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Original Research

Associations between Essential Amino Acid Intake and Functional Health Outcomes in Older Adults: Analysis of the National Health and Nutrition Examination Survey, 2001–2018☆

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A B S T R A C T

Background: The relationships between habitual essential amino acid (EAA) intake and body composition, muscle strength, and physical function in older US adults are not well defined.

Objectives: This cross-sectional study evaluated associations between usual EAA intakes and body composition, muscle strength, and physical function in US adults ≥65 y.

Methods: The Food and Nutrient Database for Dietary Studies (FNDDS) 2001–2018 was linked to the US Department of Agriculture Standard Reference database to access existing amino acid composition data for FNDDS ingredients. FNDDS ingredients without existing amino acid composition data were matched to similar ingredient codes with available data. Usual EAA, leucine, lysine, and sulfur-containing amino acid (SAA; methionine + cysteine) intakes (g/d) from National Health and Nutrition Examination Survey 2001–2018 were calculated for individuals ≥65 y ($n = 10{,}843$). Dependent variables included muscle strength measured by isometric grip test, body mass index (BMI), waist circumference, dual-energy X-ray absorptiometry-measured appendicular lean mass and whole-body fat mass, and self-reported physical function (that is, tasks of daily living). Regression analyses were used to determine covariate-adjusted relationships between EAA, leucine, lysine, and SAA intake and functional health outcomes. $P < 0.0013$ was considered significant.

Results: EAA, leucine, lysine, and SAA intakes, covaried with physical activity level and usual protein intake, were not associated with muscle strength or self-reported physical function in males or females or with body composition in males. EAA intakes were positively associated with waist circumference in females (β ± SEM, 2.1 ± 0.6 cm, $P = 0.0007$). Lysine intakes were positively associated with BMI (3.0 ± 0.7 kg/m^2, $P < 0.0001$) and waist circumference (7.0 ± 1.7 cm, $P = 0.0001$) in females.

Conclusions: Habitual EAA, leucine, lysine, and SAA intakes, covaried with physical activity level and usual protein intake, were not associated with lean mass, muscle strength, or physical function in adults ≥65 y. However, EAA intakes, particularly lysine, were positively associated with measures of adiposity in older females.

This trial was registered with the Open Science Framework (https://doi.org/10.17605/OSF.IO/25V63) as osf.io/25v63).

Keywords: NHANES, essential amino acids, muscle strength, fat mass, lean mass, physical function

Abbreviations: DXA, dual-energy x-ray absorptiometry; EAA, essential amino acid; FNDDS, Food and Nutrient Database for Dietary Studies; IUI, individual usual intake; NCHS, National Center for Health Statistics; NHB, non-Hispanic Black; NHW, non-Hispanic White; RDA, Recommended Dietary Allowance; PIR, poverty-income ratio; SAA, sulfur-containing amino acid; WWEIA, What We Eat in America.

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Introduction

Age-related changes in body composition, particularly decreased muscle mass and increased fat mass, are important factors associated with declines in physical function, reduced quality of life, and mortality [1–3]. The identification and development of strategies that preserve physical function and prolong quality years of life in aging individuals may reduce financial and logistical strain on the aging individual, their caregivers, and the healthcare system.

Consuming dietary protein at levels above the Recommended Dietary Allowance (RDA; 0.8 g/kg body weight/d or 56 g/d for a 70 kg reference male or 46 g/d for a 58 kg reference female) is suggested as a dietary strategy to maintain lean mass and physical function in older adulthood [4,5]. Perhaps, more important than the quantity of protein ingested is the quality of protein consumed in older adulthood, given both reduced energy intake and muscle anabolic resistance to ingested nutrients are associated with aging [6,7]. The quality of a specific protein source is based on its digestibility and essential amino acid (EAA) content (that is, the digestible indispensable amino acid scores [8]) which is the primary stimulus for muscle protein synthesis [9,10]. Therefore, in older adults, dietary EAA intake may be associated with health and functional outcomes, independent of total protein intake.

Findings from interventional studies are equivocal as far as chronic effects of EAA intake on body composition and physical function [11–14]. Furthermore, these interventional studies typically provide a specific dose of EAAs to participants, which is not representative of their habitual EAA intake, for a prescribed number of days, weeks, or months in addition to their normal eating pattern. Little is known about the relationship between habitual EAA intake, as a continuous variable, and body composition, muscle strength, and physical function in older US adults. The objective of the current study was to evaluate the relationship between habitual EAA intake and body composition, muscle strength, and self-reported physical function in older (≥65 y) US adults. We hypothesized greater intake of total EAAs would be associated with greater muscle strength, physical function, and appendicular lean mass and reduced whole-body fat mass, waist circumference, and BMI. Individual relationships between habitual intake of leucine, lysine, and total sulfur-containing amino acids (SAAs) and muscle strength, body composition, and physical function were also assessed. Leucine was evaluated because of its known role in stimulating muscle protein synthesis [15]. Lysine was evaluated because it is the limiting EAA in certain plant sources of protein, including wheat, rice, and nuts. The SAAs (methionine + cysteine) were evaluated together because methionine is the limiting EAA in certain plant sources of protein, including legumes, but can be spared, in part, by dietary cysteine [16]. Greater habitual leucine, lysine, or SAA intakes may be indicative of higher quality protein intake. Therefore, we hypothesized greater intakes of leucine, lysine, and SAAs would each be associated with greater muscle strength, physical function, and appendicular lean mass and reduced whole-body fat mass, waist circumference, and BMI in older adults.

Methods

The National Health and Nutrition Examination Survey (NHANES) is a combination of interviews and physical examinations used to assess the health and nutritional status of the civilian, non-institutionalized US population. The National Center for Health Statistics (NCHS), which is part of the Centers for Disease Control and Prevention, conducts the survey and releases data every 2 y [17]. The Research Ethics Review Board at the NCHS approves the survey protocol and all participants or proxies provide written informed consent. Detailed descriptions of the survey design and the data collection procedures are reported elsewhere [17]. The current trial is registered with the Open Science Framework at https://doi.org/10.17605/OSF.IO/25V63 (registration ID: osf.io/25v63). For this study, data from NHANES 2001–2018 were used to determine individual usual intakes (IUIs) of total EAAs, leucine, lysine, and SAAs in US adults ≥65 y ($n = 10{,}843$; Supplemental Figure 1). The age category was selected based on the PROT-AGE Study Group, a study group assembled to review dietary protein needs with aging, definition of an older adult [5] and Medicare eligibility (that is, ≥65 y).

Determination of usual amino acid intakes

The dietary intake assessment component of NHANES 2001–2018, What We Eat in America (WWEIA), consists of two 24-h dietary recalls collected 3–10 d apart. The first 24-h recall was collected in-person and the second over the telephone by trained staff. WWEIA data was combined with the Food and Nutrient Database for Dietary Studies (FNDDS) to generate gram amounts and determine nutrient values. The 2001–2018 FNDDS contains 3302 unique ingredients. The percentage of protein in which amino acids are directly obtained from the Standard Reference database is 88.3%, whereas the other 11.7% is obtained through matched ingredient codes. Our group has previously described a method [18] for mapping FNDDS ingredient codes with missing amino acid composition data to similar ingredients with available amino acid data to estimate amino acid composition for 100% of foods reported in WWEIA and NHANES. Total EAAs were calculated by summing dietary intakes of the 9 EAAs (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). SAAs were calculated by summing dietary intakes of methionine and cysteine. Population usual intake distributions and IUIs for amino acid intakes were estimated using the National Cancer Institute method version 2.1 [19].

Dependent variables

Muscle strength measurements were collected during the 2011–2012 and 2013–2014 NHANES cycles by isometric grip test (largest reading from each hand combined; continuous variable, range of values 11.4–169.6 kg) using a handgrip dynamometer. BMI (continuous, range 12.4–82.1 kg/m^2) and waist circumference (continuous, range 38.7–176 cm) were collected from 2001 to 2018. Body composition variables [appendicular lean mass (continuous, range 5.10–58.15 kg) and whole-body fat mass (continuous, range 3.41–102.29 kg)] measured by dual-energy x-ray absorptiometry (DXA) during cycles 2001–2002, 2003–2004, 2005–2006, 2011–2012, 2013–2014, 2015–2016, and 2017–2018 were used in the current analysis. Whole-body DXA data were not collected from 2007 to 2010. Appendicular lean mass was calculated as lean mass from both arms and both legs. Bone mineral content was not included in appendicular and whole-body lean mass.

The method of multiple imputations was used for the analysis of DXA data from 2001 to 2006, which has a large amount of missing data in a nonrandom pattern [20]. The DXA datasets for 2001–2002, 2003–2004, and 2005–2006 are multiple imputed with 5 separate imputed datasets for each cycle. This results in 5 imputed datasets for 2001 to 2006. The DXA data was available without imputation for 2011–2018. The 2011–2018 DXA dataset was appended to each of the five 2001–2006 DXA datasets resulting in 5 imputed datasets covering 2001–2006 and 2011–2018. Standard methods were used to account for the imputation component of estimated variance. This involves model fitting using each of the 5 imputation datasets and then using standard methods to combine the results to estimate means, regression coefficients, and associated standard errors, confidence intervals, and *P* values.

Self-reported physical function variables (continuous, no difficulty = 0, some difficulty = 1, much difficulty = 2, or an inability to perform the physical function = 3) are available from 2001 to 2018 and include walking for a quarter mile (that is, "by yourself and without using any special equipment, how much difficulty do you have walking for a quarter of a mile or ~2 or 3 blocks?"); walking up 10 steps without resting; lifting or carrying something as heavy as 10 pounds (like a sack of potatoes or rice); standing up from an armless straight chair; and pushing or pulling large objects like a living room chair (available 2003–2018 only).

Model covariates

Model 1 covariates included self-reported gender (categorical, male or female), race/ethnicity [categorical, Mexican American, other Hispanic, non-Hispanic White (NHW), non-Hispanic Black (NHB), other], age (continuous, years), IUI of calories (continuous, kcal/d), smoking status (categorical, smoked ≥100 cigarettes in life, yes or no), alcohol use (categorical, had ≥12 alcoholic drinks in any 1 y, yes or no), physical activity level (categorical, sedentary, moderate, or vigorous), and poverty level index (categorical, index ≤ 1.30, 1.30 < index ≤ 1.85, or index > 1.85). Model 2 included all the covariates from model 1 and the IUI of protein (continuous, g/d). Adjustment for protein intake allowed assessment of associations between specific EAAs and health/functional outcomes, independent of total protein intake. Model 2 is the fully adjusted model for BMI and waist circumference. For the dependent variables appendicular lean mass, whole-body fat mass, and muscle strength, model 3 included all the covariates from model 2 and height (continuous, m). Model 4 included all the covariates from model 3 plus whole-body fat mass (continuous, kg) when the dependent variable was appendicular lean mass, total lean mass (continuous, kg) when the dependent variable was whole-body fat mass, and BMI (continuous, kg/m^2) when the dependent variable was muscle strength. For physical function measures, model 3 included all the covariates from model 2 and BMI (continuous, kg/m^2) and model 4 included all the covariates from model 3 plus the multimorbidity score (continuous, 0–9). Model 4 is the fully adjusted model for whole-body fat mass, appendicular lean mass, muscle strength, and physical function outcomes.

For the physical activity covariate, Physical Activity Questionnaire responses to the number of d/wk in which vigorous activities were performed were separated into 3 categories: sedentary, 0–3 d; moderate, 4–6 d; and vigorous, 7 d. To characterize multimorbidity, NHANES questionnaire data for chronic health conditions (that is, "have you ever been told by a doctor or health professional you have…") was used to sum the number of "yes" responses to having received a diagnosis of any 9 chronic conditions, similar to a previous study [21]. The 9 chronic conditions were arthritis, cancer, cardiovascular disease (including congestive heart failure, coronary artery disease, heart attack, and angina), pulmonary disease (including emphysema, chronic bronchitis, chronic obstructive pulmonary disease, and asthma), stroke, chronic kidney disease, diabetes, hypertension, and depression.

Determination of food sources of lysine

Upon seeing the relationship between lysine intake and body composition, an analysis of current food sources of lysine was conducted to aid interpretation of results. In individuals ≥65 y, the greatest food sources (USDA groups: main, subgroup, and category) contributing to lysine intake were determined for each lysine intake decile using the population ratio method.

Statistical analyses

Intakes of EAAs, leucine, lysine, and SAAs are estimated IUI adjusted for total calorie intake using the residual method. IUI intake for each amino acid (mg/d) is regressed on IUI for total calories (kcal/d) allowing for an intercept. The mean IUI for the amino acid is then added to the residual from the regression to obtain the residual adjusted intake. Residual-adjusted absolute (mg/d) intakes of EAAs, leucine, lysine, and SAAs were analyzed as continuous and categorical independent variables.

Linear trends for muscle strength, the primary study outcome, were determined for EAA, leucine, lysine, and SAA intakes as continuous variables using regression analysis after adjusting for covariates (linear trend). For EAA, leucine, lysine, and SAA, least-squares means and standard errors for muscle strength were determined for each intake decile using regression analysis after adjusting for covariates (decile trend). Linear trends and decile trends also were determined for secondary outcomes, including BMI, waist circumference, appendicular lean mass, and whole-body fat mass using regression analysis and adjustment for covariates. Leucine intake during eating occasions (breakfast, morning snack, lunch, afternoon snack, dinner, and evening snack) was used to categorize individuals by the number of eating occasions in which they consumed ≥2.5 g leucine (0, 1, 2, or ≥3+). A per-meal threshold of 2.5–2.8 g leucine is recommended to stimulate anabolism in older adults [5]. Least-squares means and standard errors for muscle strength, BMI, waist circumference, appendicular lean mass, and whole-body fat mass were determined for each eating occasion category using regression analysis adjusted for covariates as described above (that is, models 1–4). Eating occasion categories (1, 2, or ≥3+) were compared with the reference (0 eating occasions) using a *t*-test. Linear trends for physical function were determined with EAA, leucine, lysine, or SAA intake as the primary independent variable using regression analysis and adjustment for covariates as described above.

Data were analyzed with the use of SAS 9.4 (Research Triangle Institute). Appropriate weighting factors were used to adjust for oversampling of selected groups, survey nonresponses of some individuals, and for the day of the week the interview was

conducted. Significance was set at a Bonferroni-adjusted α of $P < 0.0013$ [$P < 0.05$ divided by 40 (4 independent dietary amino acid intake variables (EAA, leucine, lysine, and SAA) and 10 dependent variables analyzed)]. A sample size of 1000 participants will provide 90% power to detect significance for a regression coefficient with a small effect size ($f^2 = 0.02$) and 17 predictors.

Results

Males consumed 56–111 g/d (decile 1 to decile 10) protein, 4.5–8.9 g/d leucine, 3.8–7.5 g/d lysine, 2.0–4.1 g/d SAA, and 25–49 g/d EAA, whereas females consumed 44–84 g/d protein, 3.5–6.7 g/d leucine, 3.0–5.6 g/d lysine, 1.6–3.1 g/d SAA, and 19–37 g/d EAA (Supplemental Table 1). Males with greater EAA intake were younger; less likely to be NHB; more likely to be NHW, drink alcohol, engage in vigorous physical activity, and have a poverty-income ratio (PIR) > 1.85; and consumed more total calories, total protein, leucine, lysine, and SAA. Females with greater EAA intake were younger; less likely to be NHB; more likely to be NHW, drink alcohol, engage in vigorous and moderate physical activity, and have a PIR > 1.85; and consumed more total calories, total protein, leucine, lysine, and SAA. Males with greater leucine intake were younger; less likely to be NHB; more likely to be NHW, drink alcohol, engage in vigorous physical activity, and have a PIR > 1.85; and consumed more total calories, total protein, EAA, lysine, and SAA. Females with greater leucine intake were younger; less likely to be NHB; more likely to be NHW, drink alcohol, engage in moderate physical activity, and have a PIR > 1.85; and consumed more total calories, total protein, EAA, lysine, and SAA. Males with greater lysine intake were younger; less likely to be NHB; more likely to be NHW, drink alcohol, engage in vigorous physical activity, and have a PIR > 1.85; and consumed more total calories, total protein, EAA, leucine, and SAA. Females with greater lysine intake were younger; less likely to be NHB; more likely to drink alcohol, engage in vigorous and moderate physical activity, and have a PIR > 1.85; and consumed more total calories, total protein, EAA, leucine, and SAA. Males with greater SAA intake were younger; less likely to be NHB; more likely to be NHW, drink alcohol, engage in vigorous physical activity, and have a PIR > 1.85; and consumed more total calories, total protein, EAA, leucine, and lysine. Females with greater SAA intake were younger; less likely to be NHB; more likely to be NHW, drink alcohol, engage in moderate physical activity, and have a PIR > 1.85; and consumed more total calories, total protein, EAA, leucine, and lysine.

In males, EAA, leucine, and SAA intakes were not associated with BMI for either of the statistical models (Table 1). Lysine intake was positively associated with BMI in males for model 1 but was no longer significant when we further adjusted for protein (model 2). In females, EAA and leucine intakes were not associated with BMI for any of the statistical models. SAA intake was positively associated with BMI in females for model 1 but was no longer significant when we further adjusted for protein (model 2). However, lysine intake was positively associated with BMI in females for both models 1 and 2 (linear trends only). Intakes of EAA, leucine, lysine, and SAA were not associated with waist circumference in males. Leucine and SAA intakes were not associated with waist circumference in females. Intakes of EAA and lysine were positively associated with waist circumference in females for model 2 (linear trends only).

Intakes of EAA, leucine, lysine, and SAA were not associated with whole-body fat mass in males or females (Table 1). In males, EAA, leucine, and SAA intakes were not associated with appendicular lean mass. Intakes of lysine were positively associated with appendicular lean mass in males for model 1; however, when the model was further adjusted for protein (model 2), this relationship was no longer significant. Intakes of EAA, leucine, lysine, and SAA were not associated with appendicular lean mass in females. EAA, leucine, lysine, and SAA intakes were not associated with combined grip strength in males or females.

In both males and females and for all statistical models, BMI, waist circumference, whole-body fat mass, appendicular lean mass, and combined grip strength were not significantly different in those who consumed ≥2.5 g leucine at 1, 2, or 3+ eating occasions compared with those who consumed <2.5 g leucine at all eating occasions (Supplemental Table 2).

In females and for all statistical models, self-reported physical function (that is, difficulty of walking for a quarter mile, walking up 10 steps without resting, lifting or carrying something as heavy as 10 pounds, standing up from an armless chair, and pushing or pulling large objects) was not associated with EAA, leucine, lysine, and SAA intakes (Table 2). In males, greater EAA, leucine, and SAA intakes were associated with less difficulty pushing or pulling large objects in model 1. However, when we further adjusted for protein (model 2), these relationships were no longer significant. Intakes of EAA, leucine, lysine, and SAA were not associated with any other self-reported physical function measures in males.

Due to the significant relationship identified between habitual lysine intake and adiposity, major food sources of lysine intake were investigated. For both males and females consuming the most lysine (that is, decile 10), the top 4 food sources of lysine were fish, chicken, meat mixed dishes, and beef (Figure 1).

Discussion

Some dietary recommendations [4,5] suggest older adults should consume protein at amounts above the RDA (0.8 g/kg/d) for lean mass maintenance or accrual and subsequent benefits to muscle strength. More protein or EAA intake is needed to stimulate muscle protein synthesis (MPS) to the same extent in older adults as it does in younger adults, a phenomenon known as anabolic resistance [22,23]. However, improvements in lean body mass and function are not consistently demonstrated in longer-term intervention studies supplementing protein and/or EAA in older adults [11–14]. Results of the current cross-sectional study showed: *1)* habitual EAA, leucine, lysine, and SAA intakes were not associated with measures of appendicular lean mass in older men or women; *2)* habitual EAA, leucine, lysine, and SAA intakes were not associated with handgrip strength or self-reported physical function in older men or women; and *3)* habitual lysine intake was positively associated with BMI and waist circumference in older women, but not older men.

In the current study, lysine intake (trend for EAA, leucine, and SAA; $P \leq 0.0040$) was positively associated with appendicular lean mass in males for model 1. These associations were no longer present when further adjusted for protein intake (model 2), which was adequate in all intake deciles (that is, ≥56 g/d; Supplemental Table 1). This suggests protein quantity, compared

TABLE 1
Association of absolute (g/d) usual amino acid intake with body composition and muscle strength measures by gender in adults 65 y and older: NHANES 2001–2018

			Decile of amino acid intake (mean ± SEM)			Decile trend		Linear trend	
	Model	*n*	1	5	10	β ± SE	*P*	β ± SE	*P*
Body mass index (kg/m^2)									
Essential AA (g/d)									
Male	1	4629	28.2 ± 0.3	28.6 ± 0.3	29.2 ± 0.3	0.11 ± 0.04	0.0029	0.07 ± 0.02	0.0019
	2	4629	28.9 ± 0.7	28.7 ± 0.3	28.5 ± 0.8	0.02 ± 0.10	0.8755	–0.10 ± 0.16	0.5127
Female	1	4515	28.1 ± 0.4	28.6 ± 0.4	29.3 ± 0.4	0.13 ± 0.04	0.0029	0.12 ± 0.04	0.0017
	2	4515	28.3 ± 0.8	28.6 ± 0.4	29.1 ± 0.9	0.10 ± 0.11	0.3632	0.76 ± 0.28	0.0064
Leucine (g/d)									
Male	1	4629	28.4 ± 0.3	28.6 ± 0.3	29.4 ± 0.3	0.11 ± 0.04	0.0028	0.39 ± 0.12	0.0016
	2	4629	29.2 ± 0.7	28.7 ± 0.3	28.5 ± 0.7	0.00 ± 0.09	0.9800	–0.29 ± 0.67	0.6701
Female	1	4515	28.0 ± 0.4	28.7 ± 0.5	29.3 ± 0.3	0.12 ± 0.04	0.0051	0.64 ± 0.21	0.0021
	2	4515	28.5 ± 0.7	28.8 ± 0.5	28.8 ± 0.8	0.03 ± 0.10	0.7589	2.36 ± 1.27	0.0653
Lysine (g/d)									
Male	1	4629	28.1 ± 0.3	29.2 ± 0.3	29.3 ± 0.4	0.12 ± 0.04	0.0010	0.47 ± 0.13	0.0004
	2	4629	28.4 ± 0.6	29.2 ± 0.3	29.0 ± 0.7	0.06 ± 0.08	0.4508	0.60 ± 0.53	0.2579
Female	1	4515	28.2 ± 0.4	28.5 ± 0.4	29.4 ± 0.4	0.15 ± 0.05	0.0012	0.83 ± 0.22	0.0003
	2	4515	28.1 ± 0.6	28.5 ± 0.4	29.5 ± 0.6	0.17 ± 0.09	0.0452	3.00 ± 0.69	<0.0001
Sulfur AA (g/d)									
Male	1	4629	28.4 ± 0.3	28.4 ± 0.3	29.5 ± 0.3	0.11 ± 0.04	0.0038	0.81 ± 0.26	0.0026
	2	4629	29.0 ± 0.6	28.4 ± 0.3	28.9 ± 0.6	0.02 ± 0.08	0.8520	–0.06 ± 1.05	0.9511
Female	1	4515	27.8 ± 0.3	28.6 ± 0.4	29.3 ± 0.4	0.13 ± 0.04	0.0018	1.42 ± 0.42	0.0009
	2	4515	28.0 ± 0.7	28.6 ± 0.4	29.1 ± 0.7	0.08 ± 0.09	0.3890	2.75 ± 1.57	0.0817
Waist circumference (cm)									
Essential AA g/d									
Male	1	4525	105.5 ± 0.8	105.8 ± 0.9	107.1 ± 0.8	0.17 ± 0.09	0.0745	0.11 ± 0.06	0.0551
	2	4525	106.5 ± 1.7	105.9 ± 0.9	106.0 ± 1.9	0.01 ± 0.24	0.9658	–0.14 ± 0.34	0.6783
Female	1	4332	97.8 ± 0.9	98.6 ± 1.1	99.6 ± 1.0	0.23 ± 0.11	0.0334	0.20 ± 0.09	0.0324
	2	4332	97.2 ± 1.9	98.5 ± 1.0	100.3 ± 2.2	0.38 ± 0.29	0.1889	2.17 ± 0.62	0.0006
Leucine (g/d)									
Male	1	4525	105.9 ± 0.9	105.6 ± 0.9	107.4 ± 0.8	0.17 ± 0.09	0.0616	0.61 ± 0.31	0.0491
	2	4525	106.7 ± 1.7	105.7 ± 1.0	106.5 ± 1.7	0.06 ± 0.23	0.8009	–0.09 ± 1.40	0.9501
Female	1	4332	97.7 ± 0.9	98.8 ± 1.1	99.6 ± 1.0	0.22 ± 0.11	0.0406	1.10 ± 0.51	0.0321
	2	4332	97.4 ± 1.7	98.8 ± 1.1	100.0 ± 2.0	0.27 ± 0.26	0.3062	7.83 ± 2.95	0.0089
Lysine (g/d)									
Male	1	4525	105.1 ± 0.8	107.1 ± 0.7	107.3 ± 1.0	0.18 ± 0.09	0.0524	0.77 ± 0.32	0.0185
	2	4525	105.2 ± 1.6	107.1 ± 0.7	107.2 ± 2.0	0.11 ± 0.22	0.6299	1.58 ± 1.41	0.2645
Female	1	4332	98.1 ± 1.0	97.7 ± 0.9	99.6 ± 1.1	0.29 ± 0.11	0.0140	1.48 ± 0.56	0.0096
	2	4332	97.1 ± 1.4	97.6 ± 0.9	100.6 ± 1.6	0.55 ± 0.24	0.0224	7.29 ± 1.70	<0.0001
Sulfur AA (g/d)									
Male	1	4525	105.9 ± 0.8	105.6 ± 0.8	107.6 ± 0.8	0.17 ± 0.09	0.0798	1.33 ± 0.66	0.0446
	2	4525	106.7 ± 1.4	105.7 ± 0.8	106.7 ± 1.5	0.03 ± 0.21	0.8971	1.08 ± 2.62	0.6818
Female	1	4332	97.4 ± 0.9	99.0 ± 0.9	99.6 ± 1.0	0.20 ± 0.11	0.0556	2.23 ± 1.07	0.0396
	2	4332	97.5 ± 1.7	99.0 ± 0.9	99.5 ± 1.8	0.13 ± 0.23	0.5571	4.75 ± 3.66	0.1958
Whole-body fat mass (kg)									
Essential AA (g/d)									
Male	1	1201	26.6 ± 1.2	27.0 ± 1.1	31.0 ± 2.0	0.19 ± 0.15	0.2076	0.14 ± 0.09	0.1374
	2	1201	24.6 ± 2.7	26.8 ± 1.1	33.1 ± 3.8	0.06 ± 0.39	0.8740	0.72 ± 0.56	0.1998
	3	1163	24.6 ± 2.6	26.8 ± 1.1	33.2 ± 3.4	0.02 ± 0.40	0.9617	0.68 ± 0.56	0.2264
	4	1163	25.9 ± 1.5	26.9 ± 0.7	29.8 ± 2.1	0.00 ± 0.28	0.9878	0.30 ± 0.35	0.3957
Female	1	1179	30.6 ± 2.9	30.9 ± 1.2	34.6 ± 1.6	0.36 ± 0.20	0.0810	0.33 ± 0.17	0.0513
	2	1179	28.1 ± 3.2	30.7 ± 1.2	37.3 ± 3.8	0.41 ± 0.42	0.3244	3.17 ± 2.02	0.1166
	3	1142	29.2 ± 3.3	30.9 ± 1.3	35.6 ± 3.3	0.29 ± 0.40	0.4746	2.92 ± 1.93	0.1294
	4	1142	29.6 ± 1.8	30.9 ± 0.7	34.3 ± 2.3	0.27 ± 0.30	0.3690	0.73 ± 0.65	0.2582
Leucine (g/d)									
Male	1	1201	28.0 ± 1.1	27.7 ± 1.2	31.0 ± 1.8	0.22 ± 0.14	0.1196	0.75 ± 0.50	0.1383
	2	1201	25.8 ± 2.0	27.5 ± 1.2	33.4 ± 2.9	0.34 ± 0.31	0.2623	1.71 ± 2.36	0.4674
	3	1163	26.2 ± 2.1	27.1 ± 1.2	33.0 ± 2.7	0.21 ± 0.34	0.5456	1.11 ± 2.30	0.6296
	4	1163	27.6 ± 1.6	27.2 ± 0.9	28.7 ± 1.8	0.04 ± 0.31	0.9087	–0.34 ± 1.63	0.8359
Female	1	1179	31.3 ± 2.9	31.9 ± 1.3	35.0 ± 1.7	0.33 ± 0.20	0.1074	1.84 ± 0.97	0.0566
	2	1179	30.6 ± 3.1	31.9 ± 1.3	35.7 ± 3.8	0.15 ± 0.40	0.6979	8.54 ± 6.16	0.1656
	3	1142	32.4 ± 2.9	32.1 ± 1.4	33.0 ± 3.2	–0.07 ± 0.37	0.8385	5.97 ± 5.43	0.2718
	4	1142	30.5 ± 1.6	31.2 ± 0.6	33.8 ± 2.0	0.26 ± 0.27	0.3391	1.81 ± 2.43	0.4563

(*continued on next page*)

TABLE 1 (*continued*)

			Decile of amino acid intake (mean ± SEM)			Decile trend		Linear trend	
	Model	*n*	1	5	10	β ± SE	*P*	β ± SE	*P*
Lysine (g/d)									
Male	1	1201	26.8 ± 1.2	26.4 ± 0.8	31.6 ± 2.2	0.23 ± 0.15	0.1248	0.94 ± 0.58	0.1053
	2	1201	24.9 ± 2.0	26.3 ± 0.8	33.6 ± 3.0	0.26 ± 0.28	0.3565	2.71 ± 1.75	0.1224
	3	1163	25.5 ± 1.8	26.4 ± 0.7	33.3 ± 2.8	0.24 ± 0.29	0.4178	2.54 ± 1.86	0.1712
	4	1163	27.4 ± 1.6	26.0 ± 0.7	28.5 ± 1.8	–0.02 ± 0.23	0.9265	0.26 ± 1.51	0.8654
Female	1	1179	31.2 ± 3.0	31.3 ± 1.3	32.7 ± 1.3	0.38 ± 0.21	0.0645	2.08 ± 1.06	0.0501
	2	1179	31.8 ± 3.1	31.4 ± 1.3	32.1 ± 2.6	0.46 ± 0.35	0.1912	6.09 ± 3.10	0.0493
	3	1142	31.2 ± 2.8	31.4 ± 1.3	31.9 ± 2.4	0.38 ± 0.35	0.2724	6.90 ± 2.85	0.0155
	4	1142	30.3 ± 1.2	30.9 ± 0.8	32.8 ± 1.5	0.37 ± 0.22	0.0915	3.04 ± 1.26	0.0155
Sulfur AA (g/d)									
Male	1	1201	28.0 ± 1.3	26.2 ± 0.9	30.9 ± 1.7	0.22 ± 0.15	0.1441	1.67 ± 1.09	0.1243
	2	1201	25.9 ± 2.2	25.8 ± 0.9	32.8 ± 2.3	0.29 ± 0.31	0.3454	4.27 ± 3.08	0.1657
	3	1163	26.3 ± 2.1	26.1 ± 0.8	32.8 ± 2.1	0.23 ± 0.29	0.4230	4.67 ± 2.94	0.1121
	4	1163	27.4 ± 1.8	27.2 ± 0.6	28.4 ± 1.2	0.05 ± 0.26	0.8450	1.00 ± 1.90	0.5976
Female	1	1179	28.6 ± 1.0	30.3 ± 1.0	33.4 ± 1.3	0.35 ± 0.16	0.0339	3.78 ± 1.61	0.0189
	2	1179	27.5 ± 3.4	30.1 ± 1.2	34.4 ± 3.4	0.27 ± 0.41	0.5098	8.57 ± 10.25	0.4034
	3	1142	27.7 ± 3.2	30.5 ± 1.1	33.6 ± 3.0	0.22 ± 0.39	0.5730	7.34 ± 9.49	0.4395
	4	1142	30.5 ± 1.4	31.5 ± 0.7	32.7 ± 1.6	0.12 ± 0.23	0.6038	4.19 ± 3.99	0.2939
Appendicular lean mass (kg)									
Essential AA (g/d)									
Male	1	1201	23.6 ± 0.7	23.9 ± 0.4	25.6 ± 0.8	0.18 ± 0.06	0.0040	0.12 ± 0.04	0.0027
	2	1201	23.2 ± 1.4	23.8 ± 0.5	25.9 ± 1.7	0.10 ± 0.19	0.5996	0.30 ± 0.25	0.2230
	3	1163	23.3 ± 1.1	23.8 ± 0.4	25.9 ± 1.3	0.07 ± 0.14	0.6236	0.22 ± 0.20	0.2736
	4	1163	23.9 ± 0.8	23.9 ± 0.3	24.5 ± 1.0	0.07 ± 0.11	0.5654	0.06 ± 0.15	0.6948
Female	1	1179	15.9 ± 1.0	16.3 ± 0.3	17.0 ± 0.5	0.08 ± 0.06	0.2086	0.08 ± 0.05	0.1057
	2	1179	15.7 ± 1.0	16.3 ± 0.4	17.2 ± 1.4	0.02 ± 0.13	0.8895	0.89 ± 0.64	0.1663
	3	1142	16.2 ± 0.9	16.4 ± 0.3	16.4 ± 1.1	–0.03 ± 0.13	0.8088	0.84 ± 0.59	0.1491
	4	1142	16.7 ± 0.5	16.5 ± 0.2	15.4 ± 0.8	–0.10 ± 0.10	0.3125	0.17 ± 0.19	0.3854
Leucine (g/d)									
Male	1	1201	23.5 ± 0.6	24.2 ± 0.4	25.7 ± 0.8	0.20 ± 0.06	0.0017	0.69 ± 0.23	0.0029
	2	1201	22.3 ± 1.0	24.1 ± 0.4	27.0 ± 1.3	0.28 ± 0.18	0.1153	1.70 ± 0.97	0.0779
	3	1163	22.8 ± 0.7	23.9 ± 0.4	26.6 ± 1.0	0.18 ± 0.10	0.0710	0.95 ± 0.84	0.2544
	4	1163	23.0 ± 0.6	23.9 ± 0.3	25.3 ± 0.8	0.13 ± 0.11	0.2295	0.70 ± 0.68	0.3007
Female	1	1179	16.0 ± 1.0	16.5 ± 0.4	17.1 ± 0.5	0.07 ± 0.06	0.2877	0.47 ± 0.29	0.1077
	2	1179	16.3 ± 1.0	16.5 ± 0.4	16.7 ± 1.3	–0.08 ± 0.14	0.5797	2.63 ± 2.12	0.2150
	3	1142	16.9 ± 0.8	16.6 ± 0.4	15.8 ± 1.0	–0.16 ± 0.12	0.1715	1.52 ± 1.66	0.3606
	4	1142	16.6 ± 0.5	16.5 ± 0.2	15.5 ± 0.7	–0.14 ± 0.09	0.0994	0.13 ± 0.76	0.8631
Lysine (g/d)									
Male	1	1201	23.5 ± 0.7	24.2 ± 0.4	26.1 ± 0.8	0.20 ± 0.06	0.0008	0.78 ± 0.24	0.0011
	2	1201	22.9 ± 1.2	24.1 ± 0.4	26.7 ± 1.5	0.18 ± 0.16	0.2636	1.45 ± 0.87	0.0943
	3	1163	23.1 ± 0.8	24.2 ± 0.3	26.4 ± 1.1	0.16 ± 0.12	0.1956	1.14 ± 0.66	0.0808
	4	1163	23.5 ± 0.8	24.3 ± 0.3	25.0 ± 0.9	0.11 ± 0.11	0.3383	0.56 ± 0.62	0.3626
Female	1	1179	16.1 ± 1.0	16.3 ± 0.3	16.5 ± 0.5	0.08 ± 0.06	0.2298	0.49 ± 0.33	0.1336
	2	1179	16.8 ± 1.0	16.3 ± 0.3	15.8 ± 0.9	0.00 ± 0.11	0.9858	1.20 ± 1.00	0.2328
	3	1142	16.4 ± 0.9	16.3 ± 0.4	15.9 ± 0.8	0.00 ± 0.11	0.9826	1.61 ± 0.87	0.0649
	4	1142	16.4 ± 0.4	16.3 ± 0.2	15.8 ± 0.5	–0.09 ± 0.08	0.2522	0.00 ± 0.39	0.9929
Sulfur AA (g/d)									
Male	1	1201	23.6 ± 0.8	23.2 ± 0.4	25.8 ± 0.7	0.18 ± 0.06	0.0018	1.36 ± 0.46	0.0033
	2	1201	23.0 ± 1.0	23.1 ± 0.5	26.3 ± 1.0	0.15 ± 0.14	0.2797	1.32 ± 1.43	0.3548
	3	1163	23.3 ± 0.7	23.2 ± 0.5	26.4 ± 0.8	0.13 ± 0.10	0.1619	1.76 ± 1.20	0.1434
	4	1163	23.5 ± 0.7	23.5 ± 0.4	25.1 ± 0.6	0.08 ± 0.10	0.4323	0.69 ± 0.89	0.4353
Female	1	1179	15.1 ± 0.4	15.8 ± 0.3	16.6 ± 0.4	0.08 ± 0.05	0.1059	0.92 ± 0.47	0.0493
	2	1179	14.9 ± 1.2	15.8 ± 0.4	16.7 ± 1.2	0.04 ± 0.13	0.7626	2.00 ± 3.31	0.5455
	3	1142	15.0 ± 1.0	16.0 ± 0.3	16.5 ± 0.9	0.02 ± 0.11	0.8784	1.45 ± 2.88	0.6143
	4	1142	15.9 ± 0.4	16.2 ± 0.2	16.0 ± 0.5	–0.03 ± 0.07	0.6060	–0.26 ± 1.31	0.8428
Combined grip strength (kg)									
Essential AA (g/d)									
Male	1	861	72.9 ± 2.3	76.0 ± 2.0	74.0 ± 1.2	–0.06 ± 0.20	0.7567	0.01 ± 0.11	0.9270
	2	861	74.5 ± 4.5	76.2 ± 2.1	72.4 ± 3.4	–0.52 ± 0.57	0.3735	0.62 ± 1.09	0.5718
	3	849	74.0 ± 3.4	76.1 ± 2.1	72.2 ± 3.0	–0.15 ± 0.52	0.7703	–0.18 ± 0.97	0.8531
	4	848	74.0 ± 3.4	75.9 ± 2.0	72.6 ± 3.0	–0.19 ± 0.51	0.7183	0.04 ± 0.98	0.9683
Female	1	866	44.0 ± 1.1	45.2 ± 0.7	47.2 ± 1.5	0.34 ± 0.12	0.0096	0.24 ± 0.12	0.0635
	2	866	41.7 ± 2.9	45.0 ± 0.8	49.8 ± 3.0	0.62 ± 0.44	0.1690	1.15 ± 0.83	0.1752
	3	859	42.2 ± 2.4	45.1 ± 0.7	49.2 ± 2.6	0.51 ± 0.36	0.1638	0.61 ± 0.79	0.4414

(*continued on next page*)

TABLE 1 (*continued*)

	Model	*n*	Decile of amino acid intake (mean ± SEM)			Decile trend		Linear trend	
			1	5	10	β ± SE	*P*	β ± SE	*P*
	4	856	41.9 ± 2.3	45.1 ± 0.7	49.6 ± 2.5	0.55 ± 0.35	0.1210	0.61 ± 0.79	0.4474
Leucine (g/d)									
Male	1	861	73.4 ± 2.3	76.4 ± 2.2	73.8 ± 1.3	–0.04 ± 0.19	0.8338	0.09 ± 0.63	0.8833
	2	861	74.8 ± 3.7	76.6 ± 2.3	72.5 ± 2.9	–0.33 ± 0.61	0.5942	4.21 ± 4.04	0.3052
	3	849	73.1 ± 2.7	76.8 ± 1.8	72.8 ± 2.5	0.09 ± 0.46	0.8467	1.97 ± 3.59	0.5879
	4	848	73.5 ± 2.6	76.6 ± 1.7	72.7 ± 2.2	0.07 ± 0.46	0.8725	2.19 ± 3.60	0.5472
Female	1	866	44.1 ± 1.1	44.1 ± 1.1	46.8 ± 1.5	0.33 ± 0.12	0.0109	1.28 ± 0.68	0.0690
	2	866	42.8 ± 2.8	43.9 ± 1.1	48.3 ± 2.8	0.52 ± 0.41	0.2166	2.98 ± 3.40	0.3870
	3	859	43.2 ± 2.2	44.1 ± 0.9	47.9 ± 2.4	0.45 ± 0.34	0.1864	0.43 ± 3.06	0.8887
	4	856	43.0 ± 2.1	44.1 ± 0.9	48.3 ± 2.4	0.50 ± 0.33	0.1404	0.29 ± 3.09	0.9256
Lysine (g/d)									
Male	1	861	72.3 ± 1.7	77.6 ± 2.3	74.5 ± 1.3	–0.14 ± 0.21	0.5024	–0.18 ± 0.66	0.7919
	2	861	73.7 ± 2.9	77.7 ± 2.3	73.1 ± 3.0	–0.74 ± 0.52	0.1686	–1.94 ± 2.78	0.4900
	3	849	75.2 ± 2.9	77.8 ± 2.1	72.0 ± 3.0	–0.67 ± 0.47	0.1675	–4.16 ± 2.28	0.0776
	4	848	74.8 ± 2.9	77.6 ± 2.0	72.6 ± 3.1	–0.68 ± 0.47	0.1605	–3.83 ± 2.30	0.1056
Female	1	866	43.5 ± 1.1	46.5 ± 1.3	45.9 ± 1.1	0.35 ± 0.13	0.0090	1.48 ± 0.72	0.0487
	2	866	43.0 ± 2.0	46.4 ± 1.4	46.5 ± 1.9	0.50 ± 0.35	0.1703	3.27 ± 2.03	0.1164
	3	859	43.4 ± 1.7	46.7 ± 1.2	46.7 ± 1.7	0.40 ± 0.31	0.2058	2.93 ± 1.74	0.1017
	4	856	43.2 ± 1.6	46.7 ± 1.2	46.7 ± 1.7	0.41 ± 0.30	0.1781	2.58 ± 1.77	0.1548
Sulfur AA (g/d)									
Male	1	861	72.8 ± 2.0	75.8 ± 1.5	73.9 ± 1.4	0.01 ± 0.20	0.9756	0.61 ± 1.35	0.6525
	2	861	72.3 ± 3.2	75.7 ± 1.6	74.3 ± 3.1	0.05 ± 0.50	0.9201	10.07 ± 6.14	0.1106
	3	849	73.1 ± 2.8	75.8 ± 1.4	73.3 ± 3.1	–0.05 ± 0.47	0.9150	2.19 ± 6.19	0.7256
	4	848	73.4 ± 3.0	75.9 ± 1.3	73.2 ± 3.3	–0.12 ± 0.48	0.8053	2.50 ± 6.59	0.7065
Female	1	866	43.9 ± 1.0	46.2 ± 1.4	48.3 ± 1.7	0.41 ± 0.13	0.0031	3.39 ± 1.53	0.0344
	2	866	40.4 ± 2.3	45.9 ± 1.5	52.4 ± 3.0	0.88 ± 0.38	0.0282	13.42 ± 4.30	0.0038
	3	859	40.7 ± 2.1	45.7 ± 1.2	52.3 ± 2.6	0.83 ± 0.33	0.0151	13.46 ± 4.38	0.0043
	4	856	41.0 ± 2.0	45.5 ± 1.1	52.1 ± 2.6	0.81 ± 0.31	0.0145	11.83 ± 4.27	0.0092

Abbreviations: AA, amino acid; SE, standard error; SEM, standard error of the mean; Sulfur AA, sulfur-containing amino acid.
Body composition and muscle strength values are presented as mean ± SEM. Individual usual intakes for amino acids and dietary covariates were derived from 2 nonconsecutive 24-h recalls using the National Cancer Institute method [19]. Linear and decile trends were determined using regression analysis after adjusting for covariates. Model 1: age, gender, ethnicity (Mexican American, other Hispanic, White, Black, and other), energy (kcal), alcohol use, smoking, physical activity level, poverty–income ratio level; model 2: model 1 + usual protein intake; model 3: model 2 + height; model 4: model 3 + body fat (for appendicular lean mass); model 3 + total lean mass (for whole-body fat mass); and model 3 + BMI (for combined hand grip strength). Bonferroni-adjusted $P < 0.0013$ was considered significant.

with quality or specific amino acid intake, is driving the model 1 relationship with lean mass in the older male population. This is in agreement with a meta-analysis by Martin-Cantero et al. [11] showing supplementation with protein plus amino acids led to greater lean mass in 3 of the 4 studies included, which were conducted in community-dwelling adults supplemented with whey protein plus leucine.

Furthermore, in the fully adjusted model (that is, model 4), EAA, leucine, lysine, and SAA intake were not associated with appendicular lean mass in males or females. Older adults experience anabolic resistance in muscle protein synthesis and may need greater protein intake (~25–30 g per main meal containing 10–15 g EAA and 2.5–2.8 g leucine) than the current Estimated Average Requirement (EAR) or RDA to overcome this resistance [5]. In the current study, ~50% of males met this greater intake level for protein and EAA while ~20% met the greater intake level for leucine (mean intakes: 81 g/d protein, 36 g/d EAA, and 6.5 g/d leucine; Supplemental Table 1). Approximately, 20% of females met this greater intake level for protein and EAA while none met the greater intake level for leucine (mean intakes: 62 g/d protein, 27 g/d EAA, and 5.0 g/d leucine; Supplemental Table 1). The sizable portion of the population meeting and exceeding protein and EAA recommendations and the wide distribution of intakes should facilitate the detection of a relationship between amino acid intake and lean mass if one exists. Our results contrast with the meta-analysis by Martin-Cantero et al. [11], who showed a positive effect of amino acid supplementation ($n = 5$ studies) on lean mass in older adults (≥65 y). However, the amino acid supplementation results were due to the findings of 2 studies conducted in patients with chronic obstructive pulmonary disease supplemented with EAAs [24,25] and one study supplementing L-carnitine in community-dwelling older adults [26], whereas the 2 studies supplementing leucine in community-dwelling older adults showed no effect of leucine intake on lean mass [14,27]. Specifically, Verhoeven et al. [14] and Leenders et al. [27] found no differences in whole-body or appendicular lean mass, fat mass, or muscle strength following leucine supplementation (7.5 g/d), compared with placebo supplementation, for 3 mo in healthy older males (71 ± 4 y) or for 6 mo in older males with type 2 diabetes (71 ± 1 y), respectively. These results agree with the current findings, suggesting there is no relationship between leucine intake (g/d) and appendicular lean mass, fat mass, muscle strength, and physical function in older males.

In the current study, lysine intake was associated with higher BMI ($n = 4515$, $P < 0.0001$) and waist circumference ($n = 4332$, $P < 0.0001$) in older females. However, lysine intake was not associated with whole-body fat mass ($n = 1142$). With a larger sample size, the trend between lysine intake and DXA-measured adiposity (model 2, $P = 0.015$) may have been stronger, consistent with other measures of adiposity in the current

TABLE 2
Association of self-reported physical function measures with absolute (g/d) usual amino acid intake by gender in adults 65 y and older: NHANES 2001–2018

				Essential AA (g/d)		Leucine (g/d)		Lysine (g/d)		Sulfur AA (g/d)	
	Model	*n*	Mean ± SEM	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*
Walking for a quarter mile											
Male	1	3849	1.27 ± 0.01	–0.0006 ± 0.0009	0.5372	–0.0048 ± 0.0052	0.3633	–0.0007 ± 0.0052	0.8998	–0.0021 ± 0.0113	0.8523
	2	3849	1.27 ± 0.01	–0.0007 ± 0.0012	0.5688	–0.0065 ± 0.0064	0.3141	0.0011 ± 0.0065	0.8603	0.0011 ± 0.0141	0.9357
	3	3785	1.26 ± 0.01	–0.0006 ± 0.0012	0.6360	–0.0062 ± 0.0065	0.3410	0.0019 ± 0.0067	0.7721	0.0010 ± 0.0144	0.9421
	4	3785	1.26 ± 0.01	–0.0003 ± 0.0012	0.7627	–0.0048 ± 0.0063	0.4452	0.0028 ± 0.0064	0.6585	0.0037 ± 0.0140	0.7905
Female	1	3543	1.42 ± 0.02	–0.0030 ± 0.0016	0.0538	–0.0155 ± 0.0087	0.0760	–0.0159 ± 0.0088	0.0734	–0.0364 ± 0.0178	0.0422
	2	3543	1.42 ± 0.02	0.0003 ± 0.0020	0.8595	0.0045 ± 0.0110	0.6804	0.0030 ± 0.0106	0.7755	0.0009 ± 0.0222	0.9678
	3	3484	1.41 ± 0.02	0.0014 ± 0.0019	0.4609	0.0104 ± 0.0105	0.3241	0.0076 ± 0.0101	0.4522	0.0133 ± 0.0219	0.5443
	4	3484	1.41 ± 0.02	0.0011 ± 0.0019	0.5692	0.0090 ± 0.0104	0.3851	0.0059 ± 0.0101	0.5589	0.0103 ± 0.0224	0.6461
Walking up 10 steps without resting											
Male	1	3867	1.15 ± 0.01	–0.0003 ± 0.0006	0.6457	–0.0016 ± 0.0037	0.6649	–0.0011 ± 0.0035	0.7592	–0.0027 ± 0.0079	0.7299
	2	3867	1.15 ± 0.01	0.0003 ± 0.0008	0.7250	0.0016 ± 0.0045	0.7147	0.0026 ± 0.0048	0.5902	0.0047 ± 0.0098	0.6328
	3	3805	1.14 ± 0.01	0.0003 ± 0.0008	0.7187	0.0016 ± 0.0046	0.7313	0.0026 ± 0.0049	0.6013	0.0031 ± 0.0102	0.7574
	4	3805	1.14 ± 0.01	0.0004 ± 0.0008	0.6077	0.0024 ± 0.0046	0.6044	0.0031 ± 0.0049	0.5248	0.0047 ± 0.0101	0.6407
Female	1	3576	1.28 ± 0.01	0.0003 ± 0.0013	0.8236	0.0019 ± 0.0071	0.7916	0.0025 ± 0.0068	0.7096	0.0020 ± 0.0148	0.8916
	2	3576	1.28 ± 0.01	0.0012 ± 0.0021	0.5564	0.0073 ± 0.0117	0.5367	0.0086 ± 0.0107	0.4271	0.0113 ± 0.0229	0.6244
	3	3518	1.27 ± 0.01	0.0019 ± 0.0021	0.3679	0.0110 ± 0.0119	0.3566	0.0116 ± 0.0110	0.2925	0.0199 ± 0.0234	0.3979
	4	3518	1.27 ± 0.01	0.0018 ± 0.0021	0.4034	0.0106 ± 0.0118	0.3720	0.0108 ± 0.0109	0.3235	0.0186 ± 0.0238	0.4373
Lifting or carrying objects as heavy as 10 lb											
Male	1	4718	1.23 ± 0.01	–0.0007 ± 0.0009	0.4309	–0.0046 ± 0.0050	0.3654	–0.0025 ± 0.0049	0.6164	–0.0108 ± 0.0105	0.3087
	2	4718	1.23 ± 0.01	–0.0024 ± 0.0013	0.0687	–0.0141 ± 0.0072	0.0522	–0.0105 ± 0.0071	0.1426	–0.0323 ± 0.0153	0.0363
	3	4574	1.21 ± 0.01	–0.0023 ± 0.0012	0.0496	–0.0141 ± 0.0065	0.0316	–0.0090 ± 0.0065	0.1701	–0.0300 ± 0.0143	0.0372
	4	4574	1.21 ± 0.01	–0.0019 ± 0.0011	0.0889	–0.0119 ± 0.0063	0.0612	–0.0073 ± 0.0065	0.2606	–0.0251 ± 0.0139	0.0736
Female	1	4518	1.51 ± 0.02	0.0034 ± 0.0017	0.0443	0.0194 ± 0.0094	0.0408	0.0201 ± 0.0095	0.0368	0.0324 ± 0.0187	0.0851
	2	4518	1.51 ± 0.02	0.0035 ± 0.0022	0.1079	0.0201 ± 0.0120	0.0960	0.0221 ± 0.0123	0.0744	0.0266 ± 0.0241	0.2734
	3	4387	1.48 ± 0.02	0.0043 ± 0.0022	0.0528	0.0251 ± 0.0120	0.0379	0.0245 ± 0.0124	0.0493	0.0321 ± 0.0243	0.1882
	4	4387	1.48 ± 0.02	0.0037 ± 0.0020	0.0699	0.0225 ± 0.0112	0.0469	0.0215 ± 0.0115	0.0642	0.0300 ± 0.0231	0.1967
Standing up from an armless chair											
Male	1	4766	1.27 ± 0.01	–0.0008 ± 0.0007	0.2645	–0.0048 ± 0.0042	0.2565	–0.0040 ± 0.0042	0.3381	–0.0143 ± 0.0084	0.0928
	2	4766	1.27 ± 0.01	–0.0015 ± 0.0010	0.1504	–0.0085 ± 0.0058	0.1459	–0.0072 ± 0.0055	0.1941	–0.0255 ± 0.0116	0.0292
	3	4620	1.25 ± 0.01	–0.0019 ± 0.0008	0.0222	–0.0111 ± 0.0046	0.0179	–0.0091 ± 0.0045	0.0443	–0.0300 ± 0.0094	0.0018
	4	4620	1.25 ± 0.01	–0.0016 ± 0.0008	0.0543	–0.0091 ± 0.0046	0.0485	–0.0075 ± 0.0045	0.0922	–0.0256 ± 0.0092	0.0061
Female	1	4637	1.38 ± 0.01	0.0028 ± 0.0013	0.0317	0.0153 ± 0.0073	0.0375	0.0155 ± 0.0072	0.0323	0.0270 ± 0.0142	0.0597
	2	4637	1.38 ± 0.01	0.0044 ± 0.0017	0.0099	0.0235 ± 0.0095	0.0148	0.0247 ± 0.0092	0.0079	0.0394 ± 0.0185	0.0347
	3	4500	1.35 ± 0.01	0.0049 ± 0.0017	0.0048	0.0264 ± 0.0096	0.0067	0.0262 ± 0.0091	0.0046	0.0422 ± 0.0186	0.0252
	4	4500	1.35 ± 0.01	0.0045 ± 0.0016	0.0054	0.0249 ± 0.0090	0.0064	0.0244 ± 0.0086	0.0055	0.0414 ± 0.0180	0.0234
Pushing or pulling large objects											
Male	1	4141	1.43 ± 0.02	–0.0035 ± 0.0011	0.0011	–0.0207 ± 0.0061	0.0009	–0.0176 ± 0.0059	0.0037	–0.0473 ± 0.0128	0.0003
	2	4141	1.43 ± 0.02	–0.0037 ± 0.0016	0.0196	–0.0218 ± 0.0089	0.0158	–0.0169 ± 0.0081	0.0388	–0.0524 ± 0.0189	0.0065
	3	4061	1.41 ± 0.02	–0.0038 ± 0.0015	0.0126	–0.0227 ± 0.0085	0.0091	–0.0168 ± 0.0079	0.0345	–0.0515 ± 0.0185	0.0062
	4	4061	1.41 ± 0.02	–0.0032 ± 0.0015	0.0317	–0.0190 ± 0.0083	0.0233	–0.0136 ± 0.0078	0.0818	–0.0434 ± 0.0180	0.0173

(*continued on next page*)

TABLE 2 (*continued*)

	Model	*n*	Mean ± SEM	Essential AA (g/d)		Leucine (g/d)		Lysine (g/d)		Sulfur AA (g/d)	
				β ± SE	*P*	β ± SE	*P*	β ± SE	*P*	β ± SE	*P*
Female	1	3830	1.81 ± 0.02	0.0016 ± 0.0020	0.4237	0.0093 ± 0.0112	0.4103	0.0079 ± 0.0116	0.4979	0.0213 ± 0.0230	0.3565
	2	3830	1.81 ± 0.02	0.0016 ± 0.0024	0.5199	0.0089 ± 0.0132	0.4992	0.0067 ± 0.0146	0.6478	0.0216 ± 0.0270	0.4259
	3	3757	1.79 ± 0.02	0.0020 ± 0.0025	0.4404	0.0109 ± 0.0138	0.4336	0.0088 ± 0.0151	0.5622	0.0247 ± 0.0282	0.3816
	4	3757	1.79 ± 0.02	0.0018 ± 0.0023	0.4475	0.0104 ± 0.0124	0.4043	0.0068 ± 0.0138	0.6229	0.0263 ± 0.0264	0.3216

Abbreviations: AA, amino acid; SE, standard error; SEM, standard error of the mean; Sulfur AA, sulfur-containing amino acid.
Self-reported physical function values (no difficulty = 0, some difficulty = 1, much difficulty = 2, or an inability to perform the physical function = 3) are presented as mean ± SEM. Physical function measures include walking for a quarter mile; walking up 10 steps without resting; lifting or carrying something as heavy as 10 pounds; standing up from an armless straight chair; and pushing or pulling large objects like a living room chair. Individual usual intakes for amino acids and dietary covariates were derived from 2 nonconsecutive 24-h recalls using the National Cancer Institute method [19]. Linear trends were determined using regression analysis after adjusting for covariates. Model 1: age, gender, ethnicity (Mexican American, other Hispanic, White, Black, and other), energy (kcal), alcohol use, smoking, physical activity level, poverty–income ratio level; model 2: model 1 + usual protein intake; model 3: model 2 + BMI; and model 4: model 3 + multimorbidity count. Bonferroni-adjusted $P < 0.0013$ was considered significant.

analysis. In adults 19 y and older, the EAR and RDA for lysine is 31 and 38 mg/kg/d, respectively [28]. This corresponds to lysine intakes of 1.8 and 2.2 g/d, respectively, for a reference 58 kg female to meet intake requirements, indicating our study population is meeting and exceeding lysine intake requirements (that is, deciles 1–10, Supplemental Table 1). Recently, lysine requirements were estimated using the Indicator Amino Acid Oxidation technique in older (>60 y) females [29] and younger females in different phases of their menstrual cycle [30]. Older females had a requirement of 29 mg/kg/d, whereas younger females in the follicular phase and the luteal phase had greater requirements (35–38 mg/kg/d), suggesting a relationship between female sex hormones and lysine metabolism.

Lysine is the fourth most abundant amino acid and one of the most variable amino acids across foods and dietary patterns [31]. Lysine is a limiting amino acid in certain plant proteins, such as grains, with higher lysine intake indicative of greater animal protein intake [32,33]. A recent study also using NHANES data found obesity prevalence was positively associated with histidine, alanine, glycine, lysine, and methionine and inversely with tryptophan, phenylalanine, valine, serine, asparagine, aspartate, glutamine, and glutamate intake in US adults [31]. Our study agrees with the finding of a positive relationship between lysine intake and adiposity, despite differences in how amino acids in foods were determined for those lacking amino acid composition data, the way lysine intake was expressed in statistical models (g/d compared with g/g total AA), and age and gender of the study populations. In the current study, in those consuming the most lysine (that is, decile 10), the top 4 food sources of lysine were fish, chicken, meat mixed dishes, and beef. Future studies should evaluate whether the absolute quantity of lysine in the diet or dietary patterns with high lysine content is driving the relationship with adiposity.

In previous NHANES analyses performed by our group [34,35], we found total protein intake and protein intakes from both animal and plant sources were inversely associated with adiposity in US adults, contrary to the positive relationship between lysine intake and adiposity reported here. The variability in amino acid composition within both animal and plant protein sources and the aforementioned findings of both positive and negative associations between obesity and various amino acids may be responsible, in part, for the discrepant findings. Another reason for differences between these analyses may be the way protein (g/kg actual body weight/d) compared with amino acid (g/d) intakes were expressed. The manner in which nutrient intake is expressed can influence relationships with adiposity and metabolic health, particularly when a measure of body weight is in the denominator of an independent variable (for example, g/kg body weight) [36]. However, in the current study, when lysine intake was expressed as mg/kg ideal bodyweight or mg/kg actual bodyweight, the relationships with adiposity measures were stronger and more abundant (Supplemental Tables 3–6). Finally, age may be the biggest driver of our discrepant findings. Waist circumference and age were positively correlated in the ≥19 y population but inversely in our ≥65 y population. BMI and age were not correlated in the ≥19 y population but were inversely related in our ≥65 y population. It should be considered that greater adiposity in the ≥65 y population may be protective, as higher body mass in older age has been associated with lower mortality rates [37,38].

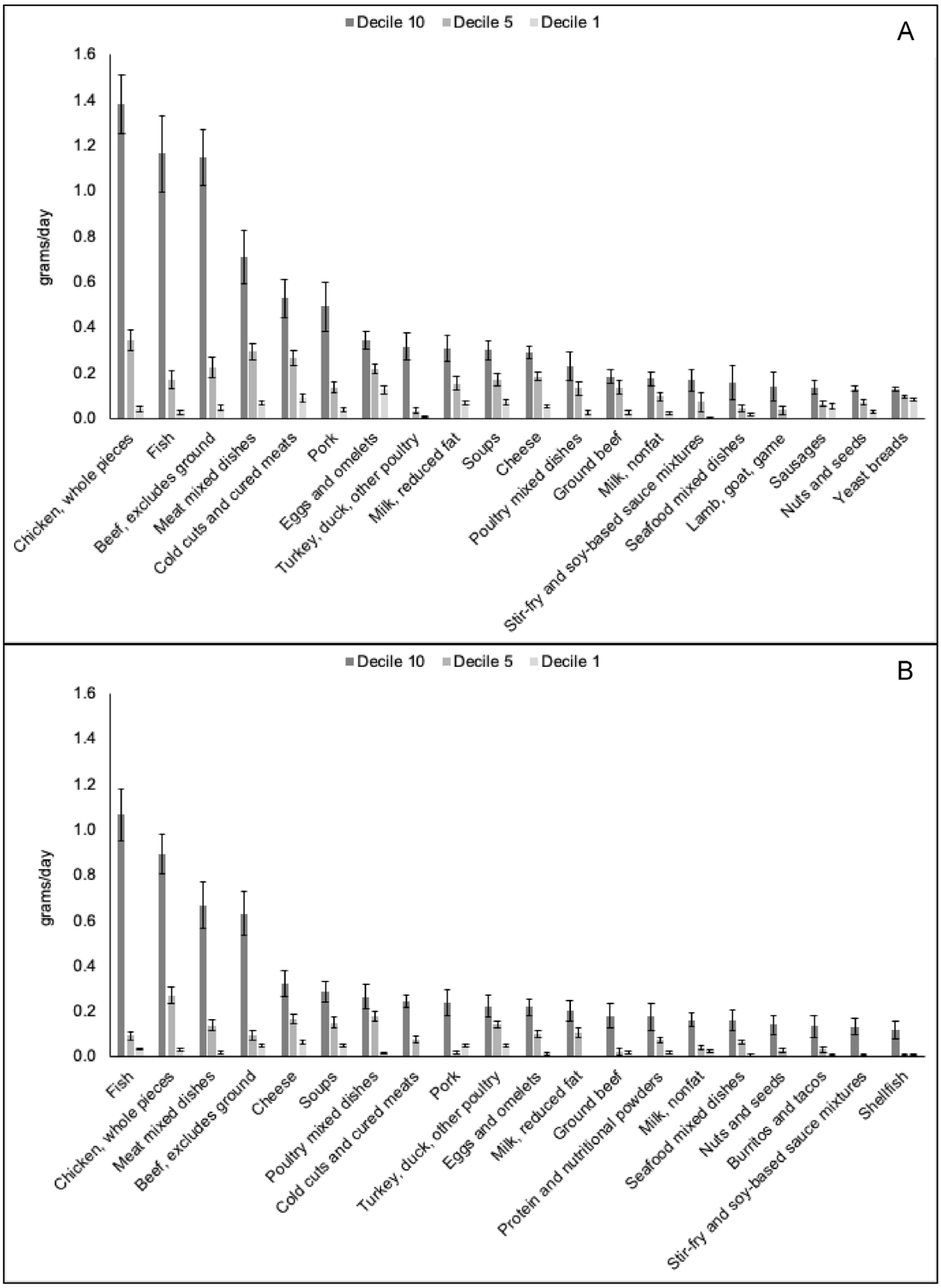

FIGURE 1. Mean lysine intakes in deciles 1, 5, and 10 for the top 20 lysine food sources consumed by males (A) and females (B) in decile 10.

Furthermore, in those ≥19 y, age was positively associated with plant protein intake and negatively associated with animal protein intake. Although animal and plant protein intake (g/kg actual body weight) were both inversely related to waist circumference and BMI in females ≥19 y, the negative slope for animal protein intake and adiposity was not pronounced until ~decile 5. It is possible most of the ≥65 y population was in deciles 1–5 in our former analysis. Discrepancies between analyses lend justification for precision nutrition efforts.

Limitations of the present study include incomplete or missing data for DEXA-measured body composition in the years 2007–2010 and the use of self-reported dietary intake data, which is based on memory and subject to errors of misreporting. Furthermore, the only muscle strength measure in NHANES is handgrip strength, and other measures of muscle strength, such as leg press or knee extension, may have yielded different findings. The use of a conservative Bonferroni-adjusted $P < 0.0013$ to reduce the occurrence of type I errors may have inflated the type II error rate, causing us to overlook real relationships when interpreting the data. Due to the cross-sectional nature of the current study, changes over time could not be investigated. Also, the addition of usual protein intake as a covariate in statistical models (that is, model 2) led to large increases in beta coefficient variability (that is, the standard error), which was likely a consequence of multicollinearity between independent variables. Future analyses should focus on the use of dimensionality reduction techniques (for example, principal components analysis) to resolve multicollinearity between independent variables while maintaining variation in the data. Strengths of the study include the characterization of missing amino acid profiles in foods, the study of habitual amino acid intake, adjustments for multiple covariates, and the presentation of multiple statistical models.

In conclusion, habitual EAA, leucine, lysine, and SAA intakes, covaried with physical activity level and usual protein intake, were not associated with lean mass, muscle strength, or physical function in older adults (≥65 y). However, EAA intakes, particularly lysine, were positively associated with measures of adiposity in older females.

Author contributions

The authors' responsibilities were as follows – HRL, SMP, VLF III, CEB: designed the research; VLF III: analyzed the data; SNC, HRL, SMP, VLF III, CEB: interpreted the data; SNC, CEB: wrote the manuscript; CEB: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

Conflict of interest

VLF performs consulting and database analyses for various food and beverage companies and related entities as Senior Vice President of Nutrition Impact. All the other authors report no conflicts of interest.

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Data availability

Data described in the manuscript are publicly available without restriction at the NHANES website: https://www.cdc.gov/nchs/nhanes/Default.aspx; the code book and analytic code described in the manuscript will be made available upon request pending application and appropriate regulatory approval.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cdnut.2024.104411.

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