Cysteine, homocysteine and bone mineral density: A role for body composition?

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Introduction

Body total lean mass and fat mass are recognized as key determinants of bone mineral density (BMD) [1], and the WHO has identified low body mass index (BMI) as a risk factor for osteoporosis [2]. We recently reported in a large population-based study that plasma total cysteine (tCys) and homocysteine (tHcy) are associated with body composition, which in turn affects bone mineral density (BMD). This relationship between aminothiols and body composition is consistent with the thin and underweight marfanoid phenotype characterizing cystathionine beta synthase (CBS) deficiency [5–7], in which a transsulfuration block results in a high tHcy and low tCys. Homocystinuria due to CBS deficiency is also characterized by osteoporosis, which occurs in 50–70% of cases by age 20 years [8], and has hitherto mainly been attributed to hyperhomocysteinemia. The possible role for decreased tCys in osteoporosis of CBS deficiency warrants further investigation, as osteoporosis has not been reported in other genetic homocystinurias in which cysteine synthesis is normal [9].

A recent study in postmenopausal women reported a strong association between decreased tCys and osteoporosis [10]. The authors proposed that this may be due to either reduced availability of cysteine for collagen formation, or increased cysteine utilization by...
proteases in the osteoclastic hyper-activity underlying the osteoporotic process. In the present study we sought to investigate a third possibility, namely whether any effects of tCys and/or tHcy on BMD are mediated through their influence on body fat mass, a recognized determinant of BMD [1].

Subjects and methods

Study population

The first Hordaland Homocysteine Study (HHS-I) was conducted in 1992–1993, on 18043 40–67 year-old residents of Hordaland county of Western Norway. In a follow-up study in 1997–1999 (HHS-II), 9187 subjects were re-invited as part of the Hordaland Health Study (HUSK); 7074 (77%) attended. Details of recruitment, outcomes and ethical approval for both studies have been reported before [11].

This study is based on data from 3009 women and 2229 men who participated in both HHS-I (baseline) and HHS-II (follow-up). Selection was based on availability of data on tCys, tHcy, weight and height at both time-points, and lean mass, fat mass and BMD at follow-up (HHS-II). Of these participants, 1237 men and 1867 women were aged 40–42 y in 1992, while 992 men and 1142 women were aged 65–67 y.

Study variables

Anthropometry and body composition

Height and weight were measured in light clothing, to the nearest 1 cm and 0.5 kg respectively, and BMI was calculated. Total body lean mass, fat mass, and total body BMD were measured using DXA [12]. Principles and details of DXA measurements in HHS-II have been described previously [13].

Lifestyle and dietary data

Self-administered questionnaires provided information on diet [14] (HHS-II only) and lifestyle (HHS-I and II). Nutrient and total energy intakes were calculated using a software system developed at the Department of Nutrition, University of Oslo, and analyzed in this study as continuous variables. Physical activity in HHS-II included 2 variables indicating heavy or light physical activity in the past year, with 4 duration categories within each variable, while in HHS-I it comprised 4 strenuousness categories. Smoking (HHS-I) and coffee consumption variables indicated the number of cigarettes or cups consumed per day. Smoking habits in HHS-II were categorized as 1—never-smoker, 2—ex-smoker, 3—pipe/cigar-smoker, 4—non-daily cigarette smoker and 5—daily cigarette smoker.

Biochemical measurements

Non-fasting plasma samples were collected in EDTA-containing tubes for tCys and tHcy analyses, which were performed using HPLC with fluorescence detection. Intra-assay coefficient of variation was lower than 4% [15]. Creatinine (HHS-II only) was measured in stored plasma using a modification of a liquid chromatography–mass spectrometry (LC–MS/MS) described previously [16].

Statistical methods

Skewed variables, namely tHcy and creatinine were log-transformed prior to regression analysis. Groups were compared by Mann–Whitney U test for gender differences and Wilcoxon signed rank test for paired differences between baseline and follow-up. Owing to previously reported gender differences in the relationship of tHcy [17] and body composition [1] with BMD, all analyses were performed separately in men and women and, in correlation and regression analyses, adjusted for age.

Age-adjusted Pearson correlation coefficients investigated simple correlations among the sulfur aminoacids, BMD, and anthropometric parameters. Multiple linear regression models were then used to assess the role of body composition as a mediator in the cross-sectional associations of tCys and tHcy with BMD. BMD was always entered as the dependent variable, with age, tCys and tHcy simultaneously as predictor variables. Various models were additionally adjusted for lean mass and/or fat mass with or without other factors selected by the strength of simple Pearson correlations or with suspected biological influence on BMD.

We similarly investigated whether baseline values or changes in tCys and tHcy were associated with BMD at follow-up (6 years later) by linear regression, and whether body weight acts as a mediator in these associations. Since body composition was not measured at baseline, these linear regression models were adjusted for relevant BMI variables.

Table 1

Population distribution for selected parameters at follow-up

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>Baseline</td>
<td>25.1 (21.3, 30.7)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>25.1 (21.3, 30.7)</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>Baseline</td>
<td>57.6 (47.2, 68.9)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>57.6 (47.2, 68.9)</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>Baseline</td>
<td>19.9 (8.6, 36.3)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>19.9 (8.6, 36.3)</td>
</tr>
<tr>
<td>Total body BMD, g/cm²</td>
<td>Baseline</td>
<td>1.192 (1.035, 1.346)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>1.192 (1.035, 1.346)</td>
</tr>
<tr>
<td>tCys, μmol/L</td>
<td>Baseline</td>
<td>282 (232, 342)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>282 (232, 342)</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>Baseline</td>
<td>11.0 (7.8, 17.5)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>11.0 (7.8, 17.5)</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>Baseline</td>
<td>80 (61, 108)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>80 (61, 108)</td>
</tr>
<tr>
<td>Calcium intake, mg/d</td>
<td>Baseline</td>
<td>8.4 (2.3, 30.0)</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>8.4 (2.3, 30.0)</td>
</tr>
</tbody>
</table>

1 Unless otherwise noted. Data presented as median (5th–95th percentiles). All parameters are significantly different in men vs. women by Mann–Whitney U test.

*Significantly different compared to follow-up within the same gender by Wilcoxon signed rank test.

1 tCys, plasma total cysteine; tHcy, plasma total homocysteine.
Body composition as a possible mediator in the cross-sectional associations of tCys and tHcy with BMD at follow-up

Table 3 shows the effect of adjustment for different anthropometric parameters on the age-adjusted associations of tCys and tHcy with BMD (as outcome variable) in a linear regression model. Similar to previous findings for total hip BMD in the same population [17], tHcy was not a significant predictor of total body BMD in men, while in women, it showed an inverse association with BMD, after controlling for age and tCys. This association remained robust after adjustment for lean mass and fat mass (Table 3), and with further adjustment for height, diet (intakes of calcium, vitamin D, protein, and coffee), plasma creatinine, smoking habits and physical activity (partial r = −0.09, p < 0.001).

Controlling for age and tHcy, tCys was positively associated with BMD in both men (partial r = 0.07, p = 0.001) and women (partial r = 0.11, p < 0.001), but these associations were abolished by adjusting for lean mass and fat mass. When tested separately, it was fat mass rather than lean mass that markedly weakened the association of tCys with BMD (Table 3). When fat mass and lean mass were excluded from the model, adjusting for height and intakes of calcium, vitamin D, protein, and coffee, as well as smoking habits, physical activity and plasma creatinine, had minor effects on the association of tCys with BMD in men (partial r for tCys = 0.06, p = 0.018) and women (partial r for tCys = 0.09, p < 0.001).

Discussion

We investigated the role of body mass and composition as mediators in the associations of tCys and tHcy with BMD. We report

Effect of changes in tCys, tHcy and BMI during 6 years on BMD at follow-up

Changes in tCys and tHcy were not associated with BMD at follow-up in a multiple linear regression model adjusted for age, baseline BMI and changes in BMI. Baseline BMI was a powerful positive predictor of BMD in the same model (partial r = 0.30 and 0.28 in men and women respectively, p < 0.001 for both). Change in BMI over 6 years also correlated directly with BMD (partial r = 0.06, p = 0.009 in men and partial r = 0.07, p < 0.001 in women) despite adjustment for baseline BMI; i.e. weight gain was associated with higher BMD and weight loss predicted a lower BMD at follow-up independent of initial body weight and changes in tCys and tHcy.

BMI as a possible mediator in the associations of tCys and tHcy at baseline with BMD at follow-up

Fig. 1 shows significant positive and negative associations respectively, of baseline tCys and tHcy, with BMD at follow-up in both men and women, with reciprocal adjustment for tCys or tHcy. After controlling for baseline BMI, only the tHcy associations persisted while the tCys associations became non-significant. Conversely, baseline BMI showed strong associations with follow-up BMD (partial r = −0.29 in men, and partial r = −0.28 in women, p < 0.001 for both), that were not attenuated after adjustment for tCys (data not shown).

In both men and women, the relationship of baseline tHcy with follow-up BMD was stronger than the cross-sectional association at follow-up. The prospective negative tHcy-BMD association was virtually unchanged after adjustment for smoking habits, coffee consumption, and physical activity at baseline as well as plasma creatinine and dietary intakes of calcium, vitamin D, and protein at follow-up (partial r for tHcy = −0.09, p = 0.002 in women, partial r = −0.08, p = 0.003 in men).

In summary, tCys at baseline and follow-up showed positive associations with BMI that were markedly weakened or abolished after adjustment for fat mass or BMI. In contrast, the negative association of tHcy with BMD persisted after controlling for fat mass or BMI.

Table 3

Effect of adjustment for body weight and composition on cross-sectional age-adjusted partial correlation coefficients of tCys and tHcy with total body BMD

<table>
<thead>
<tr>
<th>Model (additional covariates)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tCys</td>
<td>Log tHcy</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----</td>
<td>---------</td>
</tr>
<tr>
<td>1 (body weight)</td>
<td>0.07</td>
<td>−0.02</td>
</tr>
<tr>
<td>(p = 0.001)</td>
<td>(p = 0.29)</td>
<td>(p = 0.001)</td>
</tr>
<tr>
<td>2 (lean mass)</td>
<td>0.04</td>
<td>−0.03</td>
</tr>
<tr>
<td>(p = 0.078)</td>
<td>(p = 0.21)</td>
<td>(p = 0.001)</td>
</tr>
<tr>
<td>3 (fat mass)</td>
<td>0.02</td>
<td>−0.01</td>
</tr>
<tr>
<td>(p = 0.29)</td>
<td>(p = 0.55)</td>
<td>(p = 0.041)</td>
</tr>
<tr>
<td>4 (lean mass and</td>
<td>0.03</td>
<td>−0.03</td>
</tr>
<tr>
<td>fat mass)</td>
<td>(p = 0.23)</td>
<td>(p = 0.24)</td>
</tr>
<tr>
<td>5 (BMI)</td>
<td>0.00</td>
<td>−0.01</td>
</tr>
<tr>
<td>(p = 0.82)</td>
<td>(p = 0.50)</td>
<td>(p = 0.23)</td>
</tr>
<tr>
<td>6 (body weight)</td>
<td>−0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>(p = 0.70)</td>
<td>(p = 0.36)</td>
<td>(p = 0.69)</td>
</tr>
</tbody>
</table>

4 At follow-up, using linear regression with BMI as outcome variable. tCys, plasma total cysteine; tHcy, plasma total homocysteine; BMD, bone mineral density. N = 2229 men and 3009 women.

5 All models simultaneously include age, tCys and log tHcy as independent variables, and are adjusted for different anthropometric measures as indicated.
for the first time a prospective association of tCys with BMD measured 6 years later. However, our cross-sectional and prospective data suggest that tCys is a positive predictor of BMD only in so far as it predicts a higher body weight. Conversely, the negative association of tHcy with BMD in the present study was independent of body mass and composition.

We previously reported in HHS that the negative impact of tHcy on total hip BMD was significant only in women [17]; the same was observed in the present study using total body BMD. However, in the present study, the prospective association of tHcy at baseline with BMD measured 6 years later was stronger than the cross-sectional associations and was also significant in men. Other studies investigating this issue simultaneously in men and women either found no gender difference or that the relationship was stronger in men (epidemiologic studies and possible pathophysiologic mechanisms reviewed in [18]). That the baseline tHcy concentrations were stronger predictors of BMD than follow-up concentrations may point to the slow and long-standing nature of homocysteine-related pathology. This is evidenced also by our observation that 6-year changes in tHcy were not associated with BMD at follow-up when baseline concentrations were taken into account. Consistent with this, several homocysteine-lowering trials with relatively short durations (1–2 years) failed to document an improvement in bone turnover markers or BMD [19,20].

The tCys-BMD association in our dataset, largely mediated via body fat mass, was stronger in women, consistent with stronger tCys-fat mass [3] and fat mass-BMD [1] associations in women. Conversely, the tCys-BMD association reported by Baines et al. [10] in 328 post-menopausal women remained significant after adjustment for body weight, a discrepancy that may be partly related to their use of BMD measured at the os calcis, versus the total body BMD measurements used in the present study. Moreover, in their analysis of the non-smoking subgroup of 271 women, the association of tCys with BMD was non-significant after controlling for body weight, though we did not observe a similar interaction with smoking in our dataset.

Taking into account the accumulating evidence pointing to tCys as a causal determinant of increased body weight [21], our findings can be interpreted in the context of fat mass being on the causal pathway from tCys to BMD. At least two non-mutually-exclusive mechanisms are currently recognized which explain the relationship of fat mass with BMD. One mechanism involves the mechanical effect of weight-related gravitational forces in increasing bone density. Alternatively, or additionally, recent reviews on fat–bone relationships discuss various cytokines and hormones that may provide the chemical signal by which a higher body weight promotes a protective increase in bone density [22,23]. One such candidate, though not mentioned in these reviews, is of particular relevance here, as it is up-regulated in adipose tissue hyperplasia and adipogenesis [24], positively correlates with various cytokines and hormones that may provide the chemical signal by which a higher body weight promotes a protective increase in bone density [22,23]. One such candidate, though not mentioned in these reviews, is of particular relevance here, as it is up-regulated in adipose tissue hyperplasia and adipogenesis [24], positively correlates with various cytokines and hormones that may provide the chemical signal by which a higher body weight promotes a protective increase in bone density [22,23]. One such candidate, though not mentioned in these reviews, is of particular relevance here, as it is up-regulated in adipose tissue hyperplasia and adipogenesis [24], positively correlates with various cytokines and hormones that may provide the chemical signal by which a higher body weight promotes a protective increase in bone density [22,23].

Although it is uncertain how far plasma cysteine availability can limit SPARC secretion, cysteine is known to limit the synthesis of glutathione, a vital antioxidant containing one cysteine residue per molecule [27,28]. It is thus plausible that SPARC synthesis may be positively influenced by tCys concentrations, thus simultaneously promoting fat deposition and bone thickening. We hypothesize that SPARC secretion may be decreased in CBS-deficient homocystinuria patients with decreased tCys, thus contributing to the combination of thinness and osteoporosis observed in these patients. Yet this is unlikely to be the only mechanism involved, and it remains possible that other factors may link cysteine to bone health independent of adiposity.

In summary, we have demonstrated that previously reported negative correlations of tHcy with BMD are independent of body mass.
composition, while tCys positively correlates with BMD mainly through its influence on body fat mass. We propose oстеонектин as a potential link between cysteine availability, fat deposition and bone density that warrants further investigation. One practical implication of the present study and that by Baines et al. [10], is that cysteine deficiency may be more relevant to the pathogenesis of osteoporosis in CBS deficient-homocystinuria than is currently believed. This would support the rationale of treatment strategies involving cysteine supplementation in homocystinuria, which are ‘accepted but not widely used’ [29]. However, further work is required to establish to what extent osteoporosis in these patients correlates with the negative impact of low cysteine on their body weight.

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Author contributions

Elshorbagy: Concept, design, statistical analysis and interpretation, preparation of the manuscript.

Gjesdal: Data collection, critical revision of the manuscript.

Nurk: Interpretation and critical revision of the manuscript.

Tell: Planning, design, conduct and data collection of the Hordaland Health Study (HUSK) and the HHS II. Interpretation, comments and critical revisions of the manuscript drafts.

Ueland: Data collection, critical revision of the manuscript.

Vollset: Data collection, critical revision of the manuscript.

Smith: Critical revision of the manuscript.

Refsum: Concept, design, data collection, analysis, critical revision of the manuscript.

References