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ORIGINAL ARTICLE

Association of serum levels of phenylalanine and tyrosine with hip fractures and frailty in older adults: The cardiovascular health study

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Abstract

Summary This study examined if the amino acids phenylalanine or tyrosine contribute to risk of hip fracture or frailty in older adults. We determined that neither phenylalanine nor tyrosine are important predictors of hip fracture or frailty. We suggest advice on protein intake for skeletal health consider specific amino acid composition.

Purpose Protein is essential for skeletal health, but the specific amino acid compositions of protein may have differential associations with fracture risk. The aim of this study was to determine the association of serum levels of the aromatic amino acids phenylalanine and tyrosine with risk for incident hip fractures over twelve years of follow-up and cross sectional associations with frailty.

Methods We included 131 older men and women from the Cardiovascular Health Study (CHS) who sustained a hip fracture over twelve years of follow-up and 131 men and women without an incident hip fracture over this same period of time. 42% of this cohort were men and 95% were Caucasian. Weighted multivariable Cox hazards molecules were used to estimate the hazard ratios (HR) and 95% confidence intervals (CI) of incident hip fracture associated with a one standard deviation (SD) higher serum level of phenylalanine or tyrosine. Relative risk regression was used to determine the cross-sectional association of these amino acids with Freid's frailty index.

Results Neither serum levels of phenylalanine (HR 0.85 (95% CI 0.62–1.16) or tyrosine (HR 0.82 (95% CI 0.62–1.1) were significantly associated with incident hip fractures or cross sectionally with frailty (frail compared with prefrail/not frail) (HR 0.92 (95% CI 0.48–1.76) and HR (0.86 (95% CI 0.46–1.61) respectively.

Conclusion Phenylalanine and tyrosine are not significant contributors to hip fractures or frailty in older men and women.

Keywords Phenylalanine · Tyrosine · Fractures · Frailty

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Introduction

One in three women and one in twelve men will suffer a hip fracture in their lifetime [1]. The overwhelming majority (86%) of these fractures will occur in individuals aged 65 years and older [2]. Older individuals are also at high risk for frailty [3], and frailty predisposes to fragility fractures [4]. Fractures and frailty are associated with substantial morbidity, hospitalizations, healthcare costs and excess mortality [2, 4, 5]. By 2030, one in five Americans is expected to be age 65 or older [6]. With this secular trend toward an increasingly aging United States (U.S.) population, safe and cost-effective interventions to prevent fractures, in particular hip fractures, and frailty, are of significant public health interest [7, 8]. An important component of bone and muscle health is diet, and some authors have argued for increased dietary protein intake with age for prevention of fractures [9, 10] and frailty [11–13].

Tryptophan, phenylalanine and tyrosine are three aromatic amino acids (AAA) involved in protein synthesis. AAAs increase circulating levels of insulin-like growth factor-1 (IGF-1) and may increase calcium absorption [14]. Tryptophan and phenylalanine are essential amino acids, meaning they must be acquired in the diet, but tyrosine is not as phenylalanine can be converted to tyrosine through hydroxylation [15]. A number of experimental studies in animals [16–18] and humans [19, 20] suggest that tryptophan may have benefits for skeletal health. However, association of the other AAAs, phenylalanine and tyrosine, with skeletal health is less clear. In mice, higher plasma levels of phenylalanine were associated with increased bone turnover [21]. A recent systematic review reported that there was no consistent relationship between serum phenylalanine levels and bone mineral density (BMD) of the lumbar spine [22]. In one report including older community dwelling men and women in Hong Kong, neither phenylalanine nor tyrosine was associated with changes in BMD or ten-year fracture risk [23]. However, genetically increased serum tyrosine levels have been inversely associated with total body BMD [24].

The purpose of this study was to utilize stored serum from the biorepository of the Cardiovascular Health Study (CHS), a longitudinal study of community-dwelling men and women aged 65 years and older from four U.S. sites, to measure serum levels of phenylalanine and tyrosine and determine their longitudinal association with incident hip fractures and cross-sectional association with frailty status.

Study participants

The CHS is a longitudinal study of community-dwelling adults aged 65 years and older from four U.S. sites drawn from Health Care Finance Administration (HCFA) Medicare eligibility lists in: Sacramento County, California; Washington County, Maryland; Forsyth County, North Carolina, and Allegheny County (Pittsburgh), Pennsylvania [25, 26]. The original cohort of 5,201 participants, whom were selected from for these analyses, was enrolled in 1989–1990. Institutional review boards approved the study at all sites and all participants provided written informed consent. From 1989 to 1999, participants underwent annual clinic examinations including blood samples. From 1999 to June 30, 2015, participants were surveyed biennially for incident hospitalizations, diagnoses, and medications.

Sufficient funding was available to measure serum in 262 participants. We selected from these original cohort of CHS participants at years 1992–1993 131 individuals who did not sustain a hip fracture in the previous three years and 131 individuals with an incident hip fracture for whom complete information was available on covariates including age, BMI (kg/m^2), weight (kg), sex, race, clinic site, self-reported health status (excellent, very good, good, fair, poor), history of diabetes, smoking status (current, former, never), highest education level completed ($\geq$ 12th or < 12th grade), renal function, current alcohol use (0 -$\leq$ 7 drinks/week, > 7 drinks/week), and medication use. Individuals were weighted by sex, race and hip fracture status to represent the whole CHS population at years 1992–1993.

Measurements of phenylalanine and tyrosine

Serum samples collected from the 1992–1993 CHS visit after a minimum 8 -hour fast were frozen at -80 degrees Celsius and measured using liquid chromatography-mass spectrometry (LC–MS). Separation of amino acids was performed using a Phenomenex Kinetex C18 column (100 × 2.1 mm, 1.7um) on a Shimadzu Nexera Ultra High-Performance Liquid Chromatography (UHPLC) system with a gradient of 5 to 40% acetonitrile (0.1% formic acid) over 6 min at a flow rate of 0.2 ml/min and a column temperature of 40 °C. The effluent was ionized using positive ion electrospray on a Thermo Scientific TSQ Quantiva triple quadrupole mass spectrometry. The optimal fragments (Q3), collision energy (V) and RF lens (V) were determined using purchased standards. After LC–MS analysis, the raw data were imported into Skyline software (V20.0) to calculate the integrated peak areas of

these transitions for all standards and samples. All measurements are reported in (fmol/ul). The intra and interassay coefficients of variation (CV) for phenylalanine were 2.1 and 8.6% respectively and for tyrosine were 16.5 and 14.6% respectively. We analyzed phenyalanine and tyrosine separately, and not as a combined analysis, because while phenylalanine is an essential amino acid, tyrosine is not. Phenylalanine can be oxidized to tyrosine by oxidation, and although this is the predominant pathway for phenyalanine breakdown, in the fasting state phenylalanine reflects protein breakdown and oxidation provides approximately 15% of the tyrosine [27].

Outcomes

Hip fractures

Hospitalizations were identified by participant (or proxy) report every six months and confirmed by review of medical records. Hospital claims and hospitalization discharge summaries were also reviewed to capture any hip fractures not reported by the participants [28]. Hip fractures were then identified in CHS by screening these hospitalization date for ICD-9 codes (820.xx). Pathological fractures (ICD-9 code 773.1x) and fractures from motor vehicle accidents (E810.xx-E825.xx) were not included in these analyses.

Frailty

Freid's frailty index was used to define frailty for these analyses. Frailty included at least three of the following five outcomes: slow walking speed during a 4.5 m walk, muscle weakness, low physical activity, weight loss, and self-reported exhaustion. Prefrail was defined as one or two of these conditions and not frail had none of these five criteria [29]. These five frailty parameters were defined as follows: For men, walking speed was considered "slow" if it took ≥ 7 or ≥ 6 s to walk 4.5 m for men with a height of ≤ 173 cm or > 173 cm, respectively. For women, walking speed was considered "slow" if it took ≥ 7 or ≥ 6 s for women with a height of ≤ 159 cm or > 159 cm, respectively. Low muscle weakness was determined by grip strength, which was adjusted for gender and body mass index (BMI). Grip strength was measured with a Jamar hydraulic hand dynamometer. The best result of three attempts was taken as the result. For men, low grip strength was defined as ≤ 29 kg for men with a BMI of ≤ 24, ≤ 30 kg for men with a BMI between 24.1 and 28, and ≤ 32 kg for men with a BMI of > 28. For women, low grip strength was defined as ≤ 17 kg for women with a BMI of ≤ 23, ≤ 17.3 kg for women with a BMI between 23.1 and 26, ≤ 18 kg for women with a BMI between 26.1 and 29, and ≤ 21 kg for women with a BMI of > 29. Low physical activity was determined by the patient answering "less" to the question, "Are you more, less, or equally active compared to men and women of your age?" Weight loss was defined as unintentional weight loss of > 5 kg during the last year or a BMI of < 18.5. Self-reported exhaustion was determined by the patient answering "no" to the question, "Do you feel full of energy?" We assessed frailty cross sectionally at the 1992–1993 visit.

Assessment of covariates

We examined the following covariates obtained from the 1992–1993 CHS visit: age, BMI (kg/m^2), weight (kg), sex, race, clinic site, self-reported health status (excellent, very good, good, fair or poor), history of diabetes, highest education level obtained (≥ 12th or < 12th grade), smoking status: (current, former, never), current alcohol use (0 =≤ 7 drinks/week, > 7 drinks/week), and renal function from estimated glomerular filtration rate (eGFR) from combined creatinine-cystatin C equation [30]. Medications were determined via direct examination of medication bottles and included calcium and vitamin D supplements, selective estrogen receptor modulators, estrogens, bisphosphonates, oral corticosteroids, loop diuretics, thiazide diuretics, selective reuptake inhibitors, anticonvulsants, benzodiazepines, sedative/hypnotics, proton pump inhibitors, thiazolidinediones, and thyroid medications.

Statistical analyses

Weighted multivariable Cox hazards models were used to estimate the hazard ratios (HR) and 95% confidence intervals (CI) of incident hip fracture associated with a standard deviation higher exposure of phenylalanine and tyrosine. We censored participants at death, loss of follow up, or after 12 years of follow-up. Robust standard errors were used due to the weighting of the participants. Cross sectionally, weighted Poisson regression models with robust standard errors were used to estimate the relative risk of frailty associated with a standard deviation higher exposure. The frailty index included categories of frailty, prefrail and not frail which were converted to binary indexes. We used nested models adjusting for factors as follows: minimally adjusted: age, sex, race, clinic site; and fully adjusted: age, BMI (kg/m^2), weight (kg), sex, race, clinic site, self-reported health status (excellent, very good, good, fair or poor), history of diabetes, highest education level obtained (≥ 12th or < 12th grade), smoking status: (current, former, never), current alcohol use (0 =≤ 7 drinks/week, > 7 drinks/week), renal function and medication use.

Table 1 Weighted descriptives overall and by hip fracture status at follow-up

Characteristics			
	All	Censored before hip fx	Hip fx during follow-up
Age (yrs.)	75 ± 4.7	75 ± 4.6	76 ± 5.1
BMI (kg/m2)	26 ± 4.3	26 ± 4.3	26 ± 4
Weight (kg)	70 ± 14.4	70 ± 14.5	67 ± 16.5
Sex (%)			
Male	42	43	28
Female	58	57	72
Race (%)			
Black	5	5	2
White	95	95	98
Health Status (%)			
Excellent	5	4.7	2.9
Very Good	36	36.6	27.7
Good	47	46.9	48.3
Fair	12	11.8	18.7
Poor	0	0	2.4
Diabetes (%)	14	14	12
Smoking History (%)			
Current	8	8	6
Former	48	49	37
Never	44	43	57
Clinic Site (%)			
Bowman Gray NC	25	24	33
Davis CA	24	25	17
Hagerstown MD	25	25	30
Pittsburg PA	26	26	20
Education (%)			
< 12th grade	55	55	53
> 12th grade	45	45	47
Renal Function[a]	72.8 ± 19.2	72.8 ± 19.4	72.8 ± 16.5
Current Alcohol Use (%) (drinks/week)			
0—≤ 7	88	88	95
> 7	12	12	5
Medication Use (%)			
Osteoporosis Medication Use [b]	1	1	3
Bone Medication Use [c]	30	29	40
Thyroid Medication Use [d]	9	9	13
Frailty Status (%)			
Not Frail	42	51	29
Pre Frail	46	42	54
Frail	12	7	17

Mean ± SD (OR %)

[a]Estimated glomerular filtration rate calculated from serum creatinine and Cystatin C

[b]Defined as use of calcium, vitamin D, selective estrogen receptor modifiers (SERMs), estrogens, or bisphosphonates

[c]Defined as use of oral corticosteroids, loop diuretics, thiazide diuretics, selective serotonin reuptake inhibitors, anticonvulsants, benzodiazepines, sedatives/hypnotics, proton pump inhibitors, thiazolidinediones

[d]Defined as use of thyroid medications

Results

Demographic and clinical characteristics of the study population are shown in Table 1**.** The mean age of the study population at baseline was 75 years (range 67–92)42% were men and 95% were Caucasian. The mean weight was 70 (range 39–134 kg) and BMI 26 (range 14–44).The majority (74%) were prefrail. In multivariable adjusted models there was no significant association between phenylalanine (HR 0.85 (95% CI 0.62–1.16)) and tyrosine (HR 0.82 (95% CI 0.62–1.1)) with hip fractures (Table 2). Neither phenylalanine nor tyrosine was significantly associated with frailty status (Frail compared with prefrail or nonfrail: (HR 0.92 (95% CI 0.48–1.76)) and (HR 0.86 (95% CI 0.46–1.41)) (Table 3) and prefail compared with nonfrail (HR 0.95 (95% CI 0.82–1.14)) and (HR 0.87 (95% CI 0.74–1.04))) (Table 4).

Discussion

In elderly community dwelling men and women in the CHS, serum concentrations of the aromatic amino acids phenylalanine and tyrosine were not significantly associated with incident hip fractures or cross-sectional assessment of frailty status.

In support of our findings that there is no association of phenylalanine or tyrosine with hip fracture risk, in older community dwelling men and women in Hong Kong, neither phenylalanine nor tyrosine was associated with ten-year fracture risk [23]. Also, in agreement with these findings, we have previously published that higher dietary intakes of phenylalanine and tyrosine were not significantly associated with either BMD of the hip or hip fractures in participants in the CHS [31]. In contrast, using Mendelian randomization analyses of samples included in the Nightingale Health UK Biobank Initiative, serum levels of tyrosine were negatively associated with total body BMD; however, fracture data was not included in this report [24]. Interestingly, one cross sectional study [32] and several systematic reviews[33, 34], have reported that patients with phenylketonuria, a genetic disorder in which phenylalanine cannot be converted to tyrosine, have lower BMD than normal controls. Moreover, in one report of patients with a spinal cord injury, higher dietary intakes of phenylalanine were inversely associated with BMD of the lumbar spine [35]. However, the pathophysiology of bone loss in phenylketonuria is not well understand [36], and the pathophysiology of sublesional osteoporosis differs substantially from senile osteoporosis [37]. Our findings that serum levels of phenylalanine and tyrosine are not significantly associated with hip fractures is in contrast with our findings for tryptophan [38], suggesting the need to consider specific amino acids, and not just overall protein intake, when examining fracture risk.

Few prior studies have addressed the association of levels of phenylalanine and tyrosine with frailty. In contrast with our findings of no association of these aromatic

Table 2 Association of phenylalanine and tyrosine with hip fractures (HR (95% CI)

	Minimally Adjusted*	P-Value	Fully Adjusted**	*P*-Value
Phenylalanine	0.87 (0.65–1.16)	0.35	0.85 (0.62–1.16)	0.3
Tyrosine	0.87 (0.68–1.10)	0.24	0.82 (0.62–1.1)	0.14

*Adjusted for age, sex, race and clinic site

**Adjusted for age, BMI (kg/m^2), weight (kg), sex, race, clinic site, self-reported health status (excellent, very good, good, fair, poor), history of diabetes, smoking status (current, former, never), highest education level completed (≥ 12th or < 12th grade), renal function, current alcohol use (0 -≤ 7 drinks/week, > 7 drinks/week), and medication use

Table 3 Association of Phenylalanine and Tyrosine with Frailty (Frail vs. Not Frail or PreFrail) (HR (95% CI)

	Minimally Adjusted*	P-Value	Fully Adjusted**	P-Value
Phenylalanine	0.91(0.56–1.48)	0.71	0.92 (0.48–1.76)	0.8
Tyrosine	1.23(0.76–2.01)	0.41	0.86 (0.46–1.61)	0.64

*Adjusted for age, sex, race and clinic site

**Adjusted for age, BMI (kg/m2), weight (kg), sex, race, clinic site, self-reported health status (excellent, very good, good, fair, poor), history of diabetes, smoking status (current, former, never), highest education level completed (≥ 12th or < 12th grade), renal function, current alcohol use (0—≤ 7 drinks/week, > 7 drinks/week), and medication use

Table 4 Association of phenylalanine and tyrosine with frailty (Pre-Frail vs. Not Frail) (HR (95% CI)

	Minimally Adjusted*	P-Value	Fully Adjusted**	P-Value
Phenylalanine	0.96 (0.83–1.11)	0.57	0.95 (0.82–1.04)	0.5
Tyrosine	0.90 (0.76–1.04)	0.15	0.87(0.74–1.04)	0.12

*Adjusted for age, sex, race, clinic site

**Adjusted for age, BMI (kg/m^2), weight (kg), sex, race, clinic site, self-reported health status (excellent, very good, good, fair, poor), history of diabetes, smoking status (current, former, never), highest education level completed (≥ 12th or < 12th grade), renal function, current alcohol use (0 -≤ 7 drinks/week, > 7 drinks/week), and medication use

amino acids with frailty, in two reports, lower concentrations of tyrosine occurred in those with frailty [39, 40]. Our study differed from these in the ethnicity and sex of participants included, with one report confined to those of Portuguese descent [39] and the other including only men [40].

The major strength of our study lies in the use of a well-characterized community dwelling population to examine the relationship of phenylalanine and tyrosine to a number of important events in an aging population, including fractures and frailty. However, there are also a number of limitations to this work. We only had one time point for measurements of our biomarkers and levels may have changed over time. Only hip fractures, and no other fractures, were examined. We only measured serum, and not bone marrow derived, levels of these biomarkers. Our serum samples were stored over many years; however, long-term storage has not been reported to significantly impact on concentrations of these amino acids [41]and sample processing and freeze thaw cycles follow strict protocols in CHS. We were unable to include BMD measurements, as these were not done at the same time as the serum samples and were only measured in 73 of the participants included in these analyses. Due to sample size limitations, we were not able to examine changes in frailty status. In addition, there may be differences in fasting versus peak postprandial levels of amino acids that may vary in their impact on bone and muscle turnover [42, 43] and our samples were all collected in a fasting state.

In conclusion, serum levels of phenylalanine and tyrosine were not significantly associated with hip fractures or frailty in older individuals in the CHS. These aromatic amino acids are not likely to be significant contributors to musculoskeletal health in aging. Advice on protein intake for osteoporosis prevention should include consideration of specific types of amino acids and not be given as global advice to simply increase overall protein intake.

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Data availability CHS data may be accessed by qualified investigators by following CHS policies as outlined at https://chs-nhlbi.org/CHS_DistribPolicy.

Declarations

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in CHS.

Conflicts of interests Drs. Carbone, Buzkova, Robbins, Fink, Barzilay, Elam and Isales have no competing interests or conflicts of interests with this work. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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