97) 0.036	41	0.55 (0.33-0.92) 0.021
Continuous	40	1.00 (0.78-1.27) 0.993	140	0.82 (0.71-0.94) 0.006	152	0.91 (0.79-1.05) 0.208	169	0.83 (0.73-0.95) 0.007	170	0.86 (0.74-0.99) 0.034
Model 2										
Tertiles										
Lowest	12	Reference	58	Reference	70	Reference	75	Reference	81	Reference
Middle	18	1.62 (0.71-3.68) 0.252	53	0.98 (0.62-1.54) 0.935	44	0.69 (0.42-1.12) 0.134	55	0.78 (0.50-1.22) 0.273	48	0.56 (0.35-0.92) 0.021
Highest	10	0.93 (0.36-2.40) 0.878	29	0.42 (0.24-0.72) 0.001	38	0.78 (0.46-1.31) 0.348	39	0.62 (0.38-1.01) 0.054	41	0.56 (0.34-0.94) 0.027
Continuous	40	0.97 (0.76-1.25) 0.848	140	0.81 (0.70-0.94) 0.005	152	0.91 (0.78-1.05) 0.176	169	0.83 (0.73-0.96) 0.009	170	0.86 (0.74-0.99) 0.035
Model 3										
Tertiles										
Lowest	12	Reference	58	Reference	70	Reference	75	Reference	81	Reference
Middle	18	1.79 (0.77-4.11)	53	0.99 (0.63-1.56)	44	0.67 (0.41-1.11)	55	0.75 (0.48-1.18)	48	0.58 (0.35-0.95)

		0.173		0.967		0.121		0.218		0.029
Highest	10	0.92 (0.35-2.42)	29	0.42 (0.24-0.72)	38	0.81 (0.48-1.37)	39	0.62 (0.38-1.02)	41	0.55 (0.33-0.93)
		0.869		0.002		0.441		0.059		0.026
Continuous	40	0.97 (0.76-1.25)	140	0.81 (0.70-0.94)	152	0.92 (0.79-1.06)	169	0.84 (0.73-0.96)	170	0.86 (0.74-0.99)
		0.809		0.005		0.24		0.010		0.036

Notes: Tertiles and continuos variable of urinary total polyphenols (UTP) are expressed as mg GAE/24h. Number of cases (n), odds ratio (OR), confidence interval (CI), and *P*-test are estimated in Models 1 to 3. Prefrail and frail were jointly modeled using binary logistic regression. Model 1 adjusting for age, gender, body mass index, total energy intake, alcohol consumption, smoking habit, activity level, creatinine clearance. Model 2 adjusting for all variables present in Model 1 plus IL6 and CRP. Model 3 adjusting for all variables present in Model 2 plus diabetes, hypertension, dementia and renal failure. * The activity level has been excluded from the models.