Bone Metabolism in Men

BY

PETER GILLBERG
ABSTRACT


In this thesis, the importance of the growth hormone (GH)/insulin-like growth factor (IGF) system and sex steroids for male bone metabolism has been investigated, and the effects of continuous low dose GH replacement in GH deficient (GHD) adults. In a population-based sample of men, positive correlations were found between bone mineral density (BMD) and IGF-I, IGF-II, IGF binding protein (IGFBP)-3 and the testosterone/sex hormone binding globulin (SHBG) ratio. Serum IGFBP-3 and testosterone levels and weight accounted for 34% to 48% of the variation in BMD at different sites. Compared to healthy age matched controls, men with idiopathic osteoporosis had lower estradiol/SHBG ratio and higher SHBG levels. There were no differences between the groups in serum levels of IGF-I, IGFBP-3, 24 hour cumulated GH secretion or peak GH secretion. In the patients, there was a positive correlation between the estradiol/SHBG ratio and BMD in femoral neck. Treatment of patients and controls with GH 0.8 mg/day for one week resulted in similar increases in serum markers for bone turnover in both groups. Several positive correlations between indices of GH secretion and markers for bone turnover were found in the patients. Men with idiopathic osteoporosis were treated with GH, continuously (0.4 mg/day) or intermittently (0.8 mg/day for two weeks every third month), for two years followed by one year of follow-up. After two years, the BMD and bone mineral content in lumbar spine and total body and serum osteocalcin levels were increased in both groups. This increase was sustained one year post treatment. Treatment of GHD adults with a low fixed dose of GH (0.17 mg/day) for three months, resulted in increases in serum IGF-I and IGFBP-3 levels and lean body mass, and a reduction in fat mass and total and low-density lipoprotein cholesterol levels. These beneficial effects were accomplished without serious side effects. These findings indicate that: i) the sex hormone and GH/IGF systems are important in male bone metabolism, ii) a combination of subtle disturbances in these two systems could contribute to the development of male idiopathic osteoporosis, iii) GH treatment could be considered as a treatment option in this condition.

Key words: Male osteoporosis, Bone mineral density, Growth hormone, Insulin-like growth factors, Sex hormones, Growth hormone replacement.

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    In manuscript.

V. Commencing growth hormone replacement in adults with a fixed low dose. Effects on serum lipoproteins glucose metabolism, body composition and cardiovascular function.
    Gillberg P, Bramnert M, Thorén M, Werner S, Johannsson G.
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## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>24hGH</td>
<td>24-hour cumulated growth hormone secretion</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>bALP</td>
<td>Bone specific alkaline phosphatase</td>
</tr>
<tr>
<td>BIA</td>
<td>Bioelectrical impedance</td>
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<tr>
<td>BMC</td>
<td>Bone mineral content</td>
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<tr>
<td>BMD</td>
<td>Bone mineral density</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CCT</td>
<td>Combined cortical thickness</td>
</tr>
<tr>
<td>C-peptide</td>
<td>Connecting peptide</td>
</tr>
<tr>
<td>DPD</td>
<td>Deoxypyridinoline crosslinks</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual X-ray absorptiometry</td>
</tr>
<tr>
<td>FN</td>
<td>Femoral neck</td>
</tr>
<tr>
<td>FAI</td>
<td>Free androgen index</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>GHD</td>
<td>Growth hormone deficiency</td>
</tr>
<tr>
<td>GHRH</td>
<td>Growth hormone releasing hormone</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated hemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>ICTP</td>
<td>Carboxyterminal cross-linked telopeptide of type I collagen</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>IGFBP</td>
<td>Insulin-like growth factor binding protein</td>
</tr>
<tr>
<td>ITT</td>
<td>Insulin tolerance test</td>
</tr>
<tr>
<td>LBM</td>
<td>Lean body mass</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>Lipoprotein (a)</td>
</tr>
<tr>
<td>LS</td>
<td>Lumbar spine</td>
</tr>
<tr>
<td>OHPr</td>
<td>Hydroxyproline</td>
</tr>
<tr>
<td>PICP</td>
<td>Carboxyterminal propeptide of type I procollagen</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone-binding globulin</td>
</tr>
<tr>
<td>SXA</td>
<td>Single X-ray absorptiometry</td>
</tr>
<tr>
<td>TB</td>
<td>Total body</td>
</tr>
<tr>
<td>TBW</td>
<td>Total body water</td>
</tr>
<tr>
<td>UD</td>
<td>Ultra distal</td>
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INTRODUCTION

Bone Metabolism

The remodeling process

Bone is, in contrast to what is generally believed, a metabolically active organ, which is subject to continuous remodeling in order to adapt to different needs at different timepoints during life. The skeleton serves as a calcium reservoir, gives protection to our vital systems, supports the body as a whole, and has important mechanical properties in providing attachment for muscles and tendons to act as levers and thereby facilitating locomotion.

In the adult, there are two main types of bone tissue, compact or cortical bone and spongy or cancellous bone. Bones have an outer cortical part that surrounds and encloses the cancellous inner part and marrow space. The major part of adult bone mass is cortical bone, but due to the trabecular arrangement in cancellous bone, there is an 8-10 fold greater surface/volume ratio of cancellous to cortical bone. All bone surfaces are covered with cells, and the most important cells in the human skeleton are the osteoblasts, the osteoclasts, and the osteocytes.

Osteoblasts are bone-forming cells that synthesize bone matrix and have a role in the mineralization process. Some resting osteoblasts can be found on the bone surface, and these cells are termed bone-lining cells. Osteoclasts are responsible for bone resorption. These cells degrade mineralized bone by secreting lytical enzymes and acids onto the bone surface. Osteocytes are in fact osteoblasts that have been trapped in the bone matrix during the remodeling process. They are interconnected with other osteocytes and osteoblasts through long microfilament rich cell processes. The exact function of these cells is still poorly understood (1, 2).

The skeleton is subject to a continuous process of bone turnover, called bone remodeling. During this process, old bone is replaced by new, trabecular micro damage is repaired and the strength of skeleton is adjusted according to the mechanical stress to which it is subjected. Bone remodeling is a coordinated action of osteoclasts and osteoblasts which take place on the trabecular surfaces, or, in cortical bone, in Haversian systems, which are channel-like structures that surround blood vessels. Due to the larger surface/volume ratio, remodeling activity has greater impact in cancellous than in compact bone. The remodeling sequence is always the same. It starts with activation of osteoclasts in specific focal sites by mechanisms that are not fully understood. The activated osteoclast then starts bone resorption, which is a process that is estimated to last 10 days in cancellous bone. This phase, in most physiological and pathological circumstances, is followed by local osteoblastic bone formation, whereby the defect is repaired. The bone formative phase is more protracted than the resorptive and has a duration of approximately 3 months. The bone remodeling is a coupled process, but the cellular and humoral mechanisms responsible for mediating this coupling are still not clear (2-4).
Markers for bone turnover

During the process of bone resorption, the mineral phase of bone is dissolved, and the organic matrix is degraded. This results in release of calcium, pyrophosphate, a number of enzymes, and matrix degradation products that can be assayed in blood or urine.

Biochemical indices of bone resorption currently in use include hydroxyproline (OHPr), deoxypyridinoline crosslinks (DPD), and type I collagen c-terminal cross-linked telopeptide (ICTP). OHPr is released during collagen degradation and excreted in the urine. Bone turnover contributes the major component to fasting urine OHPr excretion. The most abundant quantities of DPD are found in mature bone collagen. DPD is released during collagen degradation and excreted in the urine, where the amount excreted correlate well with bone resorption. ICTP is derived from the crosslinks between collagen chains and is released during bone resorption. Serum ICTP levels correlate well with the rate of bone resorption in patients with osteoporosis (5).

During the biosynthesis of bone, precursors of bone matrix components and enzymes are produced intracellularly. These components include type I collagen, osteocalcin and alkaline phosphatase. Bone specific alkaline phosphatase (bALP) is an enzyme that is associated with osteoblastic activity and can be measured in serum. Osteocalcin is the most abundant non-collagenous protein in bone. It is associated with hydroxyapatite, and during bone formation, a small proportion of the newly synthesized osteocalcin diffuses into the circulation and can be analyzed in serum samples. The major form of collagen in bone is collagen type I. From the precursor of this form of collagen, type I procollagen, propeptides are released from the C-terminal and N-terminal ends to the circulation. The procollagen type I C-terminal propeptide (PICP) can also be measured in serum and there is a good correlation between serum PICP levels and bone formation (1, 5).

Fig. 1. Schematic drawing of the bone remodeling process
Pathogenesis of Osteoporosis

Osteoporosis is a disease of the skeleton, and is defined as “a systemic skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a subsequent increase in bone fragility and susceptibility to fractures” (6). The major clinical problem in this setting, is the risk for fractures, commonly seen in the spine, wrist and hip. The lifetime risk for one of these fractures has been estimated to 13% in men and 40% in women (7). This constitutes a major clinical problem that for the patients results in substantial morbidity and even mortality (8) and imposes a significant economic burden for the society (9).

Osteoporosis can be considered to be a type of bone atrophy. The mineral to collagen ratio is preserved in this disease which distinguishes it from osteomalacia, a disease characterized by a relative deficiency of mineral to collagen. The critical issue in osteoporosis, thus, is low bone mass. Bone mass is determined by two factors: attainment of peak bone mass and subsequent bone loss.

Peak bone mass

During puberty there is a marked increase in bone mineral density (BMD) and bone mineral content (BMC) in both men and women. In men, peak bone density in the spine is reached at about 20 years of age, and in cortical bone, such as the radius and femoral shaft, a few years later (10, 11). In women, there is a rapid increase in bone mass at puberty in association with the increase in sex hormone levels and the closure of the growth plates. Within 3 years of menarche, there is little further increase in bone mass. However, there are small yearly increases in BMD over the next 5 to 15 years, and the resulting peak bone mass is achieved between 20 and 30 years of age (12).

Peak bone mass is determined by several factors. One of the most important is the timing of puberty. Men with delayed puberty have been shown to have lower BMD than age matched normal men (13). The acquisition of bone mineral during puberty is in both genders influenced by genetic factors, and heritability is considered a major determinant for peak bone mass (14). Other factors of importance in achieving maximal peak bone mass are regular exercise and to restrain from smoking (15).

Bone loss

At tissue level, bone loss results from an imbalance between resorption and formation, with an increase in osteoclastic activity and a decrease in osteoblastic activity. In cancellous bone, this leads to thinning of the trabeculae, trabecular perforation and, as a consequence, loss of trabecular elements (16).

Normal aging is related to bone loss in both men and women (17). However, several factors can increase the rate of bone loss. In women the most well known and important of these factors is pre- and post menopausal estrogen deficiency (18).
Male Osteoporosis

In men, bone loss and subsequent development of osteoporosis is either secondary to other diseases or medications or due to aging. However, in between 40% and 60% of all cases of male osteoporosis no cause for the disease can be found, and this is termed primary or idiopathic osteoporosis (19).

Secondary osteoporosis

The most important causes of secondary osteoporosis in men are hypogonadism, glucocorticoid therapy, gastrointestinal disease and alcohol abuse (20, 21).

Hypogonadism

Male hypogonadism is associated with low bone mineral density (BMD) and osteoporosis (22). This decrease in BMD is also associated with an increased risk for fractures, both of the spine and the hip (23). In elderly men, treatment with gonadotropin-releasing hormone agonist induces hypogonadism with decreases of serum testosterone levels. This causes a high turnover accelerated bone loss during the first year of treatment, resembling what is seen in women at the menopause, and subsequently a decrease in vertebral bone density (24). There have also been several reports of increases in BMD with testosterone replacement in hypogonadal adult men (23). The cellular response to testosterone withdrawal in men seems to be complex. Gonadal insufficiency in men is followed in early stages by rapid bone loss and increased remodeling, resembling what is seen in women at the menopause. This period of rapid bone loss in men is followed by a later stage of lower remodeling rates accompanied by a relatively reduced bone formation (23). This later phase is different from the protracted accelerated bone loss associated with menopause, where there is a predominance for increased osteoclastic activity and bone resorption (3). Thus, trabecular bone mass decreases in both men and women as a result of hypogonadism, however, the bone loss differs qualitatively as trabecular perforation is more common in women (25).

Glucocorticoid therapy

Glucocorticoid excess, nearly always caused by exogenous glucocorticoid therapy, causes bone loss and is responsible for approximately 15% of all vertebral fractures in men (21). The bone loss is most marked in the first 12 months of treatment and occurs more rapidly in trabecular than cortical bone. Decrements of up to 20% of trabecular bone in the lumbar spine have been noted in patients who have received glucocorticoid treatment for 5 years (26). The primary mechanism by which glucocorticoids induce bone loss appears to be via a direct inhibitory effect on osteoblast activity (27). However, glucocorticoids affect calcium balance and bone metabolism via actions at many sites. Calcium malabsorption is common in steroid treated patients, as well as an increase in urinary calcium excretion and a reduction in renal tubular reabsorption of phosphate. Men receiving glucocorticoid treatment also have reduced serum testosterone levels, probably as a result of inhibited gonadotropin secretion (26).
Gastrointestinal disease

Diseases of the gastrointestinal tract have been associated with osteoporosis, probably as a result of calcium and vitamin D malabsorption. However, the exact etiology of gastrointestinal bone disease remains unclear. Gastrectomy and small bowel disease have been shown to be associated with low BMD in men, whereas large bowel disorders rarely have been linked to male osteoporosis (27).

Alcohol

Alcohol intake is inversely correlated to bone density in men (27) and osteoporotic fractures are common in alcoholic men (20, 28). Alcohol intake has been associated with reduced rates of bone formation in humans and experimental animals (1). Alcoholism can also result in dietary disturbances such as calcium and protein malnutrition, liver disease, decreases in serum testosterone levels and increased risk for falling which may have additional effects on the risk for osteoporotic fractures.

Other causes

In addition to the mentioned etiologies of secondary osteoporosis in men, a variety of disorders have been linked to this disease. Among other causes, primary hyperparathyroidism, hyperthyroidism or over treatment with thyroid hormone, hypercalciuria, multiple myeloma, other malignancies, anticonvulsant use and high dose chemotherapeutics have been described (25, 27).

Senile osteoporosis

Osteoporosis due to aging in men is associated with both cortical and trabecular bone loss. Histomorphometric studies have shown that bone loss with aging results from a predominant reduction in bone formation and osteoblastic activity with a preserved osteoclastic function (29). In a recent study it has been shown that in men, after the age of 60 years, markers for bone resorption increase whilst markers for bone formation remain stable (30). The result of this age related imbalance between bone formation and resorption is a net loss of bone volume which subsequently leads to the development of osteoporosis.

Senile osteoporosis in both men and women can result from an imbalance in the demands for and intake of calcium and vitamin D. There are age-related changes in calcium regulating hormones. Parathyroid hormone concentrations have been shown to increase with age, partly as a result of low dietary calcium intake, which is not compensated for by an increase in 1,25-dihydroxy vitamin D production (1). It has been shown that part of the variance in bone density in men can be explained by these factors (25). Several reports have linked calcium intake to male BMD, but there have been inconsistent results in studies on the relationship between calcium intake and fractures (27). One report has been published where elderly men and women were treated with 500 mg of calcium and 700 U of vitamin D$_3$ for three years (31). This treatment resulted in a reduction in bone loss in
both women and men, and in the whole study group a reduction in the incidence of non-vertebral fractures by 50%. These data taken together indicate a role for insufficient calcium intake in the etiology of senile male bone loss.

Aging in men is also related to a diminution of serum levels of total testosterone and estrogens (32), and an increase in sex hormone-binding globulin (SHBG) levels (33), further reducing the bioavailable fractions of these hormones. As testosterone and estradiol are linked to male bone metabolism, the age-related changes could contribute to the development of senile osteoporosis in men.

**Idiopathic Osteoporosis**

The age range of men with idiopathic osteoporosis in the few reports on this condition varies from 20-86 years of age (20, 21, 34). This age range constitutes an overlap with that for senile osteoporosis and makes it difficult to distinguish between the two. It has been suggested that the diagnosis of male idiopathic osteoporosis should be given to men under the age of 70 years, with osteoporosis that occurs without any known cause (35). The condition is relatively uncommon, with an estimated annual incidence of 0.4 cases per 100,000. In contrast to involutional osteoporosis, which has a female preponderance, idiopathic osteoporosis occurs with equal frequency in men and women. Reports of male idiopathic osteoporosis are infrequent, but reports of the female equivalent are even scarcer. The usual clinical presentation is the occurrence of multiple vertebral fractures over a period of 5-10 years, accompanied by a loss of height and in severely affected individuals unilateral or even bilateral hip fractures (36).

This group of patients is interesting with respect to possible etiologies for the disease. As these men are otherwise healthy, understanding the mechanisms underlying the development of idiopathic osteoporosis might also be of importance for understanding normal bone metabolism and the pathogenesis of bone loss in various conditions.

Bone histomorphometric analyses of these patients have shown a negative bone balance, with a reduction of bone formation that is more pronounced than the decrease in bone resorption, with a consequent reduction in mean wall thickness (37). This finding is in accordance with histomorphometric findings in men with senile osteoporosis (29). Idiopathic osteoporosis represents a state of low bone turnover and differs from findings in female postmenopausal osteoporosis, which is characterized by a high bone turnover and an increase in osteoclastic activity and bone resorption that is more pronounced than the increase in bone formation (3, 37).

The exact mechanisms for this remodeling imbalance with impaired osteoblast function in idiopathic osteoporosis remain unclear, however, disturbances in the growth hormone (GH) /insulin-like growth factor (IGF) system and the sex hormone system have been proposed as etiologic factors.
The Sex Hormone System

Androgens in men are produced in the adrenal cortex and in the testes. Testosterone is primarily produced in the Leydig cells in the testes, and the production and secretion is regulated by luteinizing hormone (LH) from the pituitary gland. Testosterone is in plasma transported bound to proteins, largely to albumin (about 50%) and to SHBG (about 45%). Only about 1 to 3 percent of the plasma testosterone is present in free and biologically active form. The major function of androgens are to regulate LH secretion, the initiation and maintenance of spermatogenesis, the formation of the male phenotype during sexual differentiation, and the induction of sexual maturation and function following puberty (38). Studies of men with hypogonadism have also shown that androgen deficiency is associated with osteoporosis, loss of muscle mass and increase in fat mass and that testosterone treatment can reverse these changes. Several studies have shown a decline in testosterone production with advancing age in men (39).

Circulating androgens, primarily androstenedione and testosterone, are converted to estrone and estradiol by an enzyme, aromatase cytochrome P450. This enzyme is found in various tissues, but the most important tissue for estrogen synthesis in males is adipose tissue. As a result, the estrogen biosynthesis increases as a function of weight, but it also increases as a function of age. The conversion of androgens to estrogens is the main source for estrogens in postmenopausal women (40). In men over 50 years of age, the serum estradiol levels have been found to be approximately 4 times higher than in postmenopausal women (41).

SHBG is a specific steroid-binding plasma glycoprotein, which is mainly synthesized in the liver. This protein reversibly and with high affinity binds biologically active circulating androgens and, to a lesser extent the active estrogen, estradiol. This high-affinity binding limits the transfer of estradiol and testosterone to target cells, and consequently their biological activity. Mean SHBG levels are higher in women than in men, and higher in children than adults. After a decline during puberty, the serum levels remain unchanged until age 40-50, when the SHBG levels again begin to increase. Serum SHBG levels correlate negatively with serum insulin and IGF-I levels. It is well established that SHBG levels increase during estrogen treatment and decrease after administration of testosterone to hypogonadal men. However, it is generally believed that factors such as nutrition, insulin, IGF-I and GH which are involved in protein synthesis, play a more active role in regulating SHBG, while sex steroids may have an indirect effect (33, 42).

The Growth Hormone/Insulin-Like Growth Factor System

Growth hormone is secreted in a pulsatile manner from the anterior part of the pituitary gland. The hormone is important in promoting linear growth in children, but is also an important anabolic factor with stimulatory effects on protein synthesis and lipolysis. The pituitary GH secretion is regulated by two hypothalamic peptides, GH-releasing hormone (GHRH) which stimulates GH synthesis and secretion, and somatostatin which inhibits the GH synthesis and secretion. Human studies have shown that GH is secreted in discrete pulses and that secretion is increased during slow wave sleep so that most GH secretion occurs at night (43). With advancing age, this secretory pattern undergoes changes, with
fewer total number of GH pulses and relatively more of the GH secretion occurring during daytime (44). GH secretion is also affected by many other factors, and increases after physical exercise and in response to hypoglycemia. Pubertal increases in plasma estradiol and testosterone also augments pulsatile GH secretion. Spontaneous and stimulated GH secretion is affected by gender and is higher in women than in men. A reduction in basal and stimulated GH secretion is seen in states of obesity, mental depression, hyperglycemia and aging (43).

GH has both direct and indirect actions on peripheral tissues. Indirect effects of GH are mediated by IGFs, mainly IGF-I. The two known IGFs, IGF-I and IGF-II, share a structural homology with insulin and act through similar cell surface receptors and share many biological properties (43). Almost every mammalian cell type can synthesize and export IGF-I and IGF-II. Circulating IGF-I is considered to be synthesized mainly in the liver and its production is GH-dependent (45). IGF-I, in turn, feeds back on the hypothalamus and the pituitary and inhibits GH release (43). There are other factors than GH affecting IGF-I concentrations in serum and tissue. The nutrient status of an individual has a profound effect on serum IGF-I, with malnutrition and catabolic states leading to reductions in serum concentrations. The age-related decline in GH secretion and sex steroid production is accompanied by decreases in serum IGF-I levels, and there is a marked gender difference in serum IGF-I concentrations, being 10-15% higher in men than in women (46). Thus, despite lower GH secretion in men than in women, men have higher serum IGF-I levels, indicating that they are more sensitive to GH than women, which has been shown in recent studies (47, 48).

In contrast to IGF-I, the production of IGF-II is not GH-dependent. IGF-II is much more active during prenatal life than IGF-I. The relative role of IGF-II, as compared with IGF-I, in stimulating non-GH-dependent growth-promoting actions in postnatal life, is incompletely characterized (49).

Circulating IGF-I and –II are to a great extent bound to IGF binding proteins (IGFBPs). Six different IGFBPs have been characterized to date and named IGFBP 1-6. They share approximately 50% sequence homology and the most abundant IGFBP is IGFBP-3 (46). IGFBPs are produced by a variety of biological tissues, and are found in various biological fluids (50). IGFBP-3 is the predominant circulating binding protein and accounts for nearly 95% of the total IGFBP activity (43). Ninety-nine percent of all IGF-I and IGF-II are bound to IGFBPs in serum, and approximately 75 % is carried in a complex with IGFBP-3 and a liver derived non-IGF binding glycoprotein component termed acid labile subunit (46, 49). The function of this complex is to prolong the half-lives of IGF-I and IGF-II in the circulation. The serum concentration of IGFBP-3 is GH-dependent (51, 52) and has together with IGF-I, been shown to reflect the spontaneous GH secretion in children (53).

IGFBP-1 has been suggested as a regulator of the bioavailability of serum IGF-I. Hepatic insulin concentrations regulate the production of IGFBP-1 in the liver and increased insulin production results in a down regulation of IGFBP-1 production. With starvation the hepatic IGF-I production is decreased. Also serum IGFBP-1 levels increase and binds more of the circulating IGF-I, thus further reducing the free biologically active fraction (54).
IGFBP-2 has been much less studied. In general the most prominent feature of this binding protein appears to be to inhibit IGF-II actions. IGFBP-4 is an inhibitor of IGF action, and its main function appears to be protection of cells from overstimulation by IGFs. This binding protein is of importance for bone metabolism. IGFBP-5 is similar to IGFBP-3 in its ability to enhance IGF actions. IGFBP-5 adheres tightly to cell membranes and thereby this protein potentiates the effect of IGF-I on DNA synthesis. Studies on IGFBP-6 are very limited. This protein may share some similarities with IGFBP-2 (49).

**Growth hormone deficiency**

GH deficiency (GHD) in adults is associated with several symptoms and metabolic consequences. Reduced vitality, diminished muscle strength and a poor quality of life are symptoms that have been reported. The metabolic consequences of prolonged GHD in adults are profound. Several studies have reported a marked increase in total body fat and an abnormal fat distribution in combination with a reduction in lean body mass (LBM), total body water and BMD (55, 56). GHD is also associated with decreased muscle strength and exercise performance. Moreover, GHD adults have an increase in total serum cholesterol levels, primarily dependent on an increase in low density lipoprotein (LDL) cholesterol (55). Furthermore, GHD in adults has been associated with impaired systolic cardiac function (57). In this view, it is not surprising that there have also been reports on reduced quality of life in GHD adults (58).

Despite this long list of clinical features, there is no single sign or symptom that is pathognomonic of GHD in adult life. The diagnosis therefore has to rest on symptoms and signs of GHD in combination with biochemical evidence. As GH secretion is pulsatile, the evaluation of spontaneous GH secretion requires frequent blood sampling over 24 hours, which in most cases is impractical. GH secretory status may be indirectly assessed by analyzes of serum levels of IGF-I and IGFBP-3, which have been considered useful in screening, but not as diagnostic tests (59). An additional way to detect diminished GH secretion is to assess the peak GH secretory response to a standard provocative stimulus. Several stimuli have been investigated for their ability to release GH. Among these are heat, insulin tolerance test (ITT), GHRH, glucagon, arginine, L-dopa and clonidine. The most reliable and preferred test in adults is ITT, whereby a hypoglycemia is induced and triggers a peak GH response (60).

**Growth hormone replacement**

GH treatment of adults with GHD has been shown to be beneficial on body composition and results in significant increase in LBM, a decrease in fat mass, and an increase in total body water (61-63). GH replacement of GHD also improves lipid metabolism, with reductions in total and LDL cholesterol levels, without causing any changes in serum high density lipoprotein (HDL) cholesterol or triglyceride levels (64). Moreover, it has been shown that GH-replacement can reverse the cardiac impairment seen in GHD adults (65, 66) and improve the psychological wellbeing of the patients (63).
GH is known to decrease insulin sensitivity (67) and acromegaly is known to be associated with insulin resistance (68), so there have been concerns that GH replacement would cause impairment in glucose metabolism, which also has been shown in several studies (62, 69, 70). This replacement-induced insulin resistance, however, seems to be dose-dependent and at least partially transient (70). However, GHD per se in adults is also associated with impaired glucose utilization and insulin resistance (71). Furthermore, GH replacement has been shown to increase serum levels of lipoprotein (a) (72), and a high serum level of this lipoprotein is an independent risk-factor for cardiovascular disease (73).

GH replacement is also associated with side-effects, such as muscle or joint stiffness, arthralgias, edemas and carpal tunnel syndrome. These GH related side-effects are mainly caused by excessive fluid retention and may disappear spontaneously within a few weeks of treatment (74).

These adverse effects of GH replacement seem to be dose dependent and could possibly be avoidable if the target dose is appropriately chosen (62, 74). At present, no consensus exists regarding individual GH dose in replacement therapy for GHD adults. The wide variation in interindividual response to GH replacement among GHD adults, however, calls for individual dose regimens (47, 75, 76). It has been recommended that GH replacement should be started at approximately 0.15-0.30 mg (0.45-0.90 U)/day and then increased gradually at monthly intervals until target dose is reached, which for younger GHD patients appear to be less than 0.5 mg (1.5 U)/m²/day and for older GHD patients less than 0.33 mg (1.0 U)/m²/day (60, 74). Target dose is determined by biochemical and clinical response, and at present, the best biochemical marker of GH effect are serum levels of IGF-I (60).

Until recently, the dose of GH in replacement therapies has been based on body surface area or body weight. More recent trials show that when considering individual responsiveness to GH in GH replacement dosage, with individualized dose titration, fewer adverse side effects but similar efficacy can be obtained (62, 76-78). Although some of the most recent studies concerning effects of GH replacement in GHD adults have started at low doses of GH (0.17-0.27 mg (0.5-0.8 U)/day) the GH dose has been titrated to higher doses (62, 76, 77). Some previous findings have suggested that continuing treatment with GH may increase the sensitivity to the hormone. During long term GH treatment reduction of the doses of GH does not result in reduced serum concentrations of IGF-I (79).

The GH/IGF System and Bone Metabolism

Several clinical and experimental studies have suggested that the GH system with IGF-I and IGF-II and their binding proteins are important factors in bone metabolism (80, 81).

Most tissues produce IGF-I and IGF-II. In bone, they are produced by osteoblasts and act by stimulating bone formation and collagen synthesis (80, 81). IGF-I also stimulates osteoclast formation and action (82). It has been shown that concentrations of IGF-I in the bone matrix of women are positively associated with histological estimates of bone formation and resorption. There is also evidence to indicate an inverse relationship between IGF-I concentrations in bone and age. Furthermore, it has been established that
the IGF-II concentration in female bone matrix is positively correlated with osteoblastic surface measured by bone histomorphometry (83).

IGFBP-1 and IGFBP-2 can be produced by osteoblast-like cells and can inhibit IGF binding to IGF receptors (50, 84, 85). Only a few clinical studies have investigated the role of IGFBP-1 and IGFBP-2 in bone metabolism. Elevated serum levels of IGFBP-2 and a negative correlation with BMD have been shown in postmenopausal women with osteoporosis (86, 87).

IGFBP-3 is produced by osteoblasts and the synthesis is stimulated by GH (88) in addition to other factors. Local IGFBP-3 in bone has been suggested to enhance the anabolic effects of IGFs (89). A possible mechanism for the anabolic effect of circulating IGFBP-3 is to potentiate the effect of IGF-1 by prolonging its half-life in serum (90). Affirmation of this view is derived from several independent studies indicating positive correlations between serum levels of IGF-I and IGFBP-3 (91-93).

IGFBP-4 is the major IGFBP produced by osteoblasts and is a potent inhibitor of IGF-I actions. Parathyroid hormone has been shown to stimulate the production of IGFBP-4 in osteoblasts, suggesting a mechanism whereby this hormone could attenuate the effect of IGF-1 (49). IGFBP-5, in contrast, has a high affinity for hydroxyapatite and binds strongly to bone matrix. This binding protein thereby can potentiate the effects of IGFs on bone remodeling by binding them to the bone surface (81). Serum levels of IGFBP-4 and IGFBP-5 have been shown to be decreased and positively correlated to BMD in GHD adults (94).

Studies on IGFBP-6 are scarce. This binding protein has been shown to bind IGF-II stronger than IGF-I and is a relatively specific inhibitor of IGF-II actions (95). IGFBP-6 has been identified in osteosarcoma cells (81), but its regulation and action in bone metabolism must be further investigated.

Previous studies on GH treatment in adults, both GH-deficient patients and non-GH-deficient subjects, have shown increased bone turnover, with enhancement of both bone resorption and bone formation markers after variable treatment periods (96-98). Increases in BMD and BMC have also been observed in GHD adults that receive GH replacement (47, 99).

A few studies have been reported concerning GH treatment of osteoporotic subjects. In two studies short-term GH treatment was given to postmenopausal osteopenic and osteoporotic women (100, 101), and in one study, men with idiopathic osteoporosis were treated with GH for one week (34). In all these studies, GH treatment resulted in significant elevations of biochemical markers for both bone resorption and bone formation. In fact, a prolonged effect on serum osteocalcin levels have been observed in GH treatment of osteoporotic subjects, with persistent increases for 30-177 days posttreatment. The effect on bone resorption markers seems to be more transient (100) (34). In two recent studies, women with osteoporosis have been treated with GH for one year. Neither of these studies could demonstrate any positive effects on BMD (102, 103). However, in one of the studies, the BMD in lumbar spine and distal radius increased significantly one year after cessation of treatment (102). A similar further increase in BMD and BMC 12-18 months after discontinuation of GH replacement has been noted in
adult GHD men (104, 105). These data could support the theory that GH treatment initializes bone remodeling cycles with a relatively greater enhancement of bone formation, as indicated by bone histomorphometric analyzes performed on GHD men treated with GH for one year (106).

Characteristics of Male Idiopathic Osteoporosis

**The GH/IGF system**

Several investigators have found significantly reduced IGF-I levels in men with idiopathic osteoporosis (107, 108). It has also been shown that serum IGF-I levels correlate positively with BMD in healthy men (91). It is not known whether circulating IGF-I and IGF–II level represent skeletal concentrations of these factors. However, positive correlations between serum levels of IGF-I and IGF–II and BMD have been reported in several studies (86, 107-109). Moreover, there is evidence that treatment with IGF-I in men with idiopathic osteoporosis results in an increase in markers for bone formation and resorption, indicating that systemically derived IGF-I has an effect on bone metabolism (34). Still, the relative contributions of systemically and bone derived IGF-I to the bone remodeling process are unknown.

The serum level of IGFBP-3 has been reported to be an age-independent predictor for BMD at different anatomical sites in healthy men (91), and it has been observed that men with idiopathic osteoporosis have lower levels of IGFBP-3 than healthy controls (37). Because serum levels of IGF-I and IGFBP-3 are GH-dependent, the positive relationship seen between serum levels of IGF-I and IGFBP-3 and BMD could also reflect the GH secretory status of the subjects (53). It has recently been shown that patients with idiopathic osteoporosis have a higher degree of polymorphism in the IGF-I gene causing, independently from GH, lower IGF-I concentrations than in controls (110).

**The sex hormone system**

Hypogonadism is an important cause for secondary osteoporosis in both men and women. Androgens have been shown to have a strong anabolic effect on bone (23), on human osteoblastic cells in vitro (111) and in eugonadal men (112) in vivo. It has been suggested that this effect, at least in part, is due to aromatization of testosterone or androstenedione to estrogens (112, 113). This conversion in men predominantly takes place in adipose tissue and is catalyzed by the enzyme, aromatase cytochrome P450, and the aromatization of androgens to estrogens therefore increases with increased fat mass (40). In two recent studies (32, 41) it has been shown that the estradiol/SHBG ratio, which reflects the free and metabolically active fraction of serum estradiol (42) is an independent predictor of BMD in healthy men. These findings, in combination with clinical observations of severe osteoporosis in one man with defective estrogen receptor (114) as well as in two men with aromatase deficiency (115, 116), suggest that not only androgens, but also estrogens, are important for maintenance of male bone mass.
AIMS OF THE INVESTIGATION

The specific aims of the thesis were:

- To investigate the importance of the sex hormone system and the GH/IGF system for bone metabolism in a population based sample of Swedish men (paper I)

- To investigate the importance of the sex hormone system and the GH/IGF system for bone metabolism in men with idiopathic osteoporosis (papers II and III)

- To investigate the effects of GH treatment on bone metabolism in men with idiopathic osteoporosis (papers III and IV)

- To investigate if a low fixed dose of growth hormone in growth hormone deficient adults may reduce the frequency of side-effects without loss of short-term efficacy (paper V)
MATERIALS AND METHODS

Diagnosis of Male Idiopathic Osteoporosis

The diagnosis of idiopathic osteoporosis in papers II, III and IV was based on a BMD in lumbar spine (L2-L4) and/or in the femoral neck of $\leq -2.5$ SD of the mean BMD in a young male reference population and/or $\geq 1$ osteoporotic fracture. Secondary osteoporosis was excluded after thorough clinical and laboratory investigations. Bone mineralization defects were excluded in all subjects by transiliac bone biopsies after tetracycline labeling.

Study Populations and Designs

**Paper I**

A random selection of 61 men with years of birth between 1910 and 1970 was performed from the population register in Uppsala County, Sweden (117). About 50% agreed to participate after the first invitation. Thereafter, we continued to send new invitations to randomly selected inhabitants until all years of birth within the defined range were represented. Each subject was evaluated by a questionnaire, where family and medical history and dietary intake of calcium was evaluated. Six men were excluded due to a history of a disease or medication known to influence bone and calcium metabolism. The final sample comprised 55 men between the ages 22 and 85 years (52±18, mean±SD). They had a mean daily intake of 592±295 grams of calcium. Four of these men had, according to the questionnaire, suffered a fracture due to low energy trauma.

Fasting morning blood samples were collected and dual-energy X-ray absorptiometry (DXA) and single-energy X-ray absorptiometry (SX) measurements were performed approximately one week later.

**Paper II**

Twelve men with idiopathic osteoporosis age 27-55 (41.5±8.7) years were investigated and twelve healthy age-matched men, age 27-62 (43.0±10.1) years, who were recruited from a population-based register and among health care personnel, served as controls. Fasting morning blood and urine samples were obtained for analysis of sex steroids and markers for bone turnover. DXA measurements were performed later the same day to determine BMD and body composition.
Paper III

A total of twenty men, age 27-57 (45.1±8.9) years, with idiopathic osteoporosis, otherwise healthy, were included in the study. The controls used were the same as in paper II.

Sex steroids, markers for bone turnover, IGF-I and IGFBP-3 were analyzed in fasting morning blood and urine samples. Samples for serum GH determinations were then obtained at 30-min intervals for 24 hours from twelve of the patients and all controls, and cumulated GH secretion (24hGH) was derived. All the subjects that had participated in the 24 hour GH sampling then underwent an insulin tolerance test in order to provoke peak GH secretion. All subjects experienced a significant hypoglycemia.

Fourteen of the patients, and all the controls were at least four weeks later treated with human recombinant growth hormone (Genotropin, Pharmacia & Upjohn), 0.8 mg (2.4 U)/day subcutaneously, at bedtime for 7 days. In the morning of day 8, after overnight fasting, samples of blood and urine for measurement of IGF-I and markers for bone turnover were obtained.

Patients and controls were matched for age in all phases of the study.

Paper IV

Twenty-nine men with idiopathic osteoporosis age 27-62 (47.8±9.8) years were randomly assigned to treatment with recombinant human GH, either 0.4 mg (1.2 U)/day continuously (group A, n=14) or 0.8 mg (2.4 U)/day for 14 days every three months (group B, n=15) for two years with a follow-up period of one year. All patients received supplements with 500 mg of calcium and 400 U of vit D3. Samples from serum and urine were obtained after overnight fasting for analysis of IGF-I and bone markers at baseline, after 1 month and then every six months. BMD, BMC and body composition were determined by DXA every six months. A radiogram of the second metacarpal bone of the right hand was performed at baseline and after two years to evaluate changes in combined cortical thickness (CCT). Lateral spine X-rays of the thoracic and lumbar spine were obtained at baseline and after two years.

Paper V

In this multicenter study, 53 GHD adults age 18-78 (44.5±16.8) years were treated with a fixed low dose (0.17 mg (0.5 U)/day) of GH (Humatrope, Eli Lilly Co) for three months. In 9 patients the starting dose was 0.08 mg (0.25 U)/day, which was increased after 2 weeks to 0.17 mg (0.5 U)/day, and in four patients the dose was increased to 0.25 mg (0.75 U)/day and in one patient to 0.3 mg (1.0 U)/day, resulting in a mean dose of 0.18 mg (0.53 U)/day after 3 months.
Nineteen of the patients were women and thirty-four were men, and in 14 of the patients the GHD was of childhood onset. GHD was defined as a peak GH at ITT, after arginine stimulation test or after GH releasing hormone stimulation ≤ 3µg/L. In 2 patients with panhypopituitarism the diagnosis was based on multiple GH sampling and low serum IGF-I levels.

DXA was used to determine body composition and bioelectrical impedance (BIA) to assess total body water. Cardiac function was examined by echocardiographic examination and exercise capacity was measured by bicycle ergonometry. Serum lipids, IGF-I, IGFBP-3, and markers for glucose metabolism were analyzed in fasting morning blood samples. An oral glucose tolerance test was also performed. All investigations were performed at baseline and after three months.

**Analytical Methods**

**Growth hormone, insulin-like growth factors and binding proteins**

GH (22 kD) in serum was determined by autoDELFIA hGH assay, which is a solid phase, two-site fluoroimmunometric assay (Wallac OY, Turku, Finland) with intra- and interassay coefficients of variation < 5%.

IGF-I in serum was measured by an immunoradiometric assay after formic acid-ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA, paper III and IV and by a method previously described (118), paper I and V).

Serum IGFBP-1, IGFBP-2 and IGFBP-3 were also measured by radioimmunoassay after isolation from human plasma as previously described (118, 119).

**Insulin tolerance test**

The subjects were given 0.15 U/kg of short-acting insulin (Actrapid, Novo-Nordisk, Denmark) intravenously in order to provoke peak GH secretion (peakGH), and blood samples were drawn every 15 minutes from –15 to +90 minutes for analysis of GH concentrations.

**Sex steroids**

17β-Estradiol in serum was measured by DELFIA®, and sex hormone-binding globulin (SHBG) by autoDELFIA™ which are solid phase, two-site, fluoroimmuno-metric assays (Wallac OY, Turku, Finland) with intra- and interassay CV: estradiol ≤ 10% (normal range 60-150 pmol/L) and SHBG ≤ 4.7 % (normal range 1.0-6.5 mg/l or 15-80 nmol/L).
Serum total testosterone was measured using Coat-A-Count® Total Testosterone (DPC®, Los Angeles, CA, USA) which is a solid phase RIA with intra- and interassay CV of \( \leq 12 \% \) and \( \leq 9.8\% \) respectively (normal range 10-45 nmol/L).

Free androgen index (FAI) was calculated as the ratio of total testosterone to SHBG, both expressed in nmol/L.

**Markers for bone metabolism and calcium**

Osteocalcin was measured in serum by RIA using a commercial kit (CIS bio international, ORIS group, Gif-Sur-Yvette Cedex, France) with intra- and interassay variations of \( \leq 6.6\% \).

Bone-specific alkaline phosphatase (bALP) activity was calculated from total and supernatant serum alkaline phosphatase (ALP) activity after precipitating bALP with a lectin from wheat germ (commercial kit from Boehringer Mannheim, Mannheim, Germany).

Carboxyterminal cross-linked telopeptide of type I collagen (ICTP) and carboxyterminal propeptide of type I procollagen (PICP) levels were measured in serum by RIA using commercial kits (Orion Diagnostica, Espoo, Finland) with intra- and interassay variations of \( \leq 6.6\% \).

Total amount of hydroxyproline in urine was determined by an automatic amino acid analyzer after separation by HPLC, and values were expressed per mol creatinine in fasting morning second void samples.

Free deoxypyridinoline cross-links (DPD) in second void morning urine samples were measured by a competitive ELISA (Pyrilinks™-D, Metra Biosystems, Inc., Mountain View, CA, USA) where both intra- and interassay coefficients of variation were < 5%.

Serum levels of calcium, phosphate, alkaline phosphatase and urine calcium expressed per mol creatinine, were measured by an automatic analyzer (Hitachi).

**Serum lipids and markers for glucose metabolism**

Serum levels of total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, lipoprotein (a) (Lp(a)), glucose, glycosylated hemoglobin (HbA1c), connecting peptide (C-peptide) and insulin were analyzed in the multicenter study (paper V) by routine methods at the Departments of Clinical Chemistry at each investigating center. Low density lipoprotein (LDL) cholesterol concentrations were calculated according to the formula suggested by Friedewald et al (120).
Oral glucose tolerance test

After overnight fast, patients were given 75 g of glucose orally which was consumed within 5 min. Blood samples for glucose and insulin were obtained after 0, 30, 60, 90 and 120 min, and thereafter area under the curve (AUC) for serum insulin was calculated. A blood glucose value of $t_11.1 \text{ mmol/L}$ was considered pathological, i.e. diabetes mellitus.

Bone mineral density

$\text{BMD (g/cm}^2\text{)}$ was determined by dual energy X-ray absorptiometry (DXA) of the total body, the lumbar spine (L2-L4), and the proximal femur with DPX-L® equipment (Lunar Co, Madison, WI, USA). The coefficient of variation was below 1% for the DPX-L® for a period of one year.

BMD in the distal and ultradistal (UD) forearm on the non-dominant side was measured using single energy X-ray absorptiometry (SXA) with a DTX-100® bone densitometer from Hologic (Hologic, Waltham, MA, USA).

The $Z$-score denotes the BMD in standard deviations in each patient, in comparison with a group of age-matched healthy men, and the $T$-score denotes the BMD in standard deviations in each patient in comparison with normal young men, using the manufacturer’s reference values.

Body composition

Body mass index ($\text{BMI}$) was calculated as weight divided by the square of height ($\text{kg/m}^2\text{)}$.

$\text{Waist:hip ratio}$ was calculated as the circumference midway between the iliac crest margin and the lowest rib divided by the widest circumference of the gluteal region.

Bioelectrical impedance ($\text{BIA}$) was performed using the BIA Akern, Tanita and Xitron equipments. Total body water (TBW) was calculated according to the manufacturer instructions. Different BIA equipments were used at different centra in the multicenter study (paper V).

Total body bone mineral content ($\text{BMC}$), lean body mass ($\text{LBM}$) and fat mass were derived from DXA measurements as previously described (121). DPX equipments from Lunar Radiation Corp. (Madison, WI) were used, and the coefficients of variation for LBM and fat mass were 1.6% and 4 % respectively.

Radiography

$\text{Lateral spine X-rays}$ of the thoracic and lumbar spine were performed as part of the clinical routine.
The combined cortical thickness (CCT) of the second metacarpal of the right hand was calculated to evaluate changes in bone size (paper IV). Standardized conventional radiography was performed at baseline and after 24 months, and the images were analyzed identically, at the same time, by the same investigator, without knowledge of dates of examination. The CCT was calculated by subtracting the diameter of the cancellous bone from the outer diameter measured at the midcarpal transverse axis of the second metacarpal bone, according to a method described previously (122).

Exercise test and echocardiography evaluation

Exercise capacity was measured by bicycle exercise test according to a standardized protocol used at all investigating centers (paper V). Exercise was initiated at a workload of 30 W for women and 50 W for men, increased by 10 W every minute until discontinued due to symptoms of exhaustion or for safety reasons. Exercise capacity was recorded as maximal workload (watts). Heart rate and systolic blood pressure at max workload and workload at heart rate 150 bpm also were recorded.

Transthoracic echocardiography was performed according to a standardized protocol used at all investigating centers. M-mode measurements were performed according to the recommendations given by the American Society of Echocardiography (123). These measurements were used to determine cardiac dimensions. Atrio-ventricular plane motion was used as a measure for global cardiac function as described by Alam and coworkers (124). Left ventricular volumes were derived from 2 D echocardiography investigations. All echocardiographic investigations were made by one single investigator at each center.

Statistical Methods and Calculations

Descriptive values are given as the mean±SD unless otherwise stated.

Twenty-four-hour cumulated GH secretion (24hGH, paper III) was calculated from the GH values obtained at 30-min intervals with use of a numerical deconvolution technique described previously (125).

Student’s unpaired, two-tailed, t-test was used for comparing mean values of two groups whereas paired t-tests were performed to assess changes within groups, except when a high degree of non-normality in the distribution was found, when the Wilcoxon’s Signed Rank test was used. Simple regression analyses were performed to assess linear relationships between study parameters. To determine explanatory models for variation in BMD in various sites and hormonal values, stepwise multiple regression analyses were run.

Repeated measures analysis of variance (ANOVA) was used to compare treatment response within and between groups. Post hoc tests were made using the Fisher least squares difference test to determine where statistical differences occurred (paper IV).

Significance was accepted at p ≤ 0.05.
Standard deviation (SD) scores for serum levels of IGF-I and IGFBP-3 (paper V) refer to the number of SD difference from the age-related normal mean value. A serum level between –2 SD and +2 SD is considered as a normal value (74). The SD scores (SDS) for IGF-I and IGFBP-3 were calculated according to the following formulas, all provided by Eli Lilly Co:

Men, 18-28 years, IGF-I SDS:  \[
\text{log (IGF-I)} - 3.59686 + 0.07981 \times \text{age} - 0.001212 \times \text{age}^2 - 0.24928 - 0.01419 \times \text{age} + 0.0003595 \times \text{age}^2
\]

Women, 18-28 years, IGF-I SDS:  \[
\text{log (IGF-I)} - 3.64235 + 0.08376 \times \text{age} - 0.001294 \times \text{age}^2 - 0.21944 - 0.01178 \times \text{age} + 0.0003119 \times \text{age}^2
\]

Men and women, 28-70 years, IGF-I SDS:  \[
\text{log (IGF-I)} - 2.36031 + 0.002393 \times \text{age} - 0.137825 + 0.0001846 \times \text{age}
\]

Men and women, 18-70 years, IGFBP-3 SDS:  \[
\text{log (IGFBP-3)} - 0.54030 + 0.001040 \times \text{age} - 0.10093 + 0.0002605 \times \text{age}
\]
RESULTS AND DISCUSSION

Sex Steroids and the GH/IGF System in Bone Metabolism in Swedish Men (paper I)

In this study, we found positive correlations between BMD in total body, distal and ultradistal radius, and femoral neck and serum levels of IGF-I ($r=0.31$ to $0.49$), IGF-II ($r=0.32$ to $0.48$) and IGFBP-3 ($r=0.37$ to $0.53$) and negative correlations with IGFBP-1 ($r=-0.37$ to $-0.41$) and IGFBP-2 ($r=-0.29$ to $-0.41$) (Fig. 2). The relationships between BMD in femoral neck and serum IGF-I, IGF-II and IGFBP-3 persisted after adjustment for age and weight, whereas the relationships between serum IGFBP-1 and IGFBP-2 and BMD at all skeletal sites were age and weight dependent.

Fig. 2. Correlation between BMD in femoral neck and insulin-like growth factor (IGF)-I, IGF-II, IGF binding protein (IGFBP)-1, IGFBP-2 and IGFBP-3 in 55 Swedish men.

BMD in total body, distal and ultradistal radius and femoral neck also correlated positively with FAI ($r=0.32$-0.40) and BMD in femoral neck correlated positively with the estradiol/SHBG ratio ($r=0.34$) and negatively with SHBG ($r=-0.33$). However, all these relationships turned out to be dependent on age or weight according to the multiple
regression analyses. Furthermore, no linear correlations were found between total testosterone or estradiol levels and BMD. Serum SHBG levels correlated negatively with weight ($r=-0.32$), and positively with age ($r=0.65$), and consequently, the relationships observed between BMD and FAI and the estradiol/SHBG ratio could be dependent on age- and weight-related changes in serum levels of SHBG.

Negative correlations were found between serum IGF-I, IGF-II and IGFBP-3 and SHBG levels and according to multiple regression analyses, a combination of weight, IGFBP-3 and total testosterone could explain 43% of the variation in BMD in femoral neck, 34% in ultradistal radius and 48% in total body.

All these findings indicate that sex steroids and the GH/IGF system are associated with BMD, and that there is an interdependency of these systems at least in Swedish men.

**Sex Steroids and Bone Metabolism in Men with Idiopathic Osteoporosis (papers II and III)**

The estradiol/SHBG ratio and FAI were lower and SHBG concentrations were higher in the men with idiopathic osteoporosis compared to the healthy controls (Fig. 3). The patients also were leaner, with a lower BMI and a tendency to lower percentage of body fat ($p<0.10$), than the controls (paper II). Regression analyses showed that BMD in femoral neck correlated negatively with serum SHBG levels and positively with the ratio estradiol/SHBG in the patients (Fig. 4).

According to multiple regression analyses, as much as 64% of the variation in estradiol/SHBG ratio could be explained by age and BMI in the patients (paper II). As estrogens in men primarily are derived from aromatization of androgens by the enzyme aromatase in fat tissue, the lower BMI and tendency to lower percentage of fat mass in the patient group could contribute to the lower estradiol levels in these men, as indicated by the multiple regression analysis. Furthermore, the simple regression analyses emphasize the importance of estradiol and SHBG for BMD in men with idiopathic osteoporosis.

**The GH/IGF system in Bone Metabolism in Men with Idiopathic Osteoporosis (paper III)**

In this study there were no differences between men with idiopathic osteoporosis or controls in serum IGF-I or IGFBP-3 levels. Nor were there any differences between the cumulated GH secretion during 24 hours (24hGH) according to the deconvolution analysis or in peak GH secretion at insulin tolerance test (peakGH). In previous studies on male osteoporosis, the findings of low IGF-I and IGFBP-3 levels have been inconsistent, possibly due to different age ranges and sizes of the populations studied.
Fig. 3. SHBG levels, estradiol/SHBG ratio and free androgen index (FAI) in 19 men with idiopathic osteoporosis and 12 healthy controls. Bars show the mean value and 1 SD. p ≤ 0.01 compared to controls.

Fig. 4. Relationship between BMD in femoral neck and estradiol/SHBG ratio and SHBG in 12 men with idiopathic osteoporosis.
The deconvolution method for analysis of cumulated GH secretion and the ITT for analysis of peakGH are well established methods for testing of GH secretion in adults. Furthermore, there was a good correlation between these two tests in both patients and controls (r=0.72 and r=0.65 respectively). Thus, as both these tests showed no difference in GH secretory status between patients and healthy controls, there does not seem to be any GH secretory defect in the men with idiopathic osteoporosis.

At baseline, the urinary deoxypyridinoline and hydroxyproline excretion were higher in the patients than the controls. Moreover, several correlations between markers for bone turnover and indices for GH secretion were found in the patient group but not in the controls (Tab. 1). This could represent a higher degree of skeletal GH sensitivity in men with idiopathic osteoporosis than in healthy men.

### Table 1. Correlation coefficients between 24-hour cumulated GH secretion (24hGH), peak GH value at insulin tolerance test (peakGH), insulin-like growth factor (IGF)-I, and markers for bone turnover in 12 men with idiopathic osteoporosis and 12 healthy controls.

<table>
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<th>Osteocalcin</th>
<th>bALP</th>
<th>PICP</th>
<th>ICTP</th>
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<tr>
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<td>r=0.43</td>
<td>r=0.72(^a)</td>
<td>r=0.54(^c)</td>
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<tr>
<td>Patients</td>
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<td>r=0.21</td>
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<tr>
<td>Patients</td>
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<td>r=0.49</td>
<td>r=0.39</td>
<td>r=0.10</td>
</tr>
</tbody>
</table>

\(^a\) p ≤ 0.01. \(^b\) p ≤ 0.05. \(^c\) p ≤ 0.10.

bALP, bone specific ALP; PICP, carboxyterminal propeptide of type I procollagen; ICTP, carboxyterminal telopeptide of type I collagen. U-DPD/Cr, urinary free deoxypyridinoline cross-links and U-OHPr/Cr, urinary hydroxyproline excretion, both expressed per mol creatinine.
Effects of GH Treatment on Bone Metabolism in Men with Idiopathic Osteoporosis (papers III and IV)

In paper III, 14 men with idiopathic osteoporosis and 12 controls were treated with 0.8 mg (2.4 U) daily of GH for one week. Serum levels of bone markers, except for bALP, increased significantly during the treatment period in both patients and controls, with no differences in the increases between the groups. When considering doses commonly used in GH replacement of GHD adults, the relatively high doses used in this study could explain why there were no differences between patients and controls in the response to this short term treatment. The increase in urinary excretion of bone resorption markers during the GH treatment was higher in the controls than the patients. One possible explanation for this could be the greater variation in excretion of these markers at baseline in the patients than the controls.

Long term treatment of men with idiopathic osteoporosis with either 0.4 mg (1.2 U)/day continuously (group A) or 0.8 mg (2.4 U)/day intermittently, for two weeks every third month (group B) resulted in an increase in BMD in the lumbar spine by 4.1% in the continuously treated group and in total body by 2.6% in this group and by 2.7% in the intermittently treated group. After 36 months the BMD had increased further in both groups (Fig. 5). No changes were noted in any of the groups in BMD in femoral neck. There were also significant increases in BMC, which continued 12 months post treatment. After 36 months the BMC in lumbar spine and total body had increased by 6.6% and 4.4% respectively in group A and by 8.3% and 4.6% respectively in group B. There were no significant differences in the increases between the two groups. There were no changes in combined cortical thickness (CCT) of the second metacarpal in any of the groups during the study. Before treatment start, there were positive correlations between serum IGF-I levels and total body BMD and BMC (Fig. 6).

Serum osteocalcin levels were increased in both groups already after one month and remained so throughout the whole study, even after cessation of GH treatment, in both groups. The urinary deoxypyridinoline excretion was increased in both groups after one month, however, after 12 and 24 months it was only increased in group A. After 12 months the urinary deoxypyridinoline excretion was analyzed immediately after a treatment period in the intermittently treated group, and was then significantly increased compared to baseline. By 36 months the urinary deoxypyridinoline excretion had returned to baseline levels (Tab. 2).

The increases noted in BMD and BMC, which were sustained and possibly further increased one year posttreatment, in combination with the protracted effect of GH on serum osteocalcin levels, could provide support for the theory that GH treatment initializes bone remodeling cycles with a relatively greater enhancement of bone formation, as indicated by bone histomorphometric analyzes performed on GHD men treated with GH for one year (106). The reason for not finding any changes in CCT despite the indications of increased bone formation, could be due to methodological problems, with radiogrammetry being a too rough method to detect small changes in cortical width. The prolonged effect on serum osteocalcin levels as well as the absence of difference between the treatment groups in BMD and BMC increase provides a rationale for intermittent GH therapy.
Fig. 5. Percent change from baseline in bone mineral density (BMD) in the lumbar spine (LS), total body (TB) and femoral neck (FN) in men with idiopathic osteoporosis who were treated with growth hormone, 0.4 mg (1.2 U)/day continuously or 0.8 mg (2.4 U)/day for 2 weeks every third month for two years and one year of follow up. Values are mean±SEM. * p < 0.05 compared to baseline within each group.
Fig. 6. Correlation between bone mineral density (BMD) and bone mineral content (BMC) in total body and serum levels of insulin-like growth factor (IGF-I) in 29 men with idiopathic osteoporosis.

Table 2. Serum osteocalcin (OC) and urinary deoxypyridinoline crosslinks (DPD/Cr) excretion corrected for creatinine in men with idiopathic osteoporosis who were treated with growth hormone 0.4 mg (1.2 U)/day continuously (group A, n=11) or 0.8 mg (2.4 U)/day, for two weeks every third month (group B, n=14) for two years and one year post treatment. In group B, all measurements are made prior to treatment period.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>1 month</th>
<th>12 months</th>
<th>24 months</th>
<th>36 months</th>
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<tbody>
<tr>
<td><strong>OC</strong> (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>9.3±2.2</td>
<td>10.8±2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.3±4.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.5±11.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.3±9.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>9.9±3.0</td>
<td>10.8±2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.1±2.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.2±13.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.9±6.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>DPD/Cr</strong> (mmol/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4.2±2.1</td>
<td>7.6±2.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.5±2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.3±1.3</td>
</tr>
<tr>
<td>B</td>
<td>5.3±2.4</td>
<td>7.3±2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.9±1.4</td>
<td>7.1±3.7</td>
<td>5.8±1.6</td>
</tr>
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</table>

Values are expressed as mean±SD. <sup>a</sup>p≤0.05, <sup>b</sup>p≤0.01, and <sup>c</sup>p≤0.001 compared to baseline.
This low dose regimen of 0.18 mg (0.53 U)/day of GH given to adult GHD patients for three months resulted in increases in both serum IGF-I (64% increase from baseline) and IGFBP-3 (26% increase from baseline) levels. Out of 44 patients having subnormal IGF-I levels at baseline, 24 normalized their IGF-I SD score (SDS) and 7 out of 13 patients having subnormal IGFBP-3 levels at baseline, normalized their IGFBP-3 SDS (Fig 7). This indicates a physiological response to the given dose, as both IGF-I and IGFBP-3 levels can be used as dose dependent markers for GH effect.

During the three months treatment period there were reductions in both total and LDL cholesterol levels (Fig. 8), with no changes in HDL cholesterol levels or triglycerides. Similar changes in serum lipids have been observed in previous studies on GH replacement in GHD adults and are considered beneficial concerning cardiovascular risk. However, the treatment also induced an increase in Lp(a) levels, from 73 (12-1162) to 112 (19-1300) mg/L, geometric mean (range), and a high serum level of this lipoprotein has
been shown to be an independent risk factor for cardiovascular disease. Similar increases in Lp(a) have been observed in other GH replacement studies, and the overall effects of changes in lipoprotein metabolism during GH replacement remain to be elucidated.

![Graph showing changes in total and LDL cholesterol levels](image)

**Fig. 8.** Effect of a low dose of growth hormone (GH) substitution (0.17 mg (0.5 U)/day) for three months on serum total and low density lipoprotein (LDL) cholesterol levels in 53 GH deficient adults. Horizontal bars represent ±SEM for the mean value shown. * p< 0.01.

Despite the low dose used and the short treatment period, an increase in lean body mass by 2% and a decrease in total body fat mass by 7% were observed. These beneficial changes are in line with results from other investigators. There also was a tendency to an increase in total body water (40.1±8.2 vs 41.4 ± 8.2 kg, p=0.07).

There were no changes in cardiac structure or function during the study period.

Ten patients experienced side effects in this study. These consisted of musculoskeletal pain and swelling, peripheral edema, gynecomastia, headache, paresthesias, insomnia and exaggerated snoring. However, all these side effects were mild and no patient had to reduce the GH replacement dose. Moreover, the low dose of rhGH caused a significant increase in 120 min glucose value at oral glucose tolerance test (OGTT) after 3 months, but fasting serum glucose, C-peptide levels and area under the curve for serum insulin during OGTT did not change significantly.

This study shows that treatment with a low fixed dose of rhGH (0.18 mg (0.53 U)/day) for three months is sufficient to improve body composition and to reduce total and LDL-cholesterol levels, without causing any serious adverse events. Side effects in patients receiving GH treatment are dose dependent (74) and by use of this treatment regime, the frequency of side effects during commencement of GH replacement in adults, can be reduced.
SUMMARY

◆ BMD in femoral neck was positively correlated to the estradiol/SHBG ratio and negatively correlated to SHBG levels in a population based sample of Swedish men and in men with idiopathic osteoporosis

◆ Men with idiopathic osteoporosis had lower estradiol/SHBG ratio and higher serum SHBG levels than healthy men

◆ Serum levels of IGF-I and IGFBP-3 were positively correlated to BMD in a population based sample of Swedish men and in men with idiopathic osteoporosis

◆ There was no difference in GH secretory status between men with idiopathic osteoporosis and healthy men

◆ Short term treatment of men with idiopathic osteoporosis and healthy men with GH, 0.8 mg (2.4 U)/day for two weeks, increased both bone formation and bone resorption markers

◆ Two years treatment of men with idiopathic osteoporosis with GH, either 0.4 mg (1.2 U)/day as continuous treatment or with 0.8 mg (2.4 U)/day for 2 weeks every three months, resulted in an increase in BMD and BMC and in serum levels of osteocalcin which were sustained for at least one year post treatment

◆ Treatment of GHD adults with a low fixed dose of GH, 0.17 mg (0.5 U)/day, for three months resulted in improvements in body composition and lipid profile without causing serious side effects
CONCLUSIONS

- The GH/IGF system and the sex hormone system are associated with BMD in healthy men

- Male idiopathic osteoporosis is characterized by low free estradiol levels and low serum IGF-I levels despite a normal GH secretion

- GH therapy could be a treatment option in male idiopathic osteoporosis

- A low daily dose of GH has significant metabolic effects in GHD adults and a similar treatment regimen should be investigated further in male idiopathic osteoporosis
GENERAL DISCUSSION

The findings in this thesis demonstrate the importance of the GH/IGF system and the sex hormone system for bone metabolism in men, and imply a pathogenetic role for these two systems in the development of male idiopathic osteoporosis.

Men with idiopathic osteoporosis had lower BMI, estradiol levels, estradiol/SHBG ratio, and free androgen index and higher SHBG levels than healthy controls. SHBG levels were negatively correlated to BMD in femoral neck in the osteoporotic men and in the population based sample of Swedish men. The estradiol/SHBG ratio correlated positively with BMD in femoral neck in both these groups serum and SHBG levels were negatively correlated to weight in the population based study. Estrogens in men are primarily produced from aromatization of testosterone or androstenedion in fat mass and free estradiol levels have been shown to be an independent predictor for BMD in healthy men (32, 41). These findings indicate that a body composition with higher BMI and possibly also higher percentage of body fat might be beneficial for BMD in men, through an increase in conversion of androgens to estrogens and lowering of serum SHBG levels resulting in higher levels of free biologically active estradiol.

There were several correlations between markers for bone turnover and indices for GH secretion in the patients, but not in the controls. This could represent a higher degree of GH sensitivity in men with idiopathic osteoporosis. These men have lower bioavailable estradiol levels than men with normal BMD. Interestingly, there is a difference in sensitivity for GH between men and women, with premenopausal women having higher estradiol levels and lower GH sensitivity than age matched men (126).

Previous studies in men with idiopathic osteoporosis have shown lower serum IGF-I and IGFBP-3 levels in men with idiopathic osteoporosis in comparison to healthy controls. This was not seen in this present thesis. However, serum IGF-I levels correlated positively with BMD both in men with osteoporosis and in the population based group. In the latter of these groups there also was a positive correlation between serum IGFBP-3 and BMD. Moreover, in the men with idiopathic osteoporosis several positive correlations were found between markers for bone turnover and cumulated GH secretion over 24 hours, peak GH secretion at insulin tolerance test, and serum IGF-I, that were not found in the controls. These findings stress the importance for the GH/IGF system for bone metabolism in men. However, the 24 hour cumulated GH secretion and the peak GH secretion did not differ between patients and controls, which has also been confirmed by others (127). Thus, there is no GH secretory defect in men with idiopathic osteoporosis and the effects of IGF-I and IGFBP-3 on bone metabolism have to be dependent on other factors in addition to GH.

In the population based study of Swedish men and in the osteoporotic patients there were negative relationships between IGF-I and SHBG levels. Other investigators have reported similar results, and IGF-I has been suggested as a key regulator for SHBG levels in men (128). Previous studies have also shown positive correlations between gonadal steroids and circulating GH levels in both men and women and an enhanced GH secretion after androgen supplementation of hypogonadal men (129). Furthermore, in this thesis, a combination of serum levels of IGFBP-3, testosterone and weight were shown to explain between 34% and 48% of the variation in BMD at various locations in healthy men. This
suggests not only that each one of these hormone systems have an impact on bone metabolism, but also that there are interactions between the two systems that are important for the maintenance of bone mass and that age related changes in these systems could contribute to bone loss with advancing age in men.

In men with idiopathic osteoporosis, previous studies have shown a reduction in bone formation rate and reduced serum IGF-I levels. This thesis demonstrates that these men have lower estradiol/SHBG ratio and higher SHBG levels than healthy controls. All these findings are similar to previous descriptions in elderly men and male idiopathic osteoporosis thus might be viewed as a form of premature aging.

Male idiopathic osteoporosis is a disorder characterized by low bone turnover (36, 37,108). The ideal treatment of this condition would be an anabolic therapy rather than an antiresorptive drug, and GH has been suggested as a possible agent. This hormone has several advantages. It has been used for decades in the treatment of GHD in children and in the past ten years it has also been widely used for treatment of GHD in adults. The effects of GH treatment in GHD as well as in several non-GHD pathological conditions have been extensively investigated in numerous studies. The side-effects are, due to the large number of studies, well known and have generally been mild and transient.

The study where GHD adults were treated with a low dose of GH for three months (paper V) clearly demonstrated that positive effects on lipid metabolism and body composition can be achieved with considerably lower doses of GH than those usually used in GH replacement. The low dose treatment was also very well tolerated and no serious adverse effects were recorded. This could thus be a useful method to institute GH therapy in GHD adults. The short term GH treatment of the osteoporotic men and the controls (paper III) resulted in pronounced effects on both bone formation and resorption markers, with no differences between the groups in the treatment response. The treatment dose in this experiment was 0.8 mg (2.4 U)/day, i.e. more than 4 times the dose that was used in the treatment of the GHD adults.

The long term GH treatment of men with idiopathic osteoporosis (paper IV) resulted in a substantial increase in BMD and BMC in both lumbar spine and total body. There were no differences in treatment response between the intermittently and continuously treated groups. The doses used in this study were in the continuously treated group 0.4 mg (1.2 U)/day, and in the intermittently treated group 0.8 mg (2.4 U)/day, i.e., respectively, more than two and four times the dose used for GH replacement in the GHD adults. There was a further increase in BMD along with a protracted effect on the increase in serum osteocalcin levels after cessation of GH treatment. The effects of GH on bone resorption markers were more transient. In a recent placebo controlled study, postmenopausal osteoporotic women on estrogen replacement therapy were treated with GH for three years (130). Increases in BMC were seen in lumbar spine and total body compared to the placebo treated group, one year after cessation of GH treatment, which is in line with the findings in the present thesis. These findings indicate that GH has an anabolic effect on bone and that GH treatment could be considered as a treatment option in male idiopathic osteoporosis.

The findings in this thesis indicate that both the sex hormone and GH/IGF systems as well as interactions between the two are important in male bone metabolism. A combination of
subtle disturbances in these two systems could contribute to the development of male idiopathic osteoporosis. Finally, this thesis suggests that GH treatment could be considered as a treatment option in male idiopathic osteoporosis. It also provides a rationale for intermittent GH treatment of this condition, possibly with lower doses than were used in the present studies, as indicated by the positive effects achieved in the study of low dose GH treatment of GHD adults.
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