GROWTH HORMONE:

AGING

and

OSTEOPOROSIS

Medical Grand Rounds
University of Texas Southwestern
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May 21, 1992

INTRODUCTION

Until recently, the use of Growth Hormone (GH) has been confined to the treatment of GH-deficient children. Expense and limited availability of GH has largely been responsible for its restricted use. In addition, the association of Creutzfeld-Jakob disease in patients treated with GH obtained from human pituitary extracts halted its use in clinical studies in the 1980's when the National Pituitary Agency discontinued distribution of human-GH.[1,2,3] The study of the physiology of GH and its potential therapeutic application is made difficult by the diurnal and pulsatile nature of GH release, making a single measurement insufficient to assess overall GH secretion.[4,5] The most accurate method to measure GH remains an area of contention.[6,7]

In 1985, GH produced by recombinant DNA technology was approved by the Food and Drug Administration for the long-term treatment of children with growth failure due to a lack of adequate endogenous GH secretion. Today, this remains the only approved indication for GH use. Recombinant human growth hormone (rhGH) has resolved the problem of infectious contamination and provides an unlimited supply of GH. Presumably with time, the cost of treatment will be reduced from its present price range of $10,000 to $20,000 a year.

The increased availability of rhGH has made possible studies of other potentially beneficial uses of GH and its physiologic actions (see table 1). Over the coming years it can be anticipated that considerable research activity will be undertaken to study the physiology and possible therapeutic role of this hormone and its mediator insulin-like growth factor. At the same time, the use of GH in some circumstances, such as short non-GH-deficient children to augment linear growth, has raised clinical, ethical, and financial concerns regarding its use.[8,9] Similar concern and caution has been raised regarding GH therapy in the elderly.[10,11,12]

Table 1

Potential Uses of Growth Hormone in Adults	
Osteoporosis	Immunodeficiency
Turner's Syndrome	Surgical Patients
Infertility	Malnutrition
Renal Failure	Burn Patients

Considerable attention was paid by the lay and medical community to a study reported several years ago in the New England Journal of Medicine reporting that GH seemed to reverse a variety of physical signs of aging.[13] This inspired a health care writer following these developments to title a subsequent article: "Human growth hormone: The Fountain of Youth?"[14] Unfortunately, at the time of this writing the "Fountain of Youth" remains elusive.

One area of interest where GH may play a therapeutic role is in the treatment of osteoporosis. To introduce some of the issues at hand involving aging, osteoporosis and the possible role for GH, a clinical case report will be presented. This review will then provide a brief overview of normal GH physiology and a discussion of age-related changes in GH and insulin-like growth factor I (IGF-1) axis and how they may relate to a number of age-related physiological changes. Lastly, I will review the present evidence for (or against) a possible therapeutic role for GH/IGF-1 in the treatment of age-related (senile) osteoporosis.

CASE PRESENTATION

GW is a 43 year old white woman with Werner's syndrome who was referred for evaluation. Werner's syndrome is a rare autosomal recessive disorder which has many clinical features resembling advanced age. Although it would be erroneous to describe this condition as a disorder of premature aging, it could be more aptly characterized as a "caricature of aging."[15] Patients with this disease commonly develop cataracts, atherosclerosis, malignancies, and osteoporosis.[15] Because of the occurrence of these medical illnesses common to the elderly, Werner's Syndrome has been viewed with interest as possibly providing insight into age-related medical problems.

The patient's childhood was unremarkable except for graying of her hair and mild hair loss in high school. Her maximum height was 4'10". She married after high school and had two miscarriages in her late twenties. Menopause occurred at 31 years (menarche at age 9) and she was started on conjugated estrogens and progesterone a few months after her last menstrual period. At the age of 39 and 40 years she underwent extraction of posterior subcapsular cataracts in each eye. She denied using steroid eye drops or chronic oral steroid therapy. At 42 years of age she underwent surgery for the removal of "calcium deposits" on the back of her heels. During the past few years she has noted a progressive loss of skin and tissue under her feet. Six months before evaluation, she was diagnosed as having scleroderma by one physician but this diagnosis could not be confirmed by another physician due to the lack of clinical and immunological evidence. The patient had no history of Raynaud's phenomena, esophageal dysmotility, peripheral joint swelling or telangiectasias. The diagnosis of Werner's syndrome was made by the presence of typical features of the disease, including short stature, hypogonadism, early graying and alopecia, cataracts, high pitched squeaky voice, loss of subcutaneous fat and hyperkeratosis predominately affecting the feet, and soft tissue calcifications.[15,16,17] The patient has no other significant past medical history.

The patient was evaluated at the outpatient General Clinical Research Center. On physical examination the features described above were confirmed. X-rays of her spine and hips revealed marked osteopenia throughout the bones with generalized compressions of almost all thoracic and lumbar vertebrae.

In order to fully evaluate her osteoporosis, the patient was admitted to the inpatient General Clinical Research Center. Bone density (BD) measurement of the distal 1/3 of the radius (by single photon absorptiometry) was 0.628 gm/cm^2 measurements or 1.98 standard deviations below the age- and sex-matched control value. Bone density of L2-L4 spine (Hologic QDR) and right femoral neck (Hologic QDR) was 0.776 gm/cm^2 and 0.441 gm/cm^2 respectively. These values correspond to 2.38 and 3.93 standard deviations below

normal for age- and sex-matched control subjects at the lumbar spine and femoral neck respectively. The patient was placed on a fixed diet containing 800 mg Ca, 800 mg P, and 100 meq sodium/day. Serum calcium (9.4 mg/dl), phosphorus (3.8 mg/dl), glucose (105 mg/dl), alkaline phosphatase (82 IU), parathyroid hormone (31 pg/ml intact assay), thyroid hormone, 25-OH-vitamin D (35 ng/ml), $1,25(OH)_2$ vitamin D (41 pg/ml), serum estradiol, serum and urine protein electrophoresis were normal. Serum osteocalcin measured 2.7 ng/ml (normal 2.5-4.0). Twenty four hour urine for 17-hydroxycorticoids, hydroxyproline (21 mg), and protein were normal. Twenty four hour urinary calcium was 134 mg and calculated glomerular filtration rate was 61 cc/min. Urine sediment on routine analysis was normal. Serum creatinine was 0.6 mg/dl. Intestinal fractional calcium absorption determined by fecal recovery of orally administered ^{47}Ca was low normal at 40.7% (normal 40 to 60%).[6] Serum insulin-like growth factor (IGF-1) was low, measured at 86 ng/ml (normal for the patient's age 141.8 to 389.3). Because of this finding, growth hormone was assessed by arginine and L-dopa stimulation test.[18] Growth hormone release was demonstrated to be normal. Baseline serum growth hormone was 3.0 ng/ml and rose to 6.6 ng/ml 60 minutes after stimulation and returned to baseline 120 minutes after stimulation.

A trans-cortical iliac crest bone biopsy was obtained after tetracycline labeling and histomorphometric analysis was performed.[19] Although trabecular bone volume and mean trabecular thickness were reduced, the most striking finding was the very low osteoid volume and lack of any identifiable osteoblasts in the histological section. Moreover, there was absence of any double labels in the cancellous bone (as well as in the cortical bone) and very reduced uptake of tetracycline as a single label. Taken together, this histomorphometric data was consistent with a suppressed bone formation rate in the face of normal bone resorption rates.

Our findings show that the osteoporotic process in this patient is due to decreased bone formation and involves both cortical and trabecular bone similar to that described in age-related or senile osteoporosis. It is known that either excessive bone resorption or inadequate or reduced bone formation can result in osteoporosis. Although considerable individual variation and overlap exists, impaired bone formation is believed to characterize age-related (type II) osteoporosis.

Of interest, this patient had a low serum IGF-1 which is known to be important for bone formation.[20] Although her creatinine clearance is lower than expected for her age, vitamin D metabolism and PTH were normal, intestinal calcium absorption was low normal and her calculated net calcium balance was positive. Serum parameters that reflect bone turnover and resorption were all normal and markers of bone formation were low normal or reduced, in all, consistent with the histomorphometric findings suggesting reduced bone formation in the setting of normal bone resorption.

In summary, our evaluation suggests that inadequate bone formation was responsible for the development of osteoporosis in this patient with Werner's syndrome. The pathophysiology is similar to what we would anticipate in a patient suffering from age-related osteoporosis. In view of her low serum IGF-1, the potential therapeutic role of agents such as human growth hormone or IGF-1 is of particular interest.

REVIEW OF NORMAL BONE REMODELING

Bone is a dynamic tissue that undergoes continuous remodeling throughout life. To understand the pathophysiology of osteoporosis it is important to appreciate the normal sequence of bone remodeling. The basic unit that is responsible for remodeling is known as the Bone Remodeling Unit (BRU). Bone remodeling occurs in a highly ordered manner in which first bone resorption is followed by bone formation.

This process is tightly coupled so that under normal circumstances the degree of resorption and formation are equal and there is no net change in bone volume or mass. The first step is the activation of surface lining cells that probably respond to bone resorbing hormones and release proteolytic enzymes. These enzymes allow access to the mineralized bone by osteoclasts. Osteoclasts then resorb bone by dissolving bone mineral and degrading bone matrix resulting in the formation of a cavity. Osteoclasts are then replaced by osteoblasts which fill the cavity with an organic matrix (osteoid) composed primarily of collagen. This new bone is then mineralized. The entire process can take three to four months.
This highly complex sequence of events (overly simplified here) is mediated by calcium-regulating hormones, systemic hormones, local (paracrine) factors as well as mechanical and electrical forces[21] (see table 2).

Table 2

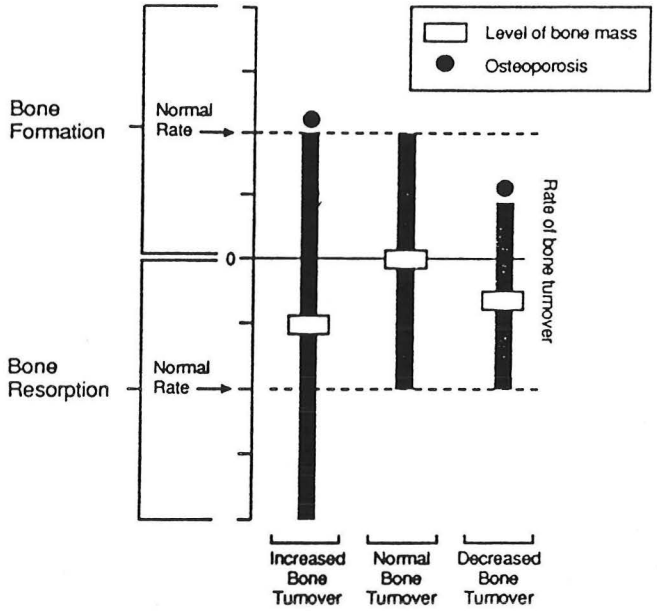
Some Factors Influencing Bone Metabolism

	ON RESORPTION	ON FORMATION
Calcium-regulating hormones		
Parathyroid hormone	+	+
1,25 Dihydroxyvitamin D	+	-.(+)
Calcitonin	-	0
Systemic hormones		
Growth hormone	0	(+)
Glucocorticoids	(+)	-
Thyroid hormones	+	+
Insulin	0	+
Estrogens	(-)	(-)
Local factors		
Prostaglandin E_2	+	+
Interleukin-1	+	-.(+)
Interferon-α	-	-
Insulin-like growth factor 1	0	+
Transforming growth factor-β	-.(+)	+

It is important to remember that bone resorption is closely coupled (presumably by local factors) to bone formation in each bone remodeling unit. If this relationship is altered we refer to it as the uncoupling of bone resorption and formation. In patients who have normal bone resorption but increased bone formation there is a net increase of bone mass and this describes the circumstance seen in normal growth and development. In osteoporosis there is a reduction of bone mass due to a proportionately greater amount of bone resorption than formation (see figure 1).

Figure 1

BONE-FORMATION/ BONE-RESORPTION COMBINATIONS



REVIEW OF THE PHYSIOLOGIC AND CLINICAL DIFFERENCES BETWEEN TYPE I (POSTMENOPAUSAL) AND TYPE II (AGE-RELATED) OSTEOPOROSIS

Riggs has postulated at least two distinct syndromes of involutional osteoporosis, type I or "postmenopausal" osteoporosis, usually affecting women within 15 to 20 years after menopause (51-75yrs), and type II or "age-related" osteoporosis.[22,23,24]

Table 3

The Two Types of Involutional Osteoporosis

	Type I	Type II
Age (yr)	51-75	>70
Sex ratio (F:M)	6:1	2:1
Type of bone loss	Mainly trabecular	Trabecular and cortical
Rate of bone loss	Accelerated	Not accelerated
Fracture sites	Vertebrae (crush) and distal radius	Vertebrae (multiple wedge) and hip
Parathyroid function	Decreased	Increased
Metabolism of 25-OH-D to $1,25(OH)_2D$	Secondary decrease	Primary decrease
Main causes	Factors related to menopause	Factors related to aging

Patients with type I osteoporosis predominantly suffer from compression fractures of the spine, and distal forearm ("Colles"). The primary etiology is thought to be estrogen deficiency. Estrogen has an inhibiting effect to the bone mobilizing influence of parathyroid hormone. In its absence, parathyroid hormone (PTH) increases bone turnover and mobilization of calcium from bone. The enhanced release of calcium from bone

subsequently lowers serum PTH which secondarily reduces the renal 1 alpha-hydroxylation of 25-hydroxyvitamin D so that gastrointestinal absorption of calcium is impaired.[23] Immunoreactive parathyroid hormone levels have been found to be lower in patients with type I osteoporosis then age matched controls.[23] Trabecular bone is particularly susceptible to estrogen deficiency and the predominant site of bone loss involves those bones with a high percentage of trabecular bone, namely the vertebral body and distal extremity. Perforation and resorption of trabecula lead to decreased "connectivity" of bone leading to structural weakness and resulting in fractures when exposed to minor forces or at times spontaneously. In general, type I osteoporosis is thought to be a high turnover state but histomorphometric studies have shown bone turnover to be high in about 25%, normal in about 45%, and low in 30%. It has been postulated in those individuals that present with a normal or low bone turnover picture may have reached a "burned out" state.[23] Although estrogen deficiency appears to be the major contributing factor in the development of type I osteoporosis there are other important factors that must be involved since not all postmenopausal women develop this disease and yet have similar estrogen levels.[25]

Type II osteoporosis affects individuals over 70 years and although it predominantly affects women, the female to male ratio (2:1) is much closer than the 6:1 ratio found in type I. The site of fractures are mainly the hip and vertebral body and less frequently the proximal humerus, tibia and pelvis. The most significant morbidity and mortality result from hip fractures. In the spine patients may be more susceptible to wedge fractures that may lead to the dorsal kyphosis commonly referred to as a dowager's hump. This is in contrast to the typical "crush" compression fracture noted in type I osteoporosis. The pathologic process in type II osteoporosis is believed to result in a low bone turnover state that results in the gradual thinning of trabecula as well as the cortices. Eventually the decline in bone mass falls to a point in which the bone strength is reduced to a level below the fracture threshold so that minor trauma results in fracture.

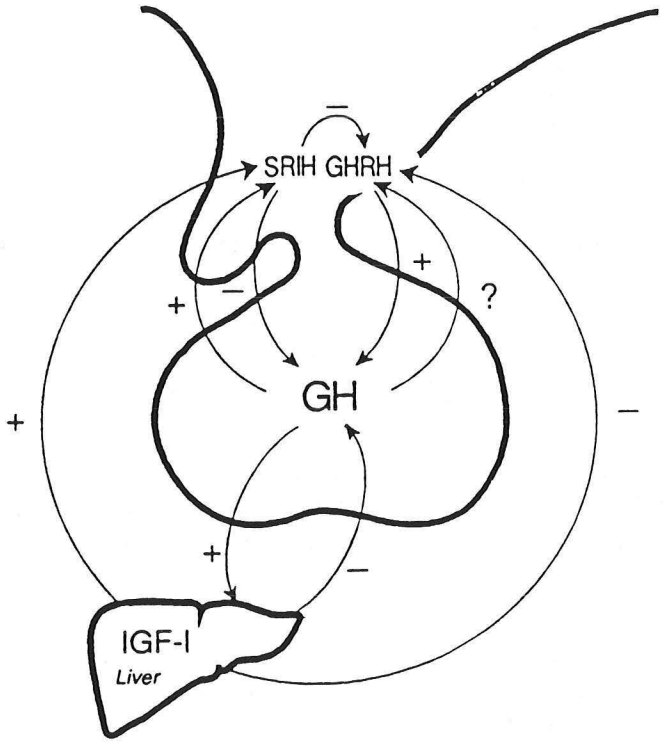
As opposed to the predominant trabecular bone loss seen in type I osteoporosis, both cortical and trabecular bone are affected in type II. The present biochemical model of changes thought to be responsible for the development of type II osteoporosis is related to the aging kidneys impaired ability to synthesize 1,25-$(OH)_2$ vitamin D because of an age-related decrease in renal 1-alpha hydroxylase activity.[26,27] The fall in 1,25-$(OH)_2$ vitamin D results in impaired intestinal absorption of calcium and phosphorus. The resulting decrease in intestinal calcium absorption stimulates PTH secretion which leads to further bone resorption especially in the absence of estrogen. Parathyroid hormone tends to affect cortical bone to an even greater degree than trabecular bone possibly playing a role in the increased incidence of hip fractures noted.[28] Osteoblasts are also known possess 1,25-$(OH)_2$ vitamin D receptors suggesting that 1,25-$(OH)_2$ vitamin D may play a direct role in the regulation of osteoblast function.

Perhaps more importantly, there appears to be an age-related decrease in osteoblast function which may play a role in uncoupling of bone remodeling. Since under the appropriate stimuli osteoblast response is normal, factors other then senescence are probably responsible. An alteration in one of the many local growth factors may play a role in uncoupling bone remodeling. Since the secretion of GH and IGF-1 has been shown to decrease in the elderly, these factors and/or age-related changes in IGF-1 binding proteins which modulate mitogenic activity of IGF-1, may contribute to a decrease in osteoblastic activity or renewal in elderly and osteoporotic patients.[21,29]

GROWTH HORMONE

Growth hormone is a single peptide chain of 191 amino acids produced in the adenohypophysis with its release regulated by the stimulatory and inhibitory action of two hypothalamic hormones: Growth Hormone Releasing Hormone (GHRH) and Somatostatin (SRIH), respectively. The release of GH fluctuates throughout the day and is secreted in pulses, which are most closely related to sleep and not to clock time.[30] Random measurements of GH are therefore of little value. Clinically, GH secretion is assessed by measuring stimulated GH levels after a provocative test such as insulin induced hypoglycemia or by L-dopa, arginine, or clonidine stimulation.[18,31] Endogenous GH secretion can also be assessed by obtaining plasma samples and measuring GH every 20 minutes for 12 to 24 hours.[4,6] The mechanism of regulatory control of GH secretion is believed to take place at both the pituitary and hypothalamic level (see figure 2). Multiple factors (endocrine, metabolic and neural) are operative in determining GHRH and SRIH secretion.[29,32] A variety of clinical states such as chronic illness, adiposity, circulating sex steroids, starvation and age can affect GH secretion.[4,33,34]

Figure 2



Schematic drawing of the GH regulatory system. (+) indicates stimulatory effect; (−) indicates inhibitory effect.

The essential role GH plays in children by promoting linear growth is well known as are the clinical sequelae due to GH deficiency. In adults, the physiological importance of GH is less clear and as yet there is no clinical syndrome of GH deficiency. GH is known to have both lipolytic and anabolic properties which appear to be mediated by IGF-1.[35,36,37] (See table 4). Insulin-like growth factor I is a single chain polypeptide of 70 amino acid residues and has about 50% structural homology to proinsulin.[38,39] Previously referred to as somatomedin C (SmC), IGF-1 and SmC are identical. IGF-1 is produced in the liver in

response to GH and appears to act on most tissue except the CNS. The identification of IGF-1 receptors in multiple extra-hepatic tissues suggests this growth factor has important paracrine and autocrine role as well as its known endocrine role.[38,40,41,42] Due to the physiologic relation of IGF-1 and GH and the formers stable daily plasma concentration, it appears to provide a reliable indicator of growth hormone secretion but considerable overlap was found in one study among normal and GH deficient children.[6,13,43,44]

Table 4

EFFECTS OF AGING AND OF GH ON SELECTED PARAMETERS OF BODY COMPOSITION AND FUNCTION

	Aging	GH*
Nitrogen balance	↓	↑
Body content of protein	↓	↑
Muscle mass as percentage of body weight	↓	↑
Body content of K	↓	↑
Bone mass	↓	↑
Body content of H_2O	↓	↑
Body content of lipid	↑	↓
Liver, spleen, pancreas size	↓	↑
Albumin synthesis	↓	↑
Cellular immunity	↓	↑
Renal Blood Flow, glomerular filtration	↓	↑

* *Direct and IGF mediated.*
(↑) *indicates increase.*
(↓) *indicates decrease.*

AGE-RELATED CHANGES IN BODY COMPOSITION

The composition of the human body changes with advancing age.[37] Lean body weight progressively decreases while adiposity increases. For example, in a healthy 30 year old women, about 65% of the body weight is fat free (mostly muscle) and 35% fat. At 80 years old a women's body weight is approximately 47% fat free and 53% fat. For men the percentage of fat is lower and lean mass higher but the relative changes over time are the same, that is, the percent of adipose tissue nearly doubles while lean mass falls almost 50% (muscle weight as a percentage of body weight changes from 30 to 15%). The mass of most organ systems change with age.

Reductions in the size of liver, spleen, and kidney vary from 20 to 50%. Reduced hepatic blood flow and reported changes in the microsomal mixed function oxidase enzyme system may have important effects on the pharmacokinetics of a variety of drugs. Changes in spleen size and shrinkage of germinal centers may be related to or reflect a variety of immunological changes associated with aging.[45,46]

With age there is a reduction in the glomerular filtration rate, renal blood flow and several tubular functions. Plasma levels of $1,25(OH)_2$ vitamin D tend to decline with aging. This is due to the aging kidneys impaired ability to synthesize $1,25\text{-}(OH)_2$ vitamin D because of an age-related decrease in renal 1-alpha hydroxylase activity. The fall in $1,25\text{-}(OH)_2$ vitamin D results in impaired intestinal absorption of calcium and phosphorus.

Bone mass also declines with age. Riggs found that the predicted mean spinal bone mineral content in women at age 90 was 47% less than at age 20 years. Bone mineral content of

the peripheral skeleton also falls but the changes are smaller. Decline in bone mineral content with age occur in men but to a much lesser degree.[47] An age-related loss of bone is largely believed due to reduced bone formation from altered osteoblastic cell activity. In 50-year-old men bone formation averages about 2% of bone mass per year and bone resorption 2.2%.[48]

AGE-RELATED CHANGES IN GROWTH HORMONE/IGF-1 AXIS

In 1981, Rudman reported progressive decline in GH secretion so that by the eighth decade 55% of healthy individuals had low IGF-1 levels which correlated with low peak waking and sleeping serum GH (<4 ng/ml).[33] He studied 94 ambulatory men and women from 21 to 86 years old, free of active or progressive disease and within 15% of ideal body weight. The estimated 95% tolerance limits for IGF-1 in the third decade was 0.64-2.2 U/ml. The number of patients with serum IGF-1 levels below the lower 2.5% tolerance limit for each decade was then determined. A subset of patients were given exogenous human growth hormone (hGH) and elemental balance studies (K,P,N) were performed in addition to measuring plasma IGF-1. They found that patients 60-79 and 20-29 years old, with IGF-1 greater than 0.64 U/ml were similarly unresponsive to hGH. Neither IGF-1 nor elemental balances changed. On the other hand, those with IGF-1 < 0.64 U/ml responded to hGH with significant positive changes in elemental balance studies and increased IGF-1. A number of correlational analyses were performed to assess which variables corresponded to impaired GH release and low IGF-1. IGF-1 was inversely proportional to adiposity and age, but both were independent of each other. Age and obesity together accounted for only 67% of the IGF-1 variability in the study population.

The exact mechanism of age-related decline of GH is unknown but appears to be related to diminished hypothalamic secretion of GHRH and augmented hypothalamic secretion of somatostatin.[30] Of note, the concentration of GH within the human pituitary does not decline with advancing age.[49,50,51]

A considerable research literature exists on the somatotropic actions of GH in animals and humans. These studies have largely demonstrated that GH tends to decrease the body content of adipose and redistribute it from a central to a peripheral sites, increase muscle mass and strength, improve exercise performance, increase body content of protein, Na, K, Ca, and P, improve immunologic parameters, improve renal function, and reduce serum cholesterol.[52,53,54,55,56] Based on the physiological changes that characterize the aging process, in association with the prevalence of impaired GH secretion with age, Rudman proposed that progressive impairment in GH secretion was operative in the senescent physiologic changes observed in about half of the elderly population.[37]

CLINICAL STUDIES OF GROWTH HORMONE IN NORMAL OLDER INDIVIDUALS

The short and long term (6-18 months) effects of GH have been studied in healthy people more than 60 years of age.[13,43] Patients in a short term study received a daily injection of rhGH for 7 days. The 12 women and 6 men received either 0.03, 0.06, or 0.12 mg/kg BW sc. The serum GH and IGF-1 concentrations were similar between men and women and did not significantly differ compared to a group of six men less than 30 years of age. However, the height of the secretory peaks and the mean integrated overnight GH concentration were significantly lower in the study group compared to the young controls. However, both serum GH and IGF-1 rose briskly in response to injections of rhGH. Serum GH levels tended to peak at about six hours and gradually fell to baseline values after 24 hours. Serum IGF-1 levels slowly climbed over the first twenty four hours and when measured on day 7 had reached a steady state. Serum GH response on day 7 resembled the pattern of response on day 1. Seven days of rhGH treatment did not alter endogenous creatinine clearance, fasting glucose (there was a change in glucose pattern following oral glucose tolerance testing and increased insulin response), and serum calcium. Serum PTH and osteocalcin increased only at the highest doses and calcitriol increased only at the mid dose. There was a significant reduction in urinary total and urea nitrogen and serum cholesterol (triglycerides increased). Serum inorganic phosphorus, urinary calcium and urinary hydroxyproline increased at all doses of rhGH. Urinary sodium fell at all dosage levels. Urinary phosphorus decreased at the mid and high dose levels. The seven day course of treatment was well tolerated with some individuals complaining of bloating and ankle swelling which resolved shortly after the study was completed. The authors of the study suggest that rhGH may attenuate or reverse the loss of muscle and bone in elderly people and elderly patients remain responsive to exogenous rhGH.

Rudman studied the effects of rhGH (initially 0.03 mg/kg sc) administered three times a week in 21 healthy men (61 to 81 years old) for six months in whom previously determined serum IGF-1 levels were low on 2 occasions.[13] Ninety-five patients were initially screened in order to find the study group that were ultimately enrolled. In all, approximately two-thirds of the elderly men who presented as potential volunteers in this study had normal IGF-1 levels. The dose of rhGH was adjusted up or down to achieve a IGF-1 level that was within the normal range.

The results of this study found no change in plasma glucose, calcium, phosphorus, creatinine, cholesterol, or alkaline phosphatase. Various measures of bone metabolism such as PTH, osteocalcin, calcitriol, urinary calcium or hydroxyproline were not reported. Lean body mass increased by 8.8 % ($P<0.0005$), adipose mass fell by 14.4% ($P<0.005$) and skin thickness increased 7.1 percent ($P=0.07$). Bone density of the radius and proximal femur did not change. The lumbar vertebral bone density increased 1.6 % $P<0.04$). In a progress report of this study, in which 45 patients are now enrolled, no change in bone density at six or twelve months at any of nine sites including the first lumbar vertebra, distal radius, or Ward's triangle were reported.[52] Significant changes at 12 months persisted in lean body mass (+6%), adipose mass (-15%), skin thickness (+4%), liver volume (+8%), and spleen volume (+23%). After rhGH was discontinued lean body and adipose mass were approaching baseline values. Overall patients tolerated rhGH well. A small percentage developed gynecomastia and carpal tunnel syndrome which resolved within 3 months of discontinuing treatment. No patients developed diabetes, hypertension, or neoplastic disease. After the carpel tunnel syndrome (CTS) developed in nine patients, subjects were

then screened for these symptoms before enrollment and excluded if present. They reported no patients developed this symptom after this modification was made in their protocol.

The authors contend that their data support the hypothesis that progressive impairment in GH secretion is responsible in part for many of the senescent changes observed in old age and that rhGH can reverse these changes. Like previous studies in growth hormone deficient children and adults, their results seem to demonstrate that exogenously administered GH in normal elderly patients increases lean body mass and decreases adipose mass. At present there is little evidence that GH may reverse age-related changes in bone density in normal elderly patients, although the design and duration of the presently available studies are unable to answer this question. Whether and/or how a decrease in the GH/IGF axis is related to senescent changes in body composition remains to be seen. In the study by Rudman et al, one-third of normal elderly patients had low IGF compared to a range defined by younger normals. This suggests that the normal distribution of IGF-1 in older adults is skewed to the left. Furthermore, as their study demonstrated, a majority of elderly individuals seem to have normal IGF levels. Although it has been suggested that individuals with a low serum IGF-1 levels may have a more rapid decline in age-related parameters this idea has not been studied.[48,57] In addition, we do not know if the men studied differed from older individuals with normal plasma IGF-1 levels in either their body composition or response to GH. Lastly, some have questioned whether the low plasma IGF-1 concentrations were the cause of body-composition changes or the results of a lowered need for IGF-1 due to a stable lean body mass.[58]

GROWTH HORMONE THERAPY IN GH DEFICIENT ADULTS

A number of studies have been carried out in adults with GH deficiency following therapy for pituitary tumors or similar disorders. In a placebo controlled trial Salomon treated 12 adults with GH deficiency for six months with daily rhGH.[34] Patients receiving rhGH had an increase in lean body mass, reduced fat mass and improved creatinine clearance. In a subsequent report from the same group, rhGH resulted in a significant increase in skeletal muscle mass and strength and improved exercise performance.[59,60] In a double-blind, placebo-controlled, crossover study Jorgensen studied 22 young adults with a history of isolated GH deficiency or multiple pituitary deficiency in which treatment with GH had been discontinued. Patients received daily injection of rhGH for four months. GH increased mean glomerular filtration rate and renal plasma flow from a subnormal level on placebo to a level comparable with that of an age-matched control group. Body weight did not change but mean muscle mass volume of the thigh by computerized tomography was higher and mean adipose tissue volume of the thigh and subcapsular skinfold thickness fell significantly during GH treatment. Serum IGF-1 levels normalized with GH treatment. Christiansen recently reviewed GH-replacement therapy in adults. He found that both GH deficiency since childhood or those rendered GH deficient in adult life were associated with: reduced muscle strength, reduced exercise capacity, subnormal kidney function and a central distribution of adipose tissue. Replacement therapy with GH improves or normalizes these abnormalities.[61] Little data on GH effects on bone metabolism in GH deficient adults is available. The study by Salomon reported a significant increase in serum calcium and phosphate but reports no other measures of bone metabolism. Kaufman reported on the bone mass in 30 men with GH deficiency during childhood which had been treated with GH until patients reached an adult bone age. Bone density was measured at the proximal and

distal forearm by single photon absorptiometry (SPA) and at the lumbar spine by dual photon absorptiometry (DPA). The men with GH deficiency of childhood onset had a significant bone mineral deficit compared to age and height matched controls. Losses were more prominent in the forearm than the lumbar spine. Patients had repeat bone mass measurements an average of 17 months following their initial study without any evidence of bone loss.[62]

Cursory information is available from a pilot study in which eight GH-deficient adults received a daily dose of rhGH for 8 to 16 weeks in a randomized double-blind cross-over trial.[63] Serum osteocalcin and urinary hydroxyproline increased significantly, while parathyroid hormone (PTH) and $1,25(OH)_2$ vitamin D did not change. In six patients, bone mineral mass by DPA of the lumbar spine increased significantly. The author hypothesized that GH therapy stimulated bone turnover, with bone formation stimulated more than bone resorption.

GROWTH HORMONE/IGF-1 AND BONE METABOLISM

Growth hormone has an effect on bone metabolism directly and indirectly via IGF-1 production. IGF-1 is produced in the liver and locally in bone as well as other tissues. GH and IGF-1 receptors have been identified on osteoblasts.[64,65]

There is growing evidence to support the role of IGF-1 in bone formation. Locally produced IGF-1 stimulates bone DNA, collagen, and noncollagen protein in cultured rat calvaria.[66,67]

In a study using fetal rat calvariae, IGF-1 stimulated bone matrix synthesis and bone cell replication.[20] When hydroxyurea was added to IGF-1 treated bones, the effects of IGF-1 on DNA synthesis were abolished, but increased bone matrix apposition induced by IGF-1 was only partly diminished. These finding suggest IGF-1 has two effects on osteoblasts. A stimulatory effect on progenitor cell replication as well as a direct effect on the differentiated cell function represented by enhanced bone collagen production. How these findings relate to adult or aged animals is not known.

Chenu found IGF-1 was secreted spontaneously in human bone cultures and its production regulated by GH and $1,25(OH)_2$ vitamin D.[68] GH stimulated the production of alkaline phosphatase activity but required the presence of $1,25(OH)_2$ vitamin D to stimulate osteocalcin secretion. High concentrations of GH inhibited osteocalcin production. Osteoblasts synthesize alkaline phosphatase which is associated with matrix mineralization. A rise in alkaline phosphatase is an early expression of commitment to the osteoblastic phenotype, whereas osteocalcin, a non-collagenous protein is a late marker of osteoblastic differentiation. $24,25(OH)_2$ vitamin D and 25(OH) vitamin D did not elicit comparable effects on alkaline phosphatase activity or on osteocalcin secretion. IGF-1 may be a local mediator of $1,25(OH)_2$ vitamin D. Although IGF-1 is a mediator of GH activity, this study suggests that $1,25(OH)_2$ vitamin D may be necessary for GH to be completely active.

Kurose, using mouse clonal osteoblastic cell lines in vitro found IGF-1 and $1,25(OH)_2$ vitamin D have a synergistic effect on alkaline phosphatase activity and an additive effect on collagen synthesis.[69] This group subsequently reported that the interaction of IGF-1

and $1,25(OH)_2$ vitamin D may be mediated by $1,25(OH)_2$ vitamin D increasing IGF-1 binding sites.[70]

Spencer studied the effect of IGF-1 on bone by infusing IGF-1 into the arterial supply of the right hindlimb of rats.[71] The contralateral limb served as a control. Histomorphometric analysis demonstrated significant cortical and trabecular bone formation. Furthermore while osteoblastic activity was stimulated there was a decrease in osteoclast number, supporting IGF-1 as a mediator of the bone forming effects of GH but not bone resorption. Furthermore, their findings suggest that the bone formation effect of IGF-1 is not coupled to bone resorption. The effects were age dependent, reported present in year old rats (not considered old) but absent in very young animals.

The only report available showing a bone resorbing effect of IGF-1 used aged ovariectomized rats that received daily intermittent PTH or continuously infused IGF-1.[72] In contrast to this study, Bak studied the effect of GH on fracture healing in old rats and found that fracture healing can be stimulated by systemic administration of GH.[73] There is some evidence that IGF-1 may decrease osteoclast number but IGF-1's effect on osteoclast activity is not known.[74]

How PTH and GH/IGF-1 interact is not clear. Most clinical studies have shown no change in serum PTH with GH administration.[75] In animal studies, PTH potentiated the effect of IGF-1 on bone formation.[76] Interestingly, both GH and IGF-1 appear to increase renal tubular resorption of phosphate and plasma $1,25\ (OH)_2$ vitamin D independent of a PTH mediated mechanism.[40] PTH enhanced local IGF-1 synthesis when studied using osteoblast-enriched cultures from fetal rat bone.[76]

Estrogen also appears to modulate IGF-1 in bone. IGF-1 synthesis from osteoblasts is enhanced by 17β estradiol.[77] Osteoblasts possess estrogen receptors but clinically they act as antiresorptive agents with little bone forming properties.[78] Interestingly, in one study IGF-1 increased bone collagen synthesis and decreased collagen degradation in cultures of intact calvariae.[79] Could estrogen play a role in local bone repair?

17β-estradiol stimulates proliferation and collagen mRNA expression in rat calvaria cells. These effects were blocked by antibodies to IGF-1 suggesting IGF-1 mediation, which was corroborated by estrogen induction of IGF-1 mRNA in these cells.[77] GH and 17B-estradiol appear to alter rat IGF-1 binding proteins suggesting a role for these hormones in bone by modulating the biological function of IGFs via their binding proteins.[80]

Weissberger and others have studied the effect of different routes of administration of estrogen replacement on the GH/IGF-1 axis.[81] Premenopausal women had higher mean 24-hour serum GH and serum IGF-1 levels compared to postmenopausal women. Postmenopausal women receiving orally administered estrogen significantly increased serum GH levels but significantly reduced circulating IGF-1 levels. In contrast women receiving transdermal estrogen increased their plasma IGF-1 to levels not significantly different from those of premenopausal women while serum GH did not change. The authors suggest that orally administered estrogen suppresses the hepatic synthesis of IGF-1 during the first pass through the portal system which results in the feedback stimulation of increased pituitary release of GH. Estrogen delivered transdermally appeared to stimulated IGF-1 production which results in feedback inhibition of GH release. An earlier study by Dawson-Hughes

also showed orally administered estrogen administration in postmenopausal women resulted in increased plasma estrogen and reduced IGF-1 levels.[82] Another study of postmenopausal woman receiving different doses of transdermal estrogen showed no change in circulating GH or IGF-1 levels. Age appeared to correlate with GH and IGF levels independent of circulating estrogen levels.[83] The lack of significant correlation of endogenous estrogen level is in contrast to another report that found age and sex appear to have a less effect on GH secretion and IGF-1 level than free circulating estradiol level.[4] In two studies of men aged 18-85, the fall in integrated GH and IGF-1 levels with age were independent of free testosterone.[4,84]

GROWTH HORMONE EXCESS AND BONE DENSITY

The effect of excess growth hormone after closure of the epiphyseal growth plates results in a striking increase in the bone mass of patients with acromegaly by virtue of increased bone volume (especially acral growth). Clinical parameters of bone metabolism (serum osteocalcin, alkaline phosphatase, urinary hydroxyproline) as well as histomorphometric studies in patients with acromegaly demonstrate that bone turnover is increased.[85] Since bone mass is increased it would seem that bone formation would exceed bone resorption. Actually, the impact on bone mineral density (BMD) is not clear. Excess GH seems to increase cortical BMD more than trabecular BMD. In a retrospective study, Diamond measured the BMD in 24 patients with acromegaly and found the forearm BMD (by SPA) increased and spinal BMD (by DPA) decreased.[86] The forearm density correlated with activity of disease (by GH and IGF-1 level) and not with gonadal status. On the other hand, lumbar bone density correlated with hypogonadism but not with GH or IGF-1 level. This study indicates that increased GH/IGF-1 was associated with an increase in BMD in the peripheral skeleton but that gonadal status (estrogen deficiency) has a greater influence on spinal density, even in the presence of excess GH. Nijs and co-workers measured the BMD of the femoral neck of patients with acromegaly by X-ray absorptiometry and found no difference (perhaps a trend of lower BMD) compared to age-and sex-specific normal range.[65]

CLINICAL STUDIES OF GROWTH HORMONE AND OSTEOPOROSIS

Few studies have been completed studying the effect of GH in patients with osteoporosis. Kruse studied one 58 year old man with severe "primary" osteoporosis (and two patients with osteogenesis imperfecta).[87] hGH was given daily for 12 months. No significant change in alkaline phosphatase, hydroxyproline, serum calcium or phosphorus was noted. Histomorphometry of paired iliac crest bone biopsy showed normal bone turnover. Osteoblastic activity increased and parameters of bone resorption decreased.

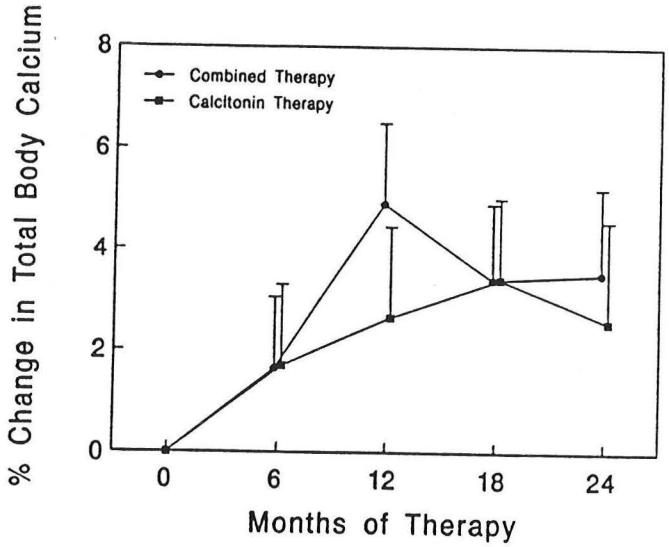
In 1976, Haas published a brief report after treating nine post-menopausal women with "overt osteoporosis" with hGH, every other day for up to one year.[88] Urinary hydroxyproline and serum phosphorus increased, while alkaline phosphatase and calcium balance studies remained unchanged. After three months of treatment the number of osteoblasts on bone biopsy increased but remained within the normal range.

In 1976 Aloia reported the results of an uncontrolled trial using GH for the treatment of

developed complications including arthralgias, edema and carpal tunnel syndrome, hypertension, and hyperglycemia. This study included seven women and one man (ages 53-76, mean 62.5). Subjects were treated for two six-month treatment periods on different doses of hGH. Outcome measures included total body calcium (TBCa) and total body potassium (TB-K) determined by neutron activation analysis (NAA), radial bone density by SPA, bone biopsy, PTH, urinary hydroxyproline, and alkaline phosphatase. TBCa reflects skeletal mass but is a rather imprecise technique. There was no overall improvement in skeletal mass by TBCa. BMC (radial) decreased in all but one patient. Evidence of increased bone turnover was indicated by a rise in serum alkaline phosphatase (four patients) and urinary hydroxyproline. Serum iPTH did not change. Paired bone biopsy in five patients showed an increase in both resorptive and formation surfaces (P=.058, .074 respectively). Half of the patients reported an improvement in symptoms.

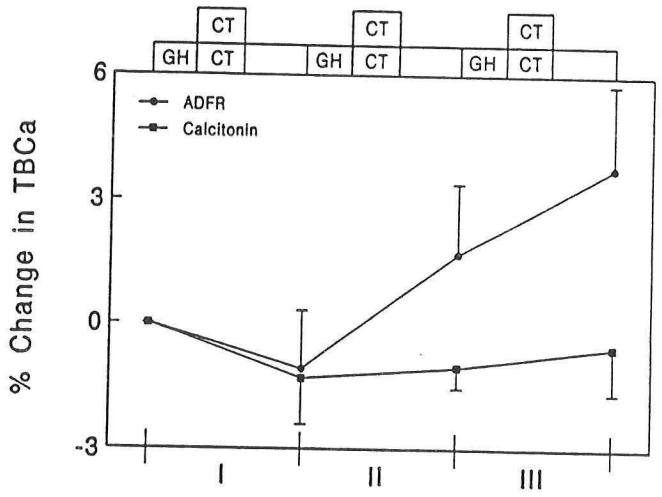
Another approach to the treatment of osteoporosis was pursed by Aloia by trying to utilize the antiresorptive effects of calcitonin in conjunction with the bone forming properties of growth hormone.[90] (see figure 3) Presumably, resorption stimulated by GH would be blunted by calcitonin. It was hoped this strategy would uncouple the bone remolding cycle by favoring bone formation and supressing bone resorption. Two groups of postmenopausal women were randomized to receive either calcitonin and hGH (combined treatment-daily subcutaneous injection of hGH three times a week and calcitonin four times a week) or calcitonin (subcutaneous injection four times a week) alone. Both groups received supplemental calcium. Patients were followed for 24 months and TBCa was monitored every six months as was radial BMC by SPA. There were significant baseline differences for both age and bone mass. The mean age in the combined treatment group was 68 1.56 (mean SE) and 60 1.7 in the calcitonin group. TBCa and BMC was greater in the calcitonin group. Over the 24 month period there was no difference in the number of further compression or new vertebral fractures. In both groups an increase in TBCa (+1.68% / yr combined, +1.33% / yr calcitonin) occurred but there was no difference between groups. The increase in TBCa was likely due to transient uncoupling of bone formation and resorption due to calcitonin's antiresorptive properties. Initially, bone formation continues at a rate which is relatively higher than resorption. Ultimately the rate of bone formation declines due to the coupling of bone formation and resorption. BMC in the combined group fell compared to the calcitonin group. There were no significant differences in laboratory chemistries between groups.

Figure 3



In yet another study by the same group, 14 women with postmenopausal osteoporosis (at least one vertebral crush fracture on x-ray) were randomized to one of two different treatment groups lasting 24 months.[1] (See figure 4.) One group (7 patients) received coherence therapy also known as ADFR (activate-depress-free-repeat). Patients in this group were given daily hGH for two months, followed by 3 months of salmon calcitonin (CT) every other day, no therapy for three months and then this sequence was repeated. The other group (7 patients) received only CT, given during the same period as the coherence group. The goal of coherence therapy is to use an agent that will stimulate bone mineral units in temporal coherence, followed by an agent that will then suppress bone resorption while bone formation continues. Calcitonin is used as to inhibit bone resorption (depressor agent) and hGH is used as the bone stimulating agent (activator). Outcome measures included TBCa by NAA and radial BMC by SPA, new fractures and blood and urine chemistries. Patients mean age in both groups were approximately 62 years. No significant side effects were reported. A new or further compression fracture occurred in one patient in the coherence group and in three patients in the CT group. TBCa increased in the coherence group +2.3%/yr compared to baseline and -0.45%/yr in the CT group. There was no significant difference between groups at two years but a positive trend was noted in the BMC in the coherence group. In the coherence group, serum 1,25 $(OH)_2$ vitamin D increased after each GH sequence and urinary hydroxyproline increased after the first sequence. Bone histomorphometry results were available from the 4 patients in each group, no significant differences were present. This study was terminated due to concern regarding the association of Creutzfeld-Jakob disease in patients treated with hGH. Although the number of patients was small and the techniques used for bone density measurements were imprecise, it is noteworthy that the TBCa in the coherence group continued to rise and did not demonstrate a plateau phase. Serum and urine bone parameter values were not reported other then alluding to an initial increase in serum 1,25 $(OH)_2$ vitamin D3 and urinary hydroxyproline with the hGH sequence. Prior studies in animals and children show conflicting results when GH is administered. One study in GH deficient children reported a decrease in serum 1,25-$(OH)_2$ vitamin D and a rise in intestinal calcium absorption following GH administration.[91] In this study calcium absorption was not measured and there was no change in urinary calcium. Measures of osteoblastic function were either not measured (osteocalcin) or were not increased (alkaline phosphatase). The increase in hydroxyproline suggests that hGH stimulated bone resorption.

Figure 4



Although associated with bone resorption, hydroxyproline is derived from the degradation of various forms of collagen and is not specific for bone collagen. Urinary hydroxyproline can also be influenced by diet. Since GH effects a variety of tissue, the source of hydroxyproline may not necessarily be of bone origin. Recently, a new class of markers of bone resorption known as pyridinium cross-links (pyridinoline and deoxypyridinoline) have become available. Whereas pyridinoline has been found in many different connective tissues, deoxypyridinoline is found in significant amounts only in bone. In a study where GH-deficient adults were given rhGH, significant increases in osteocalcin and deoxypyridinoline were measured.[92] Bone density measurements have yet to be examined. This report supports data that suggests GH stimulates both bone resorption as well as formation. How these properties affect patients with osteoporosis are still not known. The question remains is the impact on bone formation greater than resorption.

Few studies have looked at how IGF-1 correlates with bone density. Both IGF-1 and bone density fall with aging. Bennett measured serum IGF-1 in 57 women ages 30 to 57 and in 29 women with post-menopausal osteoporosis (ages 55-75).[93] Although serum IGF-1 declined with age there is considerable variability. (See figure 5.) These investigators found that IGF-1 levels did not significantly differ in women with osteoporosis from normal women. When age was held constant there was no difference in BMD and serum IGF-1. In another study that included men and women between the ages of 61 to 84 years a significant difference was observed in serum IGF-1 between control (n=27) and osteoporotic (n=17) patients.[94] (See table 5.) No bone density measurements were reported. Although the number of patients over 75 years are not reported, men and women were enrolled in this study and were older then those in Bennett's study. It is possible that the difference in results between the studies could be accounted for by a higher number of patients with type II osteoporosis in Pun's study.

Figure 5

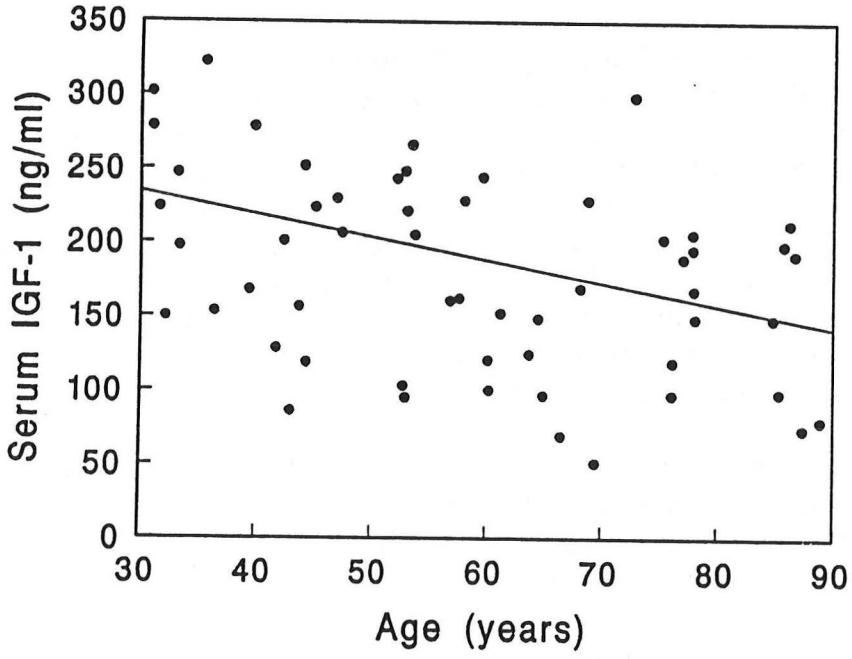


Table 5

Serum levels of osteocalcin, 25–OH–D_3, and IGFI in 44 elderly subjects aged 61 to 84.

	Control (n=27)	Spinal Fracture (n=17)	Statistical Significance
Serum Osteocalcin (nmol/l)	0.28 ± 0.10*	0.09 ± 0.09	$P < 0.001$
Serum 25-OH-D_3 (nmol/l)	70.5 ± 25.1	48.2 ± 19.9	$P < 0.005$
Serum IGFI (nmol/l)	23.8 ± 12.0	16.6 ± 7.0	$P < 0.05$

*mean ± standard deviation.

CONCLUSION

Although the relationship of GH activity and age-related changes in human physiology are intriguing, what or if any relationship exists between reduced GH secretion and health or disease are unknown. Longitudinal and better designed studies in the future will help answer these questions.

Growth hormone possesses a number of properties that are beneficial in the treatment of osteoporosis. Directly and via its mediator IGF-1, GH likely stimulates bone formation. Intestinal calcium and phosphorous absorption as well as urinary phosphorus reabsorption are increased. It is not clear whether the effects on calcium-phosphate metabolism is due to influencing vitamin D metabolism and PTH and/or due to independent mechanisms.

The ideal agent to treat age-related (type II) osteoporosis would be one that could increase bone formation and decrease or not effect bone resorption, producing structurally normal cortical and trabecular bone. Because GH appears to increase bone resorption, the only way this agent is likely to be effective (by itself) in the treatment of osteoporosis is if it's effect on bone formation is greater than that on bone resorption. Whether GH can stimulate bone formation in excess of bone resorption can not be answered with the information that is currently available. In view of the bone resorbing and forming properties of GH, a combination treatment approach may be attractive. GH used in conjunction with an antiresorbing agent such as estrogen (provided transdermally which seems to maintain plasma IGF-1 levels) would be a reasonable strategy to study.

It should be emphasized that very few studies have been performed to assess growth hormones effectiveness in the treatment of osteoporosis. The available studies included a small number of patients, used different drug regimens, monitored some outcomes fairly imprecisely, and did not fully describe the characteristics of the patients studied. The last point is important because the ideal treatment for a patient with type I osteoporosis may require a different regimen than a patient with type II osteoporosis. In other words, the response of a group of patient's whose major abnormality of bone metabolism is bone

resorption, due to excessive osteoclastic activity may be different than the patient with a primary defect in bone formation from impaired osteoblastic activity. Furthermore, the relevancy of information obtained from studies involving normal subjects or other patients to those suffering from osteoporosis may be irrelevant.

How GH interacts with other factors involved in bone metabolism is also important to understand and many questions remain to be answered. IGF-1 mediates many of the actions of GH but it also appears to be involved in the activity of PTH, estrogen, and $1,25(OH)_2$ vitamin D. In addition, IGF binding proteins are thought to play an important role in mediating the effect of IGF by determining the availability of IGF at the receptor level.[95] To this list, we need to add the interaction of the GH/IGF axis with other growth factors that are active in bone.

The availability of biosynthetically produced GH and IGF-1 with the increasing ability to measure IGF-1, IGF binding proteins and other growth factors provides exciting opportunities to better understand the physiology of bone metabolism and the pathophysiology of osteoporosis. This can only lead to new and better treatments for patients with osteoporosis.

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