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Homocysteine levels are associated with bone resorption in pre-frail and frail Spanish women: The Toledo Study for Healthy Aging

Nuria Álvarez-Sánchez[a,1], Ana Isabel Álvarez-Ríos[b,1], Juan Miguel Guerrero[a,b,c], Francisco José García-García[d], Leocadio Rodríguez-Mañas[e], Ivan Cruz-Chamorro[a,c], Patricia Judith Lardone[a,c], Antonio Carrillo-Vico[a,c,*]

[a] Instituto de Biomedicina de Sevilla, IBIS (Universidad de Sevilla, HUVR, Junta de Andalucía, CSIC), Sevilla, Spain
[b] Department of Clinical Biochemistry, Virgen del Rocío University Hospital, Seville, Spain
[c] Departamento de Bioquímica Médica y Biología Molecular e Inmunología, Facultad de Medicina, Universidad de Sevilla, Spain
[d] Division of Geriatric Medicine, Hospital Virgen del Valle, Complejo Hospitalario de Toledo, Toledo, Spain
[e] Servicio de Geriatría y Fundación para la Investigación Biomédica, Hospital Universitario de Getafe Madrid, Spain

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ABSTRACT

Background: Homocysteine (Hcy) high levels are associated with fractures, bone resorption and an early onset of osteoporosis in elderly persons; a relationship between Hcy and bone formation has also been suggested but is still controversial. Frailty, an independent predictor of fractures and decreased bone mineral density is associated with altered bone metabolism in women. However, no previous works have studied the relationship among frailty, Hcy levels and bone turnover.

Methods: We studied the association among Hcy, osteoporosis and N-terminal propeptide of type I procollagen (PINP), C-terminal telopeptide of type I collagen (β-CTX), parathyroid hormone (PTH), calcium and 25-hydroxyvitamin D (25(OH)D) in 631 Spanish women between the ages of 65–78 from the Toledo Study for Healthy Aging (TSHA) cohort, who were classified as highly functional (robust subjects) or non-robust (pre-frail or frail subjects) according to Fried's criteria.

Results: Hcy was independently associated with β-CTX in the entire population (B = 0.22; 95% CI, 0.09–0.34; $p = 0.001$) and in the non-robust group (B = 0.24; 95% CI, 0.09–0.39; $p = 0.002$). Hcy was also associated with PINP in the entire and non-robust populations, but the association was lost after including the levels of β-CTX, but not the other bone biomarkers, in the multivariate analysis. This suggests that the controversial relationship between Hcy and bone formation might be explained, at least to a certain extent, by the confounding effects of β-CTX.

Conclusions: This work highlights the important implication of frailty status in the association between Hcy and increased bone turnover in older women.

1. Introduction

With the increase in life expectancy, osteoporosis and the associated fractures are becoming a major health concern. It has been estimated that, in 2010, 27.5 million European patients had osteoporosis and sustained 3.5 million fractures with an estimated cost of €37 billion (Hernlund et al., 2013). Although bone mineral density (BMD) is used for osteoporosis diagnosis, the use of bone turnover markers (BTMs) has been proposed as they respond faster to changes in bone metabolism (Naylor and Eastell, 2012). BTMs, especially those related to bone resorption, are predictors of bone loss (Garnero et al., 1996) and fractures (Gerdhem et al., 2004). Measurement of the bone formation marker, N-terminal propeptide of type I procollagen (PINP), and the resorption marker, C-terminal cross-linked telopeptides of type I collagen (CTX), has been recommended for international standardization (Vasikaran et al., 2011).

Frailty, a geriatric syndrome characterized by an increased vulnerability to stressors due to decreased physiological reserves (Walston

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMD, bone mineral density; BMI, body mass index; BTM, bone turnover marker; CI, confidence interval; CTX, C-terminal cross-linked telopeptides of type I collagen; CV, coefficients of variation; DPD, deoxypyridinoline; Hcy, homocysteine; IQR, interquartile range; MMSE, Mini-Mental State Examination; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; PINP, N-terminal propeptide of type I procollagen; PTH, parathyroid hormone; TSHA, Toledo Study for Healthy Aging

* Corresponding author at: Institute of Biomedicine of Seville (IBiS), Virgen del Rocío University Hospital/CSIC/University of Seville, Avda. Manuel Siurot s/n, 41013 Seville, Spain.
E-mail address: vico@us.es (A. Carrillo-Vico).

[1] Both authors contributed equally to this work.

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et al., 2006), independently predicts falls (Fried et al., 2001), fractures (Kojima, 2016), decreased BMD (Sternberg et al., 2014) and reduced mobility (Garcia-Garcia et al., 2011). Since reduced mobility in older persons can augment bone turnover (Chen et al., 2006), frailty might accelerate bone loss in these subjects. Moreover, we have recently shown that increased PINP levels and a reduced concentration of 25-hydroxyvitamin D (25(OH)D) are independently associated with frailty (Alvarez-Rios et al., 2015) in elderly women.

High levels of homocysteine (Hcy) have been associated with bone alterations in humans, animals and *in vitro* models (Feigerlova et al., 2016), but also with an early onset of osteoporosis (Mudd et al., 1985) and an increased risk of osteoporotic fractures in older persons (van Meurs et al., 2004; Yang et al., 2012). Although the effect of Hcy levels on BMD is controversial, with some studies supporting a negative association (Dhonukshe-Rutten et al., 2005; Gerdhem et al., 2007) and others reporting no effect (Rumbak et al., 2012; van Meurs et al., 2004), Hcy is related to increased biochemical markers of bone resorption (calcium, CTX, cross-laps and deoxypyridinoline (DPD)) (Gerdhem et al., 2007; Herrmann et al., 2005; Nilsson et al., 2005) and with parathyroid hormone (PTH) (Gerdhem et al., 2007) in older subjects, especially women. Although some studies reported that elevated Hcy concentrations were associated with osteocalcin (Dhonukshe-Rutten et al., 2005; Gerdhem et al., 2007), others found no association between Hcy and other bone formation markers (Herrmann et al., 2005) or 25(OH)D (Gerdhem et al., 2007). The role of Hcy in bone metabolism is also supported by the association of a common polymorphism in methylenetetrahydrofolate reductase (MTHFR), which increases serum Hcy levels (Abrahamsen et al., 2003), with fractures (Abrahamsen et al., 2003; van Wijngaarden et al., 2013; Wang and Liu, 2012), decreased BMD (Abrahamsen et al., 2003; Miyao et al., 2000; Wang and Liu, 2012) and increased DPD urinary excretion (Abrahamsen et al., 2003; Miyao et al., 2000).

To the best of our knowledge, no previous studies have investigated the relationship among frailty, Hcy and bone metabolism. Thus, the objective of the present work was to study the association between Hcy, osteoporosis and bone markers in Spanish women 65 years of age or older from the Toledo Study for Healthy Aging (TSHA) classified according to Fried's criteria (Fried et al., 2001) as robust (highly functional subjects) and non-robust women, who have a higher risk of fractures and disability.

2. Methods

2.1. Study participants

This study was performed with a subsample of the TSHA participants (Garcia-Garcia et al., 2011). Participant selection and baseline data collection took place between June 2006 and September 2009 (Garcia-Garcia et al., 2011), and TSHA was conducted as described elsewhere (Alvarez-Rios et al., 2015). The present study was only conducted in women due to their higher risk of bone loss. From the 1396 women recruited, the present study was conducted with a final sample of 631 women (Fig. 1), who provided written consent. The study was approved by the Clinical Research Ethics Committee of the Hospital of Toledo.

Sociodemographic variables and comorbidities are shown in Table 1. Disability for both basic (6 items) and instrumental (8 items) activities was measured using the Katz (Katz et al., 1963) and Lawton (Lawton and Brody, 1969) indexes, respectively. Participants who were dependent for at least one activity were considered dependent in each of the scales. Cognitive status was assessed using the Mini-Mental State Examination (MMSE) with appropriate cut-off values for the population (Garcia-Garcia et al., 2011). Frailty was defined using Fried's criteria, as described elsewhere (Alvarez-Rios et al., 2015; Fried et al., 2001; Garcia-Garcia et al., 2011). The subjects were classified as robust (persons who met none of the criteria), pre-frail (individuals meeting one or two criteria) and frail (subjects meeting three or more criteria). For the analysis, the pre-frail and frail persons were pooled into the "non-robust" group due to their higher risk of osteoporosis and fractures.

All values are expressed as median and interquartile range (IQR) or as percentage. The characteristics of the subjects were compared between the robust group and the non-robust group (pre-frail or frail women) using the Mann-Whitney *U* test (for continuous variables) or the Pearson's χ^2 test (for categorical variables).

BMI: body mass index; β-CTX: C-terminal cross-linked telopeptide of type I collagen; Hcy: homocysteine; NSAIDs: non-steroidal anti-inflammatory drugs; PINP: N-terminal propeptide of type I procollagen; 25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone.

2.2. Biochemical measurements

Biomarker (PINP, β-CTX, PTH and 25(OH)D) concentrations were measured in serum from fasting blood samples as previously described (Alvarez-Rios et al., 2015), by an immunoassay chemiluminescence system (total Hcy; Dimension Vista®, Siemens, Germany) and a fully automated immunoassay electrochemiluminescence system (calcium; Cobas E 601®, Roche Diagnostics, GmbH, Germany). The total Hcy detection limit was 3 μmol/L, and the intra- and inter-assay coefficients of variation (CV) were 3.4% and 5.6%, respectively. The detection limit for calcium was 1.5 mmol/L, and the intra- and inter-assay CV were 2.0% and 5.5%, respectively.

2.3. Data analysis

The distributions of Hcy, PINP, β-CTX, calcium, 25(OH)D and PTH levels were examined using the Kolmogorov-Smirnov test and found to be skewed to the right; therefore, these data were logarithmic-transformed for all analyses. The continuous variables were expressed as medians with interquartile ranges (IQRs), whereas the categorical variables were expressed as frequencies (%) of the population. Differences between groups were analyzed using the Mann-Whitney *U* test (continuous variables) and Pearson's χ^2 test (categorical variables). Spearman's rho correlation coefficient test was used to analyze the correlations between Hcy and bone markers. Associations between Hcy levels and bone markers were assessed by multivariate linear regression, while multivariate logistic regression was performed to evaluate the association between Hcy and prevalent osteoporosis, and between a clinical history of hip fracture and Hcy. Variables that were significantly correlated with bone markers (age, body mass index (BMI), frailty, cardiovascular diseases, osteoporosis, hip fractures and use of aspirin, proton pump inhibitors, oral hypoglycemic drugs, oral anticoagulants, or drugs altering bone metabolism) or considered relevant (smoking status, history of diabetes, dementia (MMSE score)) were included in the multivariate models to control for confounding factors and to enhance precision (model II). Moreover, for each of the five bone markers assessed in the study, their association with Hcy was also adjusted for the other four bone markers (model III). Values of $p < 0.05$ were considered statistically significant. The statistical analyses were performed using SPSS software (version 23.0.02, IBM). The graphics were generated using GraphPad Prism 6.0 (GraphPad Software, USA).

3. Results

3.1. Participant characteristics

Table 1 shows the participant characteristics. A total of 631 women (the "all women" group) between the ages of 65–78 were included in this study and classified into robust and non-robust individuals, which accounted for 51.5 and 48.5% of the cohort, respectively. Non-robust women were significantly older and more dependent (as shown by the Katz and Lawton indexes), had a lower educational level and a higher