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Clinical Research Article

Low Circulating Valine Associate With High Risk of Hip Fractures

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Abstract

Context: Hip fractures constitute a major health concern. An adequate supply of amino acids is crucial to ensure optimal acquisition and remodeling of bone. Circulating amino acid levels have been proposed as markers of bone mineral density, but data on their ability to predict incident fractures are scarce.

Objectives: To investigate the associations between circulating amino acids and incident fractures.

Methods: We used UK Biobank (n = 111 257; 901 hip fracture cases) as a discovery cohort and the Umeå Fracture and Osteoporosis (UFO) hip fracture study (hip fracture cases n = 2225; controls n = 2225) for replication. Associations with bone microstructure parameters were tested in a subsample of Osteoporotic Fractures in Men Sweden (n = 449).

Results: Circulating valine was robustly associated with hip fractures in the UK Biobank (HR per SD increase 0.79, 95% CI 0.73-0.84), and this finding was replicated in the UFO study (combined meta-analysis including 3126 incident hip fracture cases, odds ratio per SD increase 0.84, 95% CI 0.80-0.88). Detailed bone microstructure analyses showed that high circulating valine was associated with high cortical bone area and trabecular thickness.

Conclusion: Low circulating valine is a robust predictor of incident hip fractures. We propose that circulating valine may add information for hip fracture prediction. Future studies are warranted to determine whether low valine is causally associated with hip fractures.

Key Words: hip fractures, biomarkers, amino acids, valine

Abbreviations: AUC, area under the receiver-operating characteristic curve; BCAA, branched-chain amino acid; BMD, bone mineral density; BMI, body mass index; CV, coefficient of variation; DXA, dual X-ray absorptiometry; FN, femoral neck; HR-pQCT, high-resolution peripheral quantitative computed tomography; MOF, major osteoporotic fracture; MrOS, Osteoporotic Fractures in Men; UFO, Umeå Fracture and Osteoporosis.

Osteoporosis—characterized by low bone mass and deteriorated bone microarchitecture—leads to enhanced bone fragility and a subsequent increase in fracture risk (1). In turn, osteoporosis-related fractures, especially those of the hip, lead to morbidity, mortality, and a substantial burden to the health care system and society in general (2, 3). The current cornerstones for fracture prediction are assessment of clinical risk factors and measurements of bone mineral density (BMD) using dual X-ray absorptiometry (DXA) (1). According to the national guidelines in many countries, the fracture prediction tool FRAX, integrating information from BMD and clinical risk factors, should be used to aid in fracture risk prediction and thereby the selection of individuals who would benefit most from osteoporosis treatment (4). The absence of signs and symptoms in the early stages of osteoporosis has prompted the search for biomarkers that could improve fracture prediction. Such biomarkers also have the potential to uncover new pathways involved in the pathophysiology of osteoporosis and may lead to new treatments.

One such group of biomarkers may be amino acids because of the many connections between bone and amino acids, individually or in the form of proteins. For instance, protein constitutes as much as 50% of bone volume, mainly as collagen but also in the form of extracellular proteins with signaling

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properties regulating bone metabolism (5). Protein also makes up a large proportion of muscle tissue. A specific type of amino acid, the branched-chain amino acids (BCAAs; valine, leucine, and isoleucine), are especially important for skeletal muscle metabolism (6). Muscle strength positively affects bone health (7), and there is intense crosstalk between muscle and bone (8). Moreover, amino acids have been implicated in calcium homeostasis and direct effects on bone cells (9). Consequently, an adequate nutritional supply of amino acids is crucial to ensure optimal acquisition and remodeling of bone across all life stages (10, 11).

Studies on associations between circulating amino acid levels and fracture risk, especially for hip fractures, are not only scarce but also small (12, 13). However, there are some studies that have evaluated either the observational or causal associations between circulating amino acids and BMD (12-17). In 1 study, circulating valine was inversely associated with 4-year decline of BMD (12), and a recent Mendelian randomization study demonstrated that high levels of genetically determined isoleucine and valine increase total body BMD (14). However, it is unknown if isoleucine or valine are causally associated with fracture risk. In addition, high circulating glycine has in some studies been associated with either osteoporosis or low BMD (13, 15-17), but the causal associations for glycine with BMD and fractures are unknown.

To reliably explore associations between circulating amino acid levels and incident fractures, large cohorts with follow-up data on incident fractures and measurements of amino acid levels using validated platforms are needed. The primary aim of the present study was to determine if any of the 9 available circulating amino acids in the large UK Biobank discovery cohort (n = 111 257; 5724 fracture cases) are reproducibly associated with risk of fracture at any bone site.

Materials and Methods

UK Biobank Cohort

From 2006 to 2010, the UK Biobank recruited around 500 000 individuals aged 37-73 years from across the United Kingdom. Participants provided biological samples, completed questionnaires, underwent assessments, and were interviewed by nurses. Blood was collected for future analysis, and the self-reported interval between consumption of food and drink and blood sampling (ie, fasting time) was recorded. Follow-up using record linkage to all health service encounters and mortality data is ongoing. The UK Biobank has ethics approval from the Northwest Multicentre Research Ethics Committee, and informed consent was obtained from all participants (18). This research was conducted using the UK Biobank resource under application number 51784.

For the present association study between circulating amino acids and fracture risk, we included 117 425 study subjects with data on amino acids, ethnicity, and fasting time. In the main analyses, only individuals who self-reported as White (n = 111 257) were included, while those who self-reported as non-White (n = 6168) were analyzed separately.

The Umeå Fracture and Osteoporosis Hip Fracture Nested Case–Control Study

The Umeå Fracture and Osteoporosis (UFO) study is a population-based, nested case–control study sampled from the Northern Sweden Health and Disease study cohort that started in 1985 and now consists of blood samples, and lifestyle and dietary data from around 135 000 unique subjects from the county of Västerbotten. During a 25-year period from 1985 to March 1, 2020, we identified 2225 hip fracture cases and 2225 nested controls matched on baseline age, sex, and fasting status who had also previously provided a blood sample to the biobank. The UFO study is approved by the Swedish Ethical Review Authority. Written consent was obtained from all participants.

The Osteoporotic Fractures in Men Sweden Cohort

The initial study population consisted of 1010 older men in Gothenburg, Sweden (n = 969 with serum valine measurements available for the present study). These men formed part of the Swedish cohort of the cross-sectional and prospective multicenter Osteoporotic Fractures in Men (MrOS) study. Study subjects were randomly selected from national population registers, contacted, and asked to participate. To be eligible for the study, the participants had to be able to walk without assistance, provide self-reported data, and sign an informed consent. The participant rate was 45%. The study was approved by the ethics committee at the University of Gothenburg (19, 20). At baseline, serum samples were collected after an overnight fast. A standardized questionnaire was used to gather information about smoking, rheumatoid arthritis, parental hip fractures, alcohol and glucocorticoid use, prevalent diseases known to induce secondary osteoporosis, and self-reported previous fractures after age 50 years. Height was measured using a Harpender stadiometer, and weight was measured with an electronic scale. Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared.

A total of 449 of the MrOS Sweden Gothenburg subjects underwent high-resolution peripheral quantitative computed tomography (HR-pQCT) imaging on the distal tibia and had serum valine analyses at a 5-year follow-up visit. For radius measurements, the number of low-quality images due to motion artifacts was high (n = 105), and there were only images from 367 subjects available for association analyses.

Identification of Fractures

In the UK Biobank cohort, the prospective period for assessing incident fractures was calculated from each study participant's inclusion date to the diagnosis of fracture, death, or the end of follow-up. Fracture cases were identified using International Classification of Diseases (ICD) codes in registries (Table S1 (21)). Major osteoporotic fracture (MOF) was defined as a fracture of the hip, clinical spine, wrist, or humerus. Censoring date was September 11, 2020.

In the UFO cohort, hip fracture cases were identified from all hospital records in the county using the ICD10-codes S72.0, S72.1, and S72.2. Cases were compared with 1 control selected from the Northern Sweden Health and Disease cohort, matched for gender, age at baseline, and fasting state (2225 hip fracture cases and 2225 matched controls).

Assessment of Estimated BMD Using Ultrasound

In the UK Biobank, quantitative ultrasound of the left heel was used to obtain a noninvasive estimated BMD (eBMD) that predicts fracture risk (22, 23). If no measurement of the left heel was available, the right heel was used. A Sahara

Clinical Bone Sonometer (Hologic Corporation, Bedford, MA) was used for quantitative assessment of calcanei in UK Biobank participants. Details of the complete protocol are publicly available on the UK Biobank website (www.ukbiobank.ac.uk/). eBMD (g/cm^2) was derived as a linear combination of speed of sound and bone ultrasound attenuation (eBMD = 0.0025926 × [bone ultrasound attenuation + speed of sound] – 3.687) (23).

Assessment of BMD Using DXA

In the UK Biobank, areal BMD (aBMD, g/cm^2) of the femoral neck (FN) was assessed at the second follow-up visit using a GE-Lunar iDXA instrument (Madison, WI, USA). If present we used the left measurement, otherwise the right measurement. Individuals were excluded if they had a difference between left and right FN-BMD of over 1 SD (24). In the Gothenburg cohort of the MrOS Sweden study, aBMD (g/cm^2) of the femoral neck was assessed at baseline by DXA using Hologic QDR 4500/A-Delphi (Hologic, Waltham, MA, USA). Because measurements in the other parts of the MrOS Sweden study were performed with different equipment, a standardized BMD (sBMD) was calculated, as previously described (19).

HR-pQCT Measurements of Cortical and Trabecular Bone Parameters Separately

In the MrOS Gothenburg study, a HR-pQCT device (XtremeCT; ScancoMedical AG, Brüttisellen, Switzerland) was used to separately measure cortical and trabecular bone parameters at the left distal tibia and radius (or nonfractured limb if prior fracture), as previously described (25). Utilizing a grading scale supported by the manufacturer, each image was ranked on a scale of 1 (optimum quality) to 5 (unacceptable). Images with a quality of 4 or 5 were discarded.

All scans were analyzed following the manufacturer's standard in vivo analysis protocol and processed according to Laib et al (26). The following parameters were analyzed in this study, presented with their corresponding coefficients of variation (CVs): cortical area (mm^2, 0.4% in tibia, 0.9% in radius), cortical volumetric BMD (mg/cm^3, 0.1% in tibia, 0.3% in radius), trabecular number (mm^{-1}, 1.6% in tibia, 3.7% in radius), trabecular thickness (µm, 0.7% in tibia, 3.7% in tibia), trabecular separation (mm, 1.4% in tibia, 3.7% in radius), and trabecular volumetric BMD (mg/cm^3, 0.2% in tibia, 0.5% in radius). Cortical porosity was assessed according to a previously described method (27) using extended cortical bone analysis incorporated in a customized version of the manufacturer's Image Processing Language (IPL v5.08b Scanco Medical AG). The CV for cortical porosity measured at the tibia was 5.5% and 15.9% at the radius.

To estimate failure load in compression, microfinite element models of the tibia and radius were created directly from the segmented HR-pQCT images by finite-element software (version V5.11/FE-V01.15) incorporated in the analysis software provided by Scanco. To estimate failure load, each bone tissue voxel was converted into an equally sized brick element (28), and all bone materials were given a Young modulus of 10 Gpa and a Poisson ratio of 0.3, as reported by Pistoia et al (29). The estimated failure load (N) was computed as earlier described (29), based on the assumption that fracture occurs when 2% of the bone elements surpass the critical limit of 7000 microstrains. The CV for failure load was 0.2% in tibia and 3.1% in radius.

Grip Strength

Grip strength was measured in kilogram at baseline in left and right hands using a Jamar hydraulic hand dynamometer in UK Biobank (J00105, Lafayette Instrument, Lafayette, IN, USA; 1 attempt/hand) (30) and in MrOS (5030J1, Sammons Preston Rolyan, Bolingbrook, IL, USA; 2 attempts/hand) (31). The maximum value was used for analysis.

Amino Acid Measurements

In the UK Biobank, 9 amino acids were quantified in baseline plasma samples using high-throughput nuclear magnetic resonance spectroscopy (Nightingale Health Plc; biomarker quantification version 2020) (32). This metabolomic panel was analyzed centrally by the UK Biobank (www.ukbiobank.ac.uk/) to identify circulating markers of disease. Concentrations below the limit of quantification were set to the lowest quantified concentration of that amino acid in our data set (Table S2 (21)).

For the hip fracture replication analysis in UFO, valine was quantified in baseline plasma samples using Metabolon's ultrahigh performance liquid chromatography-tandem mass spectroscopy platform (https://www.metabolon.com/what-we-do/our-technology). In the UFO cohort, different -omics analyses, including genomics and metabolomics, were performed to determine risk markers of hip fractures. Peaks were quantified using the area under the curve. Run day normalization was performed to correct variation resulting from instrument interday tuning differences. Each compound was corrected in run day blocks by registering the medians to equal 1 (1.00) and normalizing each data point proportionately. Valine concentrations were above the limit of quantification in all UFO samples.

In the MrOS cohort, serum valine was quantified in baseline (n = 969) and 5-year follow-up (n = 449) serum samples using the same Nightingale platform as used for the UK Biobank (32). The aim for this analysis was to identify circulating biomarkers for osteoporosis traits. Valine concentrations were above the limit of quantification in all MrOS Sweden samples.

Sample Size Selection

UK Biobank is the largest cohort with information on amino acid levels and fractures. For this cohort, we have 80% power to detect an odds ratio (OR) of 1.050 per 1 SD change in amino acid levels when there are 5725 fracture cases and 105 532 controls and an alpha of .05/9 = .0056 (due to Bonferroni adjustment of the significance level when evaluating 9 amino acids). For our replication of the valine association with hip fractures in the UFO cohort (2225 cases and 2225 controls), we have 80% power to detect an OR of 1.088 per 1 SD change in valine levels.

Statistics

Before the statistical analyses, amino acid levels were transformed using the natural logarithm and scaled to SD units (Fig. S1 (21)). Values at the tail of the distribution, defined by the mean ± 5 SD, were replaced by the respective lower/upper bound. Due to different standard deviations among men and women respectively, scaling of amino acid levels to

SD was performed separately for men and women. Associations between valine and eBMD as well as between valine and HR-pQCT parameters were investigated using linear regression models. Associations between amino acid levels and incident fractures were evaluated using Cox proportional hazard models in the UK Biobank. In the nested case–control UFO study, logistic regression was used. To be able to perform meta-analyses of incident hip fractures using both UFO and UK Biobank, we also performed logistic regression analyses in the UK Biobank. We used inverse variance weighted meta-analyses to combine associations for valine with hip fractures in the UK Biobank and UFO cohorts.

Hazard ratios (HRs) or ORs and 95% CIs were estimated from the models and expressed per SD increase in log transformed amino acid level. Regression models were adjusted for age (all cohorts), sex and fasting time (UK Biobank, UFO), assessment center (UK Biobank), and BMI and current smoking (MrOS: HR-pQCT analyses). In stratified analyses, differences between strata were investigated using Z-test. Correlations between amino acids were analyzed using Pearson's correlation. In the UK Biobank, logistic regression models using the areas under the receiver-operating characteristic curve (AUC) were used to test whether any of the other amino acids improved the prediction accuracy for fractures at any bone site beyond valine. DeLong's test was used to compare the AUCs between the different models.

Results

The primary aim of the present study was to determine whether any of the 9 available circulating amino acids (exposure variables) in the large UK Biobank discovery cohort (n = 111 257; 5724 fracture cases) are reproducibly associated with risk of fracture at any bone site, and the secondary aim was to determine the separate associations for the most robustly fracture-associated amino acid with fractures at different bone sites. For the fracture analyses, we adjusted for age and sex as these are known confounders for fractures. We further adjusted for fasting time as fasting status affects the circulating levels of amino acids (33-36). Finally, we adjusted for assessment center to avoid potential confounding due to population stratification.

Valine Levels Associated With Incident Fractures in the UK Biobank Discovery Cohort

We first used the large (n = 111 257; 5725 fracture cases) UK Biobank (Table S3 (21)) as a discovery cohort to investigate the association between circulating levels of the 9 available amino acids (alanine, glutamine, glycine, histidine, isoleucine, leucine, valine, phenylalanine, tyrosine) and incident fractures. In Cox proportional hazards models, valine was the amino acid with the most significant association with the incidence of fracture at any bone site in White participants (HR per SD increase 0.89, 95% CI 0.87-0.91, $P = 5.5 \times 10^{-18}$) (Table 1; Table S4 (21)), with similar results without adjustment for fasting (HR per SD increase 0.89, 95% CI 0.86-0.91, $P = 2.1 \times 10^{-19}$, n = 111 233). When non-White participants (n = 6167; 162 fractures) were analyzed, valine was also associated with fracture at any bone site (HR per SD increase, 0.83, 95% CI 0.71-0.97). The association between valine and fractures at any bone site was more pronounced in men than in women (P = .04, Z test), while age did not modulate the association (Fig. 1). The circulating levels of valine were higher in men (0.21 ± 0.04 mean ± SD, arbitrary units) compared with women (0.19 ± 0.04, $P < .001$, Student's t-test). There was no significant interaction between age and circulating valine levels for the association with any fractures in the UK Biobank ($P = .94$ for the interaction term age × valine), but there was a modest positive association between age and circulating valine levels (39-71 years old, β = 0.005 SD change in ln valine levels per 1 year increase, $P < .001$).

In addition to valine, 6 other amino acids were nominally ($P < .05$) associated with the risk of fracture at any bone site (Table 1; Table S4 (21)). Many of the evaluated amino acids, especially the BCAAs (valine, leucine, and isoleucine), were highly correlated (Fig. S2 (21)). Analysis of the AUC showed that valine ($P = 1.8 \times 10^{-4}$) improved fracture prediction of a model adjusted for age, sex, fasting time, and UK Biobank assessment center. However, no further improvement of the model including valine was observed by addition of any of the other available circulating amino acids. We, therefore, focused on the associations for valine in our further analyses.

The Strength of the Association Between Valine and Fracture Risk is Modestly Reduced by Adjustment for eBMD

eBMD is a robust negative predictor of fracture risk (37), and measurement of eBMD was available for a majority of the subjects with valine measurements in the UK Biobank. As we found that circulating valine was directly associated with eBMD (n = 108,548, β = .043 SD per SD increase in valine, $P = 1.1 \times 10^{-47}$; Table S5 (21)) in UK Biobank, we next evaluated whether the association between valine levels and fracture risk was changed when adjusting for eBMD. When eBMD was added to the Cox regression model, valine was still a statistically significant predictor of any fracture (HR per SD 0.91, 95% CI 0.88-0.93). The beta estimate for the association between valine and fractures at any bone site decreased only modestly (−17%) when eBMD was added to the Cox model (without eBMD ß = −.12, with eBMD β = −.10).

Valine Levels Are Inversely Associated With hip Fractures

We next evaluated the associations for valine with fractures at different bone sites in the UK Biobank (Fig. 2; Table S4 (21)). Fracture at any bone site and MOF are mixtures of fractures at different bone sites, while forearm and hip fractures are fractures at distinct anatomical locations. The association between valine levels and hip fracture risk (HR per SD increase, 0.79, 95% CI 0.73-0.84, Fig. 2), the clinically most important fracture type, was more pronounced than the association with forearm fractures (HR per SD increase, 0.88, 95% CI 0.83-0.92; $P < .01$ Z-test between hip and forearm fractures, Fig. 2).

Valine Is Associated With hip Fractures in the UFO Replication Study

We next determined if the association between circulating valine and hip fracture risk could be replicated in the UFO hip fracture study (2225 hip fracture cases and 2225 controls matched on age, sex, and fasting status, Table S6 (21)). Valine levels were inversely associated with hip fractures in

Table 1. Associations between amino acid levels and any fracture in the UK Biobank cohort

Amino acid	HR	95% CI	*P* value	n	Fractures
Valine	0.89	(0.87, 0.91)	5.5×10^{-18}	111 230	5724
Leucine	0.91	(0.88, 0.93)	3.7×10^{-13}	111 256	5725
Alanine	0.94	(0.92, 0.96)	3.2×10^{-6}	111 256	5725
Isoleucine	0.94	(0.92, 0.97)	4.4×10^{-6}	111 257	5725
Histidine	0.96	(0.93, 0.98)	1.3×10^{-3}	111 054	5710
Tyrosine	0.96	(0.93, 0.99)	2.3×10^{-3}	111 070	5712
Phenylalanine	0.97	(0.94, 0.99)	1.1×10^{-2}	111 188	5719
Glutamine	0.98	(0.95, 1.00)	9.9×10^{-2}	110 929	5699
Glycine	1.01	(0.99, 1.04)	3.5×10^{-1}	111 160	5721

Cox proportional hazards regression models adjusted for age, sex, fasting time, and UK Biobank assessment center. Hazard ratios (HR) are given with 95% CI and expressed per SD increase in ln transformed amino acid level.

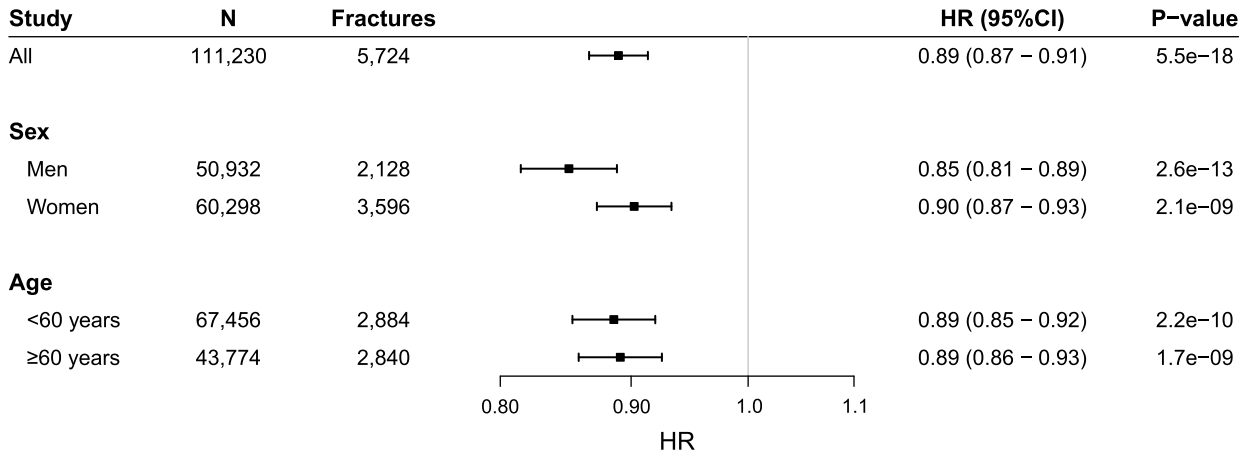

Figure 1. Associations between valine and fractures at any bone site in the UK Biobank stratified by sex and age. Cox proportional hazards regression models adjusted for age, sex, fasting time, and UK Biobank assessment center. Hazard ratios (HR) are given with 95% CI and expressed per standard deviation increase in ln transformed valine.

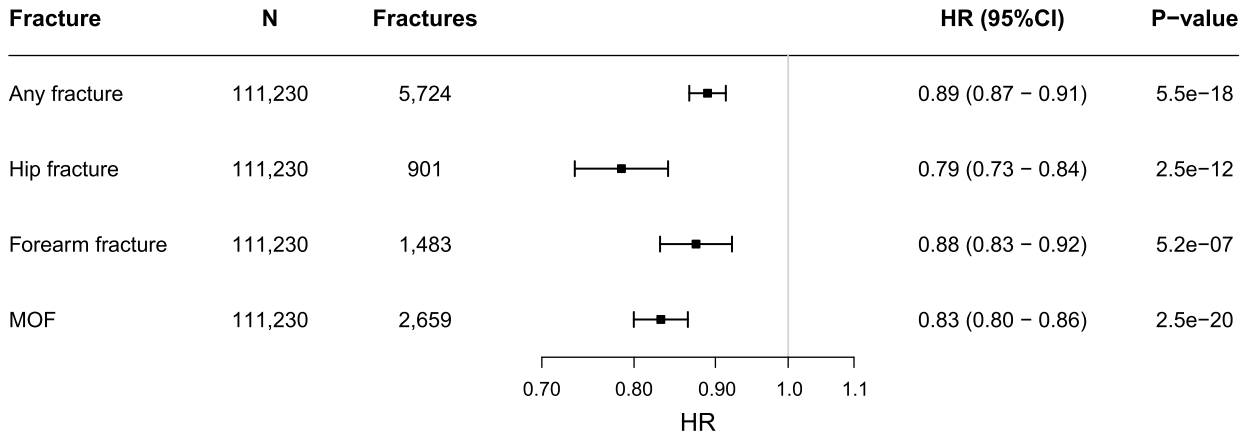

Figure 2. Associations between valine levels and fractures at different bone sites in the UK Biobank. Cox proportional hazards regression models adjusted for age, sex, fasting time, and UK Biobank assessment center. Hazard ratios (HR) are given with 95% CI and expressed per standard deviation increase in ln transformed valine. MOF, major osteoporotic fractures.

the UFO study also (Fig. 3). The large size of the UFO hip fracture cohort and the follow-up time of up to 30 years enabled stratified analyses according to follow-up time. The predictive role of valine for hip fracture risk decreased over time, with the largest role for fractures occurring 0 to 10 years after blood sampling (OR per SD increase was 0.83, 95% CI 0.75-0.92; Fig. 3). To determine the stability of valine levels in an individual over time, we determined the correlation between valine measured at baseline and the first follow-up visit (4.2 ± 0.9 years later) in a small subsample (n = 1389) of the

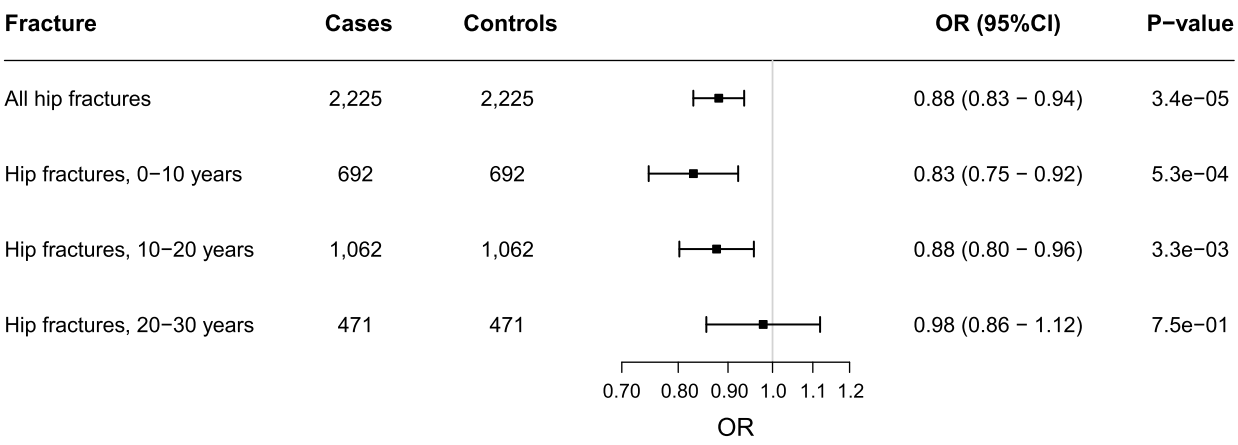

Figure 3. Associations between valine levels and hip fractures in the UFO study. Logistic regression adjusted for age, sex, and fasting status. Odds ratios (OR) are given with 95% CI per SD increase in ln transformed valine.

UK Biobank. The correlation for circulating valine between these 2 time points were 0.38 (Pearson's correlation).

Meta-analyses of data from the UK Biobank and UFO studies (3126 hip fracture cases) provided statistical evidence that circulating valine is a predictor of hip fracture risk (OR per SD increase, 0.84, 95% CI 0.80-0.88, $P = 2.3 \times 10^{-14}$, Fig. 4). Further, a meta-analysis restricted to the first 10 years of follow-up showed a slightly more pronounced association (OR per SD increase, 0.80, 95% CI 0.75-0.85, $P = 1.3 \times 10^{-12}$, Fig. 4) than when all subjects were included. Sex-stratified meta-analysis showed a stronger association between circulating valine and hip fractures in men (OR 0.78, 95% CI 0.72-0.84) compared with women (OR 0.87, 95% CI 0.22-0.91; Z-test $P = .03$ comparing the strength of the association in men and women; Fig. 4).

Circulating Valine Is Associated With Cortical and Trabecular Bone Microstructure Parameters

The associations between valine and cortical and trabecular bone microstructure parameters were investigated in a subset of the MrOS Sweden cohort with available HR-pQCT analyses and serum valine levels (Table S7-S9 (21)). Circulating valine was associated with specific bone microstructure features with the most pronounced associations observed for cortical cross-sectional bone area and trabecular bone thickness (Table 2). In addition, we observed a positive association between valine levels and the calculated bone strength parameter failure load (Table 2). The associations between circulating valine and HR-pQCT analyses in the radius were possible to test in a lower number of subjects as the number of low-quality images due to motion artifacts (n = 105) was substantially higher in the radius than the tibia (Table S8-S9 (21)). The effect sizes for the association of valine with failure load was of similar magnitude in the radius and tibia, but the association with cortical bone area was less pronounced in the radius than the tibia, while the effect estimate for the association with trabecular vBMD was more pronounced in the radius than the tibia (Table 2; Table S9 (21)).

Circulating Valine Is Associated With Grip Strength and eBMD

We next determined if circulating valine was associated with grip strength and/or DXA-derived FN-BMD in UK Biobank and MrOS (Table S10 (21)). Valine was positively associated with grip strength in the 2 cohorts (UK Biobank β = .010 95% CI 0.008-0.134, $P = 5.2 \times 10^{-4}$, n = 110 791; MrOS β= .096 95% CI 0.030-0.161, $P = .004$, n = 951, SD change in grip strength per SD increase in ln transformed circulating valine levels, models adjusted for age, sex, BMI, fasting time). Valine was also positively associated with FN-BMD in the 2 cohorts (UK Biobank β = .036 95% CI 0.016-0.055, n = 9421; MrOS β = .085 95% CI 0.020-0.151, $P = .011$, n = 959; $P = 3.2 \times 10^{-4}$, SD change in FN-BMD per SD increase in ln transformed circulating valine levels, models adjusted for age, sex, BMI, fasting time). However, it should be emphasized that the FN-BMD measurements in UK Biobank were performed on average 8.6 ± 1.7 (mean ± SD) years after the valine measurement, rendering the interpretation of the valine association with FN-BMD in the UK Biobank challenging.

Discussion

In this study, we establish low circulating valine as a robust predictor of incident hip fractures, the clinically most important fracture type. As modest positive associations were observed for valine with eBMD, FN-BMD, and grip strength, it is possible that both BMD and muscle function might contribute to the observed association between valine and hip fracture risk. However, a substantial proportion of the association for valine with fracture risk appears to be mediated via mechanisms other than what is reflected by BMD. At the bone microstructure level, we demonstrate associations between valine and specific cortical and trabecular bone microstructure parameters.

Venous blood concentrations of amino acids reflect the combination of their rate of appearance (intake, tissue release from proteolysis, and de novo synthesis) and their rate of disappearance (metabolism, incorporation in proteins, and loss in urine and feces) (34). The rate of appearance and disappearance is also affected by the gut microbiota (38). Therefore, plasma concentrations of individual amino acids, including essential amino acids, which cannot be synthesized by the body, not only reflect nutritional intake of those same amino acids (39). A wide array of mechanisms pertaining to bone, muscle, and whole-body metabolism could thus underlie our findings regarding the association between circulating valine and incident fractures, and our results must be interpreted in light of

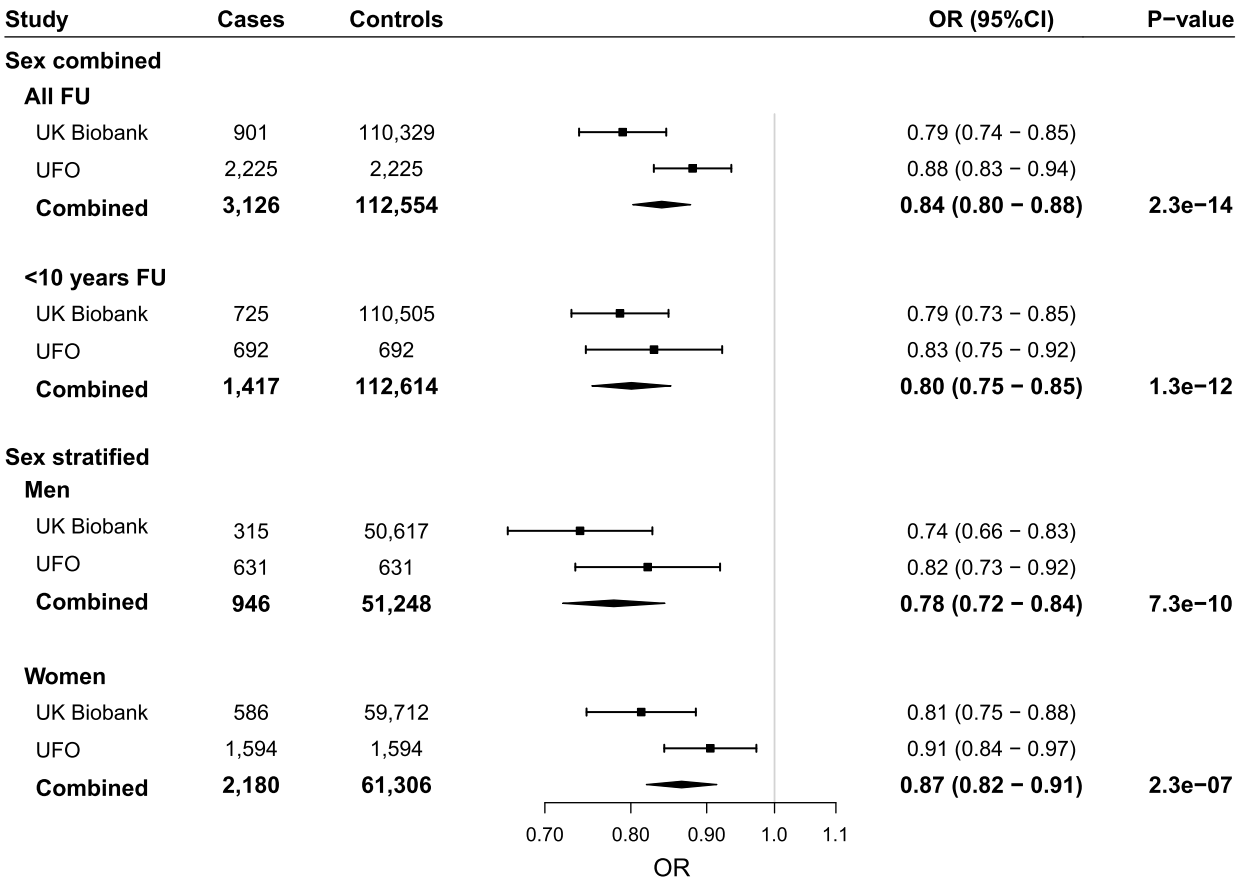

Figure 4. Meta-analysis of the association between valine levels and hip fractures in the UK Biobank and the UFO cohorts. Meta-analyses were performed using the inverse variance weighted method. Logistic regressions were adjusted for age, sex, fasting time, and assessment center in the UK Biobank and sex, age, and fasting status in the UFO study. Odds ratios (OR) are given per SD increase in ln transformed circulating valine levels. FU, follow-up time.

Table 2. Association between serum valine levels and HR-pQCT parameters in the distal tibia in a subsample of the MrOS Sweden cohort

	n	Beta (SE)	*P* value
Cortical area (mm^2)	449	.17 (0.046)	2.9×10^{-4}
Cortical vBMD (mg/cm^3)	448	.12 (0.047)	.010
Cortical porosity (%)	449	−.077 (0.048)	.11
Trabecular vBMD (mg/cm^3)	449	.057 (0.048)	.23
Trabecular number (mm^{-1})	449	−.010 (0.045)	.023
Trabecular thickness (μm)	449	.17 (0.047)	4.3×10^{-4}
Trabecular separation (mm)	449	.052 (0.046)	.26
Failure load (kN)	443	.14 (0.046)	.003

Linear regressions with serum valine as exposure and HR-pQCT parameters as outcome, adjusted for age, body mass index, and current smoking. Beta values are given as SD change of bone parameter per SD increase of ln transformed valine.
Abbreviations: HR-pQCT, high-resolution peripheral quantitative computed tomography; MrOS, Osteoporotic Fractures in Men; vBMD, volumetric bone mineral density.

this. Furthermore, even though we focused on valine based on it having the strongest association with risk of any fracture in UK Biobank, valine is highly correlated with leucine and isoleucine, and these BCAAs share pathways and mechanisms (6). It is, therefore, difficult to separate the relative importance of these 3 BCAAs for bone.

Amino acids are important for growth and maintenance of muscle and bone (10, 11). However, data on how different amino acids are associated with fractures are scarce, and the existing studies are small (12, 13). With 3126 incident hip fracture cases, the present study is by far the largest to investigate amino acids in relation to hip fractures. The large number of fractures in the present study enabled us to stratify our analyses based on sex, follow-up time, and different types of fractures. Sex-stratified analyses showed that the predictive role of valine was more prominent in men than in women, as shown for fracture at any bone site in UK Biobank and for hip fractures in our meta-analysis combining data from UK Biobank and the UFO study. The underlying mechanisms for these findings may be the sex-based differences that have been suggested in energy substrate utilization, protein and amino acid catabolism, and amino acid transport (40).

In the UFO cohort with many years of follow-up time available, the strength of the association between valine and hip fracture risk reduced after 10 years of follow-up. This may partly be explained by our finding that valine levels only display a modest stability in an individual over time. Fading relationships with outcomes have been described previously for other circulating biomarkers (41).

Furthermore, analyses stratified on fracture type showed a more prominent predictive role for valine on hip fractures than forearm fractures in the UK Biobank. While hip fractures tend to affect frail elderly persons with an increased risk of

falling and a decreased capacity to protect oneself by breaking the fall, forearm fractures are more common in younger, otherwise healthy individuals and typically occur during exercise and outdoor activities (42). Valine levels have been found to be associated with thigh muscle cross-sectional area in elderly individuals (43) and with a larger muscle mass and a reduced muscle mass loss in middle-aged patients with type 2 diabetes (44), in line with our finding that valine was modestly associated with grip strength. For the more prominent predictive role of valine on hip fractures, direct muscle effects could thus be involved, decreasing the risk of falling and increasing the likelihood of protecting oneself should a fall occur.

The predictive effect of valine for fractures may be mediated by effects on bone remodeling. For instance, a valine-deficient diet led to bone abnormalities and decreased bone ash and bone calcium in male chicks (45). Additionally, deficiency of the L-type amino acid transporter LAT1 in osteoclasts resulted in increased osteoclast activity and bone loss through decreased activation of mechanistic target of rapamycin complex 1 (mTORC1), an important stimulator of bone formation (46). Since valine is 1 of the amino acids transported intracellularly by LAT1 and since valine activates mTORC1 (47, 48), it is plausible that valine may have direct beneficial effects on bone. In line with these data, mice deficient in branched chain amino acid transaminase 1, an enzyme catabolizing BCAA, had improved bone mass and strength, likely due to impaired osteoclast activity (49). A multi-omics study provides additional support that the degradation of BCAAs is important for bone as the pathway involved in valine, leucine, and isoleucine degradation was associated with osteoporosis and also the gut microbiota composition (50). Interestingly, a recent report shows that 19% of the variance of circulating valine levels are explained by the gut microbiota composition (51). The composition of the gut microbiota changes with age, a change that has been shown to increase the gut microbial utilization of amino acids, which in turn may contribute to age-related diseases (52) such as osteoporosis. Furthermore, Mendelian randomization analyses demonstrate an effect of genetically determined circulating valine on total body BMD (14).

However, the beta estimate for the association between valine and fractures was only modestly reduced when adjusting for eBMD in the UK Biobank cohort. Apart from a possible protective effect of valine on fractures via regulation of muscle mass and strength, it is possible that valine has effects on bone properties that cannot be captured using ultrasound or DXA. HR-pQCT is a 3-dimensional image method that permits separate analyses of cortical and trabecular bone microstructure parameters. As these bone microstructure parameters have been shown to predict fracture risk independently of FN-BMD and FRAX score (53), the association between valine and the bone microstructure parameters observed in the present study may partly explain valines BMD-independent association with fracture. Moreover, circulating valine was associated with the calculated parameter failure load, which includes information on bone structure and density and predicts fractures (53). Taken together, our HR-pQCT data indicate that cortical and trabecular bone microstructure parameters may be involved in the predictive role of valine for fractures.

Additionally, it is possible that valine may provide BMD-independent effects by affecting the balance and gross motor abilities, as demonstrated in mice and humans with decreased levels of BCAA due to an inability to inhibit the rate-limiting step of BCAA catabolism (54, 55). More specifically, the deletion of the branched chain ketoacid dehydrogenase kinase (*BCKDK*) gene in mice resulted in decreased balance, locomotor activity, and rearing (55). Similarly, mutations in the *BCKDK* gene in humans was associated with delayed onset of walk (54). Thus, factors that regulate the degradation of amino acids and hence the circulating levels of amino acids seem to play a role for balance and locomotion.

The strengths of the present study are the large size of the UK Biobank discovery cohort, the ability to replicate the main finding, the strong association for valine with hip fractures in the large UFO study including as many as 2225 incident hip fracture cases, and the mechanistic insights provided by the data on cortical and trabecular bone microstructure parameters. However, the current study has several limitations. As the fractures was ascertained from ICD codes in the present study, we could not exclude fractures due to high trauma. We did not account for competing risk of death, and survival bias could therefore not be excluded. Since only 9 important amino acids were quantified in the discovery UK Biobank, associations with other amino acids may have been missed. In this study, we present associations that do not indicate causal relationships. Although we provide some mechanistic insights with the data on cortical and trabecular bone microstructure parameters, it is a limitation that we do not provide functional data evaluating the effect of valine on fracture risk. Further studies should determine the added value of circulating valine beyond FRAX and other clinically used fracture risk algorithms for hip fracture prediction.

In conclusion we have established that low circulating valine associates with high hip fracture risk. We propose that circulating valine may add information for hip fracture prediction. Future studies are warranted to determine whether low valine is causally associated with hip fractures and if valine treatment may reduce hip fracture risk in elderly subjects.

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Author Contributions

A.L.E., L.G., U.P., C.O. designed the study. L.G., A.L.E., C.O., U.P., M.N., M.L., R.J., D.M. collected and/or analyzed the data. L.G., A.L.E., C.O. performed the literature search, interpreted the data, and wrote the first draft of the manuscript. All the authors contributed to subsequent drafts of the manuscript and made the decision to submit the manuscript for publication.

Disclosures

L.G., M.N., R.J., D.M., U.P.K. have nothing to declare. A.L.E. is an employee at AstraZeneca as of June 20, 2022. C.O. is applicant on filed patent applications on the effect of probiotics on bone metabolism. M.L. has received lecture fees from Amgen, Lilly, Meda, Renapharma, and UCB

Pharma and consulting fees from Amgen, Radius Health, UCB Pharma, Renapharma, and Consilient Health.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided

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