0021-972X/99/$03.00/0
Journal of Clinical Endocrinology and Metabolism
Copyright © 1999 by The Endocrine Society
Vol. 84, No. 1
Printed in U.S.A.

Increased Cortical Bone Content of Insulin-Like Growth Factors in Acromegalic Patients*

THOR UELAND, JENS BOLLERSLEV, TONY BILL HANSEN,
EBBE NILS EBBESEN, LIS MOSEKILDE, KIM BRIXEN, ALLAN FLYVBJERG, AND
OLE DJØSELAND

Department of Endocrinology, National University Hospital (T.U., J.B., O.D.), N-0027 Oslo, Norway; the Department of Endocrinology, Odense University Hospital (T.B.H., E.N.E.), DK-5000 Odense, Denmark; and the Department of Cell Biology, University of Aarhus (L.M.), and the Department of Endocrinology (K.B.) and the Medical Research Laboratory M (A.F.), Aarhus University Hospital, DK-8000 Aarhus, Denmark

ABSTRACT

To investigate cortical bone composition and the role of the insulin-like growth factor (IGF) system in active acromegaly, iliac crest bone biopsies were obtained from 15 patients (3 women and 12 men), aged 21–64 yr (mean, 45.6 yr), and 25 age- and sex-matched controls (8 women and 17 men), aged 22–66 yr (mean, 44.6 yr). Levels of IGF-I, IGF-II, IGF-binding protein-3 (IGFBP-3), IGFBP-5, and total protein were determined in extracts obtained after ethylenediamine tetraacetate and guanidine hydrochloride extraction. Osteocalcin and calcium were determined in extracts after HCl hydrolysis. Cortical bone contents of IGF-I, IGF-II, and IGFBP-5 were significantly elevated in the acromegalic patients compared with control values [91% ($P < 0.001$), 44% ($P < 0.04$), and 115% ($P < 0.004$), respectively]. There was no significant difference in IGFBP-3, osteocalcin, protein, and calcium between patients and controls. This study suggests that the increased levels of growth factors in cortical bone from acromegalics is a reflection of local production, secondary to a chronic systemic excess of GH and IGF-I. (*J Clin Endocrinol Metab* **84:** 123–127, 1999)

GH STIMULATES bone turnover (1) in a dose-dependent manner (2), and the long lasting chronic excess in acromegaly leads to appositional growth and increased bone dimensions. Bone turnover is also increased in acromegaly, as judged by biochemical markers (3, 4), calcium kinetics (5), and histomorphometry (6).

The effects of GH on bone tissue are mediated through a complex interaction of circulating GH, insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), and locally produced IGFs and IGFBPs acting in auto- and paracrine manners (7). IGF-I and IGF-II have similar biological activities, although IGF-II is the most abundant in bone tissue (8). The IGF system has an important role in regulation of bone cell metabolism. IGFs affect the proliferation and differentiation of bone cells (9, 10) and stimulate cell activity and collagen synthesis. Moreover, IGFs and their binding proteins may be released by bone resorption to act as local determinants of site-specific coupled bone formation (11).

The aim of the present study was to investigate cortical bone composition and the role of the IGF system and osteocalcin content in cortical bone from acromegalic patients compared with those in bone from controls.

Received December 9, 1997. Revision received July 17, 1998. Rerevision received September 16, 1998. Accepted September 22, 1998.

Address all correspondence and requests for reprints to: Dr. Thor Ueland, Department of Endocrinology, National University Hospital, N-0027 Oslo, Norway.

* This work was supported by grants from the Danish Medical Research Council (Grant 9600822), the Ruth König-Petersen Foundation, the Novo Foundation, the Nordic Insulin Foundation, the Ingeborg Maibøll Foundation, and the Aarhus University-Novo Nordisk A/S Center for Research in Growth and Regeneration (Grant 9503020).

Subjects and Methods

Patients

The study comprised 15 patients (3 women and 12 men), aged 21–64 yr (mean, 45.6 yr), with active acromegaly and 25 age- and sex-matched controls (8 women and 17 men), aged 22–66 yr (mean, 44.6 yr). Activity was defined as nonsuppressible serum levels of GH (>5 mU/L) by oral glucose tolerance test. From each patient, an iliac crest bone biopsy was obtained under local anesthesia, using a modified Bordier trephine (id, 7 mm) from the standard site 2 cm below the iliac crest and 2 cm behind the anterior superior iliac spine. The specimens were frozen at −20 C immediately after removal. Still frozen, the biopsies were later sawed carefully to remove the cortical bone. Anterior-superior parts from the iliac crest were obtained in the same fashion from the controls at necropsy early after death. The controls had died in the hospital or due to accident. None of the individuals had disease interfering with bone metabolism. The study was approved by the local ethical committee and conducted according to the Declaration of Helsinki II.

Preparations of bone specimens

The cortical bone specimens were washed with water to remove soft tissue and blood. Samples were defatted in trichloroethylene for 6 days (changed every second day) at 4 C and dried by immersion in ethanol-ether (1:1, vol/vol). The samples were pulverized in a liquid nitrogen-cooled mortar and pestle, adding liquid nitrogen as necessary to keep the samples frozen. The pulverized bone particles were then passed through an 84-μm pore size sieve and stored at −40 C until use.

Extraction procedures

For determining IGF-I, IGF-II, IGFBP-3, IGFBP-5, and total protein contents, 15 mg bone powder were extracted once with 1.5 mL 0.5 mol/L ammonium ethylenediamine tetraacetate containing the protease inhibitors benzamidine (5 mmol/L), 6-aminocaproic acid (10 mmol/L), *p*-hydroxymercuribenzoic acid (100 μmol/L), and phenylmethylsulfonylfluoride (30 μmol/L), pH 6.2, and once with 1.5 mL 4 mol/L guanidinium-HCl (pH 7.4) containing the same protease inhibitors.

Extractions were carried out for 18 h at 4 C by rotation. After ex-

Downloaded from https://academic.oup.com/jcem/article/84/1/123/2866184 by guest on 21 August 2022

traction, the solution was centrifuged (12,000 × *g* for 30 min), and the supernatant was separated from the remaining bone residues. Supernatants from both extractions were combined and dialyzed (mol wt cut-off, 3500; Spectrapor, Spectrum Medical Industries, Los Angeles, CA) on an orbital shaker for 2 days at 4 C against 80 mL 0.02 mol/L acetic acid, lyophilized in a Speed-Vac concentrator (Savant Instruments, Hicksville, NY), and stored at −40 C until assayed.

For the determination of calcium and osteocalcin, 5 mg bone powder were hydrolyzed in 0.5 mL 6 mol/L HCl for 1 week at 4 C by rotation. The hydrolyzed samples were stored at −40 C after the addition of 1 mL 3 mol/L NaOH.

RIAs for IGFBP-3, IGF-I, and IGF-II

The lyophilized ammonium and guanidine extracts were redissolved in 1.0 mL 0.02 mol/L acetic acid and used to determine the cortical contents of IGF-I, IGF-II, IGFBP-3, and total protein.

IGFBP-3 was measured using a commercial RIA kit from Nichols Institute Diagnostics (Nijmegen, The Netherlands). The following modification was made: samples were lyophilized and redissolved in assay buffer, giving a dilution of 1:5 before assay. This kit has a sensitivity of 0.25 ng/mL, an intrassay variation of less than 10%, and no detectable cross-reactivity with IGFBP-1, IGFBP-2, IGF-I, or IGF-II.

IGF-I and IGF-II were measured by RIA and immunoradiometric assay (IRMA) after separation from IGFBPs by acid-gel filtration (12). IGF-I was measured by a commercial kit from Nichols Institute Diagnostics. Samples were lyophilized and redissolved in assay buffer before analysis. This kit has a sensitivity of 0.06 μg/L, an intraassay variation of less than 10%, and no cross-reactivity with IGF-II.

IGF-II was measured using a commercial IRMA kit from Diagnostics Systems Laboratories, Inc. (Webster, TX). Samples were lyophilized and redissolved in phosphate buffer before analysis. This kit has a sensitivity of 0.13 μg/L, an intraassay variation of less than 10%, and no cross-reactivity with IGFBP-2. IGFBP-3, IGFBP-4, or IGFBP-5.

Western ligand blotting (WLB) of cortical bone extracts

Cortical bone extracts (5 μg protein on each lane) were subjected to WLB to attain a semiquantitative estimate of IGFBP-5 levels. SDS-PAGE (10% polyacrylamide) and WLB were performed under nonreducing conditions as previously described (13). The electrophoresed proteins were transferred by electroelution onto nitrocellulose paper (Schleicher & Schuell, Inc., Munich, Germany), and membranes were incubated overnight at 4 C with approximately 500,000 cpm [^{125}I]IGF-I (SA, 2000 Ci/mmol) in 10 mL 10 mmol/L Tris-HCl buffer containing 1% BSA and 0.1% Tween (pH 7.4). Membranes were washed with phosphate-buffered saline, and after drying overnight, the nitrocellulose sheets were autoradiographed with Kodak X-AR film (Eastman Kodak Co., Rochester, NY) and exposed to DuPont-New England Nuclear enhancing screens (Boston, MA) at −80 C for 3–7 days. The specificity of the IGFBP bands was ensured by competitive coincubation with unlabeled IGF-I purchased from Bachem (Bubendorf, Switzerland). WLB yielded one major 28-kDa IGFBP and in some samples a faint 38-/42-kDa (doublet); the doublet band corresponded to the acid-stable IGF-binding subunit of IGFBP-3 (14) (Fig. 1). To confirm the identity of the major band (28 kDa) in cortical extracts as IGFBP-5, extracts were analyzed by immunoprecipitation followed by SDS-PAGE and WLB as described above. Cortical extracts were incubated at 4 C overnight with a specific human IGFBP-5 antibody (Austral Biologicals, San Ramon, CA) in a dilution of 1:250. Precipitation was achieved by the addition of 250 μL solid phase coated cellulose suspension (Sac-Cel, IDS, Tyne and Wear, UK) followed by incubation at 37 C for 1 h. Bound antibody was separated by centrifugation at 2800 rpm for 10 min. The precipitated proteins were washed (three times) by the addition of 1 mL phosphate-buffered saline, 0.25% BSA, and 0.1% Tween-20; centrifuged at 2800 rpm for 10 min before resuspension in 100 μL gel loading buffer; and exposed to SDS-PAGE and WLB. Immunoprecipitation with the IGFBP-5 antibody revealed a single 28-kDa band (data not shown). No detectable IGFBPs could be demonstrated when immunoprecipitation was performed with specific IGFBP-1, IGFBP-2, or IGFBP-4 antibodies (data not shown). Autoradiograms were quantified by densitometry using a Shimadzu CS-9001 PC dual wavelength flying spot scanner (Shimadzu Europe,

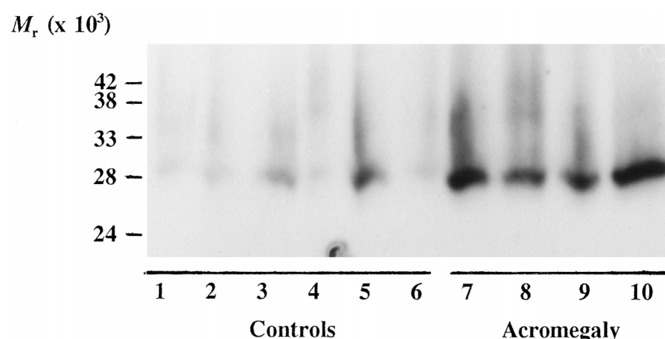

FIG. 1. Representative WLB autoradiogram of cortical bone extracts from controls (lanes 1–6) and patients with acromegaly (lanes 7–10). The identity of the major IGFBP in bone extracts (IGFBP-5) was confirmed by immunoprecipitation with a specific IGFBP-5 antibody as described in *Materials and Methods*. M_r, Mol wt.

Gmbh, Duisburg, Germany). The relative densities of the bands were measured as arbitrary absorbance units (AU) per mm^2.

Validation of extraction procedures: growth factor recoveries

To evaluate our methodology, nine samples were extracted in duplicate. Two additional extractions with guanidinium-HCl were performed, and supernatants from each extraction were kept separate and analyzed for IGF-I and IGFBP-3 contents as described above. The mean coefficients of variation for duplicate extracted samples were 12.3% and 8.8% for IGFBP-3 and IGF-I, respectively. The extraction efficiencies after the two extractions used in the study were 89 ± 2% and 99 ± 1% for IGF-I and IGFBP-3, respectively.

To evaluate whether growth factors were lost during extraction, dialysis, or acid-gel filtration, four samples were spiked with recombinant IGF-I, and two samples were spiked with IGFBP-3 and assayed according to normal protocol. The mean recoveries of samples spiked with recombinant IGF-I and IGFBP-3 were 112% (118%, 109%, 106%, and 117%) and 97% (72% and 121%), respectively.

To rule out the influence of time delay for necropsy on growth factor levels in controls, three bone samples where divided into two portions each. One was frozen immediately, and the other was left at 2–8 C for 2 days and then frozen. Samples were defatted and extracted as described, and levels of IGF-I and IGFBP-3 were measured. In the time delay experiment, we observed an increase of 6.4 ± 5.1% in IGFBP-3; the coefficient of variation between basal samples (0 days) and samples left for 2 days was 4.3%. The corresponding values for IGF-I were an increase of 9.7 ± 9.1% and a coefficient of variation of 6.1%.

Assay for osteocalcin

Osteocalcin was measured by IRMA with a commercial kit from Incstar Corp. (Stillwater, MN). The assay measures intact osteocalcin-(1–49). Samples were lyophilized and reconstituted in a BSA-borate buffer (10 mmol/L boric acid, 1% BSA, 25 mmol/L NaOH, and 25 mmol/L ethylenediamine tetraacetate, pH 8.5). Samples were diluted 1:16, as suggested by the manufacturer, because fragment interference gave poor dilution linearity. The kit has a sensitivity of 0.2 ng/mL, and an intraassay variation of less than 10%.

Calcium was determined by a colorometric method (15), using a Cobas Mira autoanalyzer (F. Hoffmann-La Roche Ltd., Basel, Switzerland) with a kit from Boehringer Mannheim GmbH (Mannheim, Germany).

Protein concentration was determined with Bio-Rad protein assay (Bio-Rad Laboratories, Inc. GmbH, Munich, Germany).

Statistical analysis

Comparisons between variables in the two groups were performed by Mann-Whitney rank-sum test for unpaired data. Relationships between variables were tested using Spearman's rank-correlation test, and the level of significance was set at $P < 0.05$. A ratio between IGF-I and

Downloaded from https://academic.oup.com/jcem/article/84/1/123/2866184 by guest on 21 August 2022

IGF-II was generated by converting their cortical bone contents from mass equivalents to molar quantities, using molecular masses of 7.7 and 7.5 kDa, respectively.

Results

As evident from Table 1, the cortical bone contents of IGF-I and IGF-II were significantly elevated in the acromegalic patients. The most pronounced increase was seen in IGF-I, with a concentration twice that found in normal controls (0.84 ± 0.10 *vs.* 0.44 ± 0.03 ng/mg dry bone; $P < 0.001$). The concentration of IGF-II was 2.28 ± 0.32 ng/mg dry bone compared with 1.58 ± 0.14 in controls ($P < 0.05$).

The mean level of IGFBP-3 expressed per mg dry bone weight was 35% higher in acromegalics than in controls, although this never reached statistical significance. When expressed per mg protein, the mean differences were less pronounced.

The major 28-kDa IGFBP detected by WLB (IGFBP-5) was significantly increased (115%) in cortical bone extracts from acromegalic patients compared with that from controls (278 ± 53 *vs.* 129 ± 26 AU/mm^2; $P < 0.01$; Table 1).

The concentrations of total protein and osteocalcin were not significantly different in the two groups.

The calcium content of cortical bone was slightly, but insignificantly, higher in patients compared with controls (3%; $P < 0.09$).

The ratio between IGF-I and IGF-II showed that cortical bone from acromegalics and controls contained 3.2 and 4.1 times, respectively, more IGF-II than IGF-I. The mean differences were insignificant ($P = 0.12$).

The age-related changes in cortical content are shown in Table 2. There was a significant age-related decrease in IGF-I in the patients when expressed per mg bone (r = −0.79; $P < 0.001$) and per mg protein (r = −0.85; $P < 0.001$). There were no relationships between age and IGF-II in acromegalics or between age and IGFs in the controls. A significant age-related increase was observed for IGFBP-3 in acromegalics when expressed per mg bone (r = 0.56; $P < 0.03$). However, the relationship was insignificant when expressed per mg protein.

TABLE 1. Cortical bone contents of IGF-I, IGF-II, IGFBP-3, IGFBP-5, osteocalcin, calcium, and protein in 15 acromegalic patients and 25 age- and sex-matched controls

	Acromegaly	Controls
IGF-I (ng/mg)[a]	0.84 ± 0.10[b]	0.44 ± 0.03
IGF-II (ng/mg)[a]	2.28 ± 0.32[d]	1.58 ± 0.14
IGFBP-3 (ng/mg)[a]	4.92 ± 0.99	3.20 ± 0.42
IGFBP-5 (AU/mm^2)	278 ± 53[b]	129 ± 26
Osteocalcin (ng/mg)[a]	76.5 ± 8.7	85.6 ± 6.8
Calcium (μg/mg)[a]	196 ± 5	190 ± 2
Protein (μg/mg)[a]	4.16 ± 0.35	4.12 ± 0.35
IGF-I/IGF-II (mol/mol)	3.2 ± 0.5	4.1 ± 0.4
IGF-I (ng/mg)[c]	240 ± 40[d]	125 ± 14
IGF-II (ng/mg)[c]	606 ± 91	424 ± 42
IGFBP-3 (ng/mg)[c]	1163 ± 175	849 ± 135
Osteocalcin (μg/mg)[c]	21.4 ± 3.6	24.3 ± 2.8

Data represent the mean ± SEM. Values are expressed per mg dry bone ([a]) and per mg protein ([c]). AU/mm^2, Arbitrary absorbance units per mm^2.
[b] $P < 0.001$ *vs.* controls.
[d] $P < 0.05$ *vs.* controls.

TABLE 2. Correlation analysis of IGF-I, IGF-II, IGFBP-3, calcium, and protein *vs.* age for the acromegalic (n = 15) and control (n = 25) groups

	Acromegaly r	Controls r
IGF-I (ng/mg)[a]	−0.789[b]	0.273
IGF-II (ng/mg)[a]	−0.023	−0.248
IGFBP-3 (ng/mg)[a]	0.564[c]	0.293
Calcium (μg/mg)[a]	−0.014	0.016
Protein (μg/mg)[a]	0.550[c]	−0.123
IGF-I (ng/mg)[d]	−0.850[b]	0.222
IGF-II (ng/mg)[d]	−0.314	−0.174
IGFBP-3 (ng/mg)[d]	0.232	0.291

Values are expressed per mg dry bone ([a]) and per mg protein ([d]).
[b] $P < 0.001$ *vs.* controls.
[c] $P < 0.05$ *vs.* controls.

A strong relationship between IGFBP-5 and the IGFs was observed in patients (r = 0.58; $P = 0.024$ and r = 0.79; $P < 0.001$ for IGF-I and IGF-II, respectively) when expressed per mg protein. This relationship was not observed in controls. Osteocalcin was highly correlated with components of the IGF system in acromegalic patients when expressed per mg protein (r = 0.70; $P = 0.003$, r = 0.80; $P < 0.001$, and r = 0.83; $P < 0.001$, for IGF-I, IGF-II, and IGFBP-5, respectively). Similar relationships were observed between osteocalcin and IGF-I (r = 0.54; $P = 0.006$) and between osteocalcin and IGF-II (r = 0.51; $P = 0.009$) in controls.

No age-related changes were observed in the cortical bone content of calcium. However, a significantly age-dependent increase in protein was observed for the patient population.

Discussion

The cortical bone contents of IGF-I and IGF-II from acromegalic patients with clinically and biochemically active disease were significantly increased compared to those in controls in this study. Increased contents of growth factors in cortical bone have previously been reported in patients with osteoarthritis in relation to slightly increased serum levels of GH (16), and the finding was related to increased bone mass and bone mineral density (17). The chronic excess of GH found in acromegalics is correlated to the increased appositional growth and bone dimensions. GH and IGF-I are well known stimulators of local osteoblastic production of growth factors (18) and stimulate bone cell differentiation, activity, and collagen synthesis in endocrine, autocrine, and paracrine manners (7–11). The excess of GH and the increased serum level of IGF-I are, therefore, probably responsible for the increased cortical bone content of IGF-I found in this study. Our results are thus in accordance with the general description of bone metabolism in acromegaly, with increased bone dimensions and turnover (3–6).

Serum levels of IGF-II have been found to be normal in acromegaly (19, 20). The bone content of IGF-II is markedly higher than that of IGF-I (8), as confirmed in this study. Moreover, the IGF-II content in cortical bone was markedly increased in our patients. The biological activity of IGF-II in bone is, however, 4–7 times less than that of IGF-I (21). Whether the increase in IGF-II in bone is related to systemic alterations in GH metabolism or is due to local alterations is unknown.

IGFBP-3 is also produced in bone tissue secondary to GH

Downloaded from https://academic.oup.com/jcem/article/84/1/123/2866184 by guest on 21 August 2022

and IGF-I stimulation, and its local production is regulated by calciotropic hormones (22). IGFBP-3 enhances the effect of IGF-I on bone (23) and may, in addition, increase the half-life of IGFs by binding and thus protecting the growth factors for later release into the local environment for biological action (8, 24). We did not find significantly increased levels of IGFBP-3 in cortical bone from acromegalic patients in this study. Recent studies have shown that IGFBP-5 is the most prominent binding protein in bone (11), and its expression is enhanced by IGFs and GH (25). IGFBP-5 has a high affinity for hydroxyapatite and may act to fixate IGFs to the skeletal matrix (11). We found significantly increased levels of IGFBP-5 in acromegalic patients, which may result in increased binding capacity and adherence of more IGFs to the bone matrix. *In vitro* studies on mouse osteoblasts (26) and human bone cells (27) have shown that IGFBP-5 can increase and potentiate the effects of IGF-stimulated bone cell proliferation, and that it also may modulate its effects via an IGF-independent mechanism. In addition, osteoblasts possess IGFBP-5-specific binding sites (28), sustaining the concept that IGFBP-5 might mediate the anabolic effects of GH on bone formation. As IGF-II is only minimally GH dependent, the elevated levels of the protein found in this study may be due to the increased binding capacity and growth-potentiating effects provided by elevated levels of IGFBP-5. In support of this, a strong relationship between IGFBP-5 and IGFs and osteocalcin was observed in the patients, but not in controls.

Osteocalcin is the major noncollagenous protein in bone and is implicated in the recruitment and differentiation of bone-resorptive cells. The protein is produced by osteoblasts and binds to hydroxyapatite (29). Serum levels of osteocalcin have been found to be normal in acromegalics (30), whereas other serum markers of bone turnover have been found to be increased (3, 4). However, the serum levels of osteocalcin in acromegalics may be explained by the increased renal blood flow secondary to increased GH and IGF-I levels and thereby the increased renal clearance of the protein (31, 32). Surprisingly, a study on the osteocalcin knockout mouse (33) suggests that the absence of osteocalcin leads to increased bone formation without impairing bone resorption or mineralization. This implies that the significance of the bone content of osteocalcin is virtually unknown.

IGF-I was negatively correlated to age in the patient population, but not in the control group. In accordance with recent studies (34, 35), the female subgroup of the controls showed an age-related decrease in IGF-I when expressed per mg protein ($r = -0.75$; $P = 0.031$). The age-related changes might reflect the age-dependent decrease in IGF-I, also found in the circulation. As the local bone content of IGF-I seems to be related to the regional bone mineral content (36), the results are in accordance with an age-dependent decrease in cortical bone volume and mineral content. IGF-II was not correlated to age in either of the groups, as also found by others (11), whereas IGFBP-3 was positively age related in patients when expressed per mg bone.

Acromegaly is characterized by appendicular skeletal growth leading to increased dimensions of bones (37). Total body calcium is increased in acromegalics (5), and osteodensitometric investigations have shown normal or increased bone mineral densities (38–40). We found an insignificantly increased calcium content of cortical bone, expressed per bone weight unit. Thus, based on this result, total bone mineral content should be increased, with a normal or slightly increased density.

In conclusion, this study shows, for the first time, increased levels of growth factors in cortical bone from acromegalics. This is, presumably, a reflection of local production, secondary to a chronic systemic excess of GH and IGF-I.

Acknowledgments

We are grateful to Mrs. K. Nyborg and K. Mathiassen for their technical assistance.

References

1. **Brixen K, Nielsen HK, Mosekilde L, Flyvbjerg A.** 1990 A short course of recombinant human growth hormone treatment stimulates osteoblasts and activates bone remodeling in normal human volunteers. J Bone Miner Res. 5:609–618.
2. **Bollerslev J, Moller J, Thomas S, Djoseland O, Christiansen JS.** 1996 Dose-dependent effects of recombinant human growth hormone on biochemical markers of bone and collagen metabolism in adult growth hormone deficiency. Eur J Endocrinol. 135:666–671.
3. **Halse J, Gordeladze JO.** 1981 Total and non-dialyzable urinary hydroxyproline in acromegalics and control subjects. Acta Endocrinol (Copenh) 96:451–457.
4. **de la Piedra C, Larranaga J, Castro N, et al.** 1988 Correlation among plasma osteocalcin, growth hormone, and somatomedin C in acromegaly. Calcif Tissue Int. 43:44–45.
5. **Aloia JF, Roginsky MS, Jowsey J, Dombrowski CS, Shukla KK, Cohn SH.** 1972 Skeletal metabolism and body composition in acromegaly. J Clin Endocrinol Metab. 35:543–551.
6. **Halse J, Melsen F, Mosekilde L.** 1981 Iliac crest bone mass and remodelling in acromegaly. Acta Endocrinol (Copenh). 97:18–22.
7. **Hayden JM, Mohan S, Baylink DJ.** 1995 The insulin-like growth factor system and the coupling of formation to resorption. Bone. 17:S93–S98.
8. **Bautista CM, Mohan S, Baylink DJ.** 1990 Insulin-like growth factors I and II are present in the skeletal tissues of ten vertebrates. Metabolism. 39:96–100.
9. **Canalis E, McCarthy T, Centrella M.** 1988 Growth factors and the regulation of bone remodeling. J Clin Invest. 81:277–281.
10. **Mohan S, Baylink DJ.** 1990 Autocrine and paracrine aspects of bone metabolism. Growth Gen Horm. 6:1–9.
11. **Nicolas V, Mohan S, Honda Y, et al.** 1995 An age-related decrease in the concentration of insulin-like growth factor binding protein-5 in human cortical bone. Calcif Tissue Int. 57:206–212.
12. **Mohan S, Baylink DJ.** 1995 Development of a simple valid method for the complete removal of insulin-like growth factor (IGF)-binding proteins from IGFs in human serum and other biological fluids: comparison with acid-ethanol treatment and C18 Sep-Pak separation. J Clin Endocrinol Metab. 80:637–647.
13. **Flyvbjerg A, Kessler U, Dorka B, Funk B, Orskov H, Kiess W.** 1992 Transient increase in renal insulin-like growth factor binding proteins during initial kidney hypertrophy in experimental diabetes in rats. Diabetologia. 35:589–593.
14. **Flyvbjerg A, Mogensen O, Mogensen B, Nielsen OS.** 1997 Elevated serum insulin-like growth factor-binding protein 2 (IGFBP-2) and decreased IGFBP-3 in epithelial ovarian cancer: correlation with cancer antigen 125 and tumor-associated trypsin inhibitor. J Clin Endocrinol Metab. 82:2308–2313.
15. **Gindler EM, King JD.** 1972 Rapid colorimetric determination of calcium in biologic fluids with methylthymol blue. Am J Clin Pathol. 58:376–382.
16. **Dequeker J, Mohan S, Finkelman RD, Aerssens J, Baylink DJ.** 1993 Generalized osteoarthritis associated with increased insulin-like growth factor types I and II and transforming growth factor β in cortical bone from the iliac crest. Possible mechanism of increased bone density and protection against osteoporosis. Arthritis Rheum. 36:1702–1708.
17. **Gevers G, Dequeker J, Geusens P, Nyssen-Behets C, Dhem A.** 1989 Physical and histomorphological characteristics of iliac crest bone differ according to the grade of osteoarthritis at the hand. Bone. 10:173–177.
18. **Stracke H, Schulz A, Moeller D, Rossol S, Schatz H.** 1984 Effect of growth hormone on osteoblasts and demonstration of somatomedin-C/IGF I in bone organ culture. Acta Endocrinol (Copenh). 107:16–24.
19. **Zapf J, Walter H, Froesch ER.** 1981 Radioimmunological determination of insulin like growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. J Clin Invest. 68:1321–1330.
20. **Jorgensen JO, Moller N, Moller J, Weeke J, Blum WF.** 1994 Insulin-like

Downloaded from https://academic.oup.com/jcem/article/84/1/123/2866184 by guest on 21 August 2022

growth factors (IGF)-I and -II and IGF binding protein-1, -2, and -3 in patients with acromegaly before and after adenomectomy. Metabolism. 43:579–583.
21. **McCarthy TL, Centrella M, Canalis E.** 1989 Regulatory effects of insulin-like growth factors I and II on bone collagen synthesis in rat calvarial cultures. Endocrinology. 124:301–309.
22. **Johansson AG, Baylink DJ, af Ekenstam E, Lindh E, Mohan S, Ljunghall S.** 1994 Circulating levels of insulin-like growth factor-I and -II, and IGF-binding protein-3 in inflammation and after parathyroid hormone infusion. Bone Miner. 24:25–31.
23. **Ernst M, Rodan GA.** 1990 Increased activity of insulin-like growth factor (IGF) in osteoblastic cells in the presence of growth hormone (GH): positive correlation with the presence of the GH-induced IGF-binding protein BP-3. Endocrinology. 127:807–814.
24. **Schmid C, Ernst M, Zapf J, Froesch ER.** 1989 Release of insulin-like growth factor carrier proteins by osteoblasts: stimulation by estradiol and growth hormone. Biochem Biophys Res Commun. 160:788–794.
25. **Ono T, Kanzaki S, Seino Y, Baylink DJ, Mohan S.** 1996 Growth hormone (GH) treatment of GH-deficient children increases serum levels of insulin-like growth factors (IGFs), IGF-binding protein-3 and -5, and bone alkaline phosphatase isoenzyme. J Clin Endocrinol Metab. 81:2111–2116.
26. **Mohan S, Nakao Y, Honda Y, et al.** 1995 Studies on the mechanisms by which insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) and IGFBP-5 modulate IGF actions in bone cells. J Biol Chem. 270:20424–20431.
27. **Andress DL, Birnbaum RS.** 1992 Human osteoblast-derived insulin-like growth factor (IGF) binding protein-5 stimulates osteoblast mitogenesis and potentiates IGF action. J Biol Chem. 267:22467–22472.
28. **Andress DL.** 1995 Heparin modulates the binding of insulin-like growth factor (IGF) binding protein-5 to a membrane protein in osteoblastic cells. J Biol Chem. 270:28289–28296.
29. **Hesp R, Tellez M, Davidson L, Elton A, Reeve J.** 1987 Trabecular and cortical bone in the radii of women with parathyroid adenomata: a greater trabecular deficit, with a preliminary assessment of recovery after parathyroidectomy. Bone Miner. 2:301–310.
30. **Ezzat S, Melmed S, Endres D, Eyre DR, Singer FR.** 1993 Biochemical assessment of bone formation and resorption in acromegaly. J Clin Endocrinol Metab. 76:1452–1457.
31. **Delmas PD, Wilson DM, Mann KG, Riggs BL.** 1983 Effect of renal function on plasma levels of bone Gla-protein. J Clin Endocrinol Metab. 57:1028–1030.
32. **Guler H, Schmid C, Zapf J, Froesch ER.** 1989 Effects of recombinant insulin-like growth factor I on insulin secretion and renal function in normal human subjects. Proc Natl Acad Sci USA. 86:2868–2872.
33. **Ducy P, Desbois C, Boyce B, et al.** 1996 Increased bone formation in osteocalcin-deficient mice. Nature. 382:448–452.
34. **Nicolas V, Prewett A, Bettica P, et al.** 1994 Age-related decreases in insulin-like growth factor-I and transforming growth factor-β in femoral cortical bone from both men and women: implications for bone loss with aging. J Clin Endocrinol Metab. 78:1011–1016.
35. **Boonen S, Aerssens J, Dequeker J, et al.** 1997 Age-associated decline in human femoral neck cortical and trabecular content of insulin-like growth factor I: potential implications for age-related (type II) osteoporotic fracture occurrence. Calcif Tissue Int. 61:173–178.
36. **Benedict MR, Ayers DC, Calore JD, Richman RA.** 1994 Differential distribution of insulin-like growth factors and their binding proteins within bone: relationship to bone mineral density. J Bone Miner Res. 9:1803–1811.
37. **Ikkos DG, Ntalles K, Velentzas C, Katsichtis P.** 1974 Cortical bone mass in acromegaly. Acta Radiol Diagn (Stockh). 15:134–144.
38. **Diamond T, Nery L, Posen S.** 1989 Spinal and peripheral bone mineral densities in acromegaly: the effects of excess growth hormone and hypogonadism. Ann Intern Med. 111:567–573.
39. **Seeman E, Wahner HW, Offord KP, Kumar R, Johnson WJ, Riggs BL.** 1982 Differential effects of endocrine dysfunction on the axial and the appendicular skeleton. J Clin Invest. 69:1302–1309.
40. **Ho PJ, Fig LM, Barkan AL, Shapiro B.** 1992 Bone mineral density of the axial skeleton in acromegaly. J Nucl Med. 33:1608–1612.

Downloaded from https://academic.oup.com/jcem/article/84/1/123/2866184 by guest on 21 August 2022