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Growth Hormone, Insulin-Like Growth Factors, and the Senescent Skeleton: Ponce de Leon's Fountain Revisited?

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Abstract As the population ages, the prevalence of osteoporosis will continue to rise. Yet, the mechanisms leading to age-related bone loss remain poorly defined. Furthermore, extensive longitudinal studies of bone mass, especially in the three decades beyond menopause, have not been completed. Although calciotropic hormones, growth hormone (GH), and insulin-like growth factor-I (IGF-I) change with age, it is not certain if these changes are responsible for age-related bone loss. Nor is it clear if the "senescent" osteoblast is fully responsive to growth factor stimulation. To complicate matters further, both circulatory and skeletal IGF regulatory systems are extremely redundant. Changes in serum IGFs may lead to compensatory alterations in IGF receptor number, IGF binding protein (IGFBP) synthesis, or IGFBP catabolism. What is measured in serum, may, in the end, be either a mirror or a mirage of skeletal IGF action! Clinical trials with "replacement" doses of GH or IGF-I are underway. But, critical evidence does not yet support the concept that a true "sommatopause" alters bone remodeling. Moreover, only scarce data exist that GH augments bone formation or prevents bone loss in the elderly. As clinicians expand the use of recombinant growth factors to elders, ethical and clinical issues surrounding administration of the new "fountain of youth" will be revisited. For basic scientists studying skeletal growth factors and their relationship to senescence, significant questions remain unanswered. New technological advances will provide clues about the basic mechanisms of skeletal aging. But, until these findings are validated, scientists and clinicians will have difficulty judging the role of growth factors in halting, or reversing, the inexorable consequences of aging.

Key words: osteoporosis, aging, growth hormone, osteoblasts, bone remodeling

"Facts are stubborn things and whatever may be our wishes, our inclinations, or the dictates of our passions, they cannot alter the state of facts and evidence."—John Adams, 2nd President of the United States

Osteoporosis is a major medical problem affecting more than 14 million Americans [Melton et al., 1992]. Elders, by far, are most susceptible to osteoporotic fractures, principally because of their low bone mass and high prevalence of falling. By age 90, the cumulative incidence of hip fractures among white women is 33%. Last year alone, 10 billion dollars were spent in the United States treating this disease. Most of that cost arose from hip fractures which are associated with extreme morbidity and an excess mortality of 20% [Meunier, 1993].

It has been estimated that over a lifetime women will lose between 35–50% of their bone mass [Turner et al., 1994]. Peak bone mass is acquired between the ages of fourteen and twenty years [Slemenda et al., 1990]. Bone loss, which begins during the fourth decade of life, continues thereafter. Since bone mineral density is the strongest predictor of future fracture, the two most critical determinants of fracture risk for any given person are the precise level of peak bone mass and the rate of bone loss over time. Recent evidence points to strong genomic regulation over bone mass acquisition. By contrast, age-related bone loss likely results from the interaction of genetic determinants with environmental and hormonal factors. One hormone secreted in lesser amounts with advancing age is growth hormone (GH). A recent study suggested that age-related changes in bone, skin, and muscle mass were related to lower amplitudes of pulsatile GH secretion, making it amenable to "replacement" therapy with GH [Rudman et al., 1990].

This paper will address three questions which are relevant to the hypothesis that age-related

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changes in growth hormone (GH) secretion cause bone loss in the elderly.

1. Is bone loss genetically regulated?
2. Is age-related osteoporosis due to defective osteoblasts?
3. Do changes in the GH/IGF-I axis affect bone remodeling and if so can administration of insulin-like growth factors or growth hormone halt or reverse age-related bone loss?

GENETICALLY PROGRAMMED BONE LOSS?

Biologic aging is a normal physiologic process, a component of the continuum associated with growth and development [Goldstein et al., 1989]. In almost every body system, homeostatic capacity maximizes during the third decade of life and then progressively declines. This decrement in function is variable but incessant whether it be nerve conduction velocity, glomerular filtration, cardiac index, or bone remodeling. More than 30 years ago Tonna defined age-associated skeletal dysfunction in this way:

"Aging of the skeleton implies intrinsic changes (biochemical and biophysical) occurring progressively within the lifespan of the system which result in the irreversible and deleterious alteration of the performance capacity of the cellular phase. In time, age changes are reflected in the matrical and the mineral phase of the skeleton."

In the 1960s, Urist suggested that skeletal aging was associated with osteocyte drop-out resulting in trabecular fragmentation and prefractures [Urist, 1964]. Both Urist and Tonna recognized that cumulative changes in cellular function ultimately resulted in biophysical design failure and fracture. In a recent histomorphometric study, Croucher et al. [1994] demonstrated that patients with osteoporosis exhibited the same structural changes in trabecular bone as did older normal subjects. This finding suggested that primary osteoporosis was a result of accelerated biological aging rather than a disease specific process. Since cellular changes in the bone ultimately lead to biomechanical failure and fracture, age-associated abnormalities must occur at the genomic level.

The functional decline in most biologic systems with aging is regulated in large part by genetic factors. This is apparent in animals, where a finite life span bespeaks the major predominance of genes over environment. However, the role of genetic factors in the development of skeletal fragility has only recently been appreciated. It appears likely that one or more sets of genes control acquisition of peak bone mass. Investigators studying twins and mother-daughter pairs have established the strong role of heredity in determining bone density in younger subjects. Seminal work by Morrison et al. [1994] has shown that specific alleles for the vitamin D receptor are very strong predictors of bone mass in one Australian population. On the other hand, lack of extensive longitudinal studies has made it exceedingly difficult to ascertain if genomic factors control age-related bone loss. For example, two recent cross-sectional studies suggested that hereditary determinants of bone mass become less important after menopause while a 3-year longitudinal study of twins in their fifth decade demonstrated that the correlation coefficient between monozygotic twins for bone density was much greater than dizygotic twins [Hansen et al., 1992; Kelly et al., 1993; McKay et al., 1994]. Although more longitudinal studies are needed, it is apparent the "one-gene one-disease" model may be too simplistic, especially when considering the multifactorial determinants of bone mass.

Lumbar bone density vs. age in blacks and whites is schematically represented in Figure 1. Although this figure is predicated on cross-sectional rather than longitudinal data, the implication is that the lower the peak bone mass, the greater the likelihood of reaching a theoretic fracture threshold. This assumes that there is a relatively constant rate of bone loss after acquisition of peak bone mass. Twenty-five years ago Newton-John proposed that the rate of bone loss is common and consistent in any individual in a population [Newton-John and Morgan, 1970]. Recent studies would seem to confirm that hypothesis, although the slope of age-related bone loss (β_1, Fig. 1) may differ for a given individual in a selected cohort.

Figure 1 represents a starting point to define the mechanisms of age-related bone loss. Certain points are clear: (1) the slope of bone loss during the fifth and sixth decades of life (β_1) is much steeper in women than in men; (2) early withdrawal of estrogen increases the slope of bone loss (β_2), thereby shortening the interval before the fracture threshold is reached; and (3) black men and women have greater peak bone mass and slower rates of bone loss (β_3) than whites.

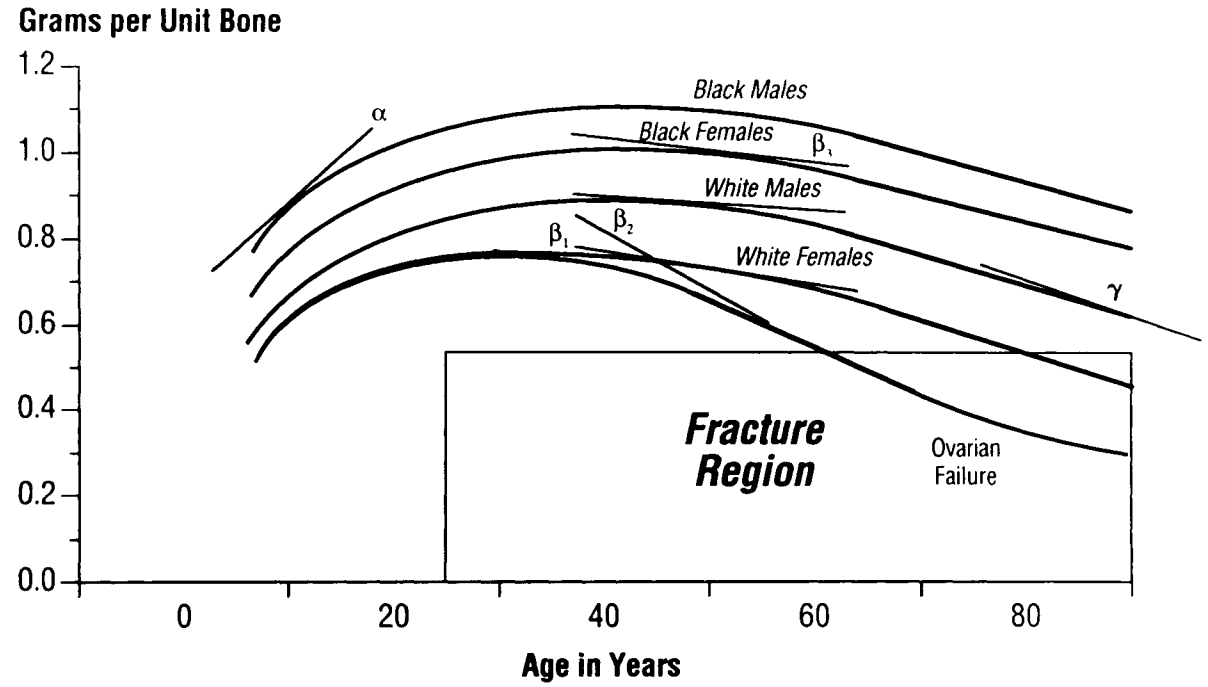

Fig. 1. Schematic representation of effects of age, sex, and race on bone mass. Note that black females and males have greater bone mass complements than either white females or white males. α represents the slope of bone mass accretion; β_1 represents the slope of bone loss during the early perimenopausal period in white females; this is in contrast to the slope in white males; β_2 demonstrates the slope of bone loss for women with premature ovarian failure; β_3 is the slope of bone loss in black females in the perimenopausal period; γ represents the slope of age-related bone loss in males.

The influence of hormonal factors on bone mass throughout life cannot be understated, especially in women. Estrogens maintain bone mass during the pre- and peri-menopausal periods. Estrogen replacement therapy prevents postmenopausal bone loss and stabilizes bone density, even in elderly women [Turner et al., 1994]. However, the mechanism whereby the absence of gonadal steroids around the time of menopause activates specific genes has not been completely delineated. There is strong evidence that estrogen deprivation (or androgen loss) can lead to enhanced stromal cell production of very powerful cytokines which recruit osteoclastic precursors from mononuclear bone marrow cells [Jilka et al., 1992; Glowacki, 1994]. Moreover, addition of estrogen (or androgen), in vitro, prevents further elaboration of these cytokines [Jilka et al., 1992]. However, with menopause, all women enter a phase of subacute estrogen deprivation. But, not all women develop osteoporosis or sustain rapid bone loss (20–30% of all postmenopausal women). Since the rate of menopausal bone loss varies among women of different races and ethnic background (Fig. 1, β_1 vs. β_3), genetic factors must interact with hormonal influences during this critical time.

Perimenopausal interpopulation variability has obscured the definition of age-related bone loss. For example, if an 80-year-old woman presents with a hip fracture and low bone density, the clinician is faced with a fundamental etiologic question: Is this due to age-related bone loss, rapid perimenopausal bone loss, reduced peak bone mass, or a combination of all these factors? Furthermore, even if the difference in bone mass over time was established for that particular individual, exclusion of other causes contributing to bone loss (e.g., dietary calcium deficiency, vitamin D depletion, renal insufficiency, etc.) would have to be considered. The same holds for investigators seeking clues to the genetic regulation of skeletal senescence. That is why other experimental models (including mice) are needed to test the hypothesis that a decline in GH secretion is responsible for age-related bone loss.

Men develop age-related osteoporosis and absolute testosterone deficiency is probably one of the strongest independent risk factors for males

[Jackson et al., 1992]. Aging leads to a gradual decline in serum testosterone. However, cortical bone mineral content falls 3–4% per decade and trabecular bone mass falls 7–12% per decade after age 40 [Jackson and Kleerokoper, 1990]. Therefore, other factors must regulate age-related changes in bone mass (Fig. 1, γ). In fact, because men do not undergo relatively sudden cessation of testosterone production, they are useful models for studying genomic and hormonal regulators of age-related bone disease. However, well controlled, cross-sectional, and longitudinal studies in men are scarce. With the increasing prevalence of osteoporosis in males, extensive longitudinal investigations are being planned.

An alternative to waiting for decade-long studies in humans is to examine bone loss in other animals. Mice provide an excellent working model for evaluating genetic regulation over age-related bone loss. Strict control of environmental factors (sunlight, exercise, diet), a shortened lifespan, and the ease of generating genetically defined recombinant inbred strains, enhance the suitability of a murine model. In addition, skeletal remodeling is the predominant feature of bone turnover in the older mouse.

Three different strategies have been employed to examine genetic control of skeletal senescence in mice: (1) utilization of the senescence-accelerated mouse (SAM); (2) development of transgenic mice; and (3) study of recombinant inbred murine strains.

In various strains of SAM mice, aging is accelerated. Bone loss begins from trabecular and cortical compartments at 4 months of age and progresses over the ensuing 20 months [Matsushita et al., 1986]. These mice also show considerable interstrain differences in peak bone density which are maintained throughout murine life. Recently it has been shown that the SAM-P/6 strain has very low femoral bone mineral density compared to other SAM strains. Moreover, TRAP activity, alkaline phosphatase, and urinary cyclic AMP are significantly higher in P/6 than in any of the other strains [Kawase et al., 1989]. These data suggest that bone resorption during senescence is accompanied by a hyperparathyroid state, a scenario similar to human aging where low bone density, high serum PTH concentrations, and low serum 25(OH) vitamin D_3 levels are often found [Rosen et al., 1994a]. Recently, Manolagas et al. [1993] reported that in the SAM-P/6 mouse, decreased osteoclastogenesis was due to impaired upregulation of cytokine production after ovariectomy. It is apparent that hormonal manipulation of these models could provide another means for studying the pathogenesis and treatment of age-related bone disease.

An alternative strategy for studying skeletal senescence is the development of transgenic and knockout models. Very recently, Lewis et al. [1993] successfully developed a transgenic mouse which inappropriately expressed the cytokine IL-4 under the direction of a specific proximal promoter for the *lck* gene. These mice developed osteopenia, curvature of the spine, and reduced bone formation. The bone remodeling cycle was slowed (similar to what is observed in age-related osteoporosis) with reduced bone resorption and impaired recruitment of pre-osteoblastic stem cells. Further use of this model may provide important clues about coupling signals in the bone remodeling unit, especially in relation to senescence.

It is now possible to precisely and accurately measure bone mass in rodents. We have recently measured bone mineral density in both mutant and recombinant strains of laboratory mice using peripheral quantitative CT imaging (sPQCT). As might be predicted, mutant hypogonadal and hyposommatropic strains have consistently lower cortical and trabecular bone mineral density (corrected for size) than age-matched controls (Donahue, personal communication). More interesting, however, is the finding that significant differences exist in bone mass among various recombinant inbred strains of normal mice, and these differences are maintained throughout murine life. This would support the hypothesis advanced by Newton-John and Morgan [1970] that bone loss with age is relatively consistent for an individual population irrespective of peak bone mass. But, even if age-related bone loss is genetically programmed, it remains unclear what genes are responsible for this, or if the process is linked to relative GH deficiency.

AGE-ASSOCIATED DEFECTIVE OSTEOBLASTS?

Osteoporosis is defined as a reduction in bone mass leading to skeletal failure and fractures. Loss of bone occurs when the balanced remodeling cycle is uncoupled (i.e., bone resorption exceeds bone formation). Postmenopausal osteoporosis (Type I by some nomenclatures) is characterized by accelerated bone resorption, due in part to enhanced osteoclastogenesis. As

noted above, this process may be related to increased synthesis and release of powerful cytokines after hormonal withdrawal. Age-related osteoporosis (Type II) is characterized by an overall decline in wall thickness of the resorption cavity [Pacifici and Avioli, 1993]. The bone remodeling cycle is imbalanced with reduced bone formation primarily because of a defect in osteoblastic function. There are three possible ways by which aging could affect the osteoblast: (1) by aberrant recruitment of osteoprogenitor cells from pluri-potent bone marrow stem cells; (2) by reduced proliferation of osteoblast-like cells due either to lower growth factor production in marrow cells or resistance to paracrine, autocrine, and endocrine growth factors; or (3) by impaired osteoblastic differentiation.

Several lines of evidence suggest pre-osteoblastic cellular proliferation may be reduced with aging. In his classic paper, Tonna [1965] noted significant age-related changes in osteoblasts. Cell proliferation was reduced, osteoblast size was smaller, and the nucleus became hyperchromic [Tonna, 1965]. In addition, osteocytes were atrophied and the matrix demonstrated loss of metachromasia in collagen fibrils, decreased matrical water, and an increase in the size of the hydroxyapatite crystal.

Recent studies have confirmed these findings. But, new questions have been raised which highlight the need for other strategies to investigate age-related bone disease. Marie et al. [1991] studied bone turnover in the syndrome of male idiopathic osteoporosis. This disorder affects middle age and older eugonadal men and may represent a good model for age-related osteoporosis since hormonal or environmental factors (PTH, vitamin D, testosterone, smoking, alcoholism) have not been identified as contributing factors [Marie et al., 1991]. In males with idiopathic osteopenia, histomorphometric indices of bone formation are significantly reduced. Moreover, cultured osteoblastic cells from these patients show significantly reduced DNA synthesis and cell proliferation indices compared to similarly age-matched control males. On the other hand, biochemical indices of osteoblastic differentiation are normal. Hence defective bone formation in eugonadal males is associated with a reduced proliferative capacity of osteoblastic cells lining trabecular bone surfaces. Marie et al. did not examine growth factor production or levels in these men. But, Ljunghall et al. [1992] studied this syndrome and found that serum IGF-I levels were lower in these men than age-matched controls. Moreover, administration of rhIGF-I to two men showed a marked increase in bone formation, suggesting that endocrine (and possibly paracrine) deficiencies in IGF-I may result in bone loss. Whether these preliminary data relate to the abnormally low bone formation of the very old is still not certain (see GH/IGF-I replacement). But these studies do highlight relevant options for studying senescent bone disease.

Production of skeletal growth factors may be normal with aging but senescent osteoblasts may be resistant to their biologic action. This defect could take the form of down-regulated growth factor receptors, impaired second messages, or production of inhibitory growth factor binding proteins. Pfeilshifter et al. [1993] studied osteoblast-like cells obtained from the femoral head and iliac crest of 50–90-year-old women. In the iliac crest samples, PTH, IGF-I, TGF-β, and PDGF increased DNA synthesis but this increase was negatively correlated with age of the donor [Pfeilshifter et al., 1993]. Furthermore, nearly 10-fold greater concentrations of these growth factors and hormones were necessary in the cultures from older donors to yield the comparable increases seen in donor osteoblasts from younger subjects. Interestingly, the same resistance to stimulation was seen with trabecular outgrowths from the femoral head but only with GH and PDGF. Hence, osteoblastic proliferation may be significantly altered with aging, although the precise mechanism responsible for this process has not been defined.

One possible cause for osteoblastic resistance to IGFs may be through the elaboration of IGF binding proteins (IGFBPs). At least three IGFBPs (-3,-4,-5) are produced in relatively large concentrations in bone [Rosen et al., 1994c]. IGFBP-3 and IGFBP-5 for the most part augment IGF (-I,-II) action in vitro, while IGFBP-4 is almost exclusively inhibitory in its actions [Mohan, 1993]. The nature of IGFBP action at the IGF receptor remains poorly defined. IGFBP-4 strongly reduces IGF mediated osteoblastic proliferation in a dose dependent manner [LaTour et al., 1990]. Furthermore, this binding protein is induced by PTH and cyclic AMP analogues. Since some elders have secondary hyperparathyroidism (possibly due to reduced calcium absorption), it is conceivable that the proliferative characteristics of "aged" osteoblasts are related to elaboration of specific growth factor antagonists. In fact, in one cross-sectional

study, serum concentrations of IGFBP-4 were directly correlated with PTH and these levels were much higher in patients with hip fractures than age-matched controls [Rosen et al., 1992]. However, IGFBP-4 is also regulated in a post-translational manner by IGFs. Recent studies suggest that IGF induced IGFBP-4 proteolysis may be a very critical process whereby growth factors control, in a non-genomic manner, their own bioavailability [Rosen et al., 1994c]. This process also closes a paracrine loop where IGFBPs can determine IGF bio-activity and IGFs can regulate IGFBPs. How this process is altered with aging will be a major question for future investigators.

Osteoblasts arise from the same progenitor cells as fibroblasts in the condensing mesenchyme [Puzas, 1993]. Senescent human diploid fibroblasts (HDFs) are now widely used to study the biologic effects of aging. A prominent characteristic of senescent HDFs is relative unresponsiveness to the action of various polypeptide growth factors including IGF-I. However, binding sites and binding affinities for IGF-I are not aberrant. Therefore, senescent fibroblast resistance to IGFs may be due to postreceptor mechanisms or prereceptor blockade. Recently Goldstein et al. [1991] showed that IGFBP-3 accumulated to very high levels in the conditioned medium of senescent HDFs. Since high concentrations of IGFBP-3 can inhibit IGF-I action in fibroblasts, one interpretation of this finding would be that senescent HDFs sequester IGFs extracellularly, thus limiting their bioavailability, and thereby contributing to senescent growth arrest or resistance.

Osteoblasts secrete IGFBPs in a manner similar to fibroblasts. Newer methods for maintaining human bone marrow cells in culture should permit more intensive investigation into the skeletal IGF regulatory system. Jilka et al. [1992] and Glowacki [1994] have used in vitro bone marrow culture techniques to study hormonal regulation of osteoclastogenesis both in mice and humans. Recently Glowacki noted an age-associated rise in IL-6 production from human bone marrow cell cultures [Glowacki, 1994]. Future studies could apply the bone marrow culture model to determine if aging alters IGF/IGFBP expression or cell responsiveness to the IGFs.

Although proliferative characteristics of the osteoblast can be modified with aging, there is also the possibility that differentiative functions of the osteoblast are changed. Indeed, alterations in synthesis of bone matrix proteins may affect skeletal composition of growth factor binding components. Fedarko demonstrated that human bone cells from older donors produce less proteoglycan, fibronectin, and thrombospondin than cells from younger controls [Fedarko, 1992]. These changes could result in the elaboration of aberrant extracellular matrices which, in turn, could alter macromolecular organization in the skeleton.

Based on this brief overview, it is clear that exploration of the mechanisms producing skeletal senescence will require novel approaches and models.

DO AGE-RELATED CHANGES IN GH/IGF-I AFFECT BONE MASS?

If age-related bone loss is genetically programmed, and the senescent osteoblast lacks either the signal or the response to insulin-like growth factor-I, then GH production in the pituitary may be the site where genetic factors regulate age-related bone loss. There is a vast body of literature supporting the hypothesis that the genome regulates aging via homeostatic and integrative mechanisms. The neuroendocrine system is the most important controlling element of homeostasis, since it regulates, to a greater or lesser degree, practically every function in the body. In addition, GH is the most important anabolic agent in the body, being absolutely essential for protein synthesis throughout life. Most (but not all) of its actions are mediated through hepatic and local synthesis of IGF-I. Hence the GH/IGF-I axis is critical to maintenance of musculoskeletal integrity across a lifetime in both humans and rodents.

Numerous studies have documented reduced amplitudes of GH secretion during senescence [Borst et al., 1994]. At least one study suggests that GH insufficiency affects approximately half of the elderly population [Rudman, 1985]. The defect appears to reside at the hypothalamic level since GH clearance is unaffected by age and, after priming, GHRH can stimulate GH release [Borst et al., 1994]. Likewise serum IGF-I, which is low in elders, can be increased by sufficient doses of GH [Rudman et al., 1990]. It is possible that increased somatostatinergic tone is responsible for the blunting of GH release with aging [Borst et al., 1994]. However, the extreme heterogeneity of the aged population dictates that the likelihood of demonstrating a

relative absence of GH production in most seniors, regardless of health, is very problematic.

Irrespective of cause, even a slight diminution in serum GH or IGF-I might be expected to have a significant impact on bone remodeling. However, like other issues in this paper, critical evidence that changes in circulating IGF-I levels predict bone turnover in the elderly is, at best, circumstantial. Acquired GH deficiency in adults is associated with low bone density and the levels of IGF-I and GH dependent binding protein, IGFBP-3, correlate closely with bone mass [Bing-you et al., 1993; Johansson et al., 1992a]. Similarly, mutant lit/lit GH deficient mice have extremely low femoral bone mineral density (Donahue, personal communication) and low IGFBP-3. Moreover, GH replacement to acquired GH deficient adults results in an increase in IGF-I, IGFBP-3, and bone mineral density (Van der veen, 1990]. On the other hand, the relationship between the GH/IGF-I axis and bone turnover in elders is not nearly as succinct.

The regression equation for age vs. IGF-I is strikingly similar to the inverse relationship between age and bone density. Yet, attempts to correlate IGF-I, as an integrated measure of GH secretion, with bone mass, have met with little success. Ten years ago, Bennett could find no relationship between serum IGF-I and lumbar bone density in a cross-sectional study of women aged 20–80 [Bennett et al., 1984]. Similarly, in elderly healthy women followed over 2 years, IGF-I was not related to bone mass at any site or at any time point [Rosen et al., 1994a]. Because some investigators believe that frail seniors produce little or no GH, the relationship between IGF-I and bone mass was also examined in a group of 30 frail elders (mean age 72 years) randomized to either GH injections, exercise, or placebo [Rosen et al., 1994b]. In that study, serum IGF-I was inversely related to total body bone density even when age and weight were held constant.

In contrast to those studies, two recent reports are noteworthy. Nicholas et al. [1994] isolated IGF-I and TGF-β from femoral cortical bone of 46 men and 20 women between the ages of 20–64. With progressive age, the skeletal content of both IGF-I and TGF-β declined ($r = -0.43$ for IGF-I vs. age). The authors calculated that there was a net loss of 60% in skeletal IGF-1 over those four decades. Conversely, Johansson et al. [1994] measured serum IGF-I and IGFBP-3 in 38 healthy men between the ages of 25–59 years of age. In that cross-sectional study, IGFBP-3 correlated better with bone density of the hip than all other parameters (e.g., VO_2 max, muscle strength, BMI). The authors concluded that IGFBP-3 and GH were important determinants of bone mass. Both investigations provided indirect evidence that circulating growth factors could mirror bone remodeling activity. But, more studies with a greater proportion of aged men, are needed before it can be concluded that serum concentrations of growth factors are reflective of skeletal activity.

The effects of GH "replacement" therapy on bone remodeling in elders are complex. GH administration can change histomorphometric indices and biochemical markers of bone resorption and formation. However, except for one paper by Rudman, no group has shown a statistically significant increase in bone mass following GH treatment in elders [Rudman et al., 1990]. Even in the Rudman study, only lumbar bone density (out of 7 skeletal sites measured) increased after GH treatment [Rudman et al., 1990]. In addition, the change after 1 year was just barely significant (+1.6%) and not different from placebo [Rudman et al., 1990]. By contrast to relatively small changes in bone mass after GH treatment, senescent osteoblasts respond to administration of rhGH by increasing the production of several markers of differentiated osteoblastic function [Marcus et al., 1990]. However, sensitive indices of bone resorption also rise, implying that the final effect on bone remodeling is the maintenance of coupling with enhancement of both the resorption and formation phases. The same holds true in preliminary trials administering rhIGF-I to normal subjects. Serum and urine markers of bone resorption and formation increase concomitantly [Eberling et al., 1993]. Even in the male osteoporosis syndrome (with IGF-I deficiency), rhIGF-I treatment was associated with an increase in the entire remodeling process (resorption and formation) [Johansson et al., 1992b]. Hence, the current data do not support the use of IGF-I or GH replacement therapy to enhance bone mass in elders. Completion of a multicenter NIA sponsored trial with these hormones in male and female seniors should provide more substantial proof of the efficacy (or lack) of growth hormone and/or IGF-I in the treatment of age-related osteoporosis.

SUMMARY

In this paper I have tried to highlight some of the salient issues surrounding age-related bone disease as it relates to growth hormone dynamics. There are no discrete answers to the questions raised in this paper. But, it is hoped that this overview will generate new questions and stimulate development of new models. Foremost among those questions is the issue of senescent osteoblastic activity. In particular, investigations into IGFBP synthesis and clearance in response to paracrine, autocrine, and endocrine factors should yield vital information about the effects of aging on bone formation. More questions will certainly surface concerning the ultimate biologic fate of osteoblasts which do not become osteocytes. For example, do osteoblasts undergo programmed cell death (PCD) and, if so, how is genomic regulation of PCD related to aging and bone loss? Or, do aged osteoblasts express a gene, like the SDI 1 gene in senescent human fibroblasts, which is upregulated during aging and inhibits further cell proliferation?

In addition to those questions, it is still unclear if the aged osteoblast can produce a full complement of growth factor signals or can respond maximally to those stimuli. Moreover, it is uncertain if osteoblastic responsiveness in the elderly is dependent on ambient skeletal IGF-I concentrations. This relates to a more subtle but critical clinical question: When GH is administered to elders, is it hormone "replacement" therapy or a pharmacological treatment? This is not a mute point since skeletal responsiveness to GH differs significantly depending on whether GH is given to acquired GH deficient adults or when administered to healthy elders. Also this issue is extremely relevant because of the inherent difficulties in determining GH deficiency in the aged. Since GH secretion is pulsatile, newer assay techniques for serum GH determinations will probably not provide a usable means for testing GH sufficiency. Recent attempts to standardize serum IGF-I measurements for the elderly population could permit clinicians to determine relative GH status. This, in turn, might lead to screening of subjects who would be eligible for possible GH (or IGF-I) treatment.

We are entering a new therapeutic era of growth factor utilization. By attempting to answer the fundamental questions of skeletal senescence, basic scientists and clinicians should be able to objectively judge the role of growth factors in halting or reversing skeletal aging.

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REFERENCES

Bennett AE, Wahner HW, Riggs BL, Hintz RL (1984): Insulin-like growth factors I and II: Aging and bone density in women. J Clin Endocrinol Metab 59:701–704.

Bing-you RG, Denis MC, Rosen CJ (1993): Low bone density in adults with previous hypothalamic-pituitary tumors. Calcified Tissue Int 52:183–187.

Borst SE, Millard WJ, Lowenthal DT (1994): GH Exercise and Aging: The future therapy for the frail elderly. J Am Geriatr Soc 42:525–535.

Croucher PI, Garrahan NJ, Compston JE (1994): Structural mechanisms of trabecular bone loss in primary osteoporosis: Specific disease mechanism or early aging? Bone Mineral 25:111–121.

Eberling PR, Jones JD, O'Fallon WM, Janes CH, Riggs BL (1993): Short term effects of rhIGF-I on bone turnover in normal women. J Clin Endocrinol Metab 77:1384–1387.

Fedarko NS (1992): Age-related changes in hyaluronin, procollagen, collagen and osteonectin synthesis by human bone cells. J Cell Physiol 151:215–227.

Glowacki J (1994): Influence of age on human marrow. In "Human Models of Skeletal Aging." Washington, DC: NIA Symposium, pp 35–37.

Goldstein S (1989): Cellular senescence. In Degroot LJ, Martini L, Potts J, Nelson D, Winegrad A, Odell W, Steinberger E, Cahill G (eds): "Endocrinology." New York: Grune and Stratton, Vol 3, pp 2525–2549.

Goldstein S, Moerman SJ, Jones RA, Baxter RC (1991): IGFBP-3 accumulates to high levels in culture medium of senescent and quiescent fibroblasts. Proc Natl Acad Sci USA 1991:9680–9684.

Hansen MA, Hassager C, Jensen SB, Christiansen C (1992): Is heritability a risk factor for postmenopausal osteoporosis? J Bone Min Res 9:1037–1043.

Jackson JA, Kleerokoper M (1990): Osteoporosis in men; diagnosis, pathophysiology and prevention. Medicine 69: 139–152.

Jackson JA, Riggs MW, Spiekerman AM (1992): Testosterone deficiency as a risk factor for hip fractures in men: A case control study. Am J Med Sci 304:4–8.

Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC (1992): Increased osteoclast development after estrogen loss: Mediation by IL-6. Science 257:88–91.

Johansson AG, Burman P, Westermark K, Ljunghall S (1992a): Bone mineral density in acquired GH deficiency correlates with circulating levels of IGF-I. J Int Med 232:447–452.

Johansson AG, Lindh E, Ljunghall S (1992b): IGF-I stimulates bone turnover in osteoporosis. Lancet 339:1619.

Johansson AG, Forslund A, Hambraeus L, Blum WF, Ljunghall S (1994): GH dependent IGFBP is a major determinant of bone mineral density in healthy men. J Bone Min Res 9:915–920.

Kawase M, Tsuda M, Matsuo T (1984): Accelerated bone resorption in SAM. J Bone Min Res 359–364.

Kelly PJ, Nguyen T, Hopper J, Pocock N, Sambrook P, Eisman J (1993): Changes in axial bone density with age. J Bone Min Res 8:11–17.

LaTour D, Mohan S, Linkhar TA, Baylink DJ, Strong DD (1990): Inhibitory IGFBP: Cloning, complete sequence and physiologic regulation. Mol Endocrinol 4:1806–1814.

Lewis DB, Liggitt D, Effmann EL, Motley ST, Teitelbaum SL, Jepsun KJ, Goldstein SA, Bonadio J, Carpenter S, Perlmutter RM (1993): Osteoporosis induced in mice by overproduction of IL-4. Proc Natl Acad Sci USA 90:11618–11622.

Ljunghall S, Johansson AG, Burman P, Kampe O, Lindh E, Karlsson FA (1992): Low plasma levels of IGF-I in male patients with osteoporosis. J Int Med 232:59–64.

Manolagas SC, Knutson S, Jilka RL (1993): The SAM-P/6 a model of senile osteoporosis exhibits decreased osteoclastogenesis and fails to upregulate this process following ovariectomy. J Bone Min Res 8(Suppl S141), p 99.

Marcus R, Butterfield G, Holloway L, Gilliland L, Baylink DJ, Hintz RL, Sherman BM (1990): Effects of short term administration of rh GH to elderly people. J Clin Endocrinol Metab 75:519–527.

Marie PJ, Vernejoul C, Connes D, Hott M (1991): Decreased DNA synthesis by cultured osteoblastic cells in eugonadal osteoporotic men with defective bone formation. J Clin Invest 88:1167–1172.

Matsushita M, Tsuboyama T, Ryuichi K, Okumura H, Yamamuro T, Higuchi K, Kohno A, Yonezu T, Utani A, Umezawa M, Takeda T (1986): Age-related changes in bone mass in the senescence-accelerated mouse (SAM). Am J Pathol 125:276–283.

McKay HA, Bailey DA, Wilkinson AA, Houston CS (1994): Famial comparison of bone mineral density at the proximal Bone Mineral 24:95–107.

Melton LJ III, Chrischilles EA, Cooper C, Lane AW, Riggs BL (1992): Perspective: How many women have osteoporosis? J Bone Min Res 7:1005–1010.

Meunier PJ (1993): Prevention of hip fractures. Hong Kong: Consensus Development Conference, March 27–April 2, 1993.

Mohan S (1993): IGFBPs in bone cell regulation. Growth Regul 3:67–70.

Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA (1994): Prediction of bone density from vitamin D receptor alleles. Nature 367:284–287.

Newton-John A, Morgan DB (1970): The loss of bone with age, osteoporosis and fractures. Clin Orthop Rel Res 71: 229–240.

Nicholas V, Prewett A, Bettica P, Mohan S, Finkelman R, Baylink DJ, Farley JR (1994): Age-related decreases in IGF-I and TGF-β in femoral cortical bone from both men and women: Implications for bone loss with aging. J Clin Endocrinol Metab 78:1011–1016.

Pacifici R, Avioli L (1993): Effects of aging on bone structure and metabolism. In Avioli CV (ed): "The Osteoporotic Syndrome." New York: Wiley Liss, pp 1–16.

Pfeilshifter J, Diel I, Pilz U, Brunotte K, Naumann A, Ziegler R (1993): Mitogenic responsiveness of human bone cells in vitro to hormones and growth factors decreases with age. J Bone Min Res 8:707–717.

Puzas JE (1993): The osteoblast. In Fauvus MJ (ed): "Primer on Metabolic Bone Diseases and Disorders of Mineral Metabolism," 2nd ed. New York: Raven Press, pp 15–20.

Rosen CJ, Donahue LR, Hunter SJ, Holick MJ, Kavookjian H, Kirshenbaum A, Mohan S, Baylink DJ (1992): The 24/25 kDa serum IGFBP is increased in elderly women with hip and spine fractures. J Clin Endocrinol Metab 74:24–27.

Rosen CJ, Morrison A, Zhou H, Storm D, Hunter SJ, Musgrave KO, Chen T, Wen-Wei W, Holick MF (1994a): Elderly women in northern New England exhibit seasonal changes in bone mineral density and calciotropic hormones. Bone Mineral 25:83–92.

Rosen CJ, Kiel DP, MacLean DB (1994b): Serum IGF-I and its relationship to bone mass in frail elders. J Bone Min Res 9 (Suppl 1):5388.

Rosen CJ, Donahue LR, Hunter SJ (1994c): Insulin-like growth factors and bone: The osteoporosis connection. Proc Soc Exp Biol Med 206:83–102.

Rudman D (1985): GH, body composition, and aging. J Am Geriatr Soc 33:800–807.

Rudman D, Feller AG, Nagraj HS, Gergens GA, Lalitha PY, Goldberg AF, Schlenker RA, Cohn L, Rudman IW, Mattson DE (1990): Effects of human GH in men over 60 years of age. N Engl J Med 323:1–8.

Slemenda CW, Hui SL, Longcope C, Wellman H, Johnston CC Jr (1990): Predictors of bone mass in peri-menopausal women. Ann Int Med 112:96–101.

Tonna EA (1965): Skeletal aging and its effects on the osteogenic potential. Clin Orthop Rel Res 40:57–81.

Turner RT, Riggs BL, Spelsberg ST (1994): Skeletal effects of estrogen. Endocr Rev 15:275–300.

Urist MR (1964): Accelerated aging and premature death of bone cells in osteoporosis. In Pearson OH, Joplin GF (eds): "Dynamic Studies of Metabolic Bone Disease." Oxford: Blackwell, pp 127–160.

Van der veen R (1990): GH replacement therapy in adults: Bone and calcium metabolism. Horm Res 33:65–68.