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The relationship between alkaline phosphatase and bone alkaline phosphatase activity and the growth hormone/insulin-like growth factor-1 axis and vitamin D status in children with growth hormone deficiency

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The relationships between bone turnover, the growth hormone/insulin-like growth factor-1 (GH/IGF-1) axis and vitamin D are complex, but still not fully explained. The GH/IGF-1 axis and vitamin D can mutually modulate each other's metabolism and influence the activation of cell proliferation, maturation, and mineralization as well as bone resorption. The aim of this study was to evaluate the reciprocal associations between bone formation markers [alkaline phosphatase (ALP), bone alkaline phosphatase (BALP)], the GH/IGF-1 axis and 25-hydroxyvitamin D [25(OH)D] in children with growth hormone deficiency at baseline and during recombinant human growth hormone (rhGH) therapy. ALP, BALP, 25(OH)D and IGF-1 levels were evaluated in 53 patients included in this prospective three-year study. ALP, BALP and IGF-1 increased during rhGH therapy. Baseline ALP activity correlated positively with baseline height velocity (HV). ALP and BALP activity at 12 months correlated positively with HV in the first year of therapy. We found positive correlations between ALP and IGF-1 at baseline and during the first year of therapy, between BALP activity at 12 months and rhGH dose in the first year of therapy, and between doses of cholecalciferol in the first year of rhGH therapy and early changes in BALP activity during rhGH therapy. Our results indicate that vitamin D supplementation enhances the effect of rhGH on bone formation process, which could improve the effects of rhGH therapy. ALP and BALP activity are useful in the early prediction of the effects of rhGH therapy, but their utility as long-term predictors seemed insufficient.

Key words: bone formation markers, vitamin D, recombinant human growth hormone treatment, children

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Abbreviations: ALP, Total alkaline phosphatase; BALP, bone alkaline phosphatase

INTRODUCTION

Total alkaline phosphatase (ALP) and bone alkaline phosphatase (BALP), measured either as protein concentration or as enzyme activity, are widely used as bone formation markers, particularly in patients with primary and secondary bone diseases and calcium-phosphorus homeostasis disturbances. Both ALP and BALP play an important role as markers of primary bone tumours and bone metastases and are helpful in early detection of bone-related cancers and monitoring of the effects of oncologic therapy (Leeming *et al*., 2006; Rao *et al*., 2017). In comparison to normal age- and sex-matched population total ALP levels are significantly higher in individuals with vitamin D deficiency, rickets, osteomalacia, hyperparathyroidism and Paget's disease. BALP levels are elevated especially during rapid physiological bone growth in children and adolescents, but also in acromegaly, which is associated with growth hormone excess. Hypervitaminosis D, hypophosphatasia, malnutrition, magnesium deficiency and hypothyroidism are associated with a decrease in bone formation and, consequently, in ALP and BALP activity (Garnero *et al*., 1993; Pruessner, 1998; Saraç *et al*., 2007; Sharma *et al*., 2014; Millán *et al*., 2016). Physiologically, ALP and BALP levels depend on age and sex. In children, ALP and BALP levels are higher than in healthy adult population, reaching the highest values in infancy and puberty, and correlate significantly with height velocity (HV). During rapid growth the percentage of BALP might reach even 90% of the total ALP, while in adults it is usually about 50% (Magnusson *et al*., 1999; Léger *et al*., 2007; Saraç *et al*., 2007; Eapen *et al*., 2008; Turan *et al*., 2011; Locatelli *et al*., 2014; Sharma *et al*., 2014). A significant difference in ALP levels between girls and boys can be observed above the age of 10. As a result of earlier onset and completion of puberty, girls reach adult-like total ALP values earlier than boys, usually at the age of 16-18 (Turan *et al*., 2011). ALP and BALP levels in children and adolescents are mainly affected by growth hormone (GH), directly or via insulin-like growth factor-1 (IGF-1), and, during puberty, also by the sex steroids (Tobiume *et al*., 1997; Turan *et al*., 2011; Locatelli *et al*., 2014). In children with growth hormone deficiency (GHD), ALP and BALP levels are decreased and rise significantly after the initiation of recombinant human growth hormone (rhGH) treatment (Juul *et al*., 1994; Ono *et al*., 1996; Tobiume *et al*., 1997; Eapen *et al*., 2008; Korpal-Szczyrska *et al*., 2008; Witkowska-Sędek *et al*., 2014). Vitamin D, which is necessary for normal calcium-phosphorus homeostasis and skeleton mineralization, also influences bone turnover during growth, but its role has not been fully explained (Barnes *et al*., 2006; Paradowska *et al*., 2007; Anderson *et al*., 2013; Koszowska *et al*., 2014; Thiering *et al*., 2015; Larijani *et al*., 2016; Ciresi *et al*., 2017; Schwetz *et al*., 2017). The effects of the GH/IGF-1 axis on bone turnover can be evaluated using the measurement of bone

turnover markers (BTMs), including ALP and BALP (Ono *et al*., 1996; Locatelli *et al*., 2014; Devesa *et al*., 2016). The GH/IGF-1 axis can modulate vitamin D metabolism and, conversely, vitamin D can influence IGF-1 concentrations (Soliman *et al*., 2008; Bogazzi *et al*., 2011; Ameri *et al*., 2013; Ciresi *et al*., 2014; Witkowska-Sędek *et al*., 2016; Ciresi *et al*., 2017; Schwetz *et al*., 2017). Activation of vitamin D receptor (VDR) localised in osteoblasts, osteocytes, chondrocytes, and osteoclasts regulates cell proliferation, maturation, and mineralization as well as bone resorption. The effects of vitamin D on bone turnover are complex, but the results of studies evaluating associations between vitamin D and BTMs are divergent (Barnes *et al*., 2006; Anderson *et al*., 2013; Thiering *et al*., 2015; Larijani *et al*., 2016; Ciresi *et al*., 2017; Schwetz *et al*., 2017).

In our study we evaluated the reciprocal associations between bone formation markers (ALP and BALP), the GH/IGF-1 axis and 25(OH)D in children and adolescents with growth hormone deficiency at baseline and during rhGH therapy, taking pubertal status into consideration.

MATERIALS AND METHODS

Study population and design. The prospective study, which included 53 children and adolescents with GHD aged 4.75–16.58 years, was conducted in the Department of Paediatrics and Endocrinology of the Medical University of Warsaw from 2013 to 2017. Data were analysed in the whole study group and in the two subgroups formed depending on the pubertal status at baseline. The study was approved by the Bioethics Committee at the Medical University of Warsaw, Poland. Informed consent was obtained from all patients and/or their parents. All procedures were performed in accordance with the Declaration of Helsinki. The prepubertal subgroup consisted of 28 children aged 4.75–13.08 years (mean 8.43 ± 2.25 years), and the pubertal subgroup consisted of 25 children aged 11.08-16.58 years (mean 13.61 ± 1.21 years). The observation started when rhGH therapy was initiated and lasted three years in 25 patients, two years in 18 patients and one year in 10 patients. The inclusion criterion was growth hormone release below 10 ng/ml in a test evaluating spontaneous growth hormone secretion at night during sleep and in two stimulation tests (with clonidine, insulin, glucagon or arginine). Maximum growth hormone release (GH max) was defined as the highest level of growth hormone in any of the three tests in a particular patient. Anthropometric measurements were taken at least 6 months before the initiation of rhGH therapy, at baseline and after 6, 12, 24 and 36 months of the treatment. Based on those measurements, HV at baseline, in the first, second and third year of rhGH treatment was calculated. Bone age was evaluated according to the Greulich and Pyle method at baseline and after each full year of rhGH treatment (Greulich *et al*., 1959). Serum ALP and BALP activity and 25(OH)D concentration were measured at baseline and after 6 and 12 months of rhGH treatment. Serum IGF-1 concentrations were measured at baseline and after 6, 12, 24 and 36 months of rhGH treatment. Mean dose of rhGH in the first year of therapy was 0.184 mg/kg/week, in the second year – 0.19 mg/kg/week and in the third year – 0.195 mg/kg/week. Apart from rhGH treatment, all the patients received a mean daily cholecalciferol dose of 984 IU during the first 12 months of rhGH therapy.

Biochemical measurements. The concentrations of GH and IGF-1 were measured by immunoassay using IMMULITE Analyzer (Siemens; Erlangen, Germany). 25(OH)D concentration was measured by immunoassay using ARCHITECT Analyzer (Abbott Diagnostics; Abbott Park, IL). ALP and BALP activity was measured by dry chemistry system using VITROS 5600 Chemistry Analyzer (Ortho-Clinical Diagnostics, USA). BALP activity was expressed as a percentage (%) of the total ALP activity.

Statistical analyses. Statistical analysis was performed using Statistica 13.1. Data normality was checked by Shapiro-Wilk normality test. For comparison of identical parameters between both studied subgroups the Mann-Whitney test was used for non-parametric data and the T-test for parametric data. Comparisons between evaluated values were conducted using the Wilcoxon signed-rank test for non-parametric data and T-test for parametric data. Correlations were evaluated using Spearman correlation analysis and Pearson correlation test, as appropriate. A p-value < 0.05 was considered significant.

RESULTS

The studied subgroups did not differ significantly in terms of GH deficiency defined as GH max and in

Table 1. The characteristics of the whole study group and prepubertal and pubertal subgroups at baseline and during the first three years of rhGH treatment

Evaluated parameters	At baseline	At 6 months	At 12 months	At 24 months	At 36 months
The whole studied group					
Height SDS	–2.53 ± 0.52	–2.19 ± 0.47	–1.96 ± 0.52	–1.63 ± 0.60	–1.27 ± 0.65
Weight SDS for height-age	0.03 ± 0.78	–0.08 ± 0.75	–0.03 ± 0.66	–0.14 ± 0.49	0.00 ± 0.66
BMI SDS for height-age	0.08 ± 1.09	–0.09 ± 0.99	0.01 ± 0.87	–0.10 ± 0.69	0.03 ± 0.82
HV (cm/year)	4.90 ± 1.45		9.07 ± 1.72	7.64 ± 1.51	6.50 ± 1.52
Bone age (years)	8.52 ± 3.31		10.09 ± 3.24	11.20 ± 2.97	11.81 ± 2.93
Prepubertal subgroup					
Height SDS	–2.60 ± 0.54	–2.20 ± 0.45	–1.99 ± 0.51	–1.64 ± 0.60	–1.20 ± 0.60
Weight SDS for height-age	–0.19 ± 0.62	–0.27 ± 0.55	–0.21 ± 0.51	–0.26 ± 0.39	–0.11 ± 0.51
BMI SDS for height-age	–0.25 ± 0.88	–0.38 ± 0.77	–0.24 ± 0.72	–0.26 ± 0.56	–0.11 ± 0.64
HV (cm/year)	4.84 ± 1.06		8.73 ± 1.41	7.49 ± 1.07	6.97 ± 1.19
Bone age (years)	5.96 ± 2.25		7.58 ± 2.34	9.28 ± 2.36	10.34 ± 2.25
Pubertal subgroup					
Height SDS	–2.45 ± 0.49	–2.18 ± 0.49	–1.92 ± 0.53	–1.63 ± 0.61	–1.41 ± 0.76
Weight SDS for height-age	0.27 ± 0.88	0.14 ± 0.89	0.18 ± 0.76	0.02 ± 0.58	0.21 ± 0.91
BMI SDS for height-age	0.45 ± 1.20	0.23 ± 1.13	0.28 ± 0.97	0.12 ± 0.80	0.33 ± 1.10
HV (cm/year)	4.97 ± 1.80		9.45 ± 1.96	7.84 ± 1.99	5.51 ± 1.72
Bone age (years)	11.40 ± 1.32		12.90 ± 1.00	13.86 ± 1.02	14.94 ± 1.18

Data are presented as means ±S.D. SDS – standard deviation score, BMI – body mass index, HV – height velocity

Table 2. The characteristics of the IGF-1 concentrations (ng/ml) at baseline and during the first three years of rhGH therapy in the whole group and in prepubertal and pubertal subgroups

Evaluated parameters	At baseline	At 6 months	At 12 months	At 24 months	At 36 months
The whole studied group	180.2 ± 136.00	371.0 ± 236.85	425.1 ± 239.22	470.1 ± 258.51	474.0 ± 179.99
Prepubertal subgroup	99.5 ± 49.75	227.6 ± 115,51	277.4 ± 150.71	341.4 ± 164.61	449.4 ± 200.67
Pubertal subgroup	270.5 ± 145.59	531.7 ± 235.92	590.4 ± 210.99	648.9 ± 261.96	526.3 ± 120.17

Data are presented as means ±S.D. IGF-1 – insulin-like growth factor-1, rhGH – recombinant human growth hormone

height deficit (height SDS) at baseline. GH max was 7.19 ± 1.87 ng/ml in the whole group, 7.23 ± 1.92 ng/ml in the prepubertal subgroup and 7.15 ± 1.84 ng/ml in the pubertal subgroup. The characteristics of selected anthropometric parameters of the whole study group and prepubertal and pubertal subgroups at baseline and during the first three years of rhGH treatment are shown in Table 1. In the first year of rhGH therapy HV increased significantly in the whole group and in both subgroups ($p < 0.0001$ for the whole group and for the prepubertal and pubertal subgroups) and did not differ significantly between the prepubertal and pubertal subgroups. In the second year of rhGH therapy HV in the whole group and in both subgroups decreased significantly ($p < 0.0001$ for the whole group, $p = 0.0015$ for the prepubertal subgroup, $p = 0.0051$ for the pubertal subgroup). In the third year of rhGH therapy HV was lower than in the second year of therapy – in the prepubertal subgroup, it was still significantly higher than the baseline values ($p < 0.0001$), but in the pubertal subgroup did not differ significantly from the baseline values. The increase in height SDS was significant as early as after the first 6 months of rhGH therapy ($p < 0.0001$) in both studied subgroups. In the next years of rhGH therapy height SDS increased significantly in the whole group and in both subgroups ($p < 0.05$ for the whole group and for the prepubertal and pubertal subgroups). Bone age increased significantly after the initiation of rhGH therapy in the whole group and both subgroups ($p < 0.0001$ for the whole group and for the prepubertal and pubertal subgroup). The differences between bone age after the first and the second year ($p < 0.0001$) and between its values after the second year and the third year of the therapy ($p < 0.0001$) were significant. Baseline IGF-1 concentrations were significantly higher in the pubertal subgroup than in the prepubertal subgroup ($p < 0.0001$). In the first year of rhGH therapy IGF-1 concentrations increased significantly ($p < 0.0001$ for the whole group, for the prepubertal subgroup and for the pubertal subgroup), and the difference was significant as early as after 6 months of therapy in the whole group and in both subgroups ($p < 0.0001$ for the whole group, for the prepubertal subgroup and for the pubertal subgroup). In the second year of observation IGF-1 concentrations increased significantly in comparison to IGF-1 levels after the first year of rhGH therapy in the whole group ($p = 0.0038$) and in the prepubertal subgroup ($p = 0.0094$), but not in the pubertal subgroup. In the third year of rhGH therapy IGF-1 concentrations changed significantly in comparison to IGF-1 levels after the second year of therapy only in the prepubertal subgroup ($p = 0.0028$). The difference was not significant for the pubertal subgroup. The characteristics of the IGF-1 concentrations at baseline and during the first three years of rhGH treatment in the whole group and in the prepubertal and pubertal subgroups are shown in Table 2. Baseline 25(OH)D concentration was 24.6 ± 7.54 ng/ml and increased significantly during the first 6 months of rhGH therapy to 28.8 ± 8.09 ng/ml ($p = 0.0027$) in the whole group. After 12 months of rhGH therapy 25(OH)D concentration was 27.2 ± 8.09 ng/ml (the difference was not significant in comparison to the level after 6 months). At baseline, the concentrations of 25(OH)D did not differ significantly between the two studied subgroups, but in the prepubertal subgroup at 6 months of rhGH therapy 25(OH)D levels were significantly higher than baseline ($p = 0.0025$), and reached a plateau at 12 months of rhGH therapy, whereas changes in 25(OH)D concentrations during the first year of rhGH therapy were not significant in the pubertal subgroup. The characteristics of 25(OH)D levels at baseline and at 6 and 12 months of rhGH therapy in the whole group and in the prepubertal and pubertal subgroups are shown in Table 3. ALP activity increased significantly at 6 months of rhGH therapy from 194.8 ± 55.37 U/L at baseline to 235.3 ± 53.57 U/L ($p < 0.0001$) in the whole group. At 12 months of rhGH therapy ALP activity in the whole group was 228.8 ± 57.17 U/L. This value was still significantly higher than the baseline ALP activity ($p = 0.00072$) and did not differ significantly from ALP activity at 6 months of rhGH therapy. ALP activity at baseline and at 6 and 12 months of rhGH therapy in the whole group is presented in Fig. 1. Similar tendencies were found in the prepubertal and pubertal subgroups. In both studied subgroups peak ALP activity was observed at 6 months of rhGH therapy, and ALP level at 6 months differed significantly from the baseline ALP ($p = 0.00073$ for the prepubertal subgroup, $p = 0.00022$ for the pubertal subgroup). At 12 months of rhGH therapy ALP activity decreased slightly, but was still higher than baseline, and did not differ significantly from ALP activity at 6 months of rhGH therapy. BALP activity increased significantly from 51.5 ± 6.74% at baseline to 55.6 ± 9.64% at 12 months of rhGH therapy ($p < 0.05$) in the whole group. BALP values in the whole group

Table 3. The characteristics of 25(OH)D concentrations (ng/ml) at baseline and during the first year of rhGH therapy in the whole group and in prepubertal and pubertal subgroups

Evaluated parameters	At baseline	At 6 months	At 12 months
The whole studied group	24.6 ± 7.54	28.8 ± 8.09	27.2 ± 8.09
Prepubertal subgroup	24.5 ± 7.44	29.5 ± 6.44	30.2 ± 9.30
Pubertal subgroup	24.7 ± 7.80	27.9 ± 9.81	24.1 ± 5.03

Data are presented as means ±S.D. 25(OH)D – 25-hydroxyvitamin D, rhGH – recombinant human growth hormone

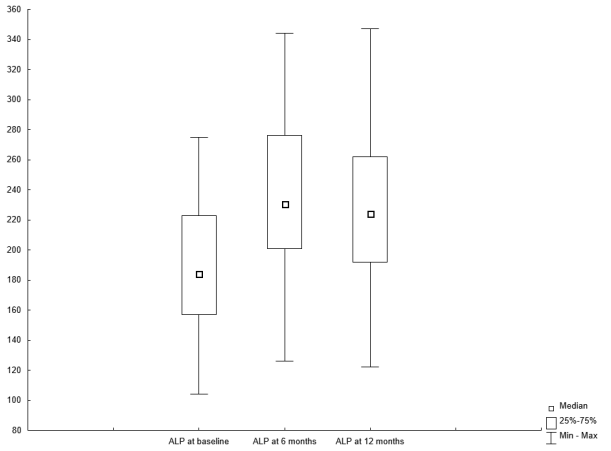

Figure 1. ALP activity (U/L) at baseline and at 6 and 12 months of rhGH therapy in the whole group
ALP – alkaline phosphatase, rhGH – recombinant human growth hormone

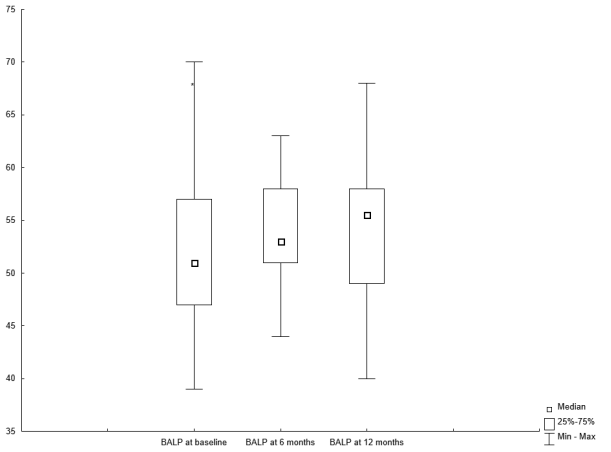

Figure 2. BALP activity (%) at baseline and at 6 and 12 months of rhGH therapy in the whole group
BALP – bone alkaline phosphatase, rhGH – recombinant human growth hormone

are presented in Fig. 2. Similar changes in BALP activity were found in the pubertal subgroup at 12 months of rhGH therapy ($p=0.014$). In the prepubertal subgroup BALP activity did not change significantly during the first year of rhGH therapy. The characteristics of ALP and BALP activity at baseline and at 6 and 12 months of rhGH therapy in the whole group and in prepubertal and pubertal subgroups are shown in Table 4.

Correlations of ALP and BALP activity with height velocity and bone age

Baseline ALP activity correlated significantly with baseline HV ($R=0.49$, $p<0.05$ for the whole group; $R=0.68$, $p<0.05$ for the pubertal subgroup) – this correlation was not found in the prepubertal subgroup. Baseline BALP activity did not correlate with baseline HV in either of the studied subgroups. ALP activity at 12 months of rhGH therapy correlated significantly with HV in the first year of therapy ($R=0.47$, $p<0.05$ for the whole group; $R=0.49$, $p<0.05$ for the pubertal subgroup) – this correlation was not found in the prepubertal subgroup. Changes in ALP activity during the first year of rhGH therapy (ALP at 12 months – ALP at baseline) did not correlate with HV in the first year of treatment. BALP activity at 12 months of rhGH therapy ($R=0.45$, $p<0.05$) and changes in BALP values during the first year of therapy (BALP at 12 months – BALP at baseline; $R=0.43$, $p<0.05$ Fig. 3) correlated significantly with HV in the first year of rhGH treatment in the whole group – we did not find either of those correlations in either of the subgroups. We also found that changes in ALP activity during the first year of rhGH therapy correlated with HV in the second year of rhGH treatment ($R=0.50$, $p<0.05$ for the whole group; $R=0.79$, $p<0.05$ for the pubertal subgroup) – this correlation was not found in the prepubertal subgroup. We did not find any significant correlations between early changes in ALP or BALP levels in the first year of rhGH therapy and HV in the third year of the treatment. In the pubertal subgroup, we also found significant positive correlations between baseline ALP and baseline bone age ($R=0.41$, $p<0.05$) and between ALP activity at 12 months of treatment and bone age at 12 months of treatment ($R=0.45$, $p<0.05$). Such correlations were not found between BALP activity and bone age in the whole group nor in either of the subgroups.

Correlations of ALP and BALP activity with IGF-1 concentration and doses of rhGH

Baseline ALP activity correlated significantly with baseline IGF-1 levels ($R=0.37$, $p<0.05$ for the whole group; $R=0.55$, $p<0.05$ for the pubertal subgroup) – this correlation was not found in the prepubertal subgroup. Baseline BALP values did not correlate with baseline IGF-1 values. We also found that changes in ALP activity during the first 6 months of rhGH therapy (ALP at 6 months – ALP at baseline) correlated significantly with the changes in IGF-1 concentrations in the first year of therapy (IGF-1 at 12 months – IGF-1 at baseline) ($R=0.34$, $p<0.05$ Fig. 4) in the whole group. BALP activity measured after 12 months of rhGH therapy correlated significantly with doses of rhGH in the

Table 4. The characteristics of ALP (U/L) and BALP (%) activity at baseline and during the first year of rhGH therapy in the whole group and in prepubertal and pubertal subgroups

Evaluated parameters	At baseline	At 6 months	At 12 months
The whole studied group (n=53)			
ALP	194.8±55.37	235.3±53.57	228.8±57.17
BALP	51.5±6.74	53.6±7.29	55.6±9.64
Prepubertal subgroup (n=28)			
ALP	183.6±52.82	222.6±51.67	219.1±50.21
BALP	51.1±6.81	54.7±7.32	53.5±6.98
Pubertal subgroup (n=25)			
ALP	207.4±56.51	250.3±53.01	238.9±63.37
BALP	51.9±6.79	52.4±7.25	58.1±11.72

Data are presented as means ±S.D. ALP – alkaline phosphatase, BALP – bone alkaline phosphatase, rhGH – recombinant human growth hormone

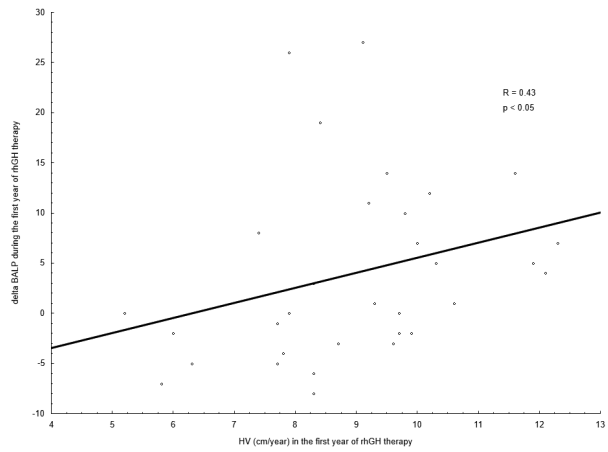

Figure 3. Correlation between changes in BALP activity (%) during the first year of rhGH therapy and height velocity (cm/year) in the first year of therapy
BALP – bone alkaline phosphatase, HV – height velocity, rhGH - recombinant human growth hormone

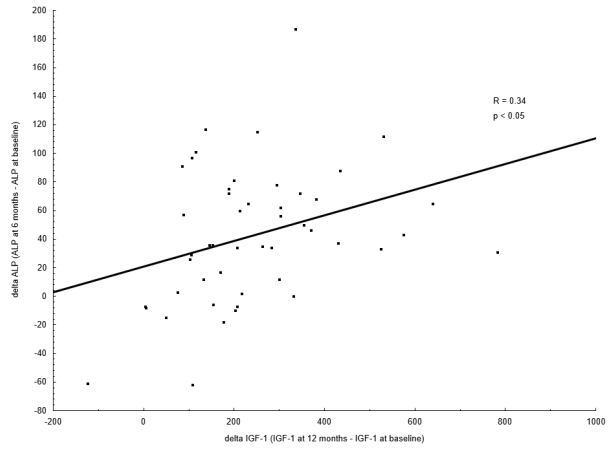

Figure 4. Correlation between changes in ALP activity (U/L) during the first 6 months of rhGH therapy and changes in IGF-1 concentrations (ng/ml) during the first year of therapy
ALP – alkaline phosphatase, IGF-1 – insulin-like growth factor 1, rhGH – recombinant human growth hormone

first year of treatment, especially in the pubertal subgroup ($R = 0.37$, $p < 0.05$ for the whole group; $R = 0.73$, $p < 0.05$ for the pubertal subgroup) – this correlation was not found in the prepubertal subgroup.

Correlations of ALP and BALP activity with 25(OH)D levels

ALP and BALP activity at baseline and at 6 and 12 months of rhGH therapy did not correlate with 25(OH)D concentrations during the first year in the whole group nor in either of the subgroups, but we found a significant positive correlation between doses of cholecalciferol in the first year of rhGH therapy and changes in BALP activity during the first 6 months of therapy (BALP at 6 months – BALP at baseline) in the pubertal subgroup ($R = 0.55$, $p < 0.05$).

DISCUSSION

Normal bone turnover is the result of a balance between two processes: bone formation and bone resorption. The intensity of those processes can be quantified by measurement of BTMs, which are widely used to diagnose bone metabolism (Leeming *et al.*, 2006; Eapen *et al.*, 2008; Orimo, 2010; Larijani *et al.*, 2016). ALP, and especially BALP, are considered to be valuable markers of bone formation. Several factors may influence the balance between the formation and resorption of the bone. The GH/IGF-1 axis is one of the main mechanisms which control bone remodelling processes in the postnatal life. In children and adolescents, GH and

IGF-1 in association with sex steroids are the most important factors which influence growth and development (Eapen *et al.*, 2008; Orimo *et al.*, 2010; Turan *et al.*, 2011; Locatelli *et al.*, 2014; Sharma *et al.*, 2014). GH directly or indirectly through IGF-1 stimulates osteoblastogenesis and chondrogenesis. IGF-1 regulates bone turnover at tissue level via enhancing osteoblast proliferation, stimulation of type I collagen production and BALP activity and via modulation of the osteoblast-osteoclast interactions IGF-1 directly regulates bone growth and density. Epidemiological studies have suggested that serum IGF-1 level correlates with fracture risk, bone density and bone mass (Rosen, 2004; Niu *et al.*, 2005; He *et al.*, 2006; Léger *et al.*, 2007; Locatelli *et al.*, 2014; Devesa *et al.*, 2016). Several authors have evaluated the association between the GH/IGF-1 axis and vitamin D status (Gómez *et al.*, 2004; Bogazzi *et al.*, 2011; Ameri *et al.*, 2013; Witkowska-Sędek *et al.*, 2016; Ciresi *et al.*, 2017; Trummer *et al.*, 2017). The study by Ameri *et al.* (2013) showed that vitamin D could increase circulating IGF-1 level in adults and better vitamin D status may facilitate the achievement of normal IGF-1 values in GHD. A similar positive correlation between 25(OH)D concentrations and serum IGF-1 concentrations was found in healthy subjects (Gómez *et al.*, 2004; Bogazzi *et al.*, 2011). It is known that an adequate vitamin D level supports normal bone turnover but the results of the studies evaluating correlations between vitamin D and BTMs are divergent (Barnes *et al.*, 2006; Lee *et al.*, 2013; Larijani *et al.*, 2016; Ciresi *et al.*, 2017; Schwetz *et al.*, 2017; Trummer *et al.*, 2017; Witkowska-Sędek *et al.*, 2017). Previously published studies by Schwetz *et al.* (2017), Wamberg *et al.* (2013) and Seamans *et al.* (2010), which enrolled, respectively, adults with arterial hypertension, obese subjects and healthy adults did not find any significant associations between vitamin D supplementation and BTMs. On the other hand, a study by Kuchuk *et al.* (2009) suggested that in older adults vitamin D could influence the BTMs only at certain serum levels of 25(OH)D. The authors found that serum 25(OH)D correlated significantly with urine deoxypyridinoline/creatinine (DPD/Cr) at serum 25(OH)D concentrations below 40 nmol/L. Data concerning healthy children and adolescents and children with short stature treated with rhGH are insufficient. Our study attempted to investigate this issue in relation to children with GHD before and during rhGH therapy. In children and adolescents with GHD, bone turnover and bone mineral density are decreased and rise significantly after the initiation of rhGH therapy. The administration of rhGH promotes bone remodelling and longitudinal bone growth and increases the bone mass. Growth hormone therapy has a significant effect on bone turnover markers in GHD children and adolescents, including ALP and BALP (Ono *et al.*, 1996; Tobiume *et al.*, 1997; Léger *et al.*, 2007; Korpal-Szczyrska *et al.*, 2008; Locatelli *et al.*, 2014; Witkowska-Sędek *et al.*, 2014). In the present study, we analysed the effects of rhGH therapy and vitamin D status on serum

ALP and BALP activity at baseline and in the first year of rhGH therapy. The enrolled patients were divided into two subgroups, according to the pubertal status at baseline, which did not differ significantly in maximum growth hormone release in diagnostic tests performed before the initiation of rhGH therapy or in height SDS at baseline. In our analysis we found a significant increase in ALP and BALP activity after the beginning of rhGH therapy. Serum ALP activity reached a peak at 6 months of therapy with a plateau within the next six months of rhGH treatment in both studied subgroups. Maximum serum BALP values were achieved significantly later than ALP and reached a peak at 12 months of therapy, but only in the pubertal subgroup. BALP values did not change significantly in the prepubertal subgroup, which could suggest the influence of other factors on bone turnover activity in the pubertal subgroup, possibly such as sex steroids. We also found significant correlations between changes in ALP and IGF-1 during the first year of rhGH therapy and between BALP activity after 12 months of rhGH therapy and rhGH doses during the first year of the treatment. Our results confirm a significant association between the GH/IGF-1 axis and bone turnover. We also confirmed the important role of bone formation markers in the prediction of rhGH therapy effects. We found both a significant correlation between baseline ALP activity and baseline HV and between ALP and BALP activity at 12 months of rhGH therapy and HV in the first year of the treatment. Those correlations further recognise the role of bone formation markers in early prediction of short-term effects of rhGH therapy. The association between changes in ALP activity during the first year of rhGH therapy and HV in the second year of rhGH therapy could suggest the role of ALP as a surrogate marker of long-term effects of rhGH therapy, but we were not able to confirm the utility of ALP and BALP activity in the prediction of rhGH effects in the third year of rhGH treatment. Early changes in the levels of bone formation markers have an important role in predicting catch-up growth after the therapy initiation but in long-term rhGH treatment, after a new balance between bone formation and bone resorption is achieved other factors, such as sex steroids or other mediators, become more significant in determining the height velocity. We also tested if vitamin D status correlates with ALP and BALP activity at baseline and during the first year of rhGH therapy. In our study we did not find any significant correlations between 25(OH)D and serum ALP or BALP activity either at baseline or at 6 or 12 months after the initiation of rhGH therapy. Several authors analysed the correlations between vitamin D and BTMs but their results are inconsistent (Barnes *et al*., 2006; Seamans *et al*., 2010; Wamberg *et al*., 2013; Larijani *et al*., 2016; Schwetz *et al*., 2017). Schwetz *et al*. (2017) evaluated the effects of cholecalciferol supplementation on the levels of BALP, osteocalcin (OC), C-terminal telopeptide (CTX) and procollagen type 1 N-terminal propeptide (P1NP) and did not find any significant changes in BTMs values after eight weeks of vitamin D supplementation. Wamberg and coworkers (2013) in obese subjects and Seamans and coworkers (2010) in a group of healthy young and elderly adults also did not find any significant effects of vitamin D supplementation on bone turnover markers. According to EFSA recommendations (2016) "more research is needed to establish the relationship between responses of bone markers to changes in vitamin D status". Our study addressed this knowledge gap on vitamin D and BTMs relationship in GH-deficient children. Although we did not find any correlations between ALP or BALP activity and 25(OH)D concentrations at baseline or during the rhGH therapy, we found a significant positive correlation between the mean doses of cholecalciferol administered in the first year of rhGH therapy and the early changes in BALP activity during the first 6 months of therapy. Those results contribute considerably to the research on the role of vitamin D in increasing bone formation and initiation of catch-up growth after the beginning of rhGH therapy and potentially suggest novel therapeutic interventions. In our opinion, supplementation of vitamin D is necessary in all children and adolescents, especially due to the widespread vitamin D deficiency (Braegger *et al*., 2013; Ciresi *et al*., 2014; Andersson *et al*., 2015). In children with GHD such supplementation is especially important and could enhance the effects of rhGH therapy. Further clinical investigation is warranted to better evaluate the influence of vitamin D on the bone turnover and the role of vitamin D in patient management during rhGH therapy. In childhood-onset GHD the initiation of rhGH therapy is associated with a significant increase in bone formation markers, which correlates with catch-up growth. ALP and BALP are useful in early prediction of rhGH therapy effects, but its utility in long-term prediction is not sufficiently proven. Vitamin D supplementation seems to enhance the effect of rhGH on the bone formation after the initiation of rhGH therapy and contributes to a significant increase in bone formation during catch-up growth.

The authors declare no conflicts of interest in relation to this manuscript.

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