Insulin-like growth factor I increases bone formation in old or corticosteroid treated rats

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We studied bone induction in subcutaneous implants of demineralized bone matrix with or without insulin-like growth factor I (IGF-I) in aged or corticosteroid-treated rats. Each rat carried one pair of implants, one control and one experiment implant, containing IGF-I dissolved in a hyaluronan solution for slow release. The rats were killed after 3 weeks and the results were evaluated by measuring the calcium content of implants. Young (6–7 weeks) and old (19–27 months) rats were used. A group of young rats was treated for 1 week with subcutaneous injections of 140 µg/kg dexamethasone daily. Old rats produced only approximately 1% as much bone as young rats. Local delivery of IGF-I did not increase bone formation in young rats. In old rats, bone formation was increased by IGF-I, 3000 ng/implant. Corticosteroids reduced bone formation in young rats. This effect was partially reversed by local administration of IGF-I.

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Submitted 96-12-19. Accepted 97-06-09

The insulin-like growth factors (IGFs) are abundant in bone matrix. They are produced by osteoblasts and regulate various osteoblast functions, e.g., proliferation and collagen expression (McCarthy et al. 1989). IGF-I stimulates differentiation and proliferation of cultured osteoblast-like cells and induces longitudinal bone growth in hypophysectomized and IGF-I-deficient rats (Loveridge and Farquharson 1993).

Using a bone induction model (Urist 1965), we have previously found IGF-I mRNA to be expressed at an increased level during an early stage of bone formation, suggesting that IGF-1 of auto- or paracrine origin is important (Prisell et al. 1993). In vivo effects of IGFs on bone are controversial since earlier studies have presented both stimulatory and non-stimulatory effects (Aspenberg et al. 1989, Baylink et al. 1993, Eriksen et al. 1993). In the present report, using a bone induction model, we investigated the local effect on bone formation of DBM implants impregnated with IGF-I in a hyaluronan solution.

Animals and methods

Human recombinant IGF-I and human recombinant GH were gifts from Pharmacia AB, Stockholm, Sweden. Iodinated IGF-I was produced by the iodogen method Fraker and Speck (1978). Human recombinant bFGF was obtained from Seios Nova, Mountain View, CA, USA, and hyaluronan (Synvisc®) was a gift from Biomatrix Inc., Ridgefield, NJ, USA. Synvisc® is a hyaluronan gel mixture, which includes cross-linked and noncross-linked hyaluronan.

Femurs and tibias from 6–7-week-old, male Sprague-Dawley rats were collected, prepared on ice and cleansed from soft tissues. Bone marrow was mechanically removed and the cortical bone was rinsed in distilled water, defatted in chloroform-methanol (1:1) for 6 h and decalcified in 0.6 M HCl for 72 h at 4 °C, rinsed in sterile water, lyophilized and weighed (Prisell et al. 1993). The first experiments used 8 mm standardized mid-diaphyseal segments of the femur, dry weight 14.2 (SD 0.6) mg. The implant weight in each pair did not differ by more than 0.5 mg. Each pair of DBM femoral segments from a single donor rat was implanted together into each recipient rat.

Various doses of IGF-I were dissolved in hyaluronan and slowly shaken for 24 h (Prisell et al. 1992). A micrometer-syringe device was constructed that could precisely deliver each dose (17 (SD 0.5) µL) of hyaluronan with or without added peptides into the former marrow cavities of the femoral DBM segment implants. In subsequent experiments, DBM from mid-parts of femurs and tibias was milled and a pow-

der with a granule size of 100–320 μm was collected. The DBM powder was mixed with hyaluronan (ratio 5:1 by weight), and compressed into pellet implants by Q-Med AB, Uppsala, Sweden. The freeze-dried weight of each pellet was 25 (SD 5) mg. When used pairwise in the experiments, the pellets did not differ by more than 1 mg dry weight. The above-described pellets were found to increase by 300% in weight when submerged in water under vacuum. This finding—i.e., the absorption of a water-containing solution into pellets—provided the basis for calculating the various IGF-I amounts in pellets. In practice, stock solutions of IGF-I were made and pellets were submerged in these solutions. After a 10-minute incubation under vacuum in IGF-I solutions or in control solutions (aqua dest), the pellets were taken out and individually placed in a sterile multicavity chamber and thereafter they were freeze-dried and kept at + 4°C until use.

Rats

In total, 193 young (6–7 weeks of age) and 53 old (19–27 months of age) Sprague-Dawley rats were obtained from B & K Universal AB, Stockholm, Sweden and Charles River AB, Uppsala, Sweden. 6 GH-deficient rats, which had been hypohysectomized at 7 weeks of age were delivered from Møllegård A/S, Skensved, Denmark.

The rats were kept under standardized conditions, fed standard diet and water ad lib. and were handled by the staff of the animal department at Huddinge University Hospital.

Hypnorm® Vet. (fentanyl citrate 0.315 mg/mL and fluanisone 10 mg/mL) was used as the anesthetic (0.5 mL/kg B.W. intramuscularly), prophylactic antibiotics wer given as a single subcutaneous dose, 0.2 mL/kg B.W. (Ethacilin Comp.® Vet., containing benzylpenicillin 200 mg/mL and dihydrostreptomycin 200 mg/mL).

The DBM segments or pellets were implanted in the subcutaneous tissue, bilaterally on either side of the abdomen.

Control experiments—evaluation of slow release and experimental models

Slow release properties were tested by mixing ^{125}I-labeled IGF-I with physiological saline, hyaluronan without cross-links (Healon®, 10 mg/mL, Pharmacia AB, Stockholm, Sweden) or hyaluronan (10 mg/mL) with cross-links. These preparations were injected subcutaneously into the dorsal part of the right hind paw in three groups totaling 37 rats. The remaining radioactivity at the site of injection after 8, 25 and 53 hours was determined using a scintillation counter, as previously described (Prisell et al. 1992).

The biological activity of growth hormone (GH) mixed in a hyaluronan solution or physiological saline was evaluated by a subcutaneous injection in hypophysectomized rats, 8 weeks of age. 3 rats received 1 mg GH-hyaluronan and 3 received 1 mg GH-saline. The increase in body weight was measured 12, 24 and 48 hours after the injections.

The effect of hyaluronan without IGF-I was tested in 7 young rats receiving paired DBM segments, of which one in each pair was treated with 17 μL hyaluronan and the other with 17 μL physiological saline. The implants were harvested after 3 weeks, and their respective Ca content was measured.

Intra- versus interanimal variation was determined, by pairwise implantation of DBM pellets containing hyaluronan for 3 weeks, in a group of 13 young rats.

The effect of cortisone was determined in two groups of 5 young rats, which were injected with dexamethasone, 14 or 140 μg/kg B.W. subcutaneously daily for 7 days, following implantation of a DBM pellet without additives. A control group of 6 rats was injected with physiological saline. The implants were harvested after 3 weeks, and their respective Ca contents were measured.

Main experiment—effects of IGF-I in young, old and in corticosteroid-treated young rats

All rats received one experiment and one control implant. The experiment implant contained either IGF-I (or bFGF as a positive control), always with hyaluronan and the control implants contained hyaluronan alone.

43 young rats, divided into 5 groups, received DBM segments with IGF-I 15, 150, 500, 1500 or 3000 ng, respectively. 49 old rats, divided into 3 groups, received DBM segment implants with IGF-I 150, 1500 or 3000 ng. DBM segment implants containing 100 ng bFGF were used in 4 old and 5 young rats.

66 young rats, were given dexamethasone (140 μg/kg Decadron®) subcutaneously for 7 days, beginning at the time of DBM pellet implantation. They were divided into 5 groups and received DBM pellets with IGF-I 0, 10, 100, 1000 or 10000 ng, respectively.

Evaluation of implants

All rats were killed 3 weeks after DBM implantation. Harvested DBM segment implants were ashed in a muffle furnace at 800 °C for 16 h, weighed and dissolved in 6 M HCl. The HCl was evaporated in a vacuum centrifuge. Samples were redissolved in a buffer. Calcium measurement was performed by an automatized colorimetric method at the Laboratory

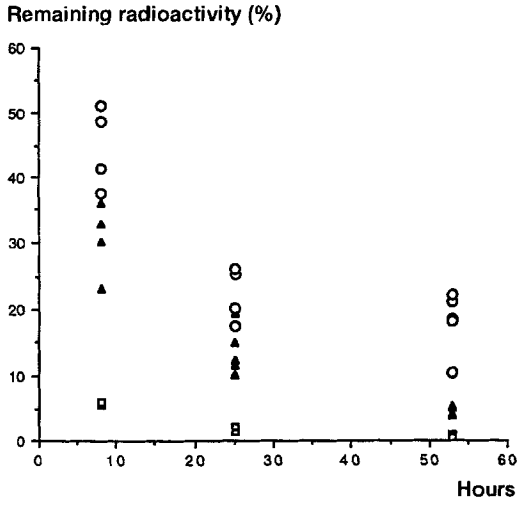

Figure 1. Slow release of ^{125}I-labeled IGF-I from different hyaluronan carriers and saline. Rats received subcutaneous hind paw injections of ^{125}I-labeled IGF-I mixed in hyaluronan (▲), cross-linked hyaluronan (○) or saline (□). The decline of radioactivity from the paw was determined. There were 3–5 rats in each group

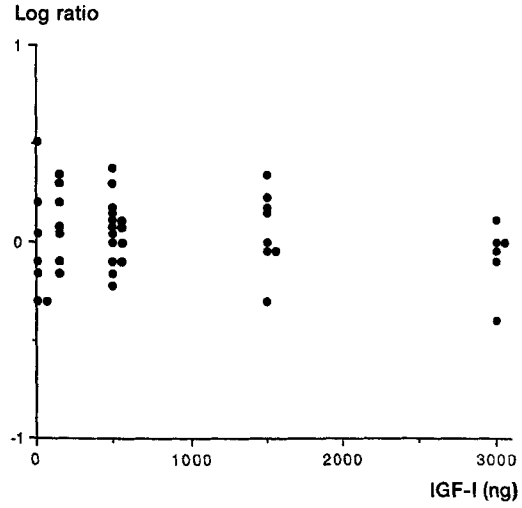

Figure 2. Bone induction in young rats; local effects of IGF-I. Pairwise ratios in calcium contents between DBM segment implants with 15, 150, 1500 or 3000 ng IGF-I and intraanimal controls. A logarithmically transformed ratio higher than zero indicates more calcium in the experimental implant. There were 6–15 animals in each group. Regression analysis of dose-response; r = 0.17, p = 0.28.

for Clinical Chemistry, Lund University Hospital, Lund, Sweden. Selected DBM segments among the old rats were histologically analyzed, as described below. The harvested implants from dexamethasone-treated rats, using DBM pellet implants as well as the selected DBM segment implants from the old rats were histologically evaluated. These implants were first fixed in 4% buffered formalin (3 h), then decalcified in 20 mL citrate (200 g/L) and formic acid (400 g/L). The Ca content of the decalcifying solution was analyzed using an atomic absorption spectrophotometer (Philips, PYE Unicam SP9) (Passey and Maluf 1992).

The local effect of the growth factor in each rat is presented as a pairwise ratio (experimental/control), mean (SEM). A ratio higher than 1 thereby indicates an increased Ca content in the experimental implant. Paraffin sections were stained with hematoxylin and eosin.

Statistical analysis was performed on logarithmically transformed ratios, using regression analysis and one factor ANOVA. The level of significance was set at $p < 0.05$.

Results

Control experiments

Both cross-linked and noncross-linked hyaluronan delayed IGF-I release when injected into the hind paw, compared to controls using IGF-I in saline. Cross-linked hyaluronan delayed the release of IGF-I more than noncross-linked hyaluronan at all evaluated times points (Figure 1).

Table 1. Biological effect of growth hormone dissolved in hyaluronan (HA). 6 hypophysectomized rats were injected with 1 mg GH mixed in 100 µL of either saline or hyaluronan (HA) and weighed at 24 and 48 hours, mean SEM

Treatment	Time (h)	Increase in body weight (g)
GH-saline	24	6.7 0.5
GH-HA	24	11.2 1.5
GH-saline	48	2.0 1.0
GH-HA	48	7.0 0.9

Dissolution of GH in hyaluronan did not impair GH bioactivity in terms of weight gain. On the contrary, in this small-scale experiment, the GH-hyaluronan mixture seemed to enhance body weight gain at 24 and 48 hours, indicating a slow release (Table 1). The addition of hyaluronan to DBM implants had no effect on calcium content, compared to saline containing implants (ratio 1.2 (SD 0.3)).

The pairwise correlation in calcium formation from paired samples (i.e., implanted into the right and left sides of each rat) of DBM pellets without added IGF-I was r = 0.92, p = 0.0001, Ca content ratio 1.1 (SD 0.1) (n 13).

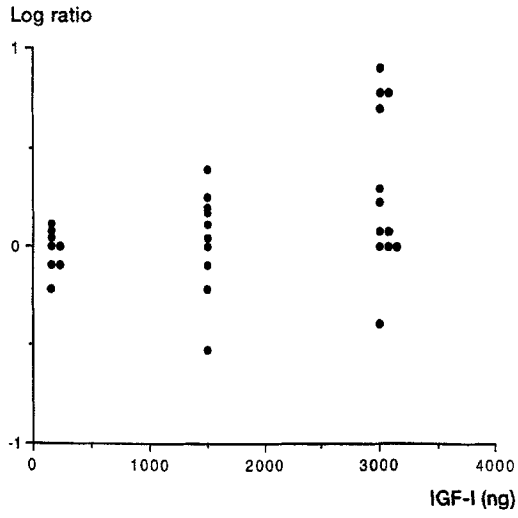

Figure 3. Bone induction in old rats; local effects of IGF-I. Pairwise ratios in calcium contents between DBM segment implants with 150, 1500 or 3000 ng IGF-I and intraanimal controls. A logarithmically transformed ratio higher than zero indicates more calcium in the experimental implant. There were 8–12 animals in each group. Regression analysis of dose-response; r = 0.4, p = 0.03.

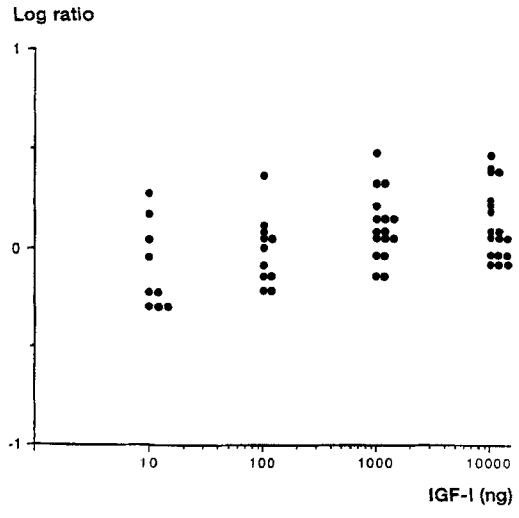

Figure 4. Local effects of IGF-I in dexamethasone-treated young rats. Pairwise ratios in calcium contents between DBM pellet implants with 10, 100, 1000 or 10000 ng IGF-I and intraanimal controls. 140 μg/kg Decadron® was injected subcutaneously for 7 days beginning at DBM implantation. A logarithmically transformed ratio higher than zero indicates more calcium in the experimental implant. There were 9–18 animals in each group. Regression analysis of dose-response; r = 0.4, p = 0.003.

Table 2. Effect of dexamethasone on bone induction in DBM pellet implants. Dexamethasone or saline was given subcutaneously once a day for 7 days following DBM implantation. Calcium content was measured after 3 weeks. Each group consisted of 5 or 6 rats. Mean SEM

Treatment	Calcium content	
Saline	200	18 [a]
Dexamethasone 14 μg/kg	90	28
Dexamethasone 140 μg/kg	82	30

[a] p = 0.01 vs. both dex. groups, respectively.

In the two dexamethasone-treated groups, the mean calcium content was less than half of that in untreated controls; mean 82 (SD 30) and 90 (SD 28), versus controls: 200 (SD 18) μg (p = 0.01, Table 2). There was no difference between the two dexamethasone doses.

Result of main experiments

12 old rats died after implantation. 7 old and 12 dexamethasone-treated rats were excluded due to lack of bone formation (defined as less than 10% of mean Ca content from respective treatment group) in one or both implants.

Young rats showed no effects of IGF-I on calcium content (Figure 2). In old rats, there was a linear dose-response relation; r = 0.4, p = 0.03 (Figure 3), indicating a stimulatory effect of IGF-I. The histological picture displayed bundles of hypertrophic chondrocytes in matrix, thereby disclosing a delayed bone formation process compared to young rats. The mean Ca content from control segments in young and old rats was 104 (SD 8) and 1.4 (SD 0.7) μg, respectively.

Basic fibroblast growth factor increased Ca content in both old and young rats with a mean ratio between experimental and control implants of 1.7 (SD 0.3), p = 0.04. No difference between young and old rats was found.

The negative effect of systemic corticosteroid treatment on calcium content was partially overcome by 1 and 10 μg IGF-I, both with a Ca content ratio of 1.4 (SD 0.2). There was a linear dose-response relation; r = 0.4, p = 0.01 (Figure 4). The histological picture displayed bone formation, but no attempts were made to quantify differences histologically.

Discussion

Expression of IGF-I is multifactorially regulated in a tissue-specific manner. Subsequently, effects of exogenous IGF-I may need to be examined under conditions where IGF-I, as opposed to systemic, is delivered locally. As a slow-release vehicle for IGF-I, we used hyaluronan, a high molecular weight glucosaminoglycan with repetitive units of N-acetylglucosamine and glucuronic acid. This is a naturally oc-

curring substance—e.g., in synovial fluid and skin. Hyaluronan has proved to be a useful vehicle for growth factors, since dissolution of IGF-I in hyaluronan generates a slow-release preparation (Prisell et al. 1992). Although several other carrier options exist that will retard release of peptides—e.g., carboxymethyl cellulose (Aspenberg and Lohmander 1989)—we considered hyaluronan a suitable vehicle because adverse effects in inflammatory responses are minimal. We have also shown that IGF-I and GH, dissolved in hyaluronan, remained bioactive. In addition, other chemical and biological properties of hyaluronan make it a useful vehicle. The release properties of peptides dissolved in hyaluronan depend on the hyaluronan concentration (Prisell et al. 1992) and presence of cross-links. Furthermore, hyaluronan is locally enriched in the early stages of wound and fracture healing (Hulth 1989).

The bone formation and mineralization process can be evaluated by the alkaline phosphatase level, ^{45}Ca incorporation into bone mineral and calcium content in sampled implants (Reddi and Huggins 1972, Reddi and Huggins 1975, Reddi and Anderson 1976, Howes et al. 1988). In order to monitor the bone formation process after various local deposits of IGF-I, an analysis of each bone-forming sample's Ca content therefore seems adequate. The atomic absorption analytic method combines high specificity with good sensitivity (Passey and Maluf 1992) and allows histological analysis to ensure that endochondral bone formation occurs and not calcification of other origin.

Bone induction in DBM implants provides certain experimental advantages regarding studies on local delivery of peptides, since a single individual experimental animal is given both a control and an experimental, e.g., IGF-I containing, implant. This is used to compensate for individual variations in osteogenic competence and bone induction capacity (Aspenberg and Lohmander 1989). The particle size of DBM implants is also important. Subcutaneous implantation of coarse bone matrix powder, size 74–420 μm, resulted in endochondral bone formation, whereas implantation of similar powder, size 44–74 μm, did not (Sampath and Reddi 1984).

One reason for attempting to influence bone formation by IGF-I was our earlier finding of a transient increase in IGF-I mRNA 3 days after implantation of DBM (Prisell et al. 1993). In the present study, we found no effect of exogenously added IGF-I in young rats. One interpretation of this finding is that a young rat forms bone under a condition where the endogenous IGF-I system is already turned on to an optimal degree. Therefore, we turned our attention to conditions where endogenous activation of IGF-I could be reduced. One such condition is endochondral bone formation in old rats, where we have previously shown that the level of IGF-I mRNA is reduced (Prisell et al. 1993). The bone-inductive capacity of DBM as well as the response to DBM implants in rats decreases with age (Irving et al. 1981, Reddi 1985). Irving et al. (1981) found similar implant reactions and sequences of bone-forming events when testing rats 6 weeks, 6 months and 2 years of age. However, the endochondral bone formation in 2-year-old rats was markedly delayed compared to younger rats; only 5 of 10 rats had bone formation at 25 days after implantation. Even after 45 days, not all rats of that age showed calcification.

In the present study, local administration of IGF-I to aged male rats increased endochondral bone formation. The finding that local delivery of IGF-I enhances bone formation in old but not in young rats may also have other explanations than a relative inability of old rats to activate local IGF-I production, considering the endocrine and nutritional differences that exist between young and old rats, which, e.g., was revealed by a weight loss in the majority of old rats during the experimental period. Serum concentrations of growth hormone and IGF-I as well as the bone content of IGF-I decline with age (D'Costa, et al. 1993, Nicolas et al. 1994).

In the present study, bone formation in old rats was only approximately 1% of that in young rats. However, it was stimulated in a dose-dependent response fashion by local addition of IGF-I. The low calcium content can be explained by the delay in bone formation in old rats (Irving et al. 1981). This was histologically revealed by the relatively large amounts of hypertrophic chondrocytes. Howes et al. (1988) found a similarly age-related response, using platelet-derived growth factor (PDGF) in a similar model. On the other hand, Aspenberg and coworkers found a positive effect of local bFGF in a similar DBM bone induction model in young rats (Aspenberg and Lohmander 1989, Aspenberg et al. 1991, Aspenberg and Wang 1993). In the present study, bFGF (100 ng) increased bone formation in both young and old rats. This might be due to different regulatory mechanisms of bFGF, compared to IGF-I (D'Costa et al. 1993, Ezzat et al. 1995, Ku and D'Amore 1995).

The positive effects of bone formation in old rats encouraged us to test other situations where formation of bone is compromised. Well established agents that reduce bone formation are corticosteroids and non-steroid anti-inflammatory drugs (NSAID) (Törnkvist et al. 1985a, b, DiCesare et al. 1991). Corticosteroid mediates anti-inflammatory effects, like depletion of the prostaglandin synthesis (Topert 1988), growth

inhibitory properties and effects on cellular differentiation, all of which are relevant to the formation of new bone. The prostaglandin PGE_2 is known to stimulate bone formation and bone marrow cells both in vivo and in vitro (Scutt and Bertram 1995) and also to stimulate IGF-I synthesis in osteoblast cultures from fetal rat bone (McCarthy et al. 1991). Corticosteroids increase plasma levels of insulin-like growth factor binding protein-1 (IGFBP-1) in humans (Conover et al. 1993). Insulin-like growth factor-I-BP-1 has mostly been found to inhibit IGF activity (for review, see Jones and Clemmons 1995).

Our findings indicate that IGF-I can stimulate endochondral bone formation in both corticosteroid-treated and aged rats. Local application of IGF-I may therefore counteract the inhibitory effects on bone formation from age and corticosteroids, which cause clinical problems in many divergent fields of medicine.

Acknowledgements

We are indebted to Ms. Marina Nyborg for assistance and help during the animal experiments, to Ms. Kristina Jønsson, Ms. Ulla Svahn for skilful technical assistance and to Dr. Elisabeth Berg, Deparmtnets of Medical Information and Educational Development at the Karolinska Institute for Statistical Counselling. We are also indebted to Dr. Bengt Ågerup at Q-Med AB, Uppsala, Sweden, for providing us with pellet implants, technical skill and know-how and to Professor Lars Hammarström for generous support and hospitality. This study was supported by grants from the Ulla and Gustaf af Uggla Foundation, the Swedish Medical Research Council (No. 8556) and the "Förenade Liv" Mutual Group Life Company.

References

Aspenberg P, Lohmander L S. Fibroblast growth factor stimulates bone formation. Bone induction studied in rats. Acta Orthop Scand 1989; 60: 473-6.

Aspenberg P, Wang J-S. Basic fibroblast growth factor and bone induction in rats. Acta Orthop Scand 1993; 5: 557-61.

Aspenberg P, Albrektsson T, Thorngren K G. Local application of growth factor IGF-1 to healing bone. Experiments with a titanium chamber in rabbits. Acta Orthop Scand 1989; 60: 607-10.

Aspenberg P, Thorngren K G, Lohmander L S. Dose-dependent stimulation of bone induction by basic fibroblast growth factor in rats. Acta Orthop Scand 1991; 62: 481-4.

Baylink D J, Finkelman R D, Mohan S. Growth factors to stimulate bone formation. J Bone Miner Res (Suppl 2) 1993; 8: 565-72.

Conover C A., Divertie G D, Lee P D. Cortisol increases plasma insulin-like growth factor binding protein-1 in humans. Acta Endocrinol 1993; 128: 140-3.

D'Costa A P, Ingram R L, Lenham J E, Sonntag W E. The regulation and mechanisms of action of growth hormone and insulin-like growth factor 1 during normal ageing. J Reprod Fertil Suppl 1993; 46: 87-98.

DiCesare P E, Nimni M E, Peng L, Yazdi M, Cheung D T. Effects of indomethacin on demineralized bone-induced heterotopic ossification in the rat. J Orthop Res 1991; 9: 855-61.

Eriksen E F, Kassem M, Brixen K. Growth hormone and insulin-like growth factors as anabolic therapies for osteoporosis. Horm Res 1993; 40: 95-8.

Ezzat S, Smyth H S, Ramyar L, Asa S L. Heterogenous in vivo and in vitro expression of basic fibroblast growth factor by human pituitary adenomas. J Clin Endocrinol Metab 1995; 80: 878-84.

Fraker P J, Speck J C, Jr. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphrenylglycoluril. Biochem Biophys Res Commun 1978; 80: 849-57.

Howes R, Bowness J M, Grotendorst G R, Martin G R, Reddi A H. Platelet-derived growth factor enhances demineralized bone matrix-induced cartilage and bone formation. Calcif Tissue Int 1988; 42: 34-8.

Hulth A. Current conceps of fracture healing. Clin Orthop 1989; 249: 265-84.

Irving J T, LeBolt S A, Schneider E L. Ectopic bone formation and aging. Clin Orthop 1981; 154: 249-53.

Jones J I, Clemmons D R. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; 16: 3-34.

Ku P T, D'Amore P A. Regulation of basic fibroblast growth factor (bFGF) gene and protein expression following its release from sublethally injured endothelial cells. J Cell Biochem 1995; 58: 328-43.

Loveridge N, Farquharson C. Studies on growth plate chondrocytes in situ: cell proliferation and differentiation. Acta Paediatr Supplement 1993; 391: 42-8.

McCarthy T L, Centrella M, Canalis E. Insulin-like growth factor (IGF) and bone. Connect Tissue Res 1989; 20: 277-82.

McCarthy T L, Centrella M, Raisz L G, Canalis E. Prostaglandin E2 stimulates insulin-like growth factor I synthesis in osteoblast-enriched cultures from fetal rat bone. Endocrinology 1991; 128: 2895-900.

Nicolas V, Prewett A, Bettica P, Mohan S, Finkelman R D, Baylink D J, Farley J R. Age-related decreases in insulin-like growth factor-I and transforming growth factor-beta in femoral cortical bone from both men and women: implications for bone loss with aging. J Clin Endocrinol Metab 1994; 78: 1011-6.

Passey R B, Maluf K C. Foundations for validation of quantitative analytical methods in the clinical laboratory. Arch Pathol Lab Med 1992; 116: 732-8.

Prisell P T, Camber O, Hiselius J, Norstedt G. Evaluation of hyaluronan as vehicle for peptide growth factors. Int J Pharm 1992; 85: 51-6.

Prisell P T, Edwall D, Lindblad J B, Levinovitz A, Norstedt G. Expression of insulin-like growth factors during bone induction in rat. Calcif Tissue Int 1993; 53: 201-5.

Reddi A H. Age-dependent decline in extracellular matrix-induced local bone differentiation. Isr J Med Sci 1985; 21: 312-3.

Reddi A H, Anderson W A. Collagenous bone matrix-induced endochondral ossification hemopoiesis. J Cell Biol 1976; 69: 557-72.

Reddi A H, Huggins C. Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. Proc Natl Acad Sci U S A 1972; 69: 1601-5.

Reddi A H, Huggins C B. Formation of bone marrow in fibroblast-transformation ossicles. Proc Natl Acad Sci U S A 1975; 72: 2212-6.

Sampath T K, Reddi A H. Importance of geometry of the extracellular matrix in endochondral bone differentiation. J Cell Biol 1984; 98: 2192-7.

Scutt A, Bertram P. Bone marrow cells are targets for the anabolic actions of prostaglandin E2 on bone: induction of a transition from nonadherent to adherent osteoblast precursors. J Bone Miner Res 1995; 10: 474-87.

Topert M. Perspectives in corticosteroid research. Drugs 1988; 5: 1-8.

Törnkvist H, Bauer F C, Lindholm T S, Nilsson O S. The osteo-inductive properties of bone matrix from rats pretreated with indomethacin. Scand J Rheumatol 1985a; 14: 197-200.

Urist M R. Bone: formation by autoinduction. Science 1965; 150: 893-9.