New markers of bone and collagen turnover in children and adults with growth hormone deficiency

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Summary: Serum bone Gla protein (BGP), a marker of osteoblastic function, serum carboxyterminal cross-linked telopeptide of type I collagen (ICTP), a marker of bone resorption, and serum aminoterminal propeptide of type III procollagen (PIIINP) levels, an index of collagen synthesis, were determined in seven children and eight adults with congenital growth hormone deficiency (GHD).

In children with GHD, serum BGP (mean ± s.e.: 12.9 ± 0.7 ng/ml), ICTP (8.3 ± 1.3 ng/ml) and PIIINP (3.5 ± 0.5 ng/ml) levels were significantly lower (P < 0.0001) than those recorded in normal children (BGP 18.9 ± 0.8 ng/ml, ICTP 14.4 ± 0.5 ng/ml and PIIINP 6.7 ± 0.7 ng/ml). Total alkaline phosphatase (184.7 ± 13.4 IU/l) and bone alkaline phosphatase (77.8 ± 4.1 IU/l) levels were also significantly lower (P < 0.0001) than in controls (338.1 ± 14.9 IU/l and 181.0 ± 7.8 IU/l, respectively). Serum BGP, ICTP and PIIINP levels were not significantly correlated with height velocity values.

In adults with GHD, mean BGP levels (3.8 ± 0.3 ng/ml) were significantly lower (P < 0.0001) than those recorded in normals (5.4 ± 0.1 ng/ml). On the contrary, serum ICTP levels were similar to those found in controls (patients: 4.7 ± 0.8 ng/ml vs normals: 4.1 ± 0.3 ng/ml), suggesting the presence of a normal resorption activity associated with a reduced osteoblastic function. This finding was also confirmed by the presence of reduced bone alkaline phosphatase levels (GHD: 44.9 ± 6.9 IU/l vs controls: 58.3 ± 2.0 IU/l; P < 0.02), while the less specific total alkaline phosphatase levels (119.5 ± 14.8 IU/l) were similar to those recorded in normal subjects (122.3 ± 4.0 IU/l). Serum PIIINP levels (3.7 ± 0.6 ng/ml) were similar to those recorded in normals (3.2 ± 0.2 ng/ml), suggesting that in adulthood the collagen turnover is not negatively influenced by the chronic GHD. No significant correlations were found between BGP/ICTP/PIIINP and IGF-I levels.

In conclusion, our data show that in children with GHD the lack of GH insulin-like growth factor-I (IGF-I) effects on bone and collagen turnover is associated with a significant reduction of bone turnover (low bone formation plus low bone resorption) and collagen synthesis. On the contrary, adult GHD seems to exert less relevant effects on bone and collagen turnover, probably due to the fact that in adult life further hormones or local factors might partially counteract the negative consequences of chronic GH-IGF-I deficiency.

Introduction

It is well known that growth hormone (GH) plays a relevant role in bone and collagen turnover, its effects being mediated at least in part by insulin-like growth factor I (IGF-I). The negative effects of GH deficiency (GHD) on bone (that is, osteopenia, low bone remodelling) and collagen turnover (that is, reduced total skin collagen content, reduced skin thickness) have been partially evaluated in children with GHD, while the importance of the metabolic effects of GH has been appreciated only recently in adults with GHD.

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Up to now, the biochemical evaluation of both bone formation/resorption and collagen synthesis has been prevented by the lack of specific and reliable markers, since the use of classical parameters (that is, total alkaline phosphatase, urinary hydroxyproline, urinary calcium/creatinine ratio) is frequently unable to clearly detect the finest modifications occurring in bone and collagen turnover. In the last few years, new specific and reliable biochemical markers of bone formation/resorption and of collagen synthesis have been discovered and usefully employed for monitoring the course of several metabolic and endocrine disorders.

In this study we determined serum bone Gla protein (BGP), a marker of osteoblastic function, serum carboxyterminal cross-linked telopeptide of type I collagen (ICTP), a marker of bone resorption, and serum aminoterminal propeptide of type

Materials and methods

Seven children (6M/1F, mean age ± s.e.: 12.1 ± 0.9 years) and eight adults (8M, mean age: 29.6 ± 1.2 years) with idiopathic GHD were admitted to the study after giving informed consent.

Children with GHD (criteria for admission to the study)

The diagnosis of GHD was based on the following criteria: (1) yearly growth velocity less than 3rd centile for age; (2) delay of bone age greater than 2 years for their chronological age; and (3) failure of plasma GH to respond (peak < 5 ng/ml) to insulin hypoglycaemia and L-dopa plus propranolol tests.

Mean height, measured with a Harpenden stadiometer, was 134.0 ± 3.9 cm (range: 121.0–147.1 cm); mean body weight was 35.0 ± 2.0 kg (range: 28.3–40.4 kg). Body mass index (BMI, kg/m²) was 19.6 ± 0.6 (range: 17.0–20.5). Mean height velocity was 2.7 ± 0.4 cm/year (range: 0.5–3.5 cm/year); bone age (BA), estimated by a single observer using the method of Tanner–Whitehouse (TW2), was 10.6 ± 0.9 year (range: 8–14 year).

Adults with GHD

The diagnosis of GHD was confirmed in each subject by means of two GH stimulation tests. All patients had earlier received GH substitution therapy during childhood; the mean duration of earlier GH treatment was 50.2 ± 3.9 months; previous GH therapy had been interrupted at least 7 years (mean: 11.4 ± 1.1 years) before the entry into the present study. Five patients had in addition multiple hormone deficiencies and therefore received standard substitution therapy of hydrocortisone, thyroxine and/or testosterone. The mean actual height was 146.1 ± 3.6 cm (range: 131.5–162 cm); mean weight was 45.9 ± 3.7 kg (range: 37–64 kg); mean BMI value was 21.6 ± 1.5 (range: 16.7–27.5).

Laboratory assays

Data obtained were compared with those recorded in two age- and sex-matched control groups (20 normal children and 28 normal adults).

Serum BGP, ICTP and PIINP levels and plasma IGF-I levels were determined using commercial RIA kits (CIS Diagnostics, Italy; Orion Diagnostica, Finland; Färmos, Finland; and Nichols Institute, USA; respectively). The intra-and inter-assay coefficients of variation of BGP, ICTP and PIINP RIA methods were lower than 6%; the sensitivity was 0.5 ng/ml (for BGP), 0.5 ng/ml (for ICTP) and 0.2 ng/ml (for PIINP).

Total alkaline phosphatase activity was measured spectrophotometrically using p-nitrophenylphosphate as substrate. Serum bone isoenzyme (lectin-precipitated) alkaline phosphatase activity was determined by the method described by Rosalky and Foo.19

Figure 1 Serum BGP (a), ICTP (b) and PIINP (c) levels in seven children with GH deficiency and in controls (age- and sex-matched control group, 20).

III procollagen (PIINP) levels, an index of collagen synthesis, in children and in adults with congenital GHD.
Serum calcium and phosphate were analysed according to standard laboratory methods.

Statistical analysis

Student's t paired and unpaired t-tests were calculated as appropriate; P values less than 0.05 were considered significant. All data are expressed as mean ± standard error (s.e.).

Results

In children with GHD, serum BGP, ICTP and PIIINP levels (BGP: 12.9 ± 0.7 ng/ml; ICTP: 8.3 ± 1.3 ng/ml; PIIINP: 3.5 ± 0.5 ng/ml) were significantly lower (P < 0.001) than those recorded in normal children (BGP: 18.9 ± 0.8 ng/ml; ICTP: 14.4 ± 0.5 ng/ml; PIIINP: 6.7 ± 0.7 ng/ml), as shown in Figure 1 (panels a–c).

Total alkaline phosphatase and bone alkaline phosphatase levels were also significantly lower (P < 0.0001) than in controls (total alkaline phosphatase: 184.7 ± 13.4 vs 338.1 ± 14.9 IU/l; bone alkaline phosphatase: 77.8 ± 4.1 vs 181.0 ± 7.8 IU/l), as shown in Figure 2 (panels a and b).

In children with GHD, serum BGP, ICTP and PIIINP levels were not significantly correlated with the values of height velocity (r = 0.29, r = 0.20 and r = 0.23, respectively).

In adults with GHD, mean BGP levels (3.8 ± 0.3 ng/ml) were significantly lower (P < 0.0001) than those recorded in normals (5.4 ± 0.1 ng/ml). On

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**Figure 2** Serum total alkaline phosphatase (T-AP; a) and bone alkaline phosphatase (B-AP; b) levels in seven children with GH deficiency and in controls (age- and sex-matched control group, 20).

**Figure 3** Serum BGP (a), ICTP (b) and PIIINP (c) levels in eight adults with GH deficiency and in controls (age- and sex-matched control group, 28).
the contrary, serum ICTP levels were similar to those found in controls (patients: 4.7 ± 0.8 ng/ml vs normals: 4.1 ± 0.3 ng/ml), suggesting the presence of a normal resorption activity associated with a reduced osteoblastic function (Figure 3, panels a and b). Serum PIIINP levels (3.7 ± 0.6 ng/ml) were similar to those recorded in normals (3.2 ± 0.2 ng/ml), suggesting that in adulthood the collagen turnover is not negatively influenced by the chronic GHD (Figure 3c).

Total alkaline phosphatase (119.5 ± 14.8 IU/l) levels were similar to those found in controls (122.3 ± 4.0 IU/l); on the contrary, bone alkaline phosphatase levels (44.9 ± 6.9 IU/l) were significantly lower (P < 0.02) than those recorded in normal subjects (58.3 ± 2.0 IU/l), as shown in Figure 4 (panels a and b). In adults with GHD, no significant correlations were found between BGP/ICTP/PIIINP and IGF-I levels (r = 0.06, r = 0.04 and r = 0.39, respectively). Mean serum calcium and phosphate levels were similar to those recorded in controls.

Discussion

Our data show that in children with GHD the effects of the lack of GH/IGF-I on bone turnover are associated with a significant reduction of BGP, total and bone alkaline phosphatase, and ICTP levels. These findings suggest the presence of a reduced bone turnover (low bone formation associated with low bone resorption) and support previous observations, which showed a relative osteopenia in children with untreated GH deficiency, both isolated and as a component of panhypopituitarism. Furthermore, GH deficiency seems to be able to cause negative effects on collagen turnover, as documented by the significant reduction of serum PIIINP levels. The presence of a low collagen synthesis might contribute to explain the skin thickness (due to a reduced skin collagen content) and the characteristic crow’s foot sign of facial wrinkling of patients with hypopituitarism.

In adults with GHD, the chronic absence of GH seems to exert less relevant effects on bone and collagen turnover, as demonstrated by the presence of a normal bone resorption activity and of a normal collagen synthesis, in the presence of a reduced bone formation.

Our finding of reduced BGP levels disagrees with those previously reported by Johansen et al., who had found normal BGP levels in adults with GHD. However, in our study the presence of a reduced osteoblastic activity was also confirmed by the finding of a significant reduction of bone alkaline phosphatase levels. The discrepancy between our data and those recorded by Johansen et al. might be probably due to their shorter withdrawal of GH treatment before the patients’ entry into the study, which might have positively influenced their osteoblastic activity. In fact, it is well known that GH treatment is able to exert prolonged effects on bone formation, which might be a long-lasting event.

In our adults with GHD, the negative equilibrium between bone formation and resorption might contribute to explain the finding that adults with GHD have a low bone mass, in particular at the forearm site. This aspect might become relevant in the view of future therapeutic trials with recombinant GH in adults with GH deficiency. Although the available data can still be considered preliminary, it seems that GH treatment might increase bone turnover, stimulating both bone formation and bone resorption (our unpublished data). However, at the moment the final meaning of this ‘substitutive’ treatment on bone mass cannot be definitely predicted.

In adulthood, the effects of chronic GH deficiency on collagen turnover appear to be less relevant than in childhood. In this respect, the finding of normal PIIINP levels might suggest that...
in adult life further hormones and/or local factors partially counteract the negative consequences of chronic GH-IGF-I deficiency on collagen synthesis.

In conclusion, our study shows that GH deficiency exerts relevant negative effects on bone and collagen turnover in childhood, while in adults it seems to cause minor consequences. However, although the metabolic effects of GH on bone and collagen turnover appear to be limited, at present it cannot be ruled out that adults with GH deficiency might have in any case a benefit from recombinant GH treatment.

References