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Original article

Serum homocysteine levels in children and adolescents with impaired bone health

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ABSTRACT

Introduction: Association between high serum homocysteine (S-Hcy) levels and low bone mineral density (BMD) and increased fracture risk in postmenopausal women has been documented. Data concerning S-Hcy and bone health in children are scarce.

Objective: Our aim was to evaluate S-Hcy in children and adolescents with impaired bone health and look for correlations with clinical and laboratory data.

Patients and methods: We assessed S-Hcy levels in 37 children and adolescents (22 boys and 15 girls; mean age 13.9 ± 3.5 years) with prevalent low-energy trauma fractures (mean 3.3 ± 2.3 per patient) and/or low spinal L1-L4 BMD (below $-2SD$ Z-score; DXA Lunar GE). We also evaluated S-ALP, serum CrossLaps, osteocalcin (S-OC), body height, weight, body mass index (BMI) and serum levels of folate and vitamin B12. At the time of assessment, the children were not taking any drugs known to influence bone metabolism. The age-dependent parameters were expressed as Z-scores \pm SD.

Results: S-Hcy Z-score was significantly higher (1.3 ± 1.5 ; $P < 0.0001$) and L1-L4 BMD Z-score was significantly lower (-1.7 ± 1.3 ; $P < 0.0001$), respectively, in comparison with reference values. S-ALP did not differ from reference values ($P = 0.88$), while S-CrossLaps and S-osteocalcin were higher (1.2 ± 1.8 and 0.4 ± 0.5 ; $P = 0.0001$ and $P = 0.001$, respectively). S-Hcy was inversely correlated to L1-L4 BMD ($r = -0.33$; $P = 0.05$) and S-ALP ($r = -0.36$; $P = 0.04$) and not related to number of prevalent fractures ($r = 0.01$), S-osteocalcin ($r = -0.22$) or S-CrossLaps ($r = 0.003$).

Conclusion: These results suggest increased bone turnover and negative influence of elevated S-Hcy on bone formation and BMD in children and adolescents with recurrent fractures.

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Níveis séricos de homocisteína em crianças e adolescentes com comprometimento da saúde óssea

R E S U M O

Palavras-chave:

Homocisteína
Densidade mineral óssea
Marcadores da remodelação óssea
Fraturas

Introdução: Foi documentada uma associação entre níveis séricos elevados de homocisteína (S-Hci) e baixa densidade mineral óssea (DMO) e aumento do risco de fratura em mulheres na pós-menopausa. São escassos os dados concernentes à S-Hci e à saúde óssea em crianças.

Objetivo: Avaliar S-Hci em crianças e adolescentes com comprometimento da saúde óssea e procurar por relações com dados clínicos e laboratoriais.

Pacientes e métodos: Avaliamos os níveis de S-Hci em 37 crianças e adolescentes (22 meninos e 15 meninas; média de idade, 13,9 ± 3,5 anos) com fraturas prevalentes por trauma de baixa energia (média 3,3 ± 2,3 por paciente) e/ou baixa DMO espinhal/L1-L4 (escore Z abaixo de -2 DP; DXA Lunar GE). Também avaliamos S-ALP, CrossLaps sérico (S-Hci-CrossLaps), osteocalcina (S-OC), altura, peso corporal, índice de massa corporal (IMC) e níveis séricos de folato e vitamina B12. Por ocasião da avaliação, as crianças não estavam tomando qualquer medicação que sabidamente influenciasse o metabolismo ósseo. Os parâmetros dependentes de idade foram expressos como escores Z ± DP.

Resultados: O escore Z para S-Hci foi significativamente mais alto (1,3 ± 1,5; P < 0,0001) e o escore Z de para DMO/L1-L4 foi significativamente mais baixo (-1,7 ± 1,3; P < 0,0001), respectivamente, em comparação com os valores de referência. S-ALP não diferiu dos valores de referência (P = 0,88), enquanto S-CrossLaps e S-osteocalcina foram mais elevados (1,2 ± 1,8 e 0,4 ± 0,5; P = 0,0001 e P = 0,001, respectivamente). S-Hci estava inversamente correlacionada com DMO/L1-L4 (r = -0,33; P = 0,05) e S-ALP (r = -0,36; P = 0,04) não tendo relação com o número de fraturas prevalentes (r = 0,01), S-osteocalcina (r = -0,22) ou S-CrossLaps (r = 0,003).

Conclusão: Esses resultados sugerem aumento na remodelação óssea e uma influência negativa da S-Hci elevada na formação óssea e na DMO em crianças e adolescentes com fraturas recorrentes.

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Introduction

Homocysteine is an amino acid which is biosynthesized from methionine by the removal of its terminal C methyl group. Furthermore, homocysteine can be recycled into methionine or converted into cysteine. The homocysteine metabolism is dependent on vitamin B6, B9, B12 status and also on serum folate concentration.^{1,2} High serum homocysteine level (S-Hcy) is associated with alterations in vascular morphology, loss of endothelial anti-thrombotic function, and induction of a procoagulant environment, and has been linked to cardiovascular and neurodegenerative disease, diabetes, thrombosis, and Raynaud's phenomenon.²⁻⁴

Associations between high S-Hcy levels and low bone mineral density (BMD) and increased fracture risk in postmenopausal women have been repeatedly documented.⁵⁻¹³ To date, there are scarce data concerning S-Hcy and bone health in children and adolescents. In our recent pilot study involving 19 children (12 boys and 7 girls; mean age 14.9 ± 3.3 years) with recurrent fractures and low BMD, we observed elevated S-Hcy (Table 1) with high inverse and significant correlations between S-Hcy and BMD (r = -0.66; P = 0.01) and S-Hcy and serum alkaline phosphatase activity (S-ALP) (r = -0.56; P = 0.03), respectively.¹⁴ S-Hcy was not related to number of prevalent fractures (r = 0.11) or S-CrossLaps (r = -0.14).¹⁴

Therefore, we further analysed S-Hcy levels together with indices of bone turnover and several clinical and biochemical parameters in a group of 37 children and adolescents with low BMD and/or prevalent low-energy trauma fractures.

Patients and methods

Patients

We evaluated the charts of children and adolescents who attended our Pediatric Bone Clinic at The Department of Pe-

Table 1 – Patient data from previous pilot study (n = 19),¹⁴ expressed as Z-scores ± SD.

Parameter	Mean	SD	P ^a
S-homocysteine	1.4	1.8	0.001
L1-L4 BMD	-2.1	1.3	0.0001
S-ALP	0.03	1.0	0.91
S-CrossLaps	1.5	1.9	0.001
Height	-0.06	1.0	0.27

BMD, bone mineral density; S-ALP, serum alkaline phosphatase activity.

^aCompared to reference data.

diagnostics in Pardubice in the years 2006-2012. The inclusion criteria were: (i) age between five and 20 years; (ii) at least two prevalent low-energy trauma fractures in personal history; or (iii) low spinal L1-L4 BMD (below -2 SD Z-score); or (iv) a combination of both.

Four children with less than two prevalent fractures and low BMD were included. In these four children, the BMD measurement was performed due to the following reasons: in one boy with no prevalent fracture, the BMD measurement was performed due to presence of bone fragility risk factors (lifelong immobilisation after intracranial hemorrhage); in two girls without fractures, BMD was measured due to estimation of low bone density on the X-ray of the extremities performed by a surgeon after soft tissue injuries; and in one girl due to presence of one low-energy trauma femoral fracture.

The subjects were not eligible for evaluation if they were taking any drugs known to influence bone metabolism (alfacalcidol, calcitriol, dihydroxycholesterol, bisphosphonates, glucocorticosteroids, anabolic steroids, antiepileptics, thiazides, furosemide, thyroid hormones, growth hormone, heparin, warfarin) at the time of the assessment or at any time before. The subjects with diabetes mellitus, celiac disease, inflammatory bowel disease, autoimmune disorders, Cushing syndrome, chronic renal failure, hypercalciuria, urolithiasis, hyper- and hypothyroidism, hyper- and hypoparathyroidism were also excluded.

All subjects were on a standard central European diet, consisting mostly of meat and carbohydrates. None was on a diet poor in vitamin B, nor was receiving doses of vitamin B exceeding the recommended daily allowances. One obese boy was treated for hypertension with enalapril. Others were normotensive. Therefore, we included 37 children and adolescents (22 boys and 15 girls; age range 7-20 years; mean age 13.9 ± 3.5 years).

Procedures

We evaluated the following data in all 37 patients: S-Hcy, markers of bone formation: total S-ALP and serum osteocalcin (S-OC), marker of bone resorption serum Crosslaps, serum calcium (S-Ca), serum phosphate (S-P), serum levels of folate and vitamin B12, body height, weight, body mass index (BMI), and L1-L4 spinal BMD. S-cholesterol was also evaluated. The biochemical parameters were assessed from a single morning blood draw in fasting patients.

Materials and methods

Body height was measured on the day of the relevant blood draw to the nearest ± 0.5 cm on a calibrated stadiometer.

Body weight was measured on the same day on a calibrated scale to ± 0.5 kg.

The BMI was calculated using the equation $BMI = \text{weight (kg)}/\text{height}^2$ (m).

BMD was measured at spine (L1-L4) with dual energy X-ray absorptiometry (DXA) Lunar GE at the day of the blood draw. Measurement precision, expressed as coefficient of variation, was 1.0%.

S-homocysteine level was evaluated by chemiluminescence (Immolute 2500 immunoassay system, Siemens Health-

care Diagnostics, Germany) and expressed in $\mu\text{mol/L}$. The interassay variation was 2.06% in samples with S-Hcy concentration 7.43 $\mu\text{mol/L}$; 1.99% with S-Hcy 10.31 $\mu\text{mol/L}$, and 1.72% with S-Hcy 22.25 $\mu\text{mol/L}$, respectively. S-Ca and S-P were assessed by colorimetric assay and expressed in mmol/L . S-ALP was measured by colorimetric assay and expressed in $\mu\text{kat/L}$.

Serum CrossLaps and S-OC were assessed by means of electrochemiluminescence immunoassay – ECLIA on Elecsys-Cobas analyzers and expressed in ng/L and ng/mL , respectively.

Serum folic acid and serum vitamin B12 (S-B12) were assessed by chemiluminescence on Access analyzer (Beckman Coulter) and expressed in $\mu\text{g/L}$ and ng/L , respectively.

Statistics

To eliminate the influence of age, the obtained results of body height, weight, BMI, S-Hcy, S-ALP, S-CrossLaps, and S-OC were calculated as standard deviation scores (SDS) or Z-scores by the equation $SDS = (\text{actual individual value} - \text{mean value for age and sex})/\text{standard deviation for age and sex}$. The BMD reference data (concerning European paediatric population) were supplied by the manufacturer within the DXA software package. Previously published results served as reference data: Czech anthropometric parameters from a 2001 survey,¹⁵ previously obtained Hcy values of healthy Czech paediatric population,¹⁶ S-ALP values of Czech children,¹⁷ and S-CTX levels of healthy British population.¹⁸ The normal S-OC range was determined in 77 children aged 7-19 years without evidence of bone, hepatic, renal, gastrointestinal or endocrine disorders. For statistical analysis, Sigmaplot 2.0 and Systat programme were used. The statistical analysis was performed by unpaired t-test. The linear regression analysis was performed to compare the relationship among respective parameters. For all results, $P < 0.05$ was required for statistical significance.

Results

The mean number of prevalent low-energy trauma fractures was 3.3 ± 2.3 (SD) per patient. The mean S-homocysteine level was $10.7 \mu\text{mol/L} \pm 2.9$ SD; range 6.7-20 $\mu\text{mol/L}$. There was a significant positive correlation with age ($r = 0.47$, $P < 0.01$) (Fig. 1).

Seven patients (19%) had S-homocysteine level above +2 SD, 30 patients (81%) had S-homocysteine levels in the range of ± 2 SD.

When converted to Z-scores and compared with reference values, the S-Hcy Z-score was significantly higher and L1-L4 BMD Z-score was significantly lower, respectively, in comparison with reference values. S-ALP Z-score did not differ from reference values, while S-CrossLaps were higher, same as S-OC (Table 2).

The mean levels of S-folate and B12 were $7.5 \mu\text{g/L} \pm 4.2$ SD and $413 \text{ ng/L} \pm 143$ SD, which fall within normal reference ranges of 2.3-17 $\mu\text{g/L}$ and 180-914 ng/L , respectively.

S-Ca and S-P levels were within normal reference range (mean \pm SD: 2.33 ± 0.10 mmol/L ; range 2.13-2.54; and 1.45 ± 0.23 mmol/L ; range 0.78-1.99, respectively). Mean S-cholesterol level was 4.21 ± 0.96 mmol/L (range 3.06-5.59 mmol/L), therefore within the normal reference range of 2.7-5.6 mmol/L .

S-homocysteine

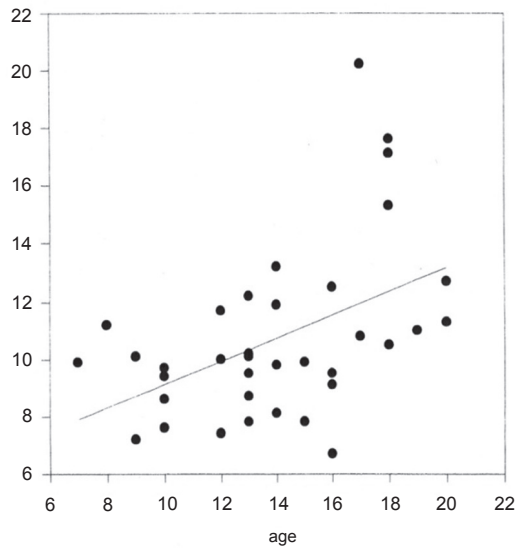


Fig. 1 – S-homocysteine ($\mu\text{mol/L}$) vs. age (years). $R = 0.47$, $P < 0.01$.

S-homocysteine

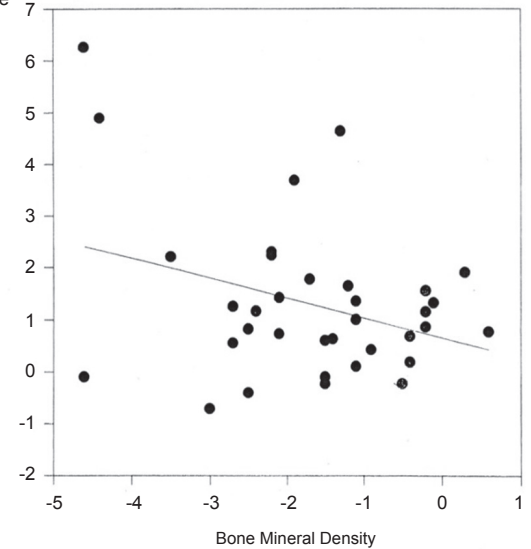


Fig. 2 – S-homocysteine (Z-score) vs. bone mineral density (Z-score). $R = -0.33$, $P = 0.05$.

Table 2 – Patient data from currently presented study (n = 37) expressed as Z-scores \pm SD.

Parameter	Mean	SD	P ^a
S-homocysteine	1.31	1.49	< 0.0001
L1-L4 BMD	-1.74	1.32	< 0.0001
S-ALP	0.02	1.01	0.88
S-CrossLaps	1.21	1.79	0.0001
S-osteocalcin	0.38	0.53	0.001
Height	-0.05	1.31	0.82
Weight	0.18	1.62	0.50
BMI	0.48	1.88	0.12

BMD, bone mineral density; S-ALP, serum alkaline phosphatase activity; BMI, body mass index.

^aCompared to reference data.

Mean body height, body weight, and BMI Z-score did not differ from reference values (Table 2), however in five subjects the BMI Z-score exceeded + 2 SD. The body height, weight and BMI Z-scores positively correlated with L1-L4 BMD Z-score ($r = 0.44$, 0.53 and 0.50 , respectively; $P = 0.02$).

S-Hcy was inversely correlated to L1-L4 BMD (Fig. 2) and S-ALP (Fig. 3), respectively, and not related to number of prevalent fractures ($r = 0.11$), S-osteocalcin ($r = -0.22$) or S-CrossLaps ($r = 0.003$).

There was significant inverse correlation between S-Hcy and vitamin B12 ($r = -0.36$, $P = 0.05$) and no correlation between S-Hcy and serum level of folic acid ($r = 0.05$).

Furthermore, we found positive correlations between S-osteocalcin and S-CTX ($r = 0.36$, $P = 0.05$), S-ALP and S-OC ($r = 0.58$, $P = 0.01$), and S-CTX and S-ALP ($r = 0.39$, $P = 0.05$), respectively.

Discussion

In our previous pilot study of 19 children with prevalent fractures and low BMD, the obtained results suggested negati-

S-homocysteine

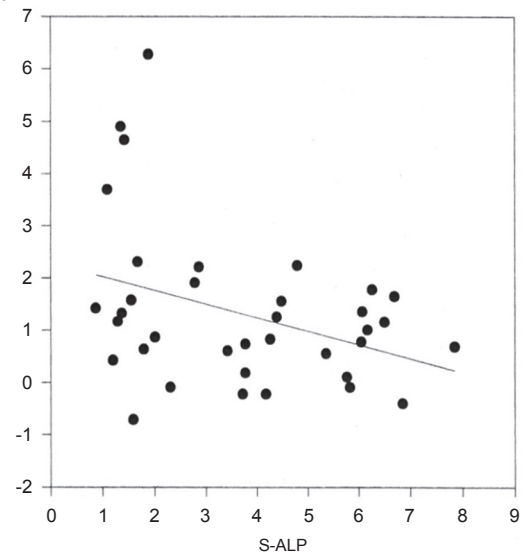


Fig. 3 – S-homocysteine (Z-score) vs. S-alkaline phosphatase activity (Z-score). $R = -0.36$, $P = 0.04$.

ve influence of elevated S-Hcy on bone formation and BMD, while bone resorption was increased in this group of pediatric patients.¹⁴ In an expanded group of 37 children with impaired bone status, we obtained very similar results. S-Hcy was elevated and inversely correlated with S-ALP and BMD. Furthermore, we found indices of increased bone turnover in our patients, as S-OC and S-Crosslaps were elevated in comparison to the reference data, and there were positive mutual correlations among the bone turnover markers. The low BMD in our patients was not influenced by stunted growth, as the body height did not differ from reference value, however our results confirm dependence of BMD on basic anthropometric parameters. As there is a proven as-

sociation between low bone density and fractures in children,^{19,20} hyperhomocysteinemia might represent an important risk factor for children's bone health. Furthermore, in adults, an association between high S-Hcy and low BMD was reported by several authors,^{6,11} same as high S-Hcy in osteoporotic postmenopausal women with no relationship to BMD values.⁷ In addition, further research revealed that high homocysteine levels are associated with an increased risk of hip fracture in elderly population.¹⁰

From the pathophysiological point of view, osteoporosis and higher fracture risk in patients with high S-Hcy are currently explained by accumulation of homocysteine in bone, resulting in a distinct reduction of cancellous bone and a drop in bone strength.⁹ Furthermore, homocysteine stimulates osteoclast activity.²¹ A reduced methylation capacity of bone cells might contribute as well.²²

As we did not find any relationship between the number of prevalent fractures and S-Hcy in our patient group, we may hypothesize that the study was not powered enough to detect significant relationship between these two parameters. Concerning the cardiovascular risk factors, the S-cholesterol and BMI were not significantly elevated in our patients, and only one subject was hypertensive.

Anyway, our results further confirm the negative effect of elevated S-Hcy on bone health, even in pediatric population. The developmental aspects of various disease states of the elderly (i.e. osteoporosis, high blood pressure, hypercholesterolemia, obesity) are currently being given special attention, as these disorders usually originate in childhood. It seems likely that the effect of homocysteine on bone quality is this case as well.

Conclusion

Our results further suggest that elevated S-Hcy could be a risk factor of impaired bone health in children and adolescents.

Conflicts of interest

The authors declare no conflicts of interest.

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