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SHORT COMMUNICATION

# Associations of homoarginine with bone metabolism and density, muscle strength and mortality: cross-sectional and prospective data from 506 female nursing home patients

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**Abstract**
*Summary* In female nursing home patients, homoarginine was associated with lower bone turnover, higher bone density, lower mortality and, by trend, with muscle strength.
*Introduction* Homoarginine, a cationic amino acid, may be relevant for muscusloskeletal health because it inhibits alkaline phosphatases (AP) and is involved in nitric oxide and energy metabolism. We aimed to evaluate whether homoarginine serum concentrations are associated with bone density and metabolism, muscle strength, fractures and mortality.
*Methods* We examined a cohort of female nursing home patients that underwent quantitative bone ultrasound (QUS) measurements and assessments of knee extensor strength. Measurements of serum homoarginine, C-terminal telopeptide cross-links (β-CTxs) and osteocalcin were also performed at baseline. Thereafter, patients were followed-up with respect to fractures and mortality.
*Results* Serum homoarginine concentrations were determined in 506 female study participants (mean age: 83.9±6.0 years). Homoarginine was inversely correlated with β-CTxs ($r$=−0.26; $p$<0.001) and osteocalcin ($r$=−0.21; $p$<0.001), and these associations remained significant in multiple regression analyses. Multivariate regression analyses showed that homoarginine is significantly associated with calcaneus stiffness

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(beta coefficient=0.11; $p$=0.020) and by trend with knee extensor strength (beta coefficient=0.09; $p$=0.065). During a mean follow-up time of 27±8 months, we recorded 119 deaths (23.5%) and 63 fractures (12.5%). In multivariate analyses, homoarginine was associated with significantly reduced risk of mortality and the combined endpoint of fractures and mortality.
*Conclusions* Whether homoarginine metabolism is critically involved into the pathogenesis of musculoskeletal diseases and fatal events warrants further studies.

**Keywords** AGAT · Amino acids · Arginine · GATM · Homoarginine · Prospective

## Introduction

Accumulating evidence supports a role of amino acids and energy metabolism in bone health [1, 2]. Homoarginine, a cationic amino acid that can be formed from lysine, may be of particular interest for bone metabolism because it is an inhibitor of bone alkaline phosphatase (BALP) and tissue-nonspecific alkaline phosphatase (TNALP) [3–6]. Previous interest in homoarginine was mainly driven by its proposed stimulatory effect on the synthesis of the endothelium-derived relaxing factor (EDRF) nitric oxide (NO), which may be involved in bone formation processes and plays a crucial role for cardiovascular health [3, 7–9]. In line with this, it was documented in both a cohort of patients referred for coronary angiography (LURIC Study) as well as in diabetic dialysis patients (4D Study), that low homoarginine is an independent risk factor for cardiovascular events and all-cause mortality [3, 10–13]. Serum homoarginine concentrations were particularly decreased in heart failure [10, 12, 13]. Involvement of homoarginine in energy metabolism might underlie this relationship because a key enzyme for homoarginine production, arginine: glycine amidinotransferase (AGAT), catalyzes the synthesis of guanidinoacetate, a precursor for creatine [3, 12, 14, 15]. Interestingly, Cullen et al. hypothesized that the upregulation of AGAT in the failing heart might be compensation to myocardial energy deficits in heart failure [15]. Considering that AGAT is also expressed in skeletal muscle and that energy metabolism is relevant for bone, it could be speculated that homoarginine metabolism might be related to musculoskeletal health [2, 15]. Therefore, we set out with the hypothesis that the relationship of homoarginine metabolism with alkaline phosphatases (AP), NO and energy metabolism could translate into clinically relevant associations of homoarginine serum concentrations with bone density and metabolism, muscle strength, fractures and mortality. To test these hypotheses, we examined a large cohort of female nursing home patients from Austria [16, 17].

## Methods

### Study population

Our study cohort was recruited from 95 nursing homes in Austria and consists of Caucasian female patients. Detailed descriptions of the study design and baseline characteristics have been published elsewhere [16, 17]. Main inclusion criterion was the ability to walk a short distance independently. We excluded subjects with malignancies, hypercalcemia, advanced kidney or liver dysfunction, bilateral hip replacements, history of total gastrectomy, decompensated heart failure, chronic alcoholism, known osteomalacia, untreated thyroid disease or chronic steroid treatment [16, 17]. Quantitative bone ultrasound (QUS) measurements were all performed on the nondominant side and have been previously described [16, 17]. In detail, patient's stiffness index (SI) at the calcaneus was determined by using the Achilles Express device (GE Lunar Corp., Madison, WI). At the distal one-third of the radius and the proximal phalanx of the third finger, we measured axially transmitted speed of sound (SOS) by the Sunlight Omnisense ultrasound bone sonometer (Sunlight Ultrasound Technologies, Ltd.; Rehovot, Israel). We used the following scoring system for the classification of mobility: score 1, walking independently and outside the institution; score 2, walking inside nursing home, majority using a walking aid but not a wheelchair; score 3, staying in bed less than 50% during daytime, majority requiring a wheelchair; and score 4, staying in bed more than 50% during daytime. Quadriceps knee extensor strength was measured on the nondominant side by use of a validated, handheld isometric device (model DPPH, Industrial Scale Inc., Houston, TX). All study participants were followed-up with respect to fractures and mortality. All study participants gave written informed consent before study entry, and we obtained approval for the study by the local ethics committees.

### Laboratory methods

Laboratory procedures have been described in previous reports [16–18]. Nonfasting blood samples were collected between 9:00 and 12:00 h and were immediately stored at −70°C until analysis. Homoarginine was determined in serum by a high-performance liquid chromatography (HPLC) method with intra- and interday coefficients of variation (CV) of 2.2 to 4.7% and 6.8 to 7.9%, respectively. C-terminal telopeptide cross-links (β-CTxs; Elecsys β-CrossLaps), osteocalcin (intact osteocalcin [1–49] and large N-MID fragments [1–43]; Elecsys N-MID Osteocalcin) and parathyroid hormone (PTH) (Elecsys intact PTH) were all measured by Elecsys systems (Roche Diagnostics, IN). 25(OH)D was measured in serum by

Radioimmunoassay (RIA) after extraction (Immunodiagnostic Systems, Boldon, UK).

### Statistical analyses

Continuous data are either presented as means ± SD (variables with a Gaussian distribution) or as medians with interquartile range (skewed variables). Categorical data are presented as percentages. We formed homoarginine quartiles according to the values of the entire study population. Differences between groups were calculated by Analysis of Variance (ANOVA) for continuous variables and by Chi-squared test for categorical data. Variables following a non-normal distribution were log(e)-transformed before using them in parametric statistical analyses. Spearman correlation coefficients were calculated to test for associations of homoarginine with continuous variables. In addition, we calculated multiple linear regression analyses with homoarginine as an independent variable and stepwise inclusion of the covariates age (years), body mass index (BMI) (kilogram per square meter), albumin (gram per deciliter), PTH (picogram per milliliter) and creatinine clearance (millilitre per minute calculated according to Cockcroft–Gault). Finally, we calculated Cox proportional hazard ratios (HR) for homoarginine (in micromole per liter) for mortality, fractures and the combined endpoint of mortality and fractures. Patients were censored at the first event (fracture or mortality). For prospective analyses on fractures, the mortality dates were considered as censoring dates. We present crude HR and performed further adjustments of the HRs as indicated. A *P* value below 0.05 was considered statistically significant, and statistical analyses were performed by SPSS version 17.0 (SPSS, Inc., Chicago, IL).

## Results

From the initial population of 1,664 study participants, 961 had a blood collection, and of these 506 (53%) had available serum samples for measurements of homoarginine and, thus, comprised the study cohort for the present work. Mean age was 83.9±6.0 years and mean serum homoarginine levels were 1.47±0.43 μmol/l. Baseline characteristics according to homoarginine quartiles are presented in Tables 1 and 2. During a mean follow-up time of 27± 8 months, we recorded 119 deaths (23.5%) and 63 fractures (12.5%) including 29 (5.7%) hip fractures. For homoarginine (in micromole per liter), the unadjusted and age-adjusted HR (with 95% CI) for mortality was 0.53 (0.34–0.82; $p=0.004$) and 0.59 (0.38–0.91; $p=0.018$), respectively. Additional adjustments for BMI, albumin, creatinine clearance and PTH attenuated this association towards a HR of 0.60 (0.38–0.95; $p=0.028$). Accordingly, the unadjusted and age-adjusted HR for fractures were 0.74 (0.40–1.35; $p=0.327$) and 0.79 (0.43–1.46; $p=0.459$), respectively. Multivariate-adjusted HR for fractures was 0.68 (0.36–1.29; $p=0.235$). For the combined endpoint of fractures and mortality, the crude and age-adjusted HR were 0.61 (0.43–0.88; $p=0.008$) and 0.67 (0.47–0.97; $p=0.033$), respectively. After additional adjustment for BMI, albumin, creatinine clearance and PTH, the multivariate-adjusted HR for this combined endpoint was 0.66 (0.45–0.96; $p=0.031$).

## Discussion

In a cohort of institutionalized elderly women, we showed that homoarginine serum concentrations were inversely correlated with bone markers and positively associated with calcaneus stiffness. There was also a nonsignificant trend towards an association of homoarginine and muscle strength, and low homoarginine emerged as an independent risk factor for mortality and for the combined endpoint of fractures and mortality.

This, to our knowledge, is the first investigation on the association of homoarginine with bone density, metabolism and muscle strength. Our data indicate that homoarginine deficiency is associated with high bone turnover. The relationship of homoarginine with bone density and muscle strength was, however, less clear, in particular, because there was only a significant inverse correlation of homoarginine with calcaneus stiffness but not with SOS at the radial or the phalangeal site. Proposed involvements of homoarginine in NO metabolism by, e.g., serving as a substrate for NO synthase, might mediate beneficial homoarginine effects on muscles and bone [3, 7, 8, 19]. In addition, homoarginine inhibition of AP may also underlie our findings regarding bone, but whether this homoarginine effect is biologically relevant is unknown [3, 5, 6]. Participation of homoarginine in energy metabolism like its contribution to creatine synthesis might also be relevant for musculoskeletal health [2, 3]. We can only hypothesize about the possible pathways linking homoarginine to parameters of bone and muscle health, but we wish to point out that our findings remained significant despite careful adjustments for possible confounders including, e.g., parameters of malnutrition (BMI and albumin) as well as renal function (creatinine clearance). Renal function is of particular interest for homoarginine metabolism because the kidneys are considered to be the major site for homoarginine synthesis from lysine and are critically involved in energy and creatine metabolism [3]. Another major finding of our work is that homoarginine is associated with reduced mortality. This observation confirms previous investigations in other cohorts and might suggest a possible role of homoarginine metabolism for overall health [10–13].

Although there exists mechanistic evidence in support of a possible causal association of homoarginine deficiency

**Table 1** Baseline characteristics according to homoarginine quartiles

| Variable<br>Range (μmol/l) | First quartile<br><1.16 | Second quartile<br>1.17–1.42 | Third quartile<br>1.43–1.69 | Fourth quartile<br>>1.69 | *P* |
|---|---|---|---|---|---|
| Number | 129 | 124 | 129 | 124 | |
| Homoarginine (μmol/l) | 0.98±0.13 | 1.29±0.07 | 1.55±0.08 | 2.06±0.33 | <0.001 |
| Age (years) | 85.3±6.0 | 83.4±5.9 | 84.0±5.4 | 83.0±6.4 | 0.008 |
| Height (cm) | 153.2±6.9 | 153.7±7.0 | 153.8±6.7 | 154.2±7.3 | 0.254 |
| Weight (kg) | 58.7±12.3 | 59.8±11.5 | 60.8±12.1 | 64.4±10.7 | <0.001 |
| Body mass index (kg/m²) | 25.0±4.8 | 25.5±4.5 | 25.7±4.9 | 27.1±4.4 | <0.001 |
| β-CTX (ng/ml) | 0.39 (0.25–0.61) | 0.31 (0.19–0.48) | 0.34 (0.20–0.48) | 0.27 (0.15–0.48) | <0.001 |
| Osteocalcin (ng/ml) | 33 (26–55) | 33 (23–44) | 33 (24–45) | 29 (19–41) | 0.003 |
| Calcaneus stiffness (z-score) | −0.52±1.03 | −0.35±1.52 | −0.23±1.36 | −0.19±1.54 | 0.053 |
| Radial SOS (z-score) | −0.95±1.51 | −0.59±1.56 | −0.49±1.64 | −0.64±1.61 | 0.136 |
| Phalangeal SOS (z-score) | −0.75±1.09 | −0.72±1.23 | −0.57±1.11 | −0.68±1.03 | 0.457 |
| Parathyroid hormone (pg/ml) | 77 (44–121) | 58 (43–81) | 60 (39–88) | 56 (37–79) | <0.001 |
| 25-Hydroxyvitamin D (ng/ml) | 6.1 (4.6–8.2) | 7.0 (5.6–10.1) | 7.5 (5.8–10.4) | 7.8 (5.6–10.6) | 0.001 |
| Serum phosphate (mg/dl) | 3.8±0.7 | 3.8±0.7 | 3.9±0.7 | 3.8±0.8 | 0.405 |
| Serum calcium (mmol/l) | 2.44±0.14 | 2.46±0.13 | 2.46±0.11 | 2.46±0.12 | 0.203 |
| Alkaline phosphatase (U/l) | 129±44 | 119±38 | 118±37 | 116±39 | 0.013 |
| Alanine aminotransferase (U/l) | 8 (7–11) | 9 (7–12) | 8 (7–11) | 9 (7–13) | 0.228 |
| Albumin (g/dl) | 3.7±0.3 | 3.8±0.3 | 3.7±0.3 | 3.8±0.2 | 0.006 |
| Creatinine clearance (ml/min) | 36.0±12.2 | 38.4±11.8 | 38.0±10.0 | 40.8±12.7 | 0.003 |
| Knee extensor strength (kp) | 12.8±5.2 | 12.1±4.7 | 13.5±5.1 | 14.0±5.3 | 0.020 |
| Mobility status (1–4) | | | | | |
| 1 (%) | 10.3 | 10.9 | 11.3 | 12.9 | 0.258 |
| 2 (%) | 6.2 | 6.9 | 7.3 | 6.7 | |
| 3 (%) | 8.9 | 6.7 | 6.3 | 4.6 | |
| 4 (%) | 0.2 | 0 | 0.4 | 0.2 | |
| HbA1c (%) | 4.8 (4.6–5.1) | 4.9 (4.6–5.3) | 4.8 (4.6–5.1) | 4.9 (4.7–5.4) | 0.043 |
| Arterial hypertension (%) | 37.2 | 35.5 | 45.7 | 46.0 | 0.189 |
| Coronary artery diseases (%) | 27.9 | 30.6 | 31.8 | 25.8 | 0.719 |

Data are presented as medians with interquartile range; means with standard deviation or as percentages. Differences across groups were calculated with Analysis of Variance with *P* for trend and with Chi-squared test

with musculoskeletal diseases and mortality, we are, of course, aware that our results could be due to reverse causality, i.e., that underlying disease contribute to low homoarginine levels. Hence, further randomized controlled trials (RCT) are required to address this issue. In this context, it should be acknowledged that although homoarginine is usually not significantly contained in nutrition, certain foods such as the grass pea which is grown and used

**Table 2** Univariate and multivariate liner regression analyses of homoarginine with bone density and parameters of bone metabolism and muscle strength

| Parameter | Univariate | Model 1 | Model 2 | Model 3 | Model 4 |
|---|---|---|---|---|---|
| | Beta coefficients (*P* value) for homoarginine | | | | |
| β-CTX (ng/ml) | −0.26 (<0.001) | −0.24 (<0.001) | −0.19 (<0.001) | −0.18 (<0.001) | −0.13 (0.003) |
| Osteocalcin (ng/ml) | −0.21 (<0.001) | −0.19 (<0.001) | −0.17 (<0.001) | −0.16 (<0.001) | −0.08 (0.039) |
| Alkaline phosphatase (U/l) | −0.16 (<0.001) | −0.15 (0.001) | −0.12 (0.008) | −0.12 (0.008) | −0.05 (0.232) |
| Calcaneus stiffness (z-score) | 0.14 (0.002) | 0.16 (0.001) | 0.14 (0.005) | 0.14 (0.005) | 0.11 (0.020) |
| Radial SOS (z-score) | 0.08 (0.099) | 0.06 (0.198) | 0.06 (0.272) | 0.06 (0.279) | 0.03 (0.526) |
| Phalangeal SOS (z-score) | 0.06 (0.231) | 0.04 (0.391) | 0.03 (0.495) | 0.03 (0.495) | 0.01 (0.931) |
| Knee extensor strength (kp) | 0.14 (0.003) | 0.11 (0.016) | 0.09 (0.059) | 0.09 (0.066) | 0.09 (0.065) |

All regression analyses have homoarginine as an independent variable. Model 1 has age as an additional covariate. Model 2 has age, BMI and albumin as additional covariates. Model 3 has age, BMI, albumin and creatinine clearance as additional covariates. Model 4 has age, BMI, albumin, creatinine clearance and PTH as additional covariates

in several countries around the world contain high levels of homoarginine and are, therefore, of great interest for homoarginine research [20].

Our data are limited by the observational design of our study that precludes any conclusions regarding causality. Considering that we measured homoarginine in nonfasting serum samples, it is conceivable that previous meals might have altered homoarginine levels. A major impact of this nonfasting state on our findings is, however, unlikely because (i) homoarginine content of a normal Austrian diet is usually negligible; (ii) even lysine, the precursor of homoarginine, is only modestly associated with homoarginine levels and (iii) homoarginine levels in our cohort were relatively low compared to previous studies [3, 10]. Apart from this, we could replicate previously shown associations of homoarginine with, e.g., age and creatinine clearance, and an impact of meals on homoarginine levels might lead to a bias towards the null which would even strengthen our findings [3, 10].

In conclusion, we observed significant associations of homoarginine with bone density and metabolism and by trend with muscle strength. Furthermore, we confirmed previously shown associations of homoarginine deficiency with increased mortality. Whether homoarginine metabolism is clinically relevant for musculoskeletal and overall health deserves further studies including RCTs with homoarginine supplementation and clinical surveys of grass pea-consuming subjects in selected populations.

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**Conflicts of interest** None.

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