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High serum total homocysteine levels accelerate hip bone loss in healthy premenopausal women and men

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ABSTRACT

Introduction: Despite extensive evidence demonstrating the direct, detrimental role of homocysteine on bone metabolism, the effects of serum total homocysteine (tHcy) on bone loss are still equivocal. In the present study, we performed a longitudinal study on healthy participants of various ages of both sexes in order to investigate the association between serum tHcy concentrations and annualized changes in bone mineral density (BMD).

Methods: A total of 460 Koreans ≥ 30 years of age received comprehensive, routine health examinations for an average period of 3 years. The BMD at proximal femur sites was measured with dual-energy X-ray absorptiometry using the same equipment at baseline and follow-up.

Results: After adjusting for potential confounders, the rates of bone loss at the proximal femur sites were significantly accelerated in a dose–response fashion across increasing tHcy concentrations in premenopausal women and men, but not in postmenopausal women. Consistently, compared with subjects in the lowest tHcy quartile, premenopausal women in the third and/or highest tHcy quartile and men in the highest tHcy quartile showed significantly higher rates of bone loss at all proximal femur sites ($p = 0.015$–0.048) and at the total femur and femur neck ($p = 0.008$–0.013), respectively. In contrast, there were no differences in terms of bone loss among the tHcy quartiles for postmenopausal women.

Conclusion: These data provide the first clinical evidence that increased tHcy levels could be an independent risk factor for the future deterioration of bone mass in premenopausal women and men.

Introduction

Many lines of evidence now indicate that elevation of homocysteine and its disulfide derivatives (tHcy) has direct detrimental effects on bone metabolism. Homocysteine may interfere with bone collagen cross-links, thereby increasing bone fragility [1]. In a murine experimental model, rats with elevated tHcy demonstrated severe trabecular bone loss, altered microarchitectural parameters, and decreased mechanical strength in the femoral neck [2]. In addition, our in vitro studies have reported that homocysteine induces apoptosis in bone marrow stromal cells via the reactive oxygen species (ROS)-mediated mitochondrial pathway and NF-kappa B activation [3], whereas it promotes bone resorption by stimulating p38 mitogen-activated protein kinase (MAPK) activity and the generation of intracellular ROS in osteoclasts [4]. The first clinically relevant evidence of these in vitro and animal findings was observed in patients with homocystinuria due to cystathionine beta-synthase deficiency, an inborn error of metabolism that is characterized by very high plasma concentrations of tHcy and, among several clinical manifestations, premature osteoporosis and fractures [5].

Based on these findings, numerous epidemiological studies have been performed to assess the role of serum tHcy as a risk factor for osteoporosis-related phenotypes. All prospective trials that enrolled >1000 patients found a significant positive relationship between tHcy and osteoporotic fractures [6–9]. In contrast, studies on the association between tHcy and bone mineral density (BMD) have yielded inconsistent results [10]. However, because almost all of these studies were cross-sectional in design [11,12], the role of tHcy in bone mass could not be appropriately investigated. Furthermore, the only two studies of longitudinal bone loss that have been previously reported were based on data from elderly patients > 75 years of age on average

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and had contradictory findings [13,14]. Thus, the effects of tHcy on bone density are still equivocal.

It is well known that the biologic differences between men and women, as well as menopause status, are important factors that influence bone metabolism, implying that the effects of various risk factors on bone health could be different depending on the sex and menopause status of the individual patient. Actually, several epidemiological studies have reported different relationships of serum tHcy levels with osteoporosis-related phenotypes in men and women [7,9,12]. In the present study, we performed a longitudinal study on healthy pre- and postmenopausal women and men in order to investigate the association between serum tHcy concentrations and annualized changes in BMD.

Materials and methods

Study participants

This was a 3-year longitudinal health promotion center-based study. The study population consisted of subjects ≥ 30 years of age who had undergone comprehensive routine health examinations at the Asan Medical Center (AMC, Seoul, Republic of Korea) in 2007 and had returned for follow-up examinations in 2010; BMD and serum tHcy concentrations were measured during these examinations. The examinations consisted of extensive screening tests for the early detection of malignancy, diabetes, osteoporosis, and other age-related diseases. Initially, 525 subjects were identified. Of these, based on the 2007 medical records, subjects with serum liver enzyme (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) activities above 2 times the upper normal limits, increased serum creatinine (> 1.4 mg/dL [> 123.8 μmol/L]), and/or abnormal thyroid function (serum thyrotropin < 0.4 mU/L or > 5.0 mU/L) were excluded from this study. In addition, subjects were excluded if they had taken drugs, such as bisphosphonates and glucocorticoids, during the study period or 12 months prior to baseline, due to their potential impacts on bone metabolism. Finally, patients that suffered from diseases such as hyperparathyroidism or rheumatoid arthritis, which might affect bone metabolism, were also excluded from the study. Some subjects met ≥ 2 exclusion criteria. The remaining 460 subjects (150 premenopausal women, 138 postmenopausal women, and 172 men) were eligible to participate in this study. Postmenopausal status was defined as the cessation of menses for ≥ 1 year, which was confirmed by a serum follicle-stimulating hormone concentration of > 30 IU/L.

Lifestyle factors and anthropometric measurements

All participants were interviewed and examined by physicians at the health promotion center. Information on medication usage and history of previous medical or surgical procedures were obtained for each subject. Smoking (never, past, or current) and drinking habits (no or yes) were categorized. Dairy product consumption and physical exercise were also categorized according to frequency (< or ≥ 3 times/week). Height (cm) and weight (kg) were measured using standardized protocols while the subject was dressed in light clothing and without shoes. Body mass index (BMI; kg/m^2) was calculated from the height and weight.

Biochemical measurements

Morning blood samples were obtained 12 h after fasting and subsequently analyzed at the certified laboratory at AMC. Serum tHcy concentrations were measured by a competitive immunoassay, using direct chemiluminescent technology (ADVIA Centaur kit; Bayer, Morristown, NJ, USA), which had intra- and interassay coefficients of variations (CVs) of 3.4–4.3% and 2.5–2.6%, respectively, and a lower limit of detection of 0.5 μmol/L. Serum calcium and phosphorus concentrations were measured by the cresolphthalein complexone and the phosphomolybdate ultraviolet methods, respectively, using the Toshiba 200FR system (Toshiba Medical System Co., Tokyo, Japan). Fasting total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, uric acid, AST, and ALT were measured using the enzymatic colorimetric method (Toshiba). Serum alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), and total bilirubin concentrations were measured using the Bowers and McComb method (Toshiba), the L-γ-glutamyl-p-nitroanilide method (Toshiba), and the vanadate oxidation method (Toshiba), respectively. Serum glucose and creatinine levels were measured by the glucose oxidase method (Toshiba) and the Jaffe method (Toshiba), respectively. The serum erythrocyte sedimentation rate (ESR) was measured using the quantitative capillary photometry method (TEST-1; ALIFAX S.P.A., Polverara, Italy). The glomerular filtration rate (GFR) was estimated using the Cockcroft–Gault formula [15]. The intra- and interassay CVs for these analyses were consistently < 3.5%.

BMD measurements

The areal BMDs (g/cm^2) of the total femur, femur neck, and trochanter were measured at baseline and on the follow-up examinations by dual-energy X-ray absorptiometry (DXA) using Lunar equipment (Prodigy; Madison, WI, USA). Repeated measurements were performed using the same instruments used for the initial measurements. The in vivo precisions of the machine were 1.08%, 1.02%, and 1.06% for the femur neck, total femur, and trochanter, respectively. These values were obtained by scanning 17 volunteers who were not enrolled in the study. Each volunteer underwent 5 scans on the same day, getting on and off the table between examinations. A quality control laboratory, certified DXA technicians, and standardized procedures for scanning were implemented in order to ensure reliable DXA measurements. The rate of change in BMD was expressed as the annualized percentage of the difference between the follow-up BMD and the initial BMD, divided by the initial BMD reflecting the examination intervals.

Statistical analysis

Continuous and categorical variables are reported as the means ± standard deviations (SDs) and percentages, respectively, unless otherwise specified. The baseline characteristics of the 3 groups were compared using one-way analysis of variance (ANOVA) for continuous variables and the Chi-square test for categorical variables. Age and GFR-adjusted least-square means (95% CI) of the tHcy levels in pre- and postmenopausal women and men were estimated and compared using analysis of covariance (ANCOVA). Tests to determine the level of interaction between variables were performed using likelihood ratio tests by comparing 2 nested models, one with the main effects only and the other with both the main effects and interaction terms. To examine the relationship between annualized BMD changes at the total femur, femur neck, and trochanter and covariates, linear univariate regression analyses were performed. Next, in order to determine the independent effects of the serum tHcy level on annualized BMD changes at various proximal femur sites, we used a multiple regression model with the annualized BMD change as a dependent variable and the serum tHcy level as an independent variable. In these analyses, the serum tHcy concentration was logarithmically transformed because the distribution was positively skewed. Confounding independent variables were selected on the basis of being clinically applicable and/or their statistical significance according to linear univariate regression models (inclusion criterion: $p < 0.2$). In addition, the same variables were forced into the multiple regression models of the 3 proximal femur sites in each study group, regardless of the significance ($p <$ or > 0.2) at the other sites in order to obtain comparable analyses. Consequently, the base adjustment model included age, BMI, baseline BMD,