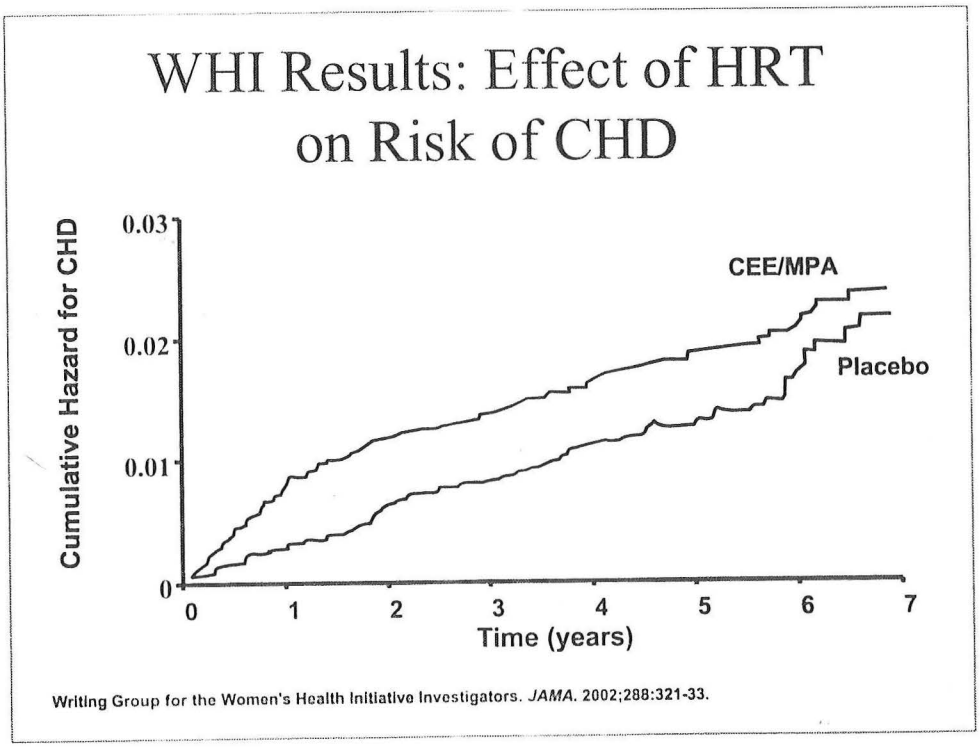
The Endocrinology of Aging

Keith L. Parker
Professor of Internal Medicine
UT Southwestern
Division of Endocrinology and Metabolism

Medicine Grand Rounds
January 23, 2003



"This is to acknowledge that Keith Parker M.D., Ph.D. is a consultant for Pharmacia and will discuss off-label uses in his presentation."

Keith L. Parker, M.D., Ph.D.
Professor of Internal Medicine
Division of Endocrinology and Metabolism

Dr. Parker is interested in the mechanisms of development of the adrenal cortex and gonads, focusing on how the production of steroid hormones is regulated. He also is studying the roles of the ventromedial hypothalamic nucleus in weight regulation, circadian rhythm, and counter-regulatory responses to hypoglycemia.

I. INTRODUCTION

In 1889, the famous physiologist Brown-Sequard—then aged 72—reported that self-administration of aqueous extracts of testes from dogs and guinea pigs was associated with striking functional improvement, including increased strength, intellectual capacity, and sexual potency (reviewed by Wilson, 1990). While one retrospective analysis indicates that the testosterone concentration in extracts prepared using his methods is four orders of magnitude below that needed to produce biological effects using modern preparations (Cussons et al., 2002), this report set the stage for a large body of reports—many either anecdotal or observational—claiming beneficial effects of hormonal supplementation in aging subjects. Life expectancy is increasing in developed nations and it is projected that more than 50 million people in the U.S. will be over 65 years old by 2020; therefore, both health care providers and the general public have increasingly sought potential therapeutic interventions that might alleviate the aging process. The similarities between manifestations of overt deficiencies of sex steroids and growth hormone in adults and the physiological changes seen with aging have led some experts to propose that the decreases in hormone levels relate causally to the aging process, such that hormone replacement to levels seen in young adults will ameliorate or even reverse the normal aging process. In the absence of conclusive scientific data, hormone replacement therapies in this setting have become very widely used, resulting in millions of dollars in health care expenses.

Two recent events have prompted increased scrutiny of the use of hormone replacement therapies in otherwise healthy individuals. The first was the recent publication of results from the Women's Health Initiative (Writing Group for the Women's Health Initiative, 2002). Despite the widespread belief and promising data from observational studies that hormone replacement therapy in postmenopausal women exerted beneficial effects on cardiovascular health, this randomized, placebo-controlled trial raised serious questions about the efficacy of hormone replacement therapy to positively impact adverse cardiovascular events, and thus about the ability to extrapolate from smaller case-controlled studies. The second was an article in the New Yorker entitled "Hormones for Men: Is male menopause a question of medicine or of marketing"? In addition to questioning the validity of the data supporting androgen replacement therapy in aging males, this article suggested that drug companies played a major role in influencing the recommendations of physician experts in support of androgen replacement therapy (Groopman, 2002).

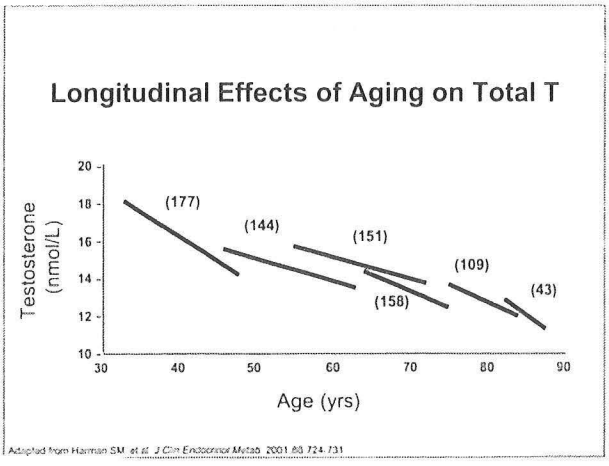
During this Grand Rounds, I will first review the available evidence regarding age-related declines in androgens in men and growth hormone in both sexes. Next, I will review the evidence for or against beneficial effects of hormone replacement in normal aging subjects, comparing and contrasting these data with those in adult subjects who have overt deficiencies of testosterone or growth

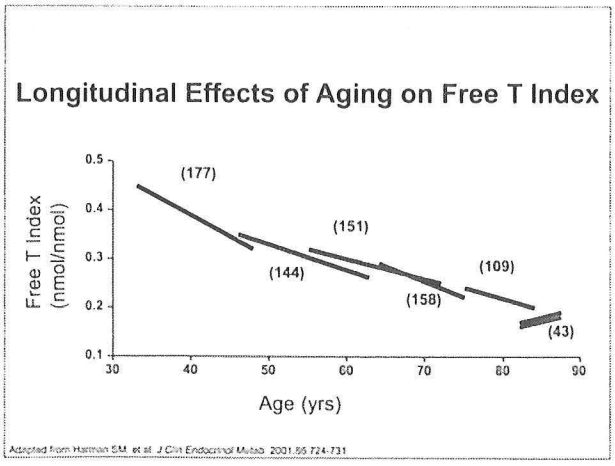
hormone. It is important to note at the outset that the number of review articles and consensus conference recommendations exceeds considerably the number of studies on which these recommendations are based; moreover, no studies to date have been sufficiently powered or of sufficient duration to provide definitive data. Finally, I will discuss emerging data with model systems that explore the molecular basis of aging, at least some of which raise concerns regarding the widespread use of hormonal replacement for aging.

II. AGING AND TESTOSTERONE: ANDROPAUSE

A. Age-related Declines in Testosterone

While not nearly as dramatic as the decline in sex steroids seen in post-menopausal women, it is generally accepted that testosterone levels in men decline with age; in association with the appropriate symptom complex, this declining androgen function is termed andropause. An appreciation of this age-related decline was hampered in part by the fact that biologically active free testosterone comprises only a small percentage of the total circulating testosterone, with most studies measuring only total testosterone. However, both cross-sectional studies (Vermuelen 1991; Harman et al. 1980) and longitudinal studies (Morley et al., 1997; Zmuda et al., 1997; Harman et al., 2001, see figure) have documented age-related declines in total and free serum testosterone, and the consensus is that free testosterone decreases by ~1-2% per year after age 30 even in otherwise healthy individuals. The concurrent age-related increase in the incidence of chronic diseases and obesity undoubtedly accelerates this decline. It is important to remember however, that most males, even the very old, are not overtly hypogonadal.





This decrease reflects both increased sex hormone binding globulin (SHBG) and diminished testosterone production secondary to decreases in episodic and stimulated gonadotropin secretion and to decreases in the number and steroidogenic capacity of Leydig cells (Mulligan et al. 1997; Veldhuis 1999).

Thus, it often is not possible to identify a discrete lesion that is the cause of hypogonadism in elderly patients because their decreased androgen levels may be due to combined hypothalamic/pituitary and testicular abnormalities.

B. Etiology of Hypogonadism in Aging Males

The etiology of hypogonadism in aging males is generally similar to that seen in younger subjects and will only be summarized briefly here (Tenover 1998). Although most elderly men diagnosed with hypogonadism have acquired disease, occasional patients with congenital disorders such as Klinefelter's syndrome may escape diagnosis until this age range. Hypogonadism can be divided into primary—caused by diseases of the testes—and secondary—resulting from pituitary or hypothalamic disorders. As noted above, hypogonadism in elderly men often reflects combined defects in both the testes and higher structures. Specific etiologies include trauma, mumps orchitis, history of chemotherapy or radiation therapy for malignancy, obesity, severe systemic illness (including HIV infection), medications (e.g., narcotics for pain relief, systemic glucocorticoids, ketoconazole), and other endocrine disorders (e.g., hyperprolactinemia, thyroid disease, or Cushing's syndrome), and it is important to consider potentially treatable causes even in older males.

C. Signs and Symptoms of Hypogonadism in Aging Males

The signs and symptoms of hypogonadism in aging men are generally the same as those seen in younger subjects, and include sexual dysfunction (loss of libido, erectile dysfunction), depression, loss of secondary sexual characteristics, osteoporosis (an increasing basis for endocrine evaluation as awareness of the frequency of osteoporosis in aging men is more widely disseminated), sarcopenia (loss of muscle mass), mild anemia, and increased adiposity (Anawalt and Merriam 2001). These manifestations are obviously very non-specific, and thus appropriate documentation of diminished testosterone with a reliable assay is essential to make the diagnosis (see below). Some experts routinely administer various questionnaires to older men in an effort to identify more subtle symptoms of andropause (for example, Morley et al. 2000), but the specificity of such questionnaire is only slightly better than 50%, and we do not routinely use them in the PMH Endocrinology Clinic. Even though erectile dysfunction in elderly men usually does not result from hypogonadism and most such patients do not improve following testosterone replacement therapy, the diagnosis of hypogonadism nonetheless should be considered in this population.

D. Diagnosis of Hypogonadism in Aging Males

Symptoms or signs of androgen deficiency at any age warrant appropriate laboratory evaluation, although the preferred diagnostic approach is subject to

debate. Testosterone levels exhibit considerable circadian variation, at least in younger men (Bremner et al. 1983), so most experts recommend an early morning sample. Given the considerable variation in testosterone levels when sampled at frequent intervals in a given subject (Spratt et al. 1988), some authorities advocate measuring testosterone on a single sample pooled from 3 separate bleeds 20 minutes apart. This approach is especially recommended when concurrent measurements of LH and FSH will be made, as gonadotropins exhibit even greater minute-to-minute variation than does testosterone. Others, based on published data that testosterone levels from a single subject are generally highly reproducible when multiple samples are analyzed (Vermuelen et al. 1992), believe that pooled samples are not necessary.

The diagnosis of androgen deficiency is essentially excluded if the total testosterone exceeds 400 ng/dL, whereas a testosterone value of <150 ng/dL is highly suggestive of androgen deficiency. Such single measurements of total testosterone form the basis for most of the published clinical trials of androgen replacement therapy in older men. In light of the known age-related increases in SHBG in older men, other experts routinely measure bioavailable (i.e. non-SHBG bound and free testosterone) or free testosterone in subjects older than 55 years old. The available assays for measuring free testosterone vary considerably in their reliability, and those that use analogue displacement rather than equilibrium dialysis are especially suspect. Finally, some experts adopt an intermediate approach, initially measuring an AM total testosterone and then checking the bioactive or free testosterone if the total testosterone is between 200-400 ng/dL

Once hypogonadism is confirmed, it is important to rule out potentially treatable causes. Any patient with a total testosterone <200 ng/dL should be evaluated for possible pituitary or hypothalamic disease by measuring LH, FSH, and prolactin. If the gonadotropins are clearly low or the prolactin is elevated, then a pituitary MRI is indicated. Other diagnostic evaluation may be indicated if signs or symptoms point to a specific diagnosis, such as hemochromatosis.

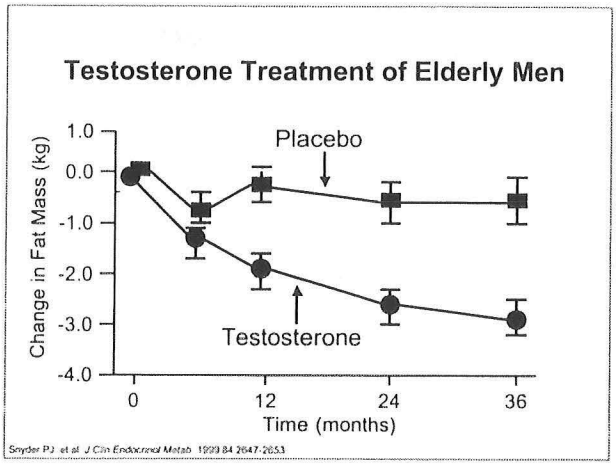
E. Beneficial Effects of Testosterone Replacement Therapy

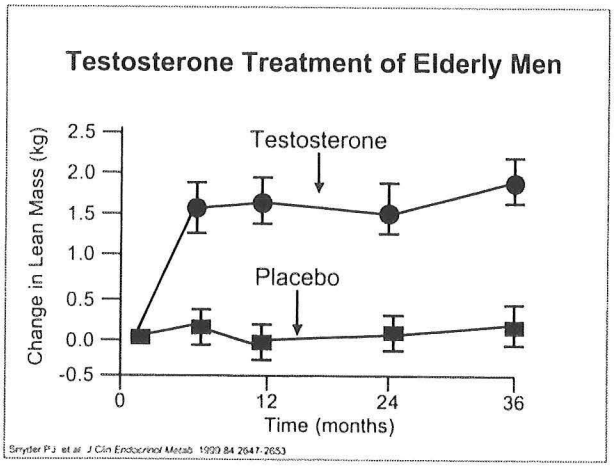
Results from clinical trials of testosterone replacement therapy in aging men are still limited, involving relatively small numbers of patients, short durations of treatment, and surrogate markers such as bone mineral density or lipids instead of clinically significant end-points such as fractures or cardiovascular events. In this setting, testosterone replacement has been associated with improved mood, decreased fatigue, increased bone mineral density, and increased lean body mass. Other studies in normal younger men have shown that pharmacologic doses of androgens can induce increased muscle mass and improved strength (Bhasin et al. 1996). To the extent that these studies will also apply to elderly

subjects, additional benefits of androgen replacement may ultimately be demonstrated.

Body Composition: There is general agreement that testosterone replacement in hypogonadal elderly men decreases adiposity and increases lean body mass, including muscle. What remains to be seen, however, is whether this increased muscle mass will translate into improved strength, or more importantly, decreased falls or disability. One randomized trial suggested that testosterone therapy of older men admitted to a rehabilitation unit at a VA Hospital was associated with improved functional performance (Bakhshi et al., 2000), although this study has not yet been confirmed in any other publications to date.

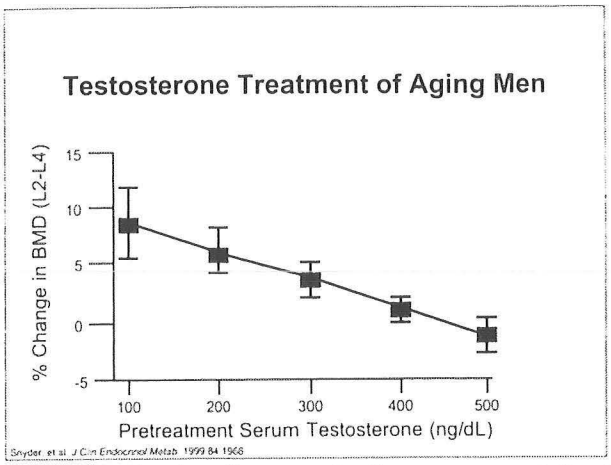
Snyder and colleagues studied 108 men age 65 or older with total testosterone < 475 ng/dL who received either testosterone by scrotal patch (6 mg/day) or placebo patch for three years (Snyder et al. 1999). Testosterone treatment was associated with increased lean body mass and decreased adiposity (see Figure), but they did not observe any changes in strength. In contrast, Tenover and colleagues studied only elderly men with total testosterone <350 ng/dL, and administered either placebo or intramuscular testosterone (150-200 mg every 2 weeks). They again noted increases in lean body mass with decreased adiposity, in this case associated with increased handgrip strength.

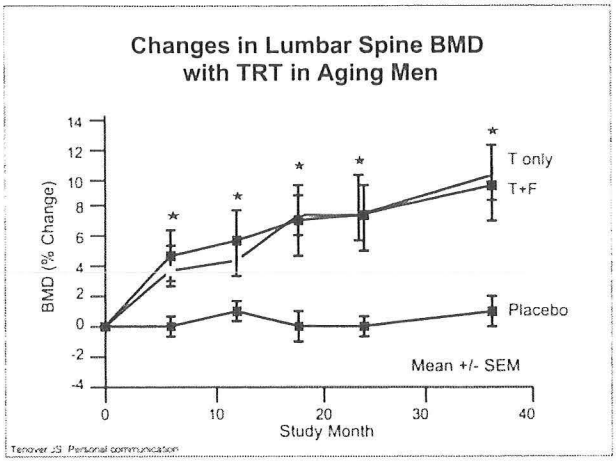




Bone Mineral Density: Again, there is general agreement that testosterone replacement therapy is associated with increased bone density in younger hypogonadal men. However, only limited clinical trials provide information about the effect of testosterone replacement in older patients. Snyder and colleagues followed bone mineral density for 3 years in men >65 years of age whose total testosterone was <475 ng/dL (Snyder et al. 1999). All men also received calcium and vitamin D. Both control and testosterone-treated groups showed significant increases in bone mineral density, such that there were no significant differences in the placebo and testosterone groups. As shown below, when the subjects with

the lowest testosterone levels were analyzed separately, there was a significant response to testosterone. In contrast, using the regimen described above, Tenover and colleagues noted that the testosterone-treated group achieved a bone mineral density significantly greater that the placebo group, and that the concurrent inhibition of 5α-reductase had no effect on bone density.





Collectively, these studies suggest that testosterone treatment of hypogonadal older males can increase bone mineral density by ~4-6% over a 2-3 year period, with the greatest benefit seen in the most severely hypogonadal subjects, but there are no data dealing with the clinically relevant end-point of fractures.

Lipids and Cardiovascular Risk: Based at least partly on the relatively higher incidence of atherosclerosis in men relative to pre-menopausal females and on the known propensity of oral androgens to increase LDL cholesterol, there is a general perception that testosterone is associated with deleterious effects on plasma lipids. The available data do not support this model (Alexandersen et al. 1996), and most epidemiologic studies have found an inverse relationship between serum testosterone and cardiovascular disease (Bagatel and Bremner, 1995). In particular, the beneficial effects of testosterone on adiposity might be expected to improve the lipid profile. Again, further studies are needed to define the long-term effect of testosterone on these parameters. As noted below, however, major adverse effects of testosterone replacement on lipid profiles or cardiovascular events have not been noted to date.

F. Therapeutic Options for Testosterone Replacement.

Several different options are now available for testosterone replacement, and additional forms are under development (Wang 2002). It should be noted that all of these preparations have received FDA approval for use in hypogonadal men, and there is no approved indication for their use in older patients with normal testosterone levels. This has not prevented the enthusiastic advocacy of testosterone replacement by both patients and physicians.

Hypogonadal men traditionally have received testosterone esters injected intramuscularly every 2 weeks. At standard doses, this modality often induces supraphysiological levels of serum testosterone in the days immediately following the injection, and low levels immediately before the next dose, often associated with symptoms due to androgen excess or deficiency. The higher testosterone levels are associated with a higher frequency of certain side effects such as polycythemia and increases in PSA, but intramuscular injections are well-tolerated by many patients and are the least expensive modality.

In an effort to achieve more consistent testosterone levels and to avoid injections, several transdermal testosterone preparations are now approved. The first such preparation was a scrotal patch, which was associated with minimal skin irritation but did not meet with great enthusiasm by patients and has largely disappeared from the market. Newer formulations are now available that are applied topically once daily, either as a transdermal patch (Androderm®) or gel (AndroGel®). At least when used as currently approved, there are differences in the levels of circulating testosterone provided by these formulations (Swerdloff et al. 2000), with the higher 10 g/day dose of Androgel® (which is estimated to deliver 10 mg/day of testosterone) producing significantly higher levels of serum testosterone. The clinical significance of these differences and the optimal regimens have not been established.

Newer preparations for testosterone replacement currently under development include the use of dihydrotestosterone, which may have a lower incidence of certain side effects. The preponderance of evidence indicates that the major regulator of bone density in men is estrogen rather than testosterone (Khosla S et al. 1998), and dihydrotestosterone, which is not subject to aromatization, may have less beneficial effects on bone mineral density. A number of pharmaceutical companies are actively trying to develop compounds—selective androgen response modulators or SARMs—that will act differentially in different androgen-responsive tissues, potentially dissociating the beneficial effects of androgens in certain tissues from the deleterious side effects. Some progress along these lines has been reported with 7α-methyl-19-nortestosterone, a synthetic androgen that is a substrate for aromatization but cannot be 5α-reduced. This compound has been reported to have relatively greater activity in vivo in exerting androgenic effects on the pituitary and prostate, with less effect on the prostate (Cummings et al. 1998).

G. Contraindications/Side Effects of Androgen Therapy

Just as the benefits of androgen therapy in older men have not been conclusively documented, we also lack definitive data regarding potential risks of therapy. Based on the concern that it may stimulate increased tumor growth, testosterone treatment is absolutely contraindicated in patients with prostate or breast cancer

and all patients should have careful evaluation (e.g. physical examination, PSA measurement) to exclude a pre-existing lesion. Given the known effect of testosterone replacement to increase hematocrit (see below), most authorities also consider a hematocrit of >54 as an absolute contraindication.

Well-documented side effects of androgen replacement therapy include: gynecomastia (due to the peripheral conversion of testosterone to estrogen), fluid retention, polycythemia, induction or exacerbation of obstructive sleep apnea, elevation of PSA, and skin irritation (seen with transdermal preparations, esp. with patches containing permeation enhancers). One comparative study suggested that the frequency of side-effects such as polycythemia and elevated PSA relates directly to the level of testosterone that is achieved, such that 18% of subjects using the 10g/day dose testosterone gel exhibited polycythemia versus 2.8% of subjects using the transdermal patch (Wang et al. 2000).

Based on studies using supraphysiologic doses of testosterone in younger males and epidemiologic differences in prevalence of coronary heart disease in males and females, there has been concern that testosterone therapy may worsen the lipid profile (especially lowering HDL). Other studies, however, suggest that testosterone therapy has beneficial effects on the lipid profile, perhaps related to decreased adiposity. Although the studies have not involved large numbers of subjects, there are no data supporting any significant adverse effect of testosterone therapy on the lipid profile. Thus, it is not anticipated that androgen replacement therapy will have major deleterious cardiovascular effects, although additional clinical trials are needed to resolve this issue.

Of greater potential concern is the possible impact of testosterone to stimulate either the initiation or growth of prostate cancer. Prostate development is androgen-dependent, treatments that decrease androgen levels diminish prostate volume in normal subjects, and hormonal therapy to remove androgen is a key component of therapy for prostate cancer. Despite this, there are no data which support the possibility that testosterone replacement can "cause" prostate cancer. Nonetheless, the concern that testosterone replacement therapy in older men may cause a latent prostate cancer to become clinically apparent is an important issue that needs to be addressed in sufficiently powered trials. In addition, the potential medical expense related to laboratory tests and evaluation of these patients is an unresolved issue that can only be addressed in large, prospective clinical trials.

H. Patient Monitoring

Current protocols to monitor elderly patients on testosterone replacement therapy derive from the known and potential side effects of androgen replacement and are largely empirical rather than based on evidence-based outcomes

(Andropause Consensus Conference, 2002). After breast examination and digital rectal examination, baseline laboratory measurements include hematocrit and PSA. Thereafter, DRE, PSA, and hematocrit should be evaluated at 3, 6, and 12 months after the initiation of therapy, and annually thereafter. An increase in hematocrit to >54 is grounds to decrease the dose of androgen replacement or to consider discontinuing the therapy. An increase in PSA of >1.4 ng/mL between two individual measurements or an increase of > 1.5 ng/ml over 2 years is an indication for urologic evaluation to rule out prostate cancer.

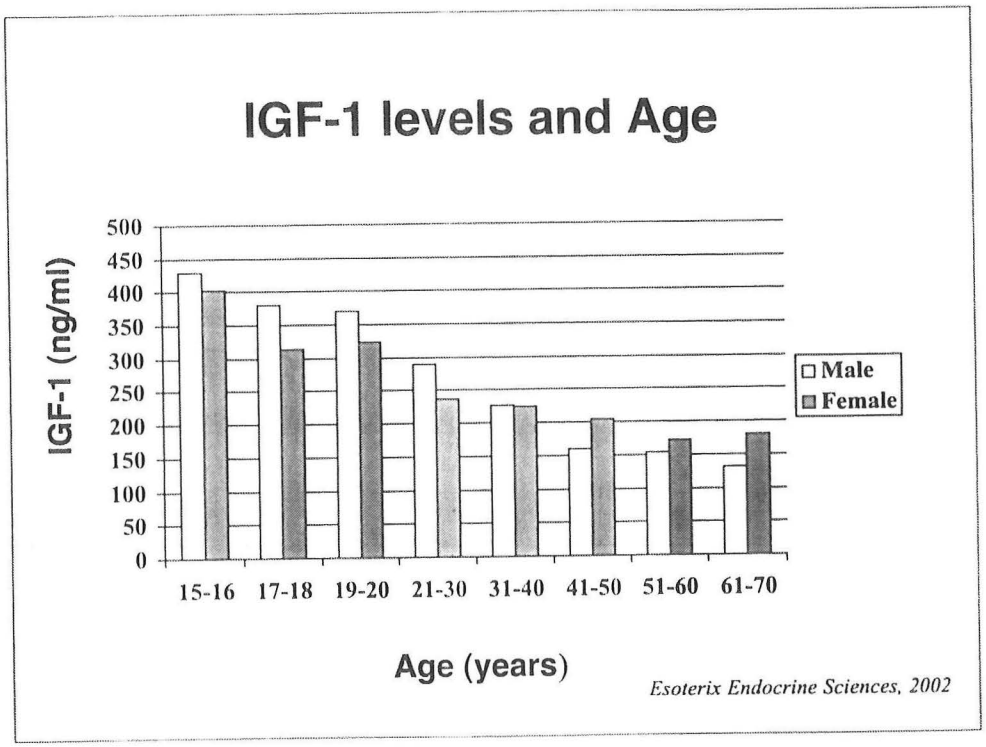
There is no consensus regarding the need to follow testosterone levels in patients receiving testosterone replacement therapy, although most experts do check testosterone levels to ensure that the dosing has not resulted in supraphysiologic levels. An improved understanding of the threshold doses for various beneficial effects of androgen therapy would facilitate a more rational approach to therapy. For increasing sexual function in hypogonadal younger men, clinical response is seen with total testosterone values in the range of 300 ng/dL, but there may be a different thresholds for beneficial effects on bone density, muscle mass, etc. (see Bhasin et al., 2001 for a consideration of the dose-response of different parameters to testosterone in healthy young men). In the absence of such data, many experts have advocated titrating the dose of testosterone to ensure that the patient is not overmedicated, shooting for testosterone levels in the mid-normal range for young healthy men. It should be noted that less cautious approaches to dosing are advocated by some of the anti-aging enthusiasts, who will often push therapy until limited by side effects.

III. AGING AND GROWTH HORMONE: SOMATOPAUSE

Although physicians traditionally believed that GH had no significant biological role in adults, the first case report suggesting an important function of GH in adults was published more than 40 years ago (Raben MS 1962). The explosion of interest in hormonal therapy to alleviate aging largely was triggered by a study by Daniel Rudman published in 1990 (Rudman et al. 1990). In an open-label trial of normal elderly men, he reported that 6 months of therapy with recombinant hGH was associated with significant changes in body composition, including increased muscle mass, increased bone mineral density, and decreased adiposity. The paper proposed that "The effects of six months of growth hormone and lean body mass and adipose tissue-mass were equivalent in magnitude to the changes incurred during 10 to 20 years of aging." This paper and others have stimulated the enthusiastic advocacy (Klatz 1997) and development of a large network of suppliers of growth hormone, as well as "growth hormone releasers" that are purported to stimulate the release of endogenous growth hormone from the somatotropes (see below).

A. Age-related Declines in the Growth Hormone Axis

GH secretion and IGF-1 levels are highest during the pubertal growth spurt and then decline progressively thereafter (see Figure below from cross-sectional studies of healthy control population). Studies have shown that the GH response to either growth hormone releasing hormone (GHRH) and arginine (Ghigo et al. 1990 or to GHRH plus a growth hormone secretogogue is normal in healthy older subjects, indicating that somatotropes can still respond with increased growth hormone secretion and that the major defect is a central one with decreased GHRH and increased somatostatin). Moreover, one study suggests that the GH secretion profile is relatively well-maintained in healthy elderly subjects (Vahl et al. 1996)), such that the associated conditions of increased adiposity and decreased physical activity may play key roles in the epidemiological observations of decreased levels of IGF-I.



B. Etiology of Adult GH Deficiency

Overt GH deficiency in adults most frequently results from pituitary tumors or the consequences of surgical or radiation treatment of these tumors. Based on this fact, most experts do not investigate the diagnosis of adult GH deficiency in the absence of documented deficits of other pituitary hormones. In contrast, GH deficiency in children is most commonly idiopathic and often is not associated with other pituitary hormone deficiencies.

C. Signs and Symptoms of Adult Growth Hormone Deficiency

The signs and symptoms of adult GH deficiency again are relatively non-specific and mimic many of those normally associated with aging. Proposed components of the adult GH deficiency syndrome include: reduced lean body mass with increased adiposity, reduced strength and exercise capacity, decreased bone mineral density, depressed mood, emotional lability, increased social isolation, and dyslipidemia. As with andropause, these symptoms and signs are remarkably similar to those associated with aging, leading to the proposal that GH therapy may have beneficial effects in GH-deficient adults as well as in otherwise healthy aging subjects.

D. Diagnosis of Adult Growth Hormone Deficiency

GH secretion is pulsatile and levels fluctuate considerably during the day, with the three major physiological stimuli for secretion being sleep, exercise, and stress. A better indicator of integrated GH secretion is insulin-like growth factor-I (IGF-I), which is secreted by the liver in response to GH and mediates many—but not all—biological effects of GH. As discussed further below, a very low IGF-I level in the setting of deficiencies of other anterior pituitary hormones strongly suggests GH deficiency, but many adults with GH deficiency have IGF-I levels that fall within the normal range. Thus, dynamic testing of somatotrope function generally is needed in diagnosing GH deficiency. It also is important to note that not all assays for IGF-I are of equal quality, and the use of a sensitive and reliable immunoassay is essential.

The traditional gold standard test at academic centers has been the insulin tolerance test. Following the adminstration of insulin (0.1 U/kg by IV bolus) to decrease blood glucose to <40 mg/dL, a peak GH response of <5 ng/mL (polyclonal radioimmunoassay) or <2.5 ng/mL (immunochemiluminometric assay) is considered indicative of an impaired response. This procedure is very labor intensive, as the physician must be in attendance throughout the test, and is contraindicated in patients with coronary artery or cerebrovascular disease or a seizure disorder. The coexistence of these disorders in elderly patients (e.g. age>60-65 years old) is sufficiently high that many experts do not use the insulin tolerance test in this population. Therefore, endocrinologists have sought alternative tests with similar diagnostic utility. Recent studies suggest that combined stimulation with GHRH and arginine has similar sensitivity and specificity (Biller et al. 2002). To perform this test, GEREF (a synthetic GHRH analog is administered at a dose of 1μg/kg, followed by an infusion of 30 g of arginine over 30 minutes. The normal response is a peak GH of >9-10 ng/mL using a radioimmunoassay or 4-5 ng/mL with chemiluminescent assay.

Based on the observation that the GH axis is often affected before other components of the anterior pituitary, some experts have proposed strategies for diagnosing adult GH deficiency without provocative testing. According to one report (Hartman et al. 2002), the combination of a very low IGF-1 level (<84 μg/L with the Esoterix assay) and impairment of at least three other components of pituitary function (hypogonadotrophic hypogonadism, secondary hypothyroidism, secondary adrenal insufficiency, central diabetes insipidus) provides a positive predictive value of 95% (89% specificity and 69% sensitivity) for GH deficiency (Hartman et al. 2002). Given the expense of GH replacement and the current lack of published corroboration of this approach, we continue to perform provocative testing in nearly all patients before making a diagnosis of GH deficiency in adults.

E. Beneficial Effects of GH Therapy in Adults

As with androgen therapy in men, the largest body of evidence relates specifically to GH deficiency rather than the issue of possible benefits of GH in healthy aging subjects. Due to the ethical considerations in clinical trials, studies with GH treatment in the elderly population have generally been restricted to those who are in good health—possibly by definition the population that stands to benefit the least from any beneficial effects.

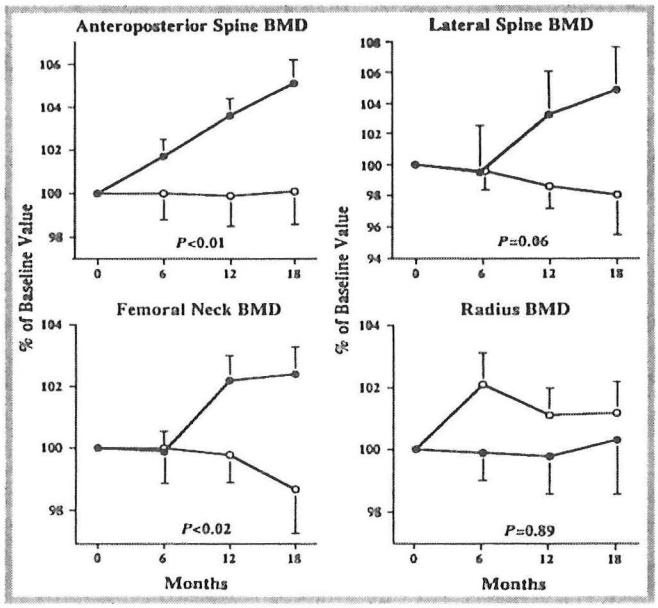
Mortality: There remains considerable debate among endocrinologists about the benefits of GH replacement, even in patients with documented GH deficiency (Frohman 2002). One side in this argument-- contends that patients with GH deficiency have increased morbidity and mortality associated with diminished quality of life, at least partly due to the consequences of growth hormone deficiency. Moreover, GH replacement causes beneficial changes in parameters such as adiposity, bone density, and psychosocial well-being (reviewed by Carroll et al., 1998). Based on these facts, many experts argue that GH replacement should be routinely offered to GH deficient adults (Cook 2002).

Others argue that most GH-deficient patients in these studies have had other pituitary hormone deficiencies, such that the increased mortality may at least partly reflect overmedication with thyroid hormone or glucocorticoids or under-replacement with sex steroids. In addition, many of the patients may have received radiation therapy for pituitary tumors or craniopharyngiomas, treatment which is known to have deleterious vascular effects. Finally, these experts argue that the long-term side effects of GH therapy have not been fully documented and that it may be more cost-effective to treat GH deficient patients with drugs such as bisphosphonates and statins for complications such as decreased bone density and dyslipidemia (Islay et al. 2002).

In balance, there is a pressing need for additional studies to define the potential benefits and risks of GH therapy in GH-deficient adults. Given that many endocrinologists contend that the benefits of GH replacement are definitively proven, such that it would be unethical to withhold therapy in this setting, these important studies will be very difficult to perform,

Body Composition: Studies in GH-deficient adults show that GH replacement is associated with increased lean body mass and decreased adiposity, particularly central adiposity. In the healthy elderly, the initial observation by Rudman was largely responsible for the surge of interest in GH as an anti-aging therapy (Rudman et al. 1990). Several well-controlled trials have supported the ability of GH to decrease adiposity and increase lean body mass in elderly subjects (Papadakis et al. 1996; Thompson et al. 1995). However, efforts to show clear-cut benefits in strength have been mixed at best, and the effect of GH on falls or rehabilitation have not been established.

Bone Mineral Density: As with androgen replacement, there are no published data defining any benefits of GH therapy on clinically relevant aspects of bone mineral density such as fractures. Nonetheless, the data in GH-deficient adults support a significant increase in bone mineral density (Carroll et al. 1998), although there initially may actually be a transient decrease in bone mass due to initial bone resorption, followed by a sustained increase reflecting increased bone formation. The data from one appropriately controlled trial for 18 months are shown below (Baum et al., 1996). An important caveat is that no studies to date have compared the beneficial effects of GH versus more established (and cheaper) agents such as bisphosphonates.



Despite the probable positive effect of GH replacement in GH-deficient adults, the evidence for positive effects of GH on bone mineral density in normal elderly subjects is much less striking. There have been a few studies looking at small numbers of subjects with osteoporosis, some of which have shown benefits of GH therapy, but this is an area that needs further investigation.

Again, the available evidence derives largely from studies of GH-deficient adults rather than elderly subjects. In this setting, the preponderance of evidence supports a beneficial effect of GH therapy to lower LDL cholesterol, whereas most studies suggest that HDL cholesterol and triglycerides are not significantly affected. On the other hand, GH replacement causes increased levels of lipoprotein (a), an independent risk factor for the development of atherosclerosis.

Psychological Well-being and Quality of Life: With more widespread attention to possible effects of GH deficiency in adults, it has become apparent that these patients perceive problems with depressed mood, social isolation, and decreased energy (Carroll et al. 1998). There is evidence from double-blinded, placebo controlled trials that GH replacement can lead to significant improvement in mood and energy level (McGauley, 1989), although this has not been universally observed (Baum et al. 1998) and there clearly is a large placebo effect with GH therapy.

F. Therapeutic Options for Growth Hormone Replacement

The availability of recombinant human GH identical in sequence to that released by the pituitary made it possible to treat a much larger group of patients than the severely GH-deficient children treated initially with cadaveric growth hormone. As noted by one endocrinologist, this resulted in a shift from "too many patients chasing too little hormone to too much hormone chasing too few patients" (cited by Jorgensen and Christiansen 2002). As a peptide hormone, however, recombinant GH is subject to proteolysis following oral administration, and all approved GH preparations are administered parenterally. There are no significant differences in the pharmacology of the various GH preparations, although varying injection devices, preservatives, etc. may influence patient preference. All are injected subcutaneously, generally at night to mimic the normal nocturnal secretion of GH, and all require daily injections for maximal effect. An encapsulated form of GH that is injected subcutaneously once or twice per month (Nutropin Depot®) is also approved by the FDA and is now under evaluation as a more convenient agent for long-term GH replacement (Cook et al. 2002).

Sermorelin (GEREF®), a synthetic derivative of GHRH, also is FDA-approved for GH replacement. It is somewhat less expensive than GH, but has also been less effective in clinical trials. Given the high frequency of pituitary disease in adult

GH deficient patients, it obviously is mandatory to document a response to a test dose of sermorelin before initiating therapy. As noted above, sermorelin also is used diagnostically in provocative testing for GH deficiency. Although there have been a few reports using intranasal administration of GHRH (for example, Khalfallah et al. 1990), such preparations have not found widespread clinical use.

The concept of "growth hormone secretogogues" arose from the observation that peptide derivatives of enkephalins stimulated growth hormone secretion. Further exploration of these compounds led to the demonstration of a specific G-protein coupled receptor—termed the growth hormone secretogue receptor—expressed by somatotropes and growth hormone releasing hormone (GHRH) neurons in the arcuate nucleus. Most recently, an endogenous ligand for the growth hormone secretogue receptor was cloned and shown to encode an acylated peptide made primarily in the stomach termed ghrelin (Kojima et al., 1999). In addition to its potent induction of GH secretion, Ghrelin also induces lactotrope and corticotrope secretion, stimulates appetite (Nazakato et al., 2001), and exerts potent metabolic, cardiovascular and anti-proliferative effects (Broglio et al. 2002). These pleiotropic actions of Ghrelin raise some concerns that growth hormone secretogogues may not be the selective, orally active inducers of GH secretion that they initially seemed, and several pharmaceutical companies have decreased their focus on these agents as therapeutic agents to increase GH secretion.

G. Contraindications/Side Effects of Growth Hormone Therapy

Absolute contraindications to GH treatment include: active malignancy, benign intracranial hypertension, carpal tunnel syndrome, and proliferative or pre-proliferative diabetic retinopathy. Although an increased incidence of malignancy with GH therapy has not been observed in large-scale trials of GH-deficient subjects, the contraindication to GH use in patients with active malignancy is based on the potential growth promoting effects of IGF-I, as well as the fact that epidemiologic studies have found a positive association between IGF-I levels and breast cancer (Hankinson et al., 1998) and prostate cancer (Chan et al., 1998).

Current GH preparations are identical in sequence to GH released from the somatotropes and the side effects therefore reflect those associated with normal actions of GH. Most of these reflect GH action to cause fluid retention, and therefore include edema, arthralgias, myalgias, and carpal tunnel syndrome. Initial studies with GH replacement in GH deficient adults used doses similar to those use in pediatrics, and the incidence of side effects was as high as 33%. Obese and elderly subjects were especially likely to develop symptoms when using dosing regimens based on body weight. At least at these higher doses, the anti-insulin action of GH also has been associated with worsened glucose tolerance or the precipitation of overt diabetes mellitus in some subjects. As

noted above, lower starting doses are now employed, and the frequency of side effects has diminished considerably. It is important to keep in mind that many of the studies that have shown benefits of growth hormone have employed relatively high doses that likely provide pharmacologic, rather than physiologic, effects.

H. Patient Monitoring with GH Replacement

Not surprisingly, there also is no consensus regarding the optimal dosing for patients with adult GH deficiency. Based on the relatively high frequency of side effects with regimens used previously, especially in elderly subjects, many experts favor a lower starting dose (150-300 μg/day) with gradual titration of dose based on patient tolerance and IGF-I levels at monthly intervals until side-effects develop or until IGF-I levels are in the mid-normal range adjusted for age and sex. Very rarely will this dose exceed 1 mg/day in patients age 35 or older. Once the appropriate dose has been established, it is reasonable to monitor IGF-I levels at least twice yearly (AACE Clinical Practice Guidelines for growth hormone use in adults and children 1998). Women who are taking oral estrogens often exhibit a less robust stimulation of IGF-I levels and diminished improvement in body mass parameters, presumably secondary to first pass effects on hepatocytes (O'Sullivan et al. 1998).

Given the lack of consensus about the ability of GH replacement to impact clinically-relevant endpoints such as cardiovascular disease and fractures, it seems prudent to base decisions about long-term therapy on objective parameters (e.g. lipids, bone mineral density, etc.) as well as the subjective well-being of the patient. If the patient doesn't feel that the GH is having a significant effect, then the expense and inconvenience of the therapy will often lead them to stop the therapy.

IV. COMBINATION THERAPY

Despite the absence of definitive evidence supporting the use of growth hormone or androgens alone in aging subjects, several groups have initiated studies to explore the effects of combination therapy (Blackman et al., 2002; Chrismas et al., 2002; Munzer et al., 2001; Brill et al., 2002). In a placebo-controlled trial in which healthy elderly individuals were treated with GH, sex steroids, or a combination for 6 months (Munzer et al., 2001; Blackman et al., 2002), GH therapy was associated with increased lean body mass and decreased adiposity in men and women and a borderline improvement in strength in men, but no change in bone mineral density in either sex. Of note, the doses of GH were pharmacologic rather than physiologic, so that edema and arthralgias were seen in ~30-40 percent of subjects receiving GH and diabetes/glucose intolerance

were also seen in a significant percentage of men. Veldhuis and colleagues also gave elderly subjects a combination of sex steroids and GH (Brill et al. 2002), examining the effects of different regimens for one month/intervention. Given the short duration of therapy, it is not surprising that they found no significant changes in strength, or percent body fat, but they did note significant increases in some functional tests such as stair-climbing. Although the data from studies examining the effects of different hormone supplements in combination are only in their infancy, such combination regimens are already widely used by many of the "Aging" clinics, and further studies are therefore clearly indicated to guide future therapeutic decisions.

E. CONCLUSIONS AND A NOTE OF CAUTION

As summarized here, the levels of testosterone and GH decline with age, and many of the features of aging resemble those exhibited by patients with deficiencies of androgen and GH. Moreover, emerging data from clinical trials—albeit limited in scope and duration—indicate that at least some of the deleterious effects seen in aging individuals can be improved by hormone replacement therapy. In light of the WHI CHD data, however, a number of important questions need to be addressed in randomized, placebo-controlled trials of sufficient duration and number of subjects before we can define the precise role of these drugs in aging individuals. Does androgen replacement to the range normally seen in younger men increase the risk of prostate cancer or its rate of progression to clinical significance? What are the consequences of diagnostic evaluation for PSA elevations in these men, both in health care expenses and emotional and physical complications? How effective will testosterone and GH be in improving clinically significant endpoints (e.g. fractures, falls, cognitive performance) as opposed to intermediate markers such as bone mineral density? Is testosterone or GH treatment more cost-effective for osteoporosis and cardiovascular risk than more standard interventions such as bisphosphonates or statins? Given the known adverse effects of GH and IGF-I excess in acromegaly, are there any deleterious effects of long term GH replacement? Finally, in view of the known ability of physical activity to induce GH secretion, to diminish adiposity (and thereby diminish the aromatization of testosterone) and to , might it not be more efficacious to encourage exercise in aging subjects rather than routinely employ expensive drugs?

It is apparent that the data currently available are woefully inadequate to make definitive recommendations about the roles of hormone replacement therapy in the elderly, despite the tremendous enthusiasm for hormonal replacement. Although a relatively large clinical trial of androgen replacement therapy in elderly men was approved for funding, it was canceled due to concerns that it was unethical to subject men to a therapy that might induce prostate cancer in a manner akin to estrogens and breast cancer (A. Matsumoto, personal

communication). With respect to patients who have demonstrable deficiencies of testosterone or GH, it is apparent that the patients who benefit most from

A final reason for caution—at least with GH replacement—derives from animal models of aging, which have linked increased longevity with defects in the GH/IGF-I axis. One such model is caloric restriction from early life, which is known to decrease insulin and IGF-1 levels (Weindruch et al. 1986). Other monogenic disorders that impair GH secretion (i.e., hypothalamic or pituitary defects, Bartke et al. 2001) or action (i.e., disruption of the GH receptor, Coschigano et al. 2000) also are associated with decreased growth, infertility, and increased longevity. Finally, studies in *Caenorhabditis elegans* and *Drosophila melanogaster* have identified genes whose loss-of-function is associated with increased longevity (Kenyon 2001). Many of these are homologs of the IGF-I receptor or components of its downstream signaling pathway, raising the unsettling possibility that diminished signaling via IGF-I is associated with increased longevity. Consistent with this model, analyses of IGF-I knockout mice have shown that haploinsufficiency for the IGF-I receptor is associated with significantly increased longevity in mice without associated growth retardation or infertility (Holzenberger et al. 2003). This study raises the possibility that insights gained from model organisms will apply also to aging in mammals, while the entire body of work raises the possibility that signaling via the IGF-I pathway is deleterious for lifespan (Lithgow GJ, Gill MS, 2003). Although the practical relevance of these studies for aging human beings remains to be established, they raise some concern that increasing IGF-I levels via GH replacement may not necessarily be advantageous in alleviating aging.

REFERENCES

Alexandersen P, Haarbo J, and Christiansen C 1996 The relationship of natural androgens to coronary heart disease in males: a review. Atherosclerosis 125:1-13.

Anawalt BD, Merriam GR 2001 Neuroendocrine aging in men: andropause and somatopause. Endocrine Metab. Cl. N. A. 30:647-669.

Bagatell CJ and Bremner WJ 1995 androgen and progestagen effects on plasma lipids. Prog. Cardiovas. Dis. 38:255-271.

Bakhshi V, Elliott M, Gentili A, Godschalk M, and Mulligan T 2000 Testosterone improves rehabilitation outcomes in ill older men. J. Am. Geriatr. Soc. 41:550-553.

Bartke A, et al. 1998 Does growth hormone prevent or accelerate aging? Exp. Gerontol. 33:675-687.

Baum HB, Biller BM, Finkelstein JS, et al. 1996 Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. A randomized, placebo-controlled trial. Ann. Intern. Med. 125:883-890

Baum HB, Katznelson L, Sherman JC, et al. 1998 Effects of physiological growth hormone (GH) therapy on cognition and quality of life in patients with adult-onset GH deficiency. J. Clin. Endocrinol. Metab. 83:3184-3189.

Bengtsson B-A, Johannsson G, Shalet SM, Simpson H, and Sonken PH 2000 Treatment of growth hormone deficiency in adults. J. Clin. Endocrinol. Metab. 85:933-942.

Bhasin S, Storer TW, Berman N, et al. 1996 The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N. Eng. J. Med. 335:1-7.

Bhasin S, Buckwalter JG 2001 Testosterone supplementation in older men: a rational idea whose time has not yet come. J. Androl. 22:718-731.

Bhasin S, Woodhouse L, Casaburi R, et al. 2001 Testosterone dose-response relationships in healthy young men. Am J. Physiol. 281:E1172-E1181.

Biller BMK, Samuels MH, Zagar A, et al. 2002 Sensitivity and specificity of six tests for the diagnosis of adult growth hormone deficiency. J. Clin. Endocrinol.Metab. 87:2067-2079.

Blackman MR, Sorkin JD, Munzer T, et al. 2002 Growth hormone and sex steroid administration in healthy aged women and men. JAMA 288:2282-2292.

Bremner WJ, Vitiello MV, Prinz PN 1983 Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J. Clin. Endocrinol. Metab. 56:1278-1281.

Brill KT, Weltman AL, Gentili, et al. 2002 Single and combined effects of growth hormone and testosterone administration on measures of body composition, physical performance, mood, sexual function, bone turnover, and muscle gene expression in healthy older men. J. Clin. Endocrinol. Metab. 87:5649-5657.

Broglio F, Arvat E, Benso A, Papotti M, Muccioli G, Deghenghi R, and Ghigo E 2002 Ghrelin: endocrine and non-endocrine actions. J. Pediatr. Endocrinol. Metab. Suppl 5:1219-1227.

Carroll PV, Christ ER, Bengtsson BA, et al. 1998 Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. J. Clin. Endocrinol. Metab. 83:382-395.

Chan JM, Stampfer MJ, Giovannucci E, et al. 1998 Plasma insulin-like growth factor-I and prostate cancer: a prospective study. Science 279:563-566.

Chrismas C, O'Connor KG, Harman SM, et al. 2002 Growth hormone and sex steroid effects on bone metabolism and bone mineral density in healthy aged women and men. J. Gerontol. A. Biol.Sci.Med.Sci. 57:M12-M18.

Cook DM 2002 Shouldn't adults with growth hormone deficiency be offered growth hormone replacement therapy? Ann. Int. Med. 137:197-201.

Cook DM, Biller BM, Vance ML, et al. 2002 The pharmacokinetic and pharcacodynamic characteristics of a long-acting growth hormone (GH) preparation (nutrotropin depot) in GH-deficient adults. J. clin. Endocriol. Metab. 87:4508-4514.

Coschigano KT, Clemmons D, Bellushi LL, and Kopchick J 2000 Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology 141:2608-2613.

Cummings DE, Kumar N, Bandin CW, Sundaram K, and Bremner WJ 1998 Prostate-sparing effects in primates of the potent androgen 7α-methyl-19-

nortestosterone: a potential alternative to testosterone for androgen replacement and male contraception. J. Clin. Endocrinol. Metab. 83:4212-4219.

Frohman LA 2002 Controversy about treatment of growth hormone-deficient adults: a commentary. Ann. Int. Med. 137:202-204.

Ghigo E, Goffi S, Nicolosi M, et al. 1990 Growth hormone (GH) responsiveness to combined administration of arginine and GH-releasing hormone does not vary with age in men. J. Clin. Endocrinol. Metab. 71:1481-1485.

Groopman J. 2002 Hormones for men: is male menopause a question of medicine or of marketing? New Yorker, issue of 7/29.

Hankinson SE, Willett WC, Colditz GA, et al. 1998 circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 351:1393-1396.

Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR 2001 Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J. Clin. Endocrinol. Metab. 86:724-731.

Harman SM, Tsitouras PD 1980 Reproductive hormones in men. I. Measurement of sex steroids, basal luteinizing hormone and Leydig cells response to human chorionic gonadotropin. J. Clin. Endocrinol. Metab. 51:35-40.

Hartman ML, Crowe BJ, Biller MKB, et al. 2002 Which patients do not require a GH stimulation test for the diagnosis of adult GH deficiency? J. Clin. Endocrinol. Metab. 87:477-485.

Holzenberger M, Dupont J, Ducos B, et al. 2003 IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature 421:182-187.

Islay WL 2002 Growth hormone therapy for adults: not ready for prime time? Ann Int. Med. 137:190-196.

Kenyon C 2001 A conserved regulatory system for aging. Cell 105:165-168.

Khosla S, Melton LJ, Atkinson EJ, O'Fallon WM, Klee GG, and Riggs BL 1998 Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men: a key role for bio-available estrogen. J. Clin. Endocrinol. Metab . 83:2266-2275.

Klatz R. Grow Young With HGH: The Amazing Medically Proven Plan to Reverse Aging. New York, NY: Harper Collins:1997.

Kojima M, Hosoda H, Data Y, Nakazato M, Matsuo H, and Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from the stomach. Nature 402:656-660.

Lamberts SWJ, van den Beld AW, van der Lely A-J 1997 The endocrinology of aging. Science 278:419-424.

Lithgow GJ, Gill MS 2003 Cost-free longevity in mice? Nature 421:125-126.

McGauley GA 1989 Quality of life assessment before and after growth hormone treatment in adults with growth hormone deficieny. Acta Paed. Scan. 356:55-59.

Morley JE, Charlton E, Patrick P, et al. 2000 Validation of a screening questionnaire for androgen deficiency in aging males. Metabolism 49:1239-1242.

Mulligan T, Iranmanesh A, Johnson ML, Straume M, Veldhuis JD 1997 Aging alters feed-forward and feedback linkages between LH and testosterone in healthy men. Am. J. Physiol. 273:R1407-R1413.

Munzer T, Harman SM, Hees P, et al. 2001 Effects of GH and/or sex steroid administration on abdominal subcutaneous and visceral fat in healthy aged women and men. J. Clin. Endocrinol. Metab. 86:3604-3610.

O'Sullivan AJ, Crampton LJ, Freund J, and Ho KKY 1998 The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. J. Clin.Endocrinol.Metab. 102:1035-1040.

Papadakis MA, Grady D, Black D, et al. 1996 Growth hormone replacement in healthy older men improves body composition but not functional ability. Ann. Int. Med. 124:708-716.

Raben MS 1962 Clinical use of human growth hormone. N. Eng. J. Med. 266:82-86.

Rudman D, Feller AG, Nagraj HS, et al. 1990 Effects of human growth hormone in men over 60 years old. N. Eng. J. Med. 323:1-6.

Sih R, Morley JE, Kaiser FE, Perry HM, Patrick P, and Ross C 1997 Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J. Clin. Endocrinol. Metab. 82:1661-1667.

Snyder PJ, Peachey H, Hannoush P, et al. 1999 Effect of testosterone treatment on bone mineral density in men over 65 years of age. J. Clin. Endocrinol. Metab. 84:1966-1972.

Snyder PJ, Peachey H, Hannoush P, et al. 1999 Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. J. Clin. Endocrinol. Metab. 84:2647-2653.

Spratt DI, O'dea LSL, Xhoenfeld D, Butler J, Rao PN, and Crowley WF, Jr. 1988 Neuroendocrine-gonadal axis in men: frequent sampling of LH, FSH, and testosterone. Am. J. Physiol. E658-E666.

Swerdloff R, Wang C, Cunningham G, et al. 2000 Long-term pharmacokinetics of transdermal testosterone gel in hypogonadal men. The Testesterone Gel Study Group. J. Clin. Endocrinol. Metab. 85:4500-4510

Takala J, Ruokonen E, Webster, et al. 1999 Increased mortality associated with growth hormone treatment in critically ill adults. N. Eng. J. Med. 341:785-792.

Tenover JL 1999 Testosterone replacement therapy in older adult men. Int. J. Androl. 22:300-306.

Tenover JL 1998 Male hormone replacement therapy including "andropause". Endocrinol. Metab. Cl. NA 27:969-987.

Thompson JL, Butterfield GE, Marcus R et al. 1995 The effects of recombinant insulin-like growth factor-I and growth hormone on body composition in elderly women. J. Clin. Endocrinol. Metab. 80:1845-1852.

Vahl N, Jorgensen JOL, Jurik AG, and Christiansen JS 1996 Abdominal adiposity and physical fitness are major determinants of the age-associated decline in stimulated GH secretion in healthy adults. J. Clin. Endocrinol. Metabl. 81:2209-2215.

Veldhuis JD 1999 Recent insights into neuroendocrine mechanisms of aging of the human male hypothalamic-pituitary-gonadal axis. J. Androl. 20:1-17.

Vermeulen A 1991 Clinical review: androgens in the aging male. J. Clin. Endocrinol. Metab. 73:221-224.

Wang C 2002 Testosteone replacement therapy choices. Proceedings of the Endocrine Society Andropause Consensus Conference. Pp 27-29.

Wang C, Swerdloff RS, Iranmenesh A, et al. 2000 Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. J. Clin. Endocrinol. Metab. 85:2839-2853.

Weindruch R, et al. 1986 The retardation of aging in mice by dietary restriction: longevity, cancer, immunity, and lifetime energy intake. J. Nutr. 116:641-654.

Wilson JD 1990 Charles-Edouard Brown-Sequard and the centennial of endocrinology. J. Clin. Endocrinol. Metab. 71:1403-1409.

Writing group for the Women's Health Initiative Investigators 2002 Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal

results from the Women's Health Initiative randomized controlled trial. JAMA 288:321-333.

Yarasheski KE, Zachwieja JJ, Campbell JA, Bier DM 1995 Effect of growth hormone and resistance exercise on muscle growth and strength in older men. Am. J. Physiol. 268:E268-276.

Zmuda JM, Cauley JA, Kriska A, et al. 1997 Longitudinal relationship between endogenous testosterone and cardiovascular disease risk factors in middle aged men: A 13-year follow-up of former Multiple Risk Factor Intervention Trial participants. Am. J. Epidemiol. 146:609-617.