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Effects of 24 Months of Growth Hormone (GH) Treatment on Serum Carboxylated and Undercarboxylated Osteocalcin Levels in GH-Deficient Adults

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Abstract. We studied the effect of growth hormone (GH) replacement on bone mineral density (BMD) and some parameters of bone metabolism, including undercarboxylated osteocalcin (ucOC), an independent predictive marker of fracture risk, which has not been previously determined or compared during GH treatment. Measurements were performed at baseline and after 6, 12, 18 and 24 months of the initiation of the GH therapy in 21 adult patients with GH deficiency. Significant increases were observed in BMD after 1 year at the lumbar spine and after 1.5 years at the femoral neck. Serum total OC and carboxylated (c) OC increased and reached the maximum at 6 months, but the values remained over the baseline at both 12 and 18 months. The ucOC:total OC ratio changed contrarily: it decreased at 6 months, then increased again and reached the baseline level during the next 18 months. Serum calcium (Ca), phosphate (P) and total alkaline phosphatase (ALP) levels increased after 6 months, thereafter the Ca and P values decreased, while the total ALP remained elevated until 12 months. Serum parathormone decreased at 12 months and increased again thereafter. GH replacement therapy is associated with improvement of ucOC, a marker of fracture risk, which in addition to the increase of BMD, might contribute to the beneficial effect of GH replacement therapy on bone metabolism.

Key words: Growth hormone replacement — Undercarboxylated osteocalcin — Bone mass

It has previously been shown that adult patients with severe growth hormone deficiency (GHD) have reduced bone mineral density (BMD) and most of them develop osteopenia, or even osteoporosis [1–4]. BMD correlates with the risk of fractures [5]. GH replacement therapy may decrease this risk [6–8], increase BMD after 12 months of treatment [9–11], and also increase the rate of bone turnover in adults [12–15]. Both the biochemical markers of bone resorption (urinary hydroxyproline, pyridinoline, deoxypiridinoline, serum type I carboxyterminal cross-linked telopeptide [CTX-I] and bone formation (total alkaline phosphatase [ALP], total osteocalcin [OC], carboxyterminal propeptides of type I collagen) have been shown to increase during GH replacement [16–19]. GH has a biphasic effect on bone remodelling: the initial increase of the resorption is followed by the increase of formation [20–22]. In addition to BMD, bone quality also seems to be dependent on other factors. One of the markers of bone formation, OC has a vitamin K-dependent gamma-carboxylation [23]. The importance of carboxylation of OC in the bone is not exactly known [24], but it may be related to bone quality [25, 26]. High serum concentration of undercarboxylated OC (ucOC) has been reportedly associated with increased risk of hip fracture and low BMD [27–29]. Thus, ucOC appears to be an independent predictor of hip fracture [23]. It has been concluded that combined measurement of the serum ucOC and BMD offers a more exact prediction of the fracture risk [30]. The risk of fracture is associated with low BMD, high serum ucOC concentration, i.e., with low carboxylated OC (cOC)/total OC ratio [24].

To explore our hypothesis that GH replacement may affect the carboxylation of OC, we studied the effects of 24 months of GH treatment in hypopituitary adults with GHD on BMD, serum total OC, cOC and ucOC levels.

Experimental Subjects

Twenty-one consecutive adult GHD patients (11 female, 10 male; ages 22–67, median 43 years) were analyzed in the present open, prospective study. Only one of the 11 female patients was postmenopausal and received sex hormone substitution. Severe GHD with one or more additional pituitary hormone deficits was determined by a peak serum GH response to insulin tolerance test (0.1–0.15 IU/kg insulin, blood glucose < 2.2 mmol/l) of less

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than 3 μg/L, and in cases of isolated GHD, the arginine test (30 g/30 min iv) was performed as well. Multiple pituitary hormone deficiencies ($n = 18$) were adequately substituted for at least 4 months before starting the study (thyroxine 11, cortisone acetate 6, sexual steroids 18, desmopressin 7 patients). Eighteen patients had adult-onset (AO) and 3 had childhood-onset (CO) GHD. The GH replacement therapy was initiated after transsphenoidal surgery of pituitary adenoma (clinically nonfunctioning 11, prolactinoma 2 patients), or craniopharyngeoma (7 patients), and in one patient the GHD was idiopathic. The diagnosis of GHD was confirmed 1–16 years (mean 6.5) prior to the start of the GH substitution. GH (Genotropin, Pharmacia, Corp. Stockholm, Sweden in 9 patients; Humatrope, Lilly GmbH, Bad Hamburg, Germany in 10 patients; Norditropin, Novo Nordisk A/S Bagsvaerd, Denmark in 2 patients) was administered as daily subcutaneous self-injections. The average dose of GH was 1.2 IU/day (0.4 mg/day), maintaining the insulin-like growth factor-I (IGF-I) levels between the median and upper end of theage and gender-related reference range during therapy.

Materials and Methods

The parameters were measured at baseline, as well as 6, 12, 18 and 24 months after initiation of GH replacement therapy. BMD was measured by dual-energy X-ray absorptiometry at the lumbar spine (L2-L4), left femoral neck, and the distal third of the nondominant radius using a Hologic QDR 4500C instrument (Hologic, Waltham, MA, USA). For analysis, software version 9.03D was used. Lumbar spine and radial BMD Z-scores and T-scores were calculated according to the Hologic-supplied reference curves. NHANES III normative data were used as a reference database for femoral bone density measurements. Quality control was maintained by daily scanning of an anthropometric spine phantom. The coefficient of variation of BMD measurements on the spine phantom over a period of 4 years in our laboratory was 0.35%.

Serum parameters were determined by specific immunoassay kits for IGF-I (Nichols, RIA; normal range in females 84–398, in males 104–450 μg/L; sensitivity 20 μg/l; intraassay coefficient of variation [CV] 2.9; interassay CV 9.8%), for total OC (Roche Elecsys System; electrochemiluminescence immunoassay [ECLIA] normal range in premenopause 12–41, in postmenopause 20–48, in males 11–46 μg/L; sensitivity 0.5 μg/L; intraassay CV 4.0, interassay CV 6.5%), for CTX-I (Roche Elecsys System; ECLIA, normal range in premenopause 0.16–0.44, in postmenopause: 0.33–0.78, in males 0.16–0.44, sensitivity 0.01 μg/L, intraassay CV 1.8, interassay CV 4.3%), for parathormone (Bio-Rad, IRMA; normal range 1.0–6.5 pmol/L; sensitivity 0.4 pmol/L; intraassay CV 5.6, interassay CV 9.3%), and by usual commercial kits for serum and urine calcium (Ca), serum and urine phosphate (P), total alkaline phosphatase (ALP). The ucOC was determined after hydroxyapatite extraction of total OC (Sigma-C5267; RIA, calcium-phosphatite trifibric, intraassay CV 2.1%, interassay CV 4.3%) [31]. The cOC was calculated as the difference of total OC and ucOC. Blood samplings were performed in the morning between 0800 and 0900 after an overnight fasting, and the first samples were drawn in the same season of the year. The 24-hour urinary P and Ca excretions were determined on normal diet. Plasma samples were stored at −20°C for less than 3 months until used for the assay.

Serum IGF-I concentration was expressed as the means of IGF-I standard deviation (SD) scores, which corresponded to the number of SD differences from the age- and sex-related normal mean values. IGF-I SD score was calculated as Z-score in relation to age-specific values [32]:

$$\frac{\text{patient value} - \text{mean of control group}}{\text{standard deviation of control group}}$$

The reference population included randomly selected 2500 healthy Swedes. Treatment effects were analyzed using ANOVA with repeated measures, and in cases of significance, Tukey test was applied. P values less than 0.05 were considered significant. Correlations were calculated according to Pearson. Results were expressed as mean ± S.E.

Results

Serum total OC and cOC showed an increase at 6 months, followed by a moderate decrease, but the values remained elevated above baseline until 18 months (Fig. 1). On the other hand, the cOC:ucOC ratio and cOC:total OC ratio showed a significant increase, while the ucOC:total OC ratio showed a significant decrease after 6 months of GH replacement therapy (Table 1). The measured resorption marker CTX-I levels did not change significantly during the study. Serum Ca and P values rose at 6 months, serum total ALP at 6 and 12 months, and a transient decrease of PTH value was observed at 12 months (Table 1).

During GH therapy the serum IGF-I SD score increased significantly from abnormally low pretreatment values to the normal target range (Table 1). At the start of the therapy 9 patients had osteoporosis, 9 had osteopenia, and 3 had normal BMD - based on WHO criteria (T score < -2.5, between -2.5 and -1.0 or > -1.0, the lowest T-score of the measured regions is considered). At the end of the study only 3 subjects were osteoporotic, 12 osteopenic and the T score values of 6 patients were within the normal range.

The GH replacement therapy increased BMD at the lumbar and femoral regions (Fig. 2) and Z-score at the lumbar spine was (12 months -0.8 ± 0.27, $P < 0.001$; 18 months; -0.51 ± 0.25, $P < 0.05$; 24 months 0.44 ± 0.26 vs -1.21 ± 0.29, $P < 0.001$), and at the femoral neck (18 months 0.23 ± 0.22, $P < 0.01$; 24 months 0.30 ± 0.19 vs -0.19 ± 0.26, $P < 0.01$). The T-score at the lumbar spine was (12 months -1.04 ± 0.24, 18 months -0.98 ± 0.23, 24 months -0.81 ± 0.26 vs -1.56 ± 0.26, $P < 0.01$), and at the femoral neck increased (18 months -0.30 ± 0.22, 24 months -0.20 ± 0.19 vs -0.73 ± 0.24, $P < 0.01$). The radius values remained unchanged (6 months -1.45 ± 0.33, 12 months -1.35 ± 0.31, 18 months -1.18 ± 0.30, 24 months -1.22 ± 0.31 vs -1.43 ± 0.32).

Discussion

The uniform conclusion of more than one decennial experience of human GH therapy in adults with GHD is that untreated adulthood-onset GHD is associated with

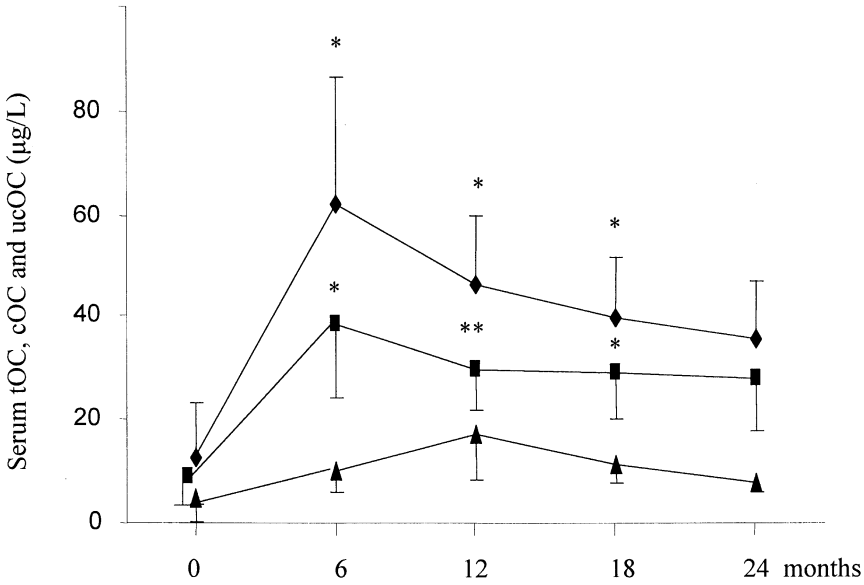

Fig. 1. Serum total (◆), carboxylated (■) and undercarboxylated (▲) osteocalcin levels at baseline and during growth hormone replacement therapy (means ± SEM), $*P < 0.05$, $**P < 0.01$ vs baseline.

Table 1. Serum carboxylated- (cOC) and undercarboxylated osteocalcin (ucOC) ratio, cOC:tOC ratio, ucOC:tOC ratio, insuline-like growth factor-I standard deviation score (IGF-I SD score), calcium (Ca), phosphate (P), C-terminal cross-linking telopeptide of type I collagen (CTX-I), parathyroid hormone (PTH), alkaline phosphatase (ALP), urinary Ca and P values, prior to and during GH therapy (mean ± SE)

	Baseline	6 months	12 months	18 months	24 months
cOC:ucOC ratio	3.6 ± 0.6	7.1 ± 1.4**	4.4 ± 0.9	5.2 ± 1.2	4.9 ± 1.4
cOC:tOC ratio	0.7 ± 0.0	0.8 ± 0.0**	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
ucOC:tOC ratio	0.3 ± 0.0	0.2 ± 0.0**	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
IGF-I SD score	−3.4 ± 0.6	0.3 ± 0.3***	−0.1 ± 0.5***	0.9 ± 0.3***	0.5 ± 0.3***
Serum Ca (mmol/L)	2.3 ± 0.0	2.4 ± 0.0*	2.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.0
Serum P (mmol/L)	1.2 ± 0.0	1.3 ± 0.0*	1.3 ± 0.1	1.2 ± 0.0	1.2 ± 0.0
CTX-I (µg/L)	0.6 ± 0.2	0.7 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
PTH (pmol/L)	3.5 ± 0.4	3.3 ± 0.3	2.8 ± 0.2*	3.5 ± 0.2	3.8 ± 0.3
ALP (IU/L)	185 ± 22	213 ± 25*	218 ± 26*	190 ± 17	163 ± 14
Urine Ca (mmol/day)	4.7 ± 1.0	6.4 ± 1.2	4.1 ± 0.9	4.4 ± 1.0	5.6 ± 1.2
Urine P (mmol/day)	33.0 ± 4.6	39.0 ± 5.0	26.1 ± 4.3	26.8 ± 4.6	27.5 ± 4.7

$*P < 0.05$ vs baseline, ** $P < 0.01$ vs baseline, *** $P < 0.001$ vs baseline

reduced bone mass assessed by BMD measurements, and that hormone replacement has a beneficial effect on bone metabolism.

The main finding of our study is that GH replacement therapy in patients with GHD resulted in potentially important changes not only in serum total OC but also cOC, ucOC levels and their ratios. Both total OC and cOC increased significantly after 6 months and decreased moderately thereafter, but both remained elevated above baseline until 18 months. Thus, the elevation of serum total OC levels does not simply indicate an improvement of cOC with significant increases between 6 and 18 months during treatment. Accordingly, the cOC:total OC ratio significantly increased at 6 months compared with pretreatment values. As cOC may be related more directly to bone formation than total OC, the changes observed in our study seem to be particularly important for the beneficial effect of GH treatment on bone metabolism. The BMD is probably the most important, but not the only predictor of the osteoporotic fracture. Recently, Wuster et al. [8] demonstrated that patients with GHD have a high risk of fractures. The quality of bone, i.e., the strength of bone, is dependent on several factors such as architecture and microdamage [33, 34]. In this respect it seems especially important that the carboxylation of OC is considered as an independent predictor of bone fragility [24, 26, 28–30].

OC is the major noncollagen protein in bone and reflects osteoblast number and function. It contains 49 amino acids but it is a product of a larger intracellular precursor molecule. OC is a marker of bone formation and turnover, although its exact role in bone metabolisms remains to be established. Posttranslational modi-

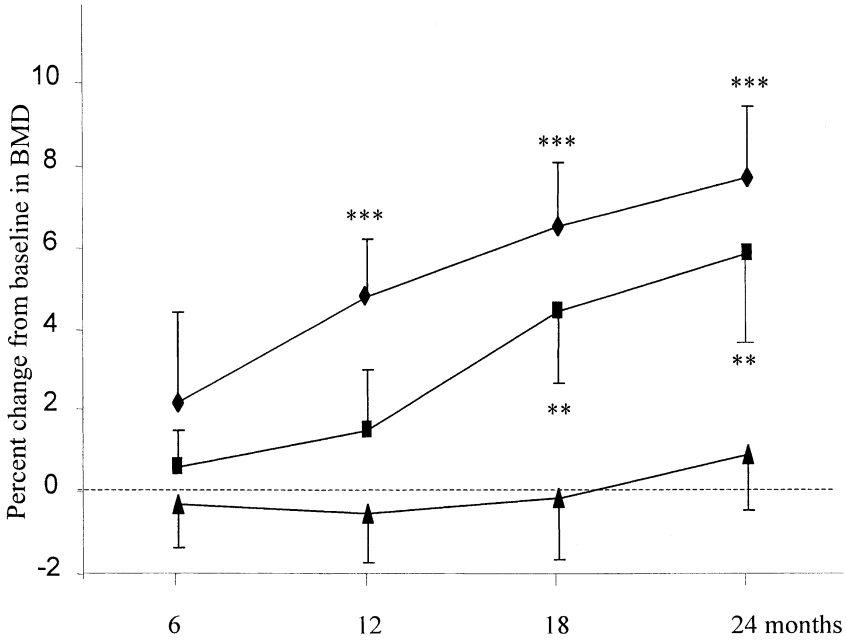

Fig. 2. Percent change from baseline in bone mineral density (means ± SEM) in response to growth hormone treatment at the lumbar spine (◆), femoral neck (■) and radius (▲), (mean ± SE), $^{**}P < 0.01$, $^{***}P < 0.001$ vs baseline.

fication of OC occurs through gamma carboxylation. Gamma carboxylation is largely responsible for Ca binding properties of OC. The fully carboxylated state of OC is required for normal bone formation. Biochemical mechanisms by which ucOC could be associated with impaired bone metabolism are not known, but it is suggested that ucOC a direct product of osteoblast can be inhibitory on osteoblast function [30, 31]. Vitamin K mediates the gamma-carboxylation of glutamyl residues of several bone proteins, including OC [35]. Vitamin K insufficiency, due to low intake or clinical use of vitamin K antagonists as anticoagulants, has been associated with higher ucOC concentration [35–39], lower BMD [28] and increased risk of fracture [27, 29, 30].

The concentrations of cOC and ucOC have not been previously determined or compared in patients with GH deficiency before and during treatment. A few earlier studies suggested somewhat higher ucOC concentrations in osteoporotic patients with vertebral or hip fractures than in healthy controls [35, 37]. However, it is not entirely clear whether treatment with drugs other than GH could influence ucOC, cOC, or their ratio in osteoporotic patients.

In conclusion, our results confirm previous data of open studies that low BMD is present in patients with GHD and that this decrease can be inversed by GH therapy. More importantly, our finding of an improvement of ucOC raises the possibility of a previously unrecognized mechanism by which GH replacement exerts its beneficial effects on bone metabolism. It is possible that the combination of ucOC and cOC measurements, and bone mass determination might improve the assessment of bone fragility in GHD patients. The effect of GH treatment on OC carboxylation and bone fracture rate should be investigated in a randomized, double-blind, placebo-controlled study.

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