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The influence of gender on the short and long-term effects of growth hormone replacement on bone metabolism and bone mineral density in hypopituitary adults: a 5-year study

W. M. Drake* J. Rodríguez-Arnao* J. U. Weaver† I. T. James* D. Coyte* T. D. Spector‡ G. M. Besser* and J. P. Monson*

**Department of Endocrinology and the Medical Professorial Unit St Bartholomew's Hospital* †*Department of Medicine Gateshead District General Hospital* ‡*Twin Research and Genetic Epidemiology Unit St. Thomas' Hospital London UK*

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Summary

OBJECTIVES The objectives of this study were to investigate the effects of GH replacement therapy in hypopituitary adults with growth hormone deficiency (GHD) on activation of bone remodelling during dose titration and on BMD over a median of 58 months of continuous therapy.

STUDY DESIGN Open label study in adult patients with GHD. rhGH was commenced at dose of 0·8 IU subcutaneously daily (0·4 IU if hypertensive or glucose tolerance impaired) with subsequent dose titration based on 2 weekly measurement of serum IGF-I until levels reached the target range (between the median and upper end of the age related reference range). In patients previously commenced on GH using weight based regimens the dose of GH was adjusted during clinical follow-up in order to maintain serum IGF-I in the target range.

PATIENTS Initial effects of GH on bone remodelling during dose titration were studied in 17 patients (8F). Long-term effects of GH were determined in a separate group of 13 GHD adults (6F) over a median period of 58 months (range 44–72).

MEASUREMENTS Osteoblastic activity was estimated by measuring serum bone specific alkaline phosphatase (S-BAP). BMD was determined at both lumbar spine (L2-L4) and femoral neck by dual energy X-ray absorptiometry (DEXA).

RESULTS During dose titration a significant increment in S-BAP was observed by 10 weeks in females but occurred later in males (12–26 weeks). In the long term treatment group there was a significant increment in S-BAP compared to baseline ($P = 0{\cdot}013$) after 6 months GH treatment. After long-term GH treatment (median 58 months) S-BAP levels decreased and were no longer statistically significantly different from baseline at the end of the study period. A similar response was observed in male and female patients. There were no significant differences in baseline BMD between male and female patients at either lumbar spine or femoral neck in the long term treatment group. No significant changes were observed in BMD after 6 months GH treatment in either lumbar spine or femoral neck but BMD increased over the remainder of the study at both sites ($P = 0{\cdot}023$ and $P = 0{\cdot}03$ respectively). When analysed by gender male patients showed a clear positive change in BMD after longer-term replacement in both lumbar spine and femoral neck ($P = 0{\cdot}01$ and $P = 0{\cdot}02$ respectively) but female patients showed no significant changes. Qualitatively similar results were observed when analysing changes in BMD expressed as Z scores.

CONCLUSION This study demonstrates an earlier onset of GH activation of bone remodelling as reflected by S-BAP in females compared to males and confirms that long-term GH treatment in hypopituitary adults with GH deficiency increases or preserves BMD both at lumbar spine and femoral neck. However male patients seem to derive the greater benefits in BMD from long-term GH replacement; in females BMD appears simply to be stabilized rather than increased. This constitutes a genuine gender difference in susceptibility given that serum IGF-I was in the upper part of the reference range in all subjects.

Correspondence: Professor John P. Monson, Department of Endocrinology, St Bartholomew's Hospital, London EC1A 7BE, UK. Fax: +44 207 6018505

Unravelling the complex nature of the regulation of GH secretion has permitted a greater understanding of normal and abnormal GH physiology. The various components of the GH-IGF-I axis have many peripheral effects on growth, activation of main cellular functions, energy metabolism and protein anabolism. With the increasing use of GH replacement therapy in GH deficient (GHD) adults (Jorgensen *et al*. 1989; Cuneo *et al*. 1992; Bengtsson *et al*. 1993) the role of GH in bone metabolism has also been recognized (Slootweg 1993; Wüster 1993; Inzucchi & Robbins 1994). Patients with untreated GH deficiency have reduced levels of osteoblastic activity that increases significantly after GH treatment (Delmas *et al*. 1986; Johansen *et al*. 1990a; Juul *et al*. 1994; Weaver *et al*. 1996). Furthermore GH deficient adults of either childhood or adult onset have reduced BMD compare to normal (Kaufman *et al*. 1992; De Boer *et al*. 1994; Holmes *et al*. 1994). Although the clinical implications of this reduction in BMD are still not fully known an increased prevalence of fractures has been described in adults with GH deficiency (Wüster *et al*. 1991; Rosén *et al*. 1997). As GH has a clear effect on bone physiology it has been suggested that GH replacement may be effective in normalizing BMD thereby decreasing the risk of fracture. This hypothesis has been tested in a number of short and long-term studies (for reviews see: Carroll *et al*. 1998; Ohlsson *et al*. 1998). Contradictory conclusions have been reported from these trials as the effects of GH on adult bone mass are complex and treatment periods exceeding 12 months are required in order to see a positive effect of GH on bone mineral density. However long-term data are limited as none of the studies published to date has exceeded 48 months (Kann *et al*. 1998). In addition the timing of onset of GH stimulation of bone remodelling during initiation of GH therapy by dose titration has not been reported previously. The aims of this study were to investigate the initial effects of GH on activation of bone remodelling and the effects of GH replacement therapy for between 44 and 72 months on bone formation and on BMD in GHD hypopituitary adults. The possibility of gender differences in susceptibility was also assessed.

Patients and methods

Patients

Two groups of patients (short- and long-term) were studied. The long-term treatment group comprised 13 patients (six females) median age 46·0 years (range 28–64 years). The short-term dose titration treatment group 17 patients (eight females) median age 44 years (range 32–62). Clinical details for the two treatment groups are shown in Table 1. The diagnosis of GH deficiency was based on a peak GH response of less than 9 mU/l after stimulation with either an insulin tolerance test (blood glucose $< 2{\cdot}2$ mmol/l) or a glucagon test (1 mg s.c.). The mean peak serum GH during provocative testing was $2{\cdot}06 \pm 2{\cdot}04$ mUl/l (range 0·5–8). The mean age at diagnosis of GH deficiency was 40·9 years (range 14–66) with all but one patient having adult onset of GH deficiency. At entry, all patients had been GH-deficient for at least 2 years before entering into the study, all had normal routine liver and renal function tests; none had diabetes mellitus and all were euthyroid. Each patient had received stable hormone replacement therapy where indicated for at least 6 months before entry into the study.

Study protocol

The design was that of an open label treatment study of the administration of rhGH (Genotropin® Pharmacia Corporation Stockholm Sweden) over 44–72 months (median 58). In the dose titration group GH was commenced at a dose of 0·8 IU subcutaneously daily (0·4 if the patients had hypertension or impaired glucose tolerance) and subsequently adjusted on the basis of 2 weekly measurements of serum IGF-I (median time to achievement of serum IGF-I between median and age-related reference range was 4 weeks in males and 8 weeks in females) (Drake *et al*. 1998). In the long term group during the first 4 weeks of treatment the GH dose was 0·125 IU/kg body weight/week given as a daily dose and the target dose thereafter was 0·25 IU/Kg body weight/week. After the first year GH replacement dose was individually adjusted to maintain serum IGF-I levels between the median and upper end of the age-related reference range (median GH dose: 1·6 IU daily; male patients 1·5 IU/daily; female patients: 2 IU/daily). Blood samples were obtained after an overnight fast via an intravenous forearm cannula with the patient sitting in a chair. Serum was separated and stored at -20 °C until further analysis. Measurements of BMD were performed at entry into the study, at 6 months and after a median of 58 months (range 44–72) of GH replacement. All patients were given written information about the study and written consent was obtained. The study was approved by the East London and City Health Authority Ethics Committee.

Laboratory measurements

Serum IGF-I was determined using an in-house radioimmunoassay with acid-ethanol extraction as previously described (Morrell *et al*. 1989). The intra-assay and inter-assay coefficient of variation were 3·1 and 10% respectively. Cross-reactivity of the assay for IGF-II was 1% and negligible for insulin and proinsulin.

Serum bone specific alkaline phosphatase (S-BAP) was determined using a direct ELISA assay (Alkphase B Metra

Table 1 Clinical characteristics of patients in the long-term treatment and dose titration groups

Patient no.	Age	Sex	Years of GHD	GH peak (MU/l)	Aetiology	Treated deficiency of			
						TSH	ACTH	LH-FSH	ADH
Long-term treatment group									
1	42	Female	11	1·1	NFPA	No	No	No	No
2	48	Female	8	2·6	NFPA	No	No	No	No
3	34	Female	8	1·2	NFPA	No	Yes	No	Yes
4	52	Female	8	2·4	NFPA	Yes	No	Yes	No
5	58	Female	3	2	Craniopharyngioma	Yes	Yes	Yes	Yes
6	35	Female	21	1·2	Granuloma	Yes	Yes	Yes	Yes
7	42	Male	28	0·5	Craniopharyngioma	Yes	Yes	Yes	Yes
8	60	Male	2	0·8	NFPA	No	Yes	Yes	No
9	34	Male	12	0·5	NFPA	Yes	Yes	Yes	No
10	44	Male	2	0·5	NFPA	Yes	Yes	Yes	No
11	42	Male	3	1·5	Craniopharyngioma	Yes	Yes	Yes	Yes
12	25	Male	2	0·5	Craniopharyngioma	Yes	Yes	Yes	Yes
13	47	Male	26	6	Idiopathic	No	No	Yes	No
Titration treatment group									
1	33	Female	5	5·2	NFPA	No	No	No	No
2	28	Female	28	0·5	Corticotrophinoma	Yes	Yes	Yes	Yes
3	54	Female	2	0·8	Corticotrophinoma	No	Yes	Yes	No
4	42	Female	5	$< 0·5$	NFPA	Yes	No	Yes	No
5	22	Female	12	0·5	Craniopharyngioma	Yes	Yes	Yes	No
6	50	Female	8	1·2	NFPA	No	Yes	No	Yes
7	68	Female	2	1·2	NFPA	Yes	Yes	No	No
8	59	Female	8	2·4	NFPA	Yes	No	Yes	No
9	49	Male	10	$< 0·5$	Corticotrophinoma	Yes	Yes	Yes	No
10	65	Male	19	$< 0·5$	NFPA	Yes	Yes	Yes	Yes
11	42	Male	2	3	PRL	Yes	No	Yes	No
12	69	Male	11	1·8	NFPA	Yes	Yes	Yes	No
13	56	Male	15	3·6	PRL	Yes	Yes	Yes	No
14	54	Male	10	1·1	Corticotrophinoma	Yes	Yes	Yes	Yes
15	58	Male	14	3	TB meningitis	Yes	Yes	Yes	No
16	56	Male	3	1·2	RT(ALL)	Yes	Yes	Yes	No
17	47	Male	11	8	Idiopathic	No	No	Yes	No

NFPH nonfunctioning pituitary adenoma; RT (ALL) radiotherapy for acute lymphoblastic leukaemia

Biosystems, Mountainview, California, USA). Briefly samples (standards and controls (20 μl)) were incubated for 3 h at room temperature with 125 μl assay buffer in anti BAP coated wells. Plates were then washed (x 3) and S-BAP activity determined after incubation for 30 minutes with 150 μl of p-nitro phenyl phosphate. The reaction was stopped by the addition of l00 μl 1 M NaOH and absorbance read at 405 nm. A quadratic calibration curve was used for the quantification of bALP in unknown samples. Intra-assay variation was 5·4% and inter-assay variation 5·8%.

Bone densitometry

Dual energy X-ray absorptiometry was used to measure BMD in femoral neck and lumbar spine (L1-L4) using an Hologic QDR-1000/W (Hologic Waltham MA USA) or a Lunar DPX-L (Lunar Corp. Madison WI USA) absorptiometer. Individual subjects had their follow-up measurements performed on the same machine throughout. Results are expressed as absolute values (g/cm^2) or Z-scores (difference expressed as SD between the individual values and the mean of age-and sex-matched healthy subjects).

Statistical analysis

Results for S-BAP are presented as the mean ± SEM. Those for BMD (absolute values and Z-scores) were not normally distributed and are expressed as median and range. The study was analysed for sequential changes from patients within a group using the Wilcoxon signed rank test. The Mann–Whitney

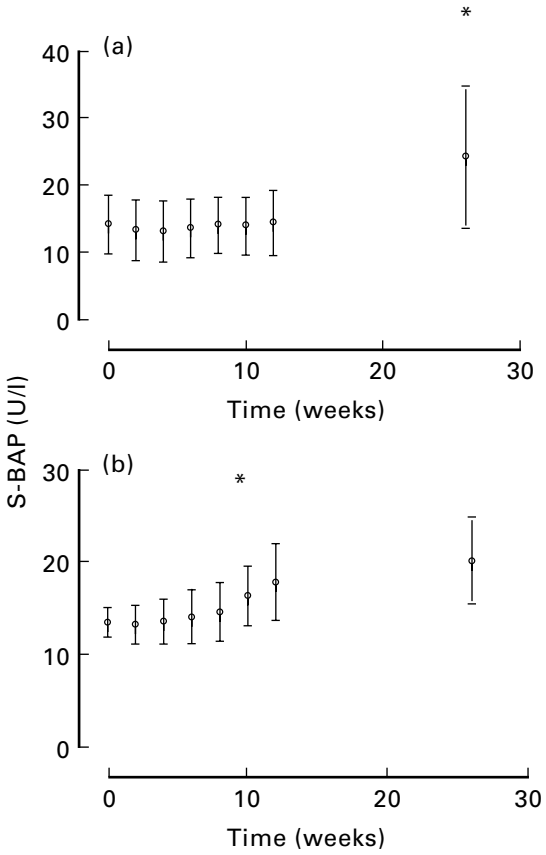

Fig. 1 Effect of GH replacement by dose titration (against serum IGF-I) on serum bone specific alkaline phosphatase (S-BAP) in (a) male and (b) female GH naïve patients.

U-test was used to compare data between groups of patients. $P < 0{\cdot}05$ was considered statistically significant. All statistical analyses were performed using SPSS (SPSS Inc. Chicago IL USA).

Results

Serum IGF-I

Prior to GH treatment serum IGF-I levels in most patients were below the lower age-related reference range. Concentrations of serum IGF-I increased significantly during GH treatment and were maintained between the median and upper end of the age-related reference range (data not shown) in all patients.

Serum bone specific alkaline phosphatase

S-BAP increased significantly in the GH naïve group; this was evident by 10 weeks in female patients and between 12 and 26 weeks in male patients (Fig. 1). In the long term treatment group there were significant changes in S-BAP compared to baseline ($26{\cdot}31 \pm 5{\cdot}22$ IU/l *vs.* $14{\cdot}16 \pm 3{\cdot}59$ IU/l; $P = 0{\cdot}013$) at 6 months. After 44–72 months (median 58) of GH therapy S-BAP levels had decreased again and were no longer significantly different from baseline ($20{\cdot}02 \pm 2{\cdot}78$ IU/l *vs.* $14{\cdot}16 \pm 3{\cdot}59$ IU/l; $P = 0{\cdot}09$). A similar response was seen in male and female patients.

Bone mineral density

Results for BMD are presented in Table 2 and Fig. 2. At baseline no significant differences were observed in the grouped data between male and female patients separately at either lumbar spine or femoral neck. Baseline median lumbar spine Z-score was higher in females than males but this apparent difference was not statistically significant. Femoral neck Z-scores were similar in males and females. In the long term treatment group no net significant changes in BMD were observed by 6 months at either lumbar spine or femoral neck but a significant increment was noted at both sites ($P = 0{\cdot}023$ and $P = 0{\cdot}03$ respectively) after a median of 58 months treatment. A gender difference in the response to GH was

Table 2 Changes in bone mineral density (BMD) expressed as g/cm^2, in men and women with GH deficiency before and after a median of 58 months of GH replacement therapy. All values represent median (range). Long-term measurements: median 58 months, range 44–72

	Baseline	6 months	Long-term
BMD femoral neck (g/cm^2)			
All patients	0·79 (0·67–1·08)	0·83 (0·68–1·16)	0·85 (0·64–1·23)*
Male patients	0·88 (0·68–1·08)	0·93 (0·69–1·16)	0·92 (0·74–1·23)†
Female patients	0·76 (0·67–0·94)	0·78 (0·68–0·95)	0·81 (0·64–0·94)
BMD lumbar spine (g/cm^2)			
All patients	1·08 (0·80–1·32)	1·07 (0·81–1·42)	1·14 (0·79–1·50)‡
Male patients	1·08 (0·80–1·32)	1·05 (0·81–1·35)	1·13 (0·94–1·5)§
Female patients	1·11 (0·86–1·21)	1·09 (0·85–1·42)	1·14 (0·79–1·22)

*$P = 0{\cdot}03$ *vs.* baseline; †$P = 0{\cdot}02$ *vs.* baseline; ‡$P = 0{\cdot}023$ *vs.* baseline; §$P = 0{\cdot}01$ *vs.* baseline.

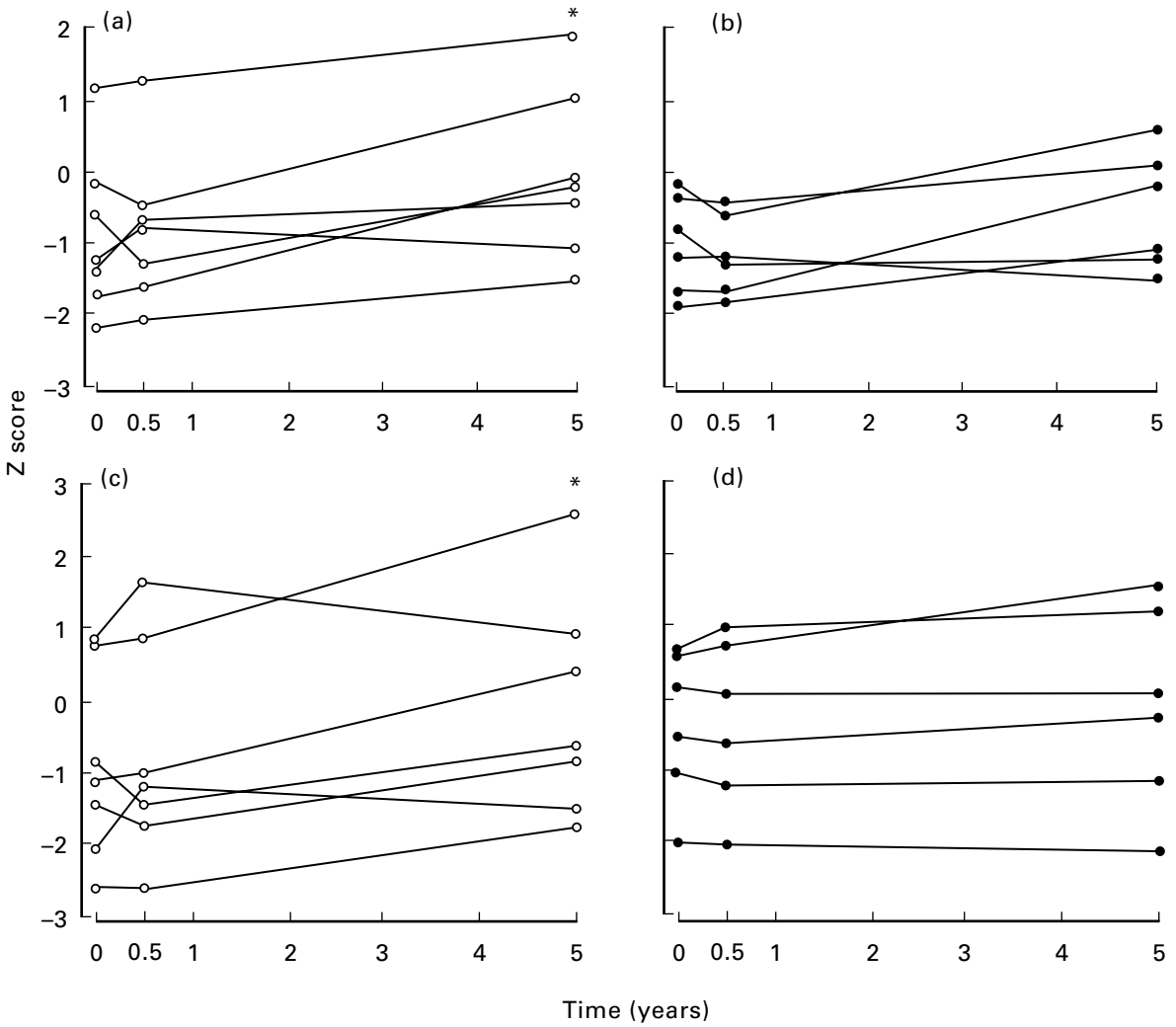

Fig. 2 Effect of GH treatment on bone mineral density (Z scores: the difference, expressed as SD, between the individual values and the mean of age- and sex-matched healthy subjects) in (a, c) male and (b, d) female hypopituitary adults on long-term GH replacement: (a, b) at the femoral neck; $*P = 0{\cdot}02$ compared to baseline. (c, d) at lumbar spine; $*P = 0{\cdot}04$ compared to baseline. The final reading, denoted 5 years in all patients, represents the median duration of GH therapy (range 44–72 months).

observed (Table 2). Male patients showed a clear improvement in BMD after long-term replacement in both lumbar spine and femoral neck ($P = 0{\cdot}01$ and $P = 0{\cdot}02$ respectively). In contrast female patients showed no significant change after long-term GH replacement in either of the areas studied. Similar changes were observed when analysing changes in BMD expressed as Z-scores (Fig. 2). There was no significant correlation between baseline BMD Z-score and the observed increment in Z-score.

Discussion

GH deficient adults of either childhood or adult onset have reduced BMD compared with age-matched healthy controls (Kaufman *et al*. 1992; De Boer *et al*. 1994; Holmes *et al*. 1994). Although the clinical implications of this reduction in BMD are still not well understood an increased prevalence of fractures has been described in adults with GH deficiency (Wüster *et al*. 1991; Rosen *et al*. 1997). While GH has a clear effect on bone physiology (Wüster 1993) there are conflicting data regarding the short-term effects of GH replacement on BMD in GH deficient patients (O'Halloran *et al*. 1993; Juul *et al*. 1994; Degerblad *et al*. 1995; Holmes *et al*. 1995; Inzucchi & Robbins 1996). The effects of GH on BMD for periods longer than two years have been previously described by three groups: Vandeweghe *et al*. (1993) Rahim *et al*. (1998)

and Kann *et al*. (1998) with a maximum duration of four years (Kann *et al*. 1998). To our knowledge the present study is the longest duration published observation of the effects of GH treatment on bone in hypopituitary adults. More physiological GH replacement at a dose adjusted to maintain normal IGF-I levels decreases the likelihood of adverse consequences but clearly is still capable of producing beneficial effects in BMD as demonstrated here. However there is a gender difference after long-term GH treatment in GHD adults (Johansson *et al*. 1999). Our data confirm that the net effects of long-term GH treatment on bone mass are more advantageous to men than to women. In contrast after initiation of GH therapy by dose titration we observed more rapid activation of bone remodelling in female patients despite the fact that females generate less IGF-I for a given dose of GH and take longer to reach maintenance dose than males (Johannsson *et al*. 1996a; Drake *et al*. 1998). Furthermore 24 h GH production analysis shows that mean daily GH production is approximately three times greater in women than in men in order to maintain an equivalent serum IGF-I level (Van der Berg *et al*. 1996). Consistent with the notion that adult males may exhibit greater sensitivity to GH it was later confirmed that in female GH deficient patients the mean increment in serum IGF-I is significantly lower than in male patients (Burman *et al*. 1997; Drake *et al*. 1998; Janssen *et al*. 1998; Thorén *et al*. 1998).

The precise mechanism underlying the gender difference in sensitivity of BMD to GH remains unclear although it seems likely that sex steroids may play a role. In women physiological oestrogen replacement augments the pituitary secretion of GH (Kerrigan & Rogol 1992) but leads to a relative resistance to the stimulatory effect of GH on IGF-I production (Juul *et al*. 1994). Although it has been suggested that there may be antagonism between oestrogen and GH at peripheral tissue level (Burman *et al*. 1997) no systematic difference has been found between GH doses and increments in IGF-I when female patients are subdivided by gonadal status and use of oestrogen replacement (Drake *et al*. 1998; Abs *et al*. 1999). Furthermore no significant differences in the percentage increase in BMD or in the incremental change in Z-scores between oestrogen-deplete and oestrogen-replete women has been found after 2 years of GH treatment (Janssen *et al*. 1998). All our male patients were on testosterone replacement. Given the previously reported synergy between GH and androgens at peripheral tissue level (Burman *et al*. 1997) it is possible that the differential effect of GH on BMD in male and female GHD patients could be explained by a positive interaction between GH and androgens in male patients together with a relative resistance to the effect of GH in female patients.

Our results are in disagreement with a previous report in which after two years GH treatment male patients showed a less pronounced increase in BMD than female patients (Johannsson *et al*. 1996b) but concur with observations by the same group that men derive greater benefit from GH than women (Johannsson *et al*. 1996a). The reason for this difference between studies is unclear. In the study of Johannsson *et al*. (1996b) at baseline women had significantly lower measurements of BMD than men. It has been demonstrated previously that the increase in BMD after GH treatment depends on baseline BMD values with the highest increment found in those patients with lowest pretreatment BMD. Therefore the differences observed in baseline BMD between male and female patients in that study could explain the discrepant results (Degerblad *et al*. 1996). In this context it should be noted that we observed similar BMD at baseline in males and females and a median Z-score for the lumbar spine which was not significantly higher in females than males. Furthermore we also observed a greater improvement in Z-score at the femoral neck in males despite a similar baseline Z-score compared with females.

Bone alkaline phosphatase (BAP) is released by osteoblasts during the mineralization process and is a well characterized biochemical marker of bone formation (Johansson *et al*. 1993; Weaver *et al*. 1996). It also provides a robust index of overall bone remodelling during GH replacement because bone formation and resorption are tightly coupled in this clinical situation. Patients with untreated GH deficiency have reduced levels of osteoblastic activity that increases significantly after GH treatment (Delmas *et al*. 1986; Johansen *et al*. 1990a; Juul *et al*. 1994). Our data indicate that this response occurs more rapidly in females than males despite the opposite gender difference in susceptibility in other respects. These changes reflect both an increase in the activity and an expansion of the osteoblast population (Johansen *et al*. 1990b; Nielsen *et al*. 1991). The mechanism of this paradoxical gender difference merits further investigation and may have implications for our understanding of the initiation of GH action on bone. However the increment in S-BAP was not sustained in the long term suggesting that the major effect of GH in promoting bone formation has occurred within the timeframe of this study.

In conclusion this study confirms that GH treatment in hypopituitary adults with growth hormone deficiency results in an increase in bone mineral density in males and stabilization of bone mineral density in females both at lumbar spine and femoral neck during a median of 58 months of continuous treatment. These changes may result in a reduction of fracture risk and this issue should be resolved by longitudinal follow up of large cohorts of patients on prolonged GH replacement. Male patients seem to derive the greater benefits from GH replacement and understanding this gender difference will be necessary in order to design optimal GH replacement.

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