# GROWTH HORMONE METABOLISM IN HYPOPITUITARY SUBJECTS AND YOUNG, MIDDLE-AGED AND ELDERLY NORMAL SUBJECTS

by

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Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
Human Nutrition and Foods

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ACKNOWLEDGMENTS

"Life is not easy for any of us - but what of that we must have perseverance and above all confidence in ourselves. We must believe we are gifted for something and that this thing at whatever cost must be attained." - Marie Curie

The author wishes to express his appreciation to Dr. Argelia Ocando who shared these remarks with him; they have been inspirational in my attempts to complete this undertaking. The author also wishes to express his appreciation for the privilege of working with two major advisors, of the University of Virginia School of Medicine, and In this regard, the author wishes to express his gratitude to and who set up a joint program between the Department of Pediatrics of the University of Virginia School of Medicine and the Department of Human Nutrition and Foods of Virginia Polytechnic Institute and State University. The author also wishes to thank the other members of his committee, Dr. S.J. Ritchey, Dr. R.E. Webb, and to Dr. Carl E. Polan for their guidance and advice. The author wishes to thank Chairman of the Department of Pediatrics of the University of Virginia School of Medicine for his support and guidance without which this research endeavor would not have been possible. Special thanks also goes to Dr. Carol Huseman who carried out a major part of the clinical responsibilities of this effort and who spent many sleepless nights unclotting the intravenous catheters. The author is indebted to the services of dietician,

and and the entire staff of the Clinical Research Center of the University of Virginia Hospital. The author would like to thank for the determinations of urinary calcium concentrations and to Dr. Frederic Westervelt for providing this technical assistance. Finally, the author would like to express his deep appreciation to those individuals who volunteered as subjects for this study and to his wife, who has made all things worthwhile.

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# CHAPTER I

## INTRODUCTION

As life expectancy increased from 47 years at the turn of the century to about 67 in 1946, and in the proceeding decades leveled off to about 70 at present (1), greater concern has developed around the phenomenon of aging. Some individuals feel that diet and decreased physical activity may be factors responsible for the failure to increase the life expectancy in the past several decades (1). A few studies have shown that increased physical activity and caloric restriction significantly increase the life expectancy of laboratory rodents (2,3).

In an effort to gain greater insight into the complexities of human aging, we have attempted to examine the aging process as related to endocrine physiology. The interest of nutritionists in age-related changes of various hormones lies in the fact that one of the first systems affected by alterations in diet is the endocrine system (4). Kreisberg et al. (4) contend that the maintenance of optimum nutrition in man is primarily dependent upon normal endocrine function and adequate nutrition. Pointing out the cyclical nature of various hormones in regulating the metabolic processes and requirements of various nutrients, and of various nutrients in stimulating the release of certain hormones, it becomes necessary to examine hormonal function as related to age. Because of the wide range of effects exhibited by growth hormone (GH) on the metabolic processing of various nutrients as well as the regulation of GH release by amino acids, free fatty acids (FFA's) and glucose, the question concerning whether or not pituitary function of GH metabolism declines with advancing age was

examined.

## CHAPTER II

## REVIEW OF LITERATURE

The purpose of this review of literature is an attempt to consider the current knowledge of the pituitary hormone, somatotropin, its metabolic interactions with various dietary components and the effects of advancing age on growth hormone metabolism.

### Growth Hormone

Growth hormone, also knows as somatotropin, is one of the seven polypeptide hormones secreted by the pituitary gland. The uniqueness of growth hormone in relation to the other pituitary hormones lies in the absence of a specific target organ as the site of GH action. Growth hormone is considered responsible for the development and maturation of all body tissues to their adult state except for the possible exceptions of the gonads and the brain. Until the 1950's, it was believed that human beings either did not require or possess GH since pituitary extracts from various animals were ineffective in the treatment of hypopituitary dwarfs. Around 1956 GH was first extracted from human pituitaries and first administered to humans in 1958 (8). Since that time it was discovered that GH is a species specific molecule (5-8). Human growth hormone (hGH) is a polypeptide chain of approximately 191 amino acids connected by two disulfide bridges (9). HGH is unique among the mammalian hormones in that it has the lowest molecular weight (5-8). With a molecular weight of about 21,000, hGH exists in both monomeric and dimeric forms recently referred to as little and big GH respectively (5-8).

Until the double antibody radioimmunoassay for hGH was developed by Utiger et al.(10), Glick et al.(11), and Hunter and Greenwood (12), a biological assay monitoring the rate of incorporation of $^{35}SO_4$ into chondroitin sulfate was used to measure different levels of hGH. This classical bioassay for GH is now used to measure somatomedin since it is now known that sulfate incorporation into chondroitin sulfate is due to the direct action of somatomedin rather than growth hormone (13). At present the most common means of determining GH concentrations in serum is the double antibody radioimmunoassay; however, a receptor assay using particulate fractions obtained from rabbit livers has recently been developed (14,15). The receptor-site assay requires less time than the radioimmunoassay and apparently is not species specific (14).

## Metabolism of Growth Hormone

The wide variety of actions attributed to GH are summarized in a number of review articles (5-8, 16-20).

### Action on carbohydrate metabolism

According to Daughaday and Kipnis (21) the inhibitory action of GH on glucose uptake and utilization by muscle tissue is partly due to its inhibitory action of insulin secretion. The interrelationships of GH and insulin appear to result in the conservation of blood glucose for the energy requirements of the brain. Gold et al. (22, 23) observed that GH administration resulted in a rise in insulin levels in insulinopenic subjects. Cerasi and Luft (24) noted that in cases of excessive GH secretion such as acromegaly, abnormal responses to oral glucose tolerance tests and hyperinsulinism are characteristic. Frohman et al.

(25) found that one of the first noticeable effects of hGH administration in hypopituitary subjects was a rise in blood sugar followed by a subsequent rise in plasma insulin.

Action on protein metabolism

The growth promoting effects of GH appear to result from its actions on protein metabolism. Wright et al. (26) found hGH administration resulted in nitrogen retention reflected by decreased urinary nitrogen excretion and a fall in blood urea nitrogen. Kostyo (27) observed that GH promoted the transport of amino acids across the cell membrane. GH has also been found to stimulate the rate of conversion of proline to hydroxyproline (28). Increased protein synthesis at the cellular level is apparently due to the increased incorporation of uridine into RNA and thymidine into DNA (28). GH's action on protein metabolism ultimately results in the stimulation of increased cell number and size. Daughaday (18) notes that the actions of GH on protein anabolism are primarily the result of GH stimulation on somatomedin secretion since somatomedin has been found to be directly responsible for most of these activities previously attributed to growth hormone.

Because GH and insulin both exhibit growth promoting effects in spite of their antagonism with regard to carbohydrate and fat metabolism, Raben (29) proposed three phases of GH - insulin interaction following the ingestion of glucose. Phase 1 is characterized by insulin excess and the absence of GH; phase 2 by the presence of both insulin and GH; and phase 3 by GH excess and the absence of insulin. The synergistic effects of GH and insulin on protein anabolism are believed to occur

during phase 2. Phase 3 is characteristic of the protein catabolic states of marasmus, kwashiorkor and anorexia nervosa (30,31).

Action on fat metabolism

Forearm studies by Rabin and Zierler (32) and Rabin et al. (33) showed that GH appeared to stimulate free fatty acid (FFA) release from adipose tissue. Body composition studies demonstrated further effects of hGH on fat metabolism. HGH levels are decreased in obese individuals concommitant with the observation of elevated insulin (34,35). While GH administration results in increased body weight of most tissues, there is a decrease in the adipose tissue mass in experimental studies on rats and dogs (36,37). Adipose tissue mass appears to be preserved in children with isolated growth hormone deficiency who show a decrease in subcutaneous fat when placed on GH therapy (38,39).

Effects on skeletal growth

Studies of somatomedin activity suggest this new molecular entity is responsible for a number of effects on skeletal growth previously attributed to GH (18). Salmon and Duvall (40) reported that somatomedin stimulated the incorporation of $^{35}SO_4$ into chondroitin sulfate and $^{3}H$ leucine into chondromucoprotein. Other studies by Salmon and Duvall (40) revealed that somatomedin stimulated the uptake of $^{3}H$ - uridine into RNA and $^{3}H$ - thymidine into DNA in rat cartilage. Whether or not somatomedin mediates all of the effects of GH on skeletal metabolism has not yet been answered; nevertheless it remains fairly certain that somatomedin is dependent upon the presence of growth hormone for

physiologic activity. Somatomedin levels appear reduced in hypopituitary subjects and elevated in acromegalics in comparison to normal control plasma (41,42,43). In addition, somatomedin levels may be reduced with aging since the mean somatomedin level in normal children was 1.79 units in comparison to an assigned value of 1 unit for pooled normal adult plasma (43). Perhaps a decline in somatomedin activity with age is related to changes in skeletal metabolism characteristic of aging such as osteoporosis.

Earlier studies reported hGH administration resulted in the stimulation of the rate of turnover of calcium and phosphorous (8). When calcium intake is adequate, calcium and phosphorous stores increase following hGH administration (44).

## Factors Affecting GH Release

Although the control of GH release is a very complex phonomenon, a great many factors have been reported to be involved in the regulation of serum GH levels (8). Sleep appears to be the most potent stimulus of hGH release since GH levels are higher 2-3 hours after the advent of REM (rapid eye movement) sleep periods which generally far exceed those obtained with other provocative stimuli (45). The stimulation of GH release appears to depend on the occurrence of slow-wave sleep (SWS) since SWS deprivation is associated with the absence of these GH peaks. Some experimental studies in cats suggest SWS is initiated by central serotinergic mechanisms since destruction of 5-hydroxytryptamine (serotonin) containing neurons of the brain stem results in insomnia (48).

States of nutritional deficiency such as anorexia nervosa, starvation, kwashiorkor, renal failure, mucoviscidosis and hepatic cirrhosis have been associated with elevated GH levels (47), however their effect on SWS induced rises in serum hGH have not been determined. Other nutritional factors such as elevated plasma free fatty acids and obesity have been found to suppress nocturnal GH release (47). Sleep induced rises in GH appear to be absent or reduced in normal subjects over 50 years of age (49,50).

Other factors besides sleep have been observed to induce rises in GH levels. Arginine, L-DOPA, 2-desoxy-d-glucose, nicotinic acid, protein ingestion, pyrogen, vasopressin, glucagon, epinephrine plus propranolol and exercise have all been noted to cause elevations in circulating GH (46,47,51).

Animal studies suggest GH secretion is under neural-endocrine control which is mediated by the ventromedial-arcuate region of the medial basal hypothalamus (47). This region of the hypothalamus appears to be the final common pathway of GH control and appears to function as the central means of regulating energy balance and food intake (47). The prevention of GH-stimulated release by insulin-hypoglycemia, exercise, vasopressin, arginine and L-DOPA by phentolamine suggests these entities regulate GH release by alpha-adrenergic stimulation or by blocking beta stimulation (51).

The number of factors affecting GH release at times seems inexhaustible. Stress induces GH secretory episodes in normal subjects at rest with no apparent change in metabolites such as glucose, amino acids and FFA's suggesting the limbic system may act in either an excitatory or

inhibitory fashion on GH release (47). Among inhibitors of GH release, GH itself may act by a short-loop negative feedback mechanism on the basis of studies in animals, monkeys and man. A fourteen amino acid tetradecapeptide called somatostatin has been isolated and identified which is capable of short-term inhibition of high GH levels observed in acromegaly (52). Attempts to isolate and purify a growth hormone releasing factor (GRF) have not been as successful. Various hormonal entities have been observed to affect hGH release. Several studies suggest androgens may promote growth by stimulating GH release while other experiments found corticosteroids, estrogens, progestins and thyroid deficiency to either inhibit GH release to provocative agents or to interfere with the peripheral action of GH (8).

Effects of Nutritional Alterations on GH Metabolism

Not only is GH metabolism under the control of various neural and hormonal influences, but diet has been found to exhibit profound alterations in GH concentrations which may far exceed changes observed with pharmacological agents according to Merimee and Rabin (17). Both acute and chronic alterations in diet can lead to changes in GH response to provocative tests.

Effects of chronic changes in diet on GH response

HGH response to intravenous administration of arginine, a standard test for the determination of normal GH release, has been observed to change with the carbohydrate content of the diet (17). A study in normal adult subjects fed a high carbohydrate diet consisting of 525

grams carbohydrate, 75 grams protein and 3600 Calories per day resulted in the suppression of GH secretion following arginine stimulation at the end of the 23 day diet period (17). At the end of this diet period, the response to arginine stimulation was 4.6 $\pm$ ng hGH/ ml compared to a mean response of 21.5 $\pm$ 3.6 ng hGH/ ml for these subjects at the beginning of the study (17). The suppression of hGH appeared to be related to the high carbohydrate content of the diet rather than the caloric intake since comparable results were obtained on a 2300 Calorie per day diet in which the proportion of fats, carbohydrates and protein was equivalent to that of the 3600 Calorie a day diet (17).

Grey and Kipnis (53) obtained comparable results with regard to the effects of changes in diet composition on insulin secretion in 10 obese female subjects with regard to the data for GH reported by Merimee and Rabin. Basal plasma insulin levels dropped 50% on an isocaloric, low carbohydrate diet (25% carbohydrate, 53% fat and 22% protein); however, in spite of a continued weight loss on a comparable isocaloric diet, when the carbohydrate content was increased from 25% to 62%, the basal plasma insulin levels returned to their original high levels (53). Of interest was the observation that on the basis of diet histories, the obese women consumed more carbohydrate in terms of the total amount eaten and the percentage of carbohydrate eaten expressed as a part of the total calories consumed in comparison to normal weight women.

Although hGH values were not determined in this study, the association of low GH levels with the obese condition (8,16) leads one to hypothesize that the high carbohydrate content of the obese person's diet and subsequent hyperinsulinemia contribute to the blunted GH response

observed in obese individuals. The mechanism of the blunted GH response may be related to an increase in cell size concomitant with no increase in the number of receptor sites which in turn distort the feedback control mechanisms regulating GH secretion. The hyperplasia of obesity correlates well with the hyperinsulinemia of this condition according to Salans et al. (54). The hypothesis may be extended to changes in GH secretion in the elderly since the incidence of diabetes increases with age; adipose tissue accumulation is characteristic of the aging process and since cellularity studies in adults indicate growth is achieved primarily by increases in cell size rather than increases in cell number.

Merimee et al. (55) recently reported a significant fall ($p < .01$) in the 24 hour secretion of hGH in 8 normal-weight men 20 - 40 years of age following a two week high carbohydrate (80%) diet from 106 $\pm$ 17 ng/ 24 hours to 67 $\pm$ 10 ng/ 24 hours. A non-significant fall in hGH from 159 $\pm$ 18 ng/ 24 hours to 145 $\pm$ 18 ng/ 24 hours was observed in a comparable group of 7 normal-weight women 20 - 40 years of age. Twenty-four four hour hGH secretion was not altered following two week study periods on either a high calorie (3600 Calories), a 70% fat or a 74% protein diet.

Effects of acute changes in diet on GH response

Acute changes in hGH levels in response to dietary components have been demonstrated by a number of studies (55 - 58). Fineberg et al.(56) found the heparin-induced rise in FFA's following a fat meal (60 grams corn oil) blunted the mean rise in arginine-stimulated hGH from 38 ng/

ml to less than 18 ng/ ml. A fat meal (60 grams corn oil) was also found to blunt the mean rise in hGH following a 300 gram meal of beef tenderloin from 8 ng/ ml to 3 ng/ ml twenty minutes after the intravenous injection of fat (intralipid) with heparin. GH levels remained depressed in these animals for more than two hours after the fat-heparin injection (57).

HGH levels have also been shown to change in response to various dietary metabolites present in blood. Luft et al. (58) have shown a fall in blood glucose results in a significant rise in hGH levels. Elevation of FFA's suppresses hGH as well as results in the inhibition of nocturnal peaks of hGH secretion according to Reichlin (16). Intravenous administration of different amino acids and ingestion of a high protein meal stimulate the release of hGH (55,56,57). A five-fold increase in hGH levels for obese subjects was observed in comparison to an eleven-fold increase observed for a group of normal weight subjects in response to an arginine stimulation test (62).

## Growth Hormone and Aging

Although a decline in the secretion of any hormone with advancing age is certain to have many implications, it is necessary that all possible factors be considered in monitoring such changes. According to Gregerman and Bierman unaltered changes in hormonal concentration do not necessarily indicate an absence of physiological changes (63). They also point out that a constant production rate of a hormone reflects not only the rate of secretion but, in addition, the concentration of specific carrier-binding proteins which in turn determine the amount of

"free" or metabolically active hormone present as well as the rate of metabolic distribution and clearance (63). One must also consider that feedback mechanisms generally associated with the secretion of releasing hormones and the sensitivity of target tissue to appropriate hormones may change with age (63). Finally, one must be able to differentiate those changes in hormone production that are age-related from those that are associated with various disease states (63). With these considerations in mind, a search of the literature was undertaken to explore the present status of information regarding changes in hGH metabolism as a result of advancing age in both animals and man.

## Age-related changes in growth hormone metabolism in animals

Studies of a growth hormone releasing factor (GRF) by Pecile et al. (64) found that senescent rats respond minimally to GRF extracts which were observed to exhibit strong GH release responses in young rats. In addition the presence of GRF was observed in young rats but not in the senescent rats (64). These observations might account for decreased responses of GH to hypoglycemia and arginine infusion observed in older human subjects according to Pecile et al. (64). Thus, age-related changes in hGH could result from raising the threshold to stimulate GH secretion.

Verzar (65) reported that the life-span of hypophysectomized animals is shortened as is their growth. On the basis of his work on the role of collagen formation with regard to aging, Verzar has hypothesized that aging is a result of the failure to renew new macromolecules over time (66). Because GH participates in collagen formation by stimulating

the turnover rate of hydroxyproline and the incorporation of mucopolysaccharrides into the collagen matrix, a decline in GH activity due to advancing age would imply a major role for GH in the etiology of aging if this hypothesis bears out.

A study on lymphocytes, hormones and aging by Fabris et al. (67) also suggests GH may play an important role in the process of aging. This study involved the administration of either 150,000 x $10^6$ peripheral lymph node lymphocytes from 40-day-old normal Snell-Bag mice or 250 ug bovine GH plus 1 ug L-thyroxine to Snell-Bag mice expressing the homozygous recessive gene for dwarfism. The dwarf mice exhibit juvenile body proportions, reduced body size, low muscular activity, sterility, juvenile body hair and a life-span of 3-5 months in comparison to a mean life-span of about 22 months for their normal littermates. In addition, the dwarf mice have few or no GH or TSH secreting cells. Parameters used to measure aging, life-span, loss of hair, cutaneous and subcutaneous atrophy, bilateral cataracts and in vivo cellular turnover monitored by measuring the rate of $^3$H-thymidine uptake, were all delayed by both treatments. The life-span of the dwarf mice by either treatment increased from 5 months to at least 14 months. The investigators concluded that GH and thyroxin were necessary for normal development of the lymphoid system, and that the action of these hormones in preventing aging occurs through the normalization of the thymus structure.

Age-related changes in growth hormone metabolism in man

Although there is a decrease of about 20% in human pituitary size with advanced age, it is not known whether there is a decrease in

pituitary content in hGH (65). Gershberg (68) reports no gross decrease in hGH content of the pituitary as a result of aging. Daughaday reports that although only a few pituitary glands have been analyzed for their content, octogenarians have nearly as much hGH as the rapidly growing child (5). Gregerman and Bierman (63), however, point out that functionally or physiologically, the changes in the release of GH in response to various stimuli is of greater importance than actual changes in pituitary hGH content.

A number of investigations have attempted to look at hGH metabolism over a 24-hour period by monitoring 24-hour integrated concentrations (69,70) or by 24-hour secretion or production rates (49,71,72). Thompson et al. (69) reported mean 24-hour concentrations of 5.6 $\pm$ 3.6 ng/ ml for 17 boys 7 - 16 years of age and 1.8 $\pm$ 1.0 ng/ ml for 16 men 30 - 50 years of age. Plotnick et al. (70) observed mean 24-hour integrated concentrations of 4.8 $\pm$ 0.4 ng/ ml for 5 prepubertal females 5 - 14 years of age and 5.1 $\pm$ 1.2 for 5 pubertal females.

Taylor et al. (71) reported a metabolic clearance rate of 125 $\pm$ 26 ml/ min/ $m^2$ (mean $\pm$ S.D.) and a production rate of 347 $\pm$ 173 ng/ min for hGH in 22 normal subjects 17 - 75 years of age. McGillivray et al. (72) observed a metabolic clearance rate of 112 $\pm$ 5 ml/ min/ $m^2$ (mean $\pm$ S.E.) and a production rate of 363 $\pm$ 105 ng/ min/ $m^2$ (mean $\pm$ S.E.) for normal males 8 - 31 years of age for hGH while they observed a metabolic clearance rate of 111 $\pm$ 10 ml/ min/ $m^2$ and a production rate of 329 $\pm$ 74 ng/ min/ $m^2$ for 8 normal females 9 - 43 years of age for hGH. Both studies (71,72) reported an absence of any sex-related or age-related differences for metabolic clearance and production rates of hGH in their subjects.

Studies of 24-hour spontaneous secretion of hGH in various age groups by Finklestein et al. (49) revealed the following changes: 4 prepubertal males secreted hGH during sleep only and exhibited a mean secretion rate of 91 ug/ 24 hours; 10 adolescents secreted hGH during both sleep and awake period with a mean secretion rate of 226 ug/ 24 hours; secretion rates in 8 adults, 21 - 41 years of age, averaged 385 ug/ 24 hours with secretory episodes occurring during both sleep and awake periods; the total hGH secretion in three older adults decreased and approached zero.

Data collected by Hunter (73) however suggests that children secrete more GH during sleep than do adults. Hunter also reports that children at rest eating normal meals exhibit higher plasma GH values than were observed for adults in a comparable study. The greater levels of GH observed for children than adults was attributed to a greater basal metabolic rate in the younger group (73). A study by Carlson et al. (50) reported an absence of a sleep peak of hGH in four of six subjects over 50 years of age. Blichert-Toft (74) observed a mean peak hGH level during sleep of 11.1 ng/ ml in 12 younger subjects 20 - 41 years of age in contrast to a mean peak hGH level of 6.4 ng/ ml in 5 elderly subjects 71 - 90 years of age. The daytime range of hGH ranged from 2.2 - 30.7 ng/ ml in the younger subjects in comparison to a daytime range of 1.6 - 10.4 for the older subjects.

Changes in hGH response to provocative stimuli in elderly subjects appear to be blunted with respect to responses observed for some tests but not for others. Root and Oski (75) observed normal responses in three elderly adult male subjects over age 65 to insulin tolerance

tests. Normal responses to oral glucose and arginine stimulation tests were observed in only two of the three subjects. Vidalon et al. (76) reported lower fasting, peak and sum levels of hGH in women 40 - 59 years old with normal glucose tolerance tests than those observed for younger women with normal glucose tolerance tests. The same relationship was observed for obese women 40 - 59 years old when compared to obese women in younger age groups (76). Sandberg et al. (77) observed significantly higher serum fasting hGH levels in 14 elderly males, 70 $\pm$ 6 years of age, (2.5 $\pm$ 1.9 ng/ ml) than they observed in 12 young males, 25 $\pm$ 9 years of age, (0.6 $\pm$ 0.3 ng/ ml) prior to an oral glucose tolerance test while serum fasting hGH levels in 8 young females, 25 $\pm$ 9 years of age (4.1 $\pm$ 2.8 ng/ ml) were higher than the fasting hGH levels observed in 7 elderly females, 70 $\pm$ 6 years of age (1.8 $\pm$ 1.3 ng/ ml). Because of the high variability of basal serum levels, these data could be questioned. The young males exhibited a greater peak in serum hGH than the elderly males, however, the elderly females exhibited a greater peak hGH than the younger females in this study in response to an oral glucose tolerance test (77). An earlier study by Danowski et al. (78) observed no age-related change in hGH response to oral glucose tolerance tests in subjects without glucose tolerance. Dudl et al. (79) observed no differences in serum hGH levels between 10 young subjects 20 - 40 years of age and 10 older subjects 60 - 80 years of age in response to a 20 gram pulse of glucose.

The response of hGH release to insulin-induced hypoglycemia in 8 elderly subjects 80 - 95 years of age in a study by Cartlidge et al. (80) were normal. The serum fasting levels ranged from 1.2 to 6.1 ng/ ml and

the peak hGH levels ranged from 27 to more than 50 ng/ ml. Another study on the response of hGH to insulin-induced hypoglycemia in the elderly by Kalk et al. (81) reported the mean hGH response in 30 elderly subjects 63 - 99 years of age was comparable to that of 26 younger subjects with a mean age of 32.7 years. Differences in blood glucose were not significantly different between the two groups. Four of the elderly subjects hGH response was either blunted or absent (81). Three of these individuals were obese. In addition, when the individuals in the elderly group were divided into either obese or non-obese groups, the obese group exhibited a significantly lower fasting hGH than the non-obese group. These data are in contrast to a report by Laron et al. (82) that the mean hGH response to insulin-induced hypoglycemia of 12 ng/ ml was "subnormal" in a group of 19 elderly subjects over 70 years of age versus 20 ng/ ml for the younger control group.

Changes in response to arginine-stimulated hGH release between young and elderly subjects have been reported by Dudl et al. (79), Root and Oski (75), and Blichert-Toft (74). There were no apparent differences in response to 0.5 grams arginine-monochloride per kg body weight between 13 elderly individuals 68 - 90 years of age and 16 younger subjects 17 - 57 years of age in the study by Blichert-Toft (74). The mean increase in hGH over fasting levels was 13.7 $\pm$ 4.3 ng/ ml for the younger subjects compared to 13.3 $\pm$ 3.3 ng/ ml for the elderly (74). Dudl et al. (79), however, reported a mean peak of 30 ng hGH sixty minutes post 0.5 g arginine monochloride in 7 subjects 20 - 40 years of age in comparison to a mean peak of 15 ng hGH sixty minutes post injection in 8 elderly subjects 60 - 80 years of age. Buckler (84) reported

that only one of 10 men under age 25 failed to respond to an oral dose of 20 g Bovril with an increase in hGH levels of at least 10 ng hGH/ ml in contrast to the failure of 18 of 19 men over age 30 to respond to the oral ingestion of Bovril.

The effects of age and exercise on growth hormone secretion have been examined in several experiments. A study of multiple hormonal responses to graded exercise in response to physical exercise (79) showed that hGH increased with work, although hGH values with moderate work loads (12.6 $\pm$ 6 ng/ ml) were greater than hGH values with heavy work loads (4.6 $\pm$ 1 ng/ ml). Similar observations were reported by Buckler (84). Hartley et al. (83) observed that physical training increased hGH levels at rest and just prior to exhaustion in 7 young male subjects 20 - 24 years of age over hGH values observed at rest and exhaustion at the beginning of a seven week physical exercise program. Ericksson et al. (85) observed no difference in peak hGH responses to prolonged exercise between boys 12 - 13 years old and men 23 - 24 years old. Slightly higher hGH responses to a standard meal were observed for the boys (11.4 ng/ ml) when compared to the men (8.7 ng/ ml) in this study (85). A recent paper at the 1974 Federation Proceedings reported the serum hGH values in response to exercise increased in a group of elderly males and in elderly females following a physical fitness training program (86).

## CHAPTER III

## EXPERIMENTAL PROCEDURE

### Design and Subjects

Growth hormone provocative testing

HGH secretion of twenty-two subjects were examined at the Clinical Research Center of the University of Virginia Hospital, Charlottesville, Virginia. The subjects were divided into the following groups (Table I) for comparison of their data: Group I: 6 hypopituitary subjects ranging in age from 9 - 29 years. The criterion for definition of hypopituitarism was the failure to respond to any of the provocative tests for GH release with an increase in hGH concentration of 5 ng/ ml or greater. All of the hypopituitary subjects were caucasian males except for a black female 12 years of age and a caucasian female 9 years of age (Table II).

Group II: 4 caucasian males ranging in age from 23 - 29 years.

Group III: 6 caucasian males ranging in age from 35 - 51 years.

Group IV: 6 caucasian subjects ranging in age from 61 - 69 years.

All of these subjects were males except for one 61 year old female. All subjects were within $\pm$ 10% of their desired body weight for height, sex and age except WH in group II and HW in group IV who were 114% and 121% of their desired body weights respectively.[1]

Pituitary function as related to hGH response to provocative tests was considered normal provided the serum hGH reached a level of 5 ng/ ml or more to at least one of the three tests. The three hGH provocative

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[1] Metropolitan Life Insurance Co., 1959 Statistical Bulletin 40:3.

TABLE I

Demographic data of subjects

| Group | Subject | Sex | Age (yrs.) | Height cm | Weight kg |
|---|---|---|---|---|---|
| I. Hypopituitary | RP | M | 15 | 123 | 24.1 |
| | AJ* | F | 12 | 115 | 19.6 |
| | EF | M | 29 | 167 | 52.0 |
| | MF | M | 18 | 137 | 37.2 |
| | WC | M | 11 | 137 | 47.5 |
| | JD | F | 9 | 97 | 13.3 |
| II. 23 - 29 year | TB | M | 25 | 170 | 66.2 |
| old males | DS | M | 29 | 169 | 56.8 |
| | JB | M | 23 | 186 | 71.1 |
| | WH | M | 25 | 181 | 80.0 |
| III. 35 - 51 year | FM* | M | 48 | 171 | 69.1 |
| old males | RB* | M | 51 | 170 | 70.0 |
| | MA* | M | 39 | 170 | 70.9 |
| | RD* | M | 35 | 174 | 69.8 |
| | JH* | M | 50 | 173 | 71.8 |
| | MV* | M | 36 | 172 | 75.0 |
| IV. 61 - 69 year | HW | F | 61 | 158 | 64.1 |
| old subjects | JW | M | 66 | 181 | 73.7 |
| | JJ | M | 69 | 185 | 71.7 |
| | GA* | M | 67 | 171 | 72.2 |
| | FB | M | 66 | 157 | 58.4 |
| | WB | M | 61 | 176 | 74.8 |

* No metabolic balance data.

TABLE II

Clinical summary of hypopituitary subjects

| Subject | Sex | Etiology | CA[1] | BA[2] | TSH Deficiency | ACTH Deficiency[3] |
|---|---|---|---|---|---|---|
| RP | M | idiopathic | 15 | 7 | - | - |
| AJ | F | idiopathic | 13 | 6 | - | + |
| JD | F | idiopathic | 9 | 8 | - | o[4] |
| EF | M | idiopathic ? traumatic delivery | 29 | 18 | + | + |
| MF | M | idiopathic ? traumatic delivery | 18 | 11 | + | + |
| WC | M | post-operative craniopharyngioma | 11 | 10 | + | + |

1 CA: chronological age

2 BA: bone age

3 As detected by metapyrone testing (91)

4 o - not adequately evaluated

tests used in our protocol (Appendix A) were exercise (87), L-DOPA (88), and insulin (89). Exercise stimulation was performed by drawing 10 ml blood samples at time 0 after overnight fasting, after 20 minutes of exercise which generally consisted of running or walking at different speeds and or grades on a treadmill during which the heart rate was monitored via an EKG machine, and after 20 minutes rest following the exercise period. The L-DOPA and insulin stimulation tests conducted in tandem consisted of drawing 5 ml blood at time 0 after overnight fasting which was immediately followed by the ingestion of 250 - 500 mg L-DOPA.[2] Blood samples were then collected at 30, 60 and 90 minutes post L-DOPA ingestion. Immediately after drawing the 90 minute post L-DOPA blood sample, 0.1 U insulin[3] per kg body weight (BW) was administered intravenously as a bolus infusion. Five ml blood samples were also drawn at these same time intervals for cortisol determinations for assessing adrenal function as part of a collaborative study of age-related changes in other endocrine organs.

Twenty-four-hour integrated hGH concentrations (I CGH) were determined at either the time of the subjects availability or on day 5 of the control period and on day 5 of the hGH administration period during the metabolic balance experiments according to the procedure of Kowarski et al. (90). Peaks in hGH associated with sleep and awake periods were examined from these data. Blood was drawn at a relatively constant rate with indwelling catheters coated with 1% TDMAC-heparin solution dried

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[2]Hoffman LaRoche, Nutley, New Jersey.

[3]Eli Lilly Company, Indianapolis, Indiana.

overnight and gas sterilized connected to withdrawal pumps[4]. Occasionally the continuous withdrawal technique was discontinued and blood samples were drawn every 30 minutes to complete the 24 hour period from an indwelling heparin-lock syringe. Twenty-four-hour pooled hGH integrated concentrations were determined from 100 ul aliquots from each half-hour sample. Integrated hGH concentrations were also determined from the sum of each of the half-hour values divided by the total number of samples. Peaks in hGH associated with sleep and awake periods as noted on the patients' activity sheet were examined from these data. Forty-eight half-hour pooled collections were used to determine integrated concentrations for testosterone, prolactin, LH, FSH and TSH as part of a collaborative study at the University of Virginia. Twenty-four-hour pooled half-hour samples were also collected and frozen for determination of integrated concentrations of somatomedin at a later date.

Short-term growth hormone metabolic balance studies

Seventeen of the twenty-two subjects also participated in the metabolic balance experiments (Appendix A) which consisted of a three day dietary adjustment period during which the provocative tests of hGH release, physical examinations, blood chemistries, chest x-ray, and EKG were administered; a 5 day control period; and a 5 day treatment period during which 0.168 U hGH/ kg $BW^{0.75}$/ day was administered. This dose was selected on the basis of dose-response studies by Rudman et al. (92) who observed this amount of hGH to exhibit feeble but nevertheless significant

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[4]Sigmamotor, Inc., Middleport, New York.

responses to hGH therapy with regard to nitrogen retention in 6 normal adults 27 - 55 years old.

Patients were made aware of the objectives and procedures of these experiments by written information and discussion. They signed written consent forms for voluntary participation in the study and for venous catheterization for constant withdrawal of blood. Due to the demands made on the patients in this study it was essential that they maintained a cooperative attitude.

Patients were weighed daily in hospital gown and undershorts at 8:00 A.M. on the first full day of the adjustment period and on successive days thereafter for the entire experimental period (Appendix A). The nurses and members of this study instructed each patient concerning the critical importance of obtaining adequate urine collections. Each patient was told to void each morning at 8:00 A.M. prior to beginning the next day's twenty-four hour urine collection. Twenty-four hour urines were collected for determination of creatinine, nitrogen, hydroxyproline and calcium. In spite of efforts by the nursing staff, the investigators and the patients, metabolic data from the urine collections of one hypopituitary subject, AJ, and one elderly subject, GA, were discarded because of apparent inadequate collection. Five ml glacial acetic acid was added to each twenty-four hour urine collection as a preservative. Total volume was measured for each twenty-four hour period and two aliquots of approximately 100 ml each were then frozen until the day of analysis.

Patients were asked to keep a 3 day dietary diary prior to admission (Appendix B). Nutritional histories and evaluations were also

conducted for each subject at the time of admission in order to eliminate any problem foods from the two daily meal patterns alternated throughout the study. The metabolic diets prepared by the clinical research dietitian and her staff were constant, low hydroxyproline diets in which the energy and protein requirements were determined for each individual according to the most recent dietary allowances (93). Dietary protein was 0.8 g per kg BW for adults and 1.2 to 1.5 g per kg BW for the hypopituitary subjects 9 - 18 years old. Energy intake was altered during the adjustment period and first day of the control period when necessary to maintain a constant weight during the control period. Dietary requirements of the hypopituitary subjects were based on the requirements of children of similar weight and height rather than chronological age and modified accordingly. The two daily menus (Appendix C) consisted of three meals and snacks of lemonade or other carbohydrate beverages depending upon the subjects energy requirements. Iron supplements were provided daily to patients exhibiting a fall in hematocrit and hemoglobin according to the physicians' discretion.

The nurses noted provided a log of daily activities and any irregularities or problems noted therein. The nurses also kept activity sheets describing the activities of each patient during each 24 hour pump study period. The dietitian maintained daily records of uneaten food for each patient.

## Biochemical methods and statistical analysis

HGH analyses in triplicate were made according to the double antibody radioimmunoassay method of Schalch and Parker (94). Our limit of

sensitivity was determined at 0.8 - 1.0 ng/ ml and our reproducibility about 0.3 ng/ ml. Concentrations greater than 40 ng/ ml were analyzed as 40 ng/ ml while concentrations less than 2 ng/ ml were analyzed as 2 ng/ ml since these concentrations were the upper and lower limits of our standards. Technicon$^{R}$ AutoAnalyzer$^{R}$ II procedures were used to determine twenty-four hour urinary excretion of creatinine (95), nitrogen (96) and hydroxyproline (97). Twenty-four hour urinary excretion of calcium was determined spectrophotometrically (98). Statistical analysis was performed by the unpaired student t test.

## CHAPTER IV

## RESULTS

### HGH Response to Provocative Testing, Sleep and 24 Hour Integrated Concentrations Before and After hGH Administration

The results of our studies are presented in Table III. None of the hypopituitary subjects were observed to release greater than 2 ng hGH/ ml in response to either exercise or L-DOPA stimulation. The mean hGH concentrations observed for the 23 - 29 year old men were consistently higher than the concentrations observed for any of the other groups with regard to the exercise, L-DOPA and insulin provocative tests as well as for peak hGH levels during sleep and their integrated 24 hour hGH concentrations prior to hGH administration. (Individual hGH values: see Appendix D).

### Exercise stimulation of hGH release

Group II (23 - 29 year old men) had elevated hGH levels ($P < 0.05$) above group I (hypopituitary subjects) and group IV (elderly subjects 61 - 69 years of age) in response to exercise stimulation. The hGH response of group III (35 - 51 year old men) was also statistically higher ($P < 0.05$) than that of groups I and IV. In spite of the younger men's greater mean response in comparison to the middle-age men's mean response, their was no statistical difference between the mean peak response to exercise of group II and III. Two of the young men, TB and DS, were observed to exhibit hGH concentrations considerably greater than 40 ng/ ml.

TABLE III

Mean maximum hGH response to provocative testing; sleep peaks; and 24 hour integrated concentrations (ICGH). (ng/ ml)

| Group | | Exercise | L-DOPA | Insulin | Sleep Peak | Control Period 24 hour ICGH | hGH Period 24 hour ICGH |
|---|---|---|---|---|---|---|---|
| I. Hypopituitary | n | 5 | 5 | 4 | - | 5 | 4 |
| | Mean | $< 2^{a}$ | $< 2^{a}$ | $< 2^{A}$ | | $< 2^{a}$ | $5.2^{**}$ |
| | SE | - | - | - | | - | 0.8 |
| II. 23 - 29 year old males | n | 4 | 4 | 4 | 4 | 4 | 4 |
| | Mean | $22^{b}$ | $23^{b}$ | $30^{B}$ | $21^{a}$ | $3.5^{b}$ | 3.7 |
| | SE | 11 | 6.5 | 9 | 6.7 | 0.7 | 1.0 |
| III. 35 - 51 year old males | n | 3 | 3 | 3 | 3 | 3 | 1 |
| | Mean | $4.0^{bc}$ | $4.9^{c}$ | $29^{B}$ | 4.8 | $< 2^{a}$ | 4.3 |
| | SE | 0.6 | 1.8 | 12 | 2.8 | - | - |
| IV. 61 - 69 year old subjects | n | | 6 | 6 | 6 | 6 | 4 |
| | Mean | $3.0^{ac}$ | $11^{d}$ | $15^{B}$ | $3.5^{b}$ | $< 2^{a}$ | $3.7^{**}$ |
| | SE | 1.1 | 1.5 | 5 | 1.2 | - | 0.4 |

abcd - Group means in each column with different small letter superscripts are statistically different ($P < 0.05$) from one another by the student t test.

AB - Group means in each column with different capital letter superscripts are statistically different ($P < 0.01$) from one another by the student t test.

** - GH administration period ICGH different from control period ($P < .01$) by the student t test.

L-DOPA stimulation of hGH release

Group II and group IV had elevated hGH levels ($P < 0.01$) above either groups I or III in response to L-DOPA stimulation. The mean peak response of all four groups were statistically different from one another ($P < 0.05$). Subjects TB and DS in group II again had the greatest magnitude of response to a hGH stimulus.

Insulin stimulation of hGH release

Although the mean peak responses to insulin stimulation of the three normal groups were not statistically different, their responses declined with the advancing age of their respective groups. Groups II, III and IV had statistically greater responses than group I ($P < 0.01$).

Sleep

Mean hGH peaks during sleep were statistically different ($P < 0.05$) between groups II and IV although the mean hGH peak during sleep declined progressively with advancing age among groups II, III and IV. The total area of the hGH peaks related to sleep were much greater in the young men than in any other subjects (Figure I). Only one of 3 subjects in group III, RB, and one of 6 subjects in group IV, JW, had a nocturnal hGH peak greater than 5 ng hGH/ ml while 3 of the 4 young men exhibited nocturnal peaks in hGH above 10 ng/ ml. HGH peaks associated with stress due to catheterization were more frequent and of greater magnitude in the young men than in either of the older groups.

Twenty-four hour integrated hGH concentrations

The mean 24 hour integrated hGH concentration of group II, 3.5 ng/ ml, was statistically greater ($P < 0.05$) than any of the other groups. The 24 hour integrated hGH concentration was less than 2 ng/ ml for all subjects in group I, III or IV. The range of the 24 hour integrated concentrations of the young men ranged from 2 to 5.2 ng/ ml. The 24 hour integrated concentrations were not statistically different from one another for group II in comparing the control period I CGH with the growth hormone period I CGH; however, the two periods were different from one another ($P < 0.01$) for groups I and IV.

Metabolic Responses to hGH Administration

The metabolic responses of our subjects to 0.168 U hGH/ kg $BW^{0.75}$/ day are presented in Tables IV and V. (Individual values are presented in Appendix E). Because only one subject, FM, in the 35 - 51 year old group was available for the two week metabolic balance period, this group was excluded from statistical analysis of the metabolic data. His metabolic responses fell within the range of the various responses observed for the 61 - 69 year old subjects.

Body Weight. Changes in body weight were variable within each experimental group. A mean weight loss of .28 kg/ day was observed in group II which was statistically different from groups I and IV who experienced a mean weight gain of .17 and .33 kg/ day respectively. Three of the four young men lost weight during the hGH administration period - in the remaining young man no change in body weight occurred. All normal subjects were within $\pm$ 10% of their desired body weight except for WH in group II and HW in group IV who were 14 and 21% over

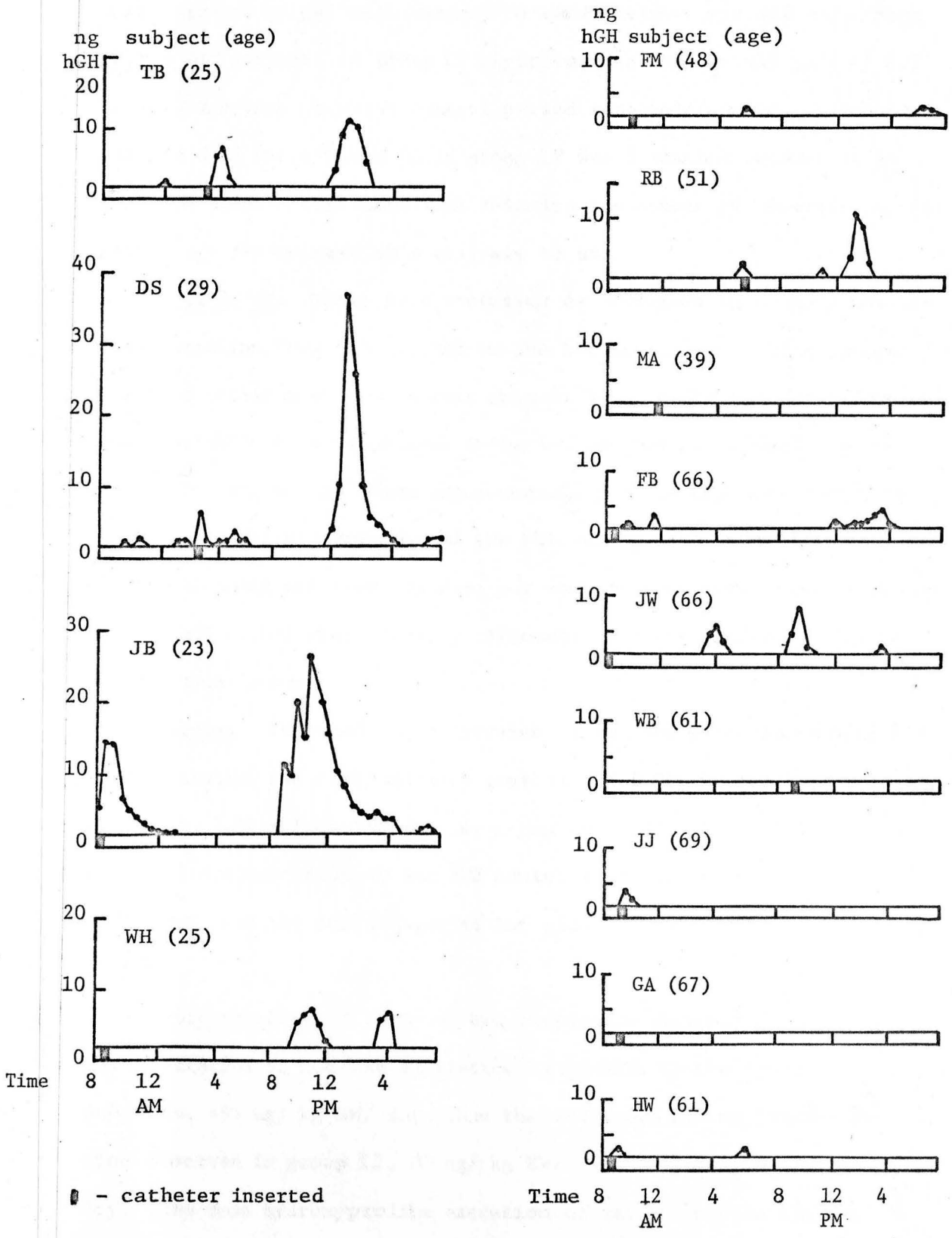



Figure 1
Twenty-four hour hGH secretion with samples every half hour

their desired weight with respect to their height, age and sex. Four of the six subjects in group IV experienced a mean weight gain of 0.7 kg in comparison to their control period mean body weight. Urinary balance data for subject GA in group IV was discarded because of incomplete urine collections thus reducing the number of observations for this group for urinary data analysis to six.

Creatinine. Daily mean increases or decreases in urinary creatinine excretion from the control to the hGH period paralleled observations of either mean body weight gain or loss in the respective groups. Wide variability in creatinine excretion between individuals was observed in each of the three experimental groups. The mean change in urinary creatinine excretion of the hGH period was not statistically different among the three groups, nor was the mean creatinine excretion of the hGH period statistically different from the control period for any of these groups.

Calcium. The mean daily increase in calcium excretion during hGH administration was statistically greater ($P < 0.01$) in the hypopituitary subjects, 3.40 mg/ kg BW/ day, or group IV, 1.05 mg/ kg BW/ day. The mean calcium excretion of the hGH administration period was statistically different from the control period for group I and IV but not for group II.

Hydroxyproline. Increased hydroxyproline excretion during the administration of hGH was statistically greater in the hypopituitary subjects, 495 ug/ kg BW/ day, than the increased hydroxyproline excretion observed in group II, 83 ug/ kg BW/ day, or group IV, 89 ug/ kg BW/ day. The mean hydroxyproline excretion of the hGH period was not

TABLE IV

Metabolic Response to Human Growth Hormone Administration[1]

| Group | | Mean Change BW (kg) | Mean Change Creatinine mg/kg BW/day | Mean Change Calcium mg/kg BW/day | Mean Change Hydroxyproline ug/kg BW/day | Mean Change Nitrogen mg/kg BW/day | Mean Change Hydroxyproline: Creatinine Ratio ug/mg |
|---|---|---|---|---|---|---|---|
| I. Hypopituitary | n | 6 | 5 | 5 | 5 | 5 | 5 |
| Mean | | +.17[a] | +1.43 | +3.40[A] | +495[A] | -38.6[a] | +0.97[A] |
| SE | | .17 | .98 | .18 | 77 | 7.3 | .43 |
| II. 23 - 29 year old men | n | 4 | 4 | 4 | 4 | 4 | 4 |
| Mean | | -.28[b] | -0.38 | +0.78[B] | + 83[B] | -20.8[ac] | +0.06[B] |
| SE | | .14 | 1.21 | .28 | 19 | 7.2 | .02 |
| IV. 61 - 69 year old subjects | n | 6 | 5 | 5 | 5 | 5 | 5 |
| Mean | | +.33[a] | +0.49 | +1.06[B] | + 89[B] | -24.5[bc] | +0.19[B] |
| SE | | .28 | .49 | .22 | 18 | 1.8 | .07 |

AB - Means in column with different capital letter superscripts are statistically different ($P < 0.01$)

abc - Means in column with different small leter superscripts are statistically different ($P < 0.05$)

[1] The level of administered hormone was 0.168 U hGH/kg $BW^{.75}$/day.

TABLE V

Comparison of urinary excretion of comparators during control and hGH administration periods

| Group | n | Creatinine mg/kg BW/day | Calcium mg/kg BW/day | Hydroxyproline ug/kg BW/day | Nitrogen mg/kg BW/day | Hydroxyproline: Creatinine Ratio ug:mg/kg BW/day |
|---|---|---|---|---|---|---|
| I. Hypopituitary | 5 | | | | | |
| Control Period | | 16.3 ± 1.7 | 2.91 ± .46 | 1333 ± 280 | 137 ± 12 | 3.58 ± 1.5 |
| hGH Period | | 17.7 ± 2.0 | 6.31 ± .83 | 1828 ± 289 | 99 ± 15* | 4.55 ± 1.9 |
| II. 23 - 29 year old men | 4 | | | | | |
| Control Period | | 26.9 ± 1.6 | 3.20 ± .20 | 403 ± 26 | 133 ± 13 | .23 ± .02 |
| hGH Period | | 26.5 ± 1.5 | 3.90 ± .39 | 486 ± 30 | 113 ± 11 | .29 ± .04 |
| IV. 61 - 69 year old subjects | 5 | | | | | |
| Control Period | | 20.3 ± 1.8 | 2.91 ± .39 | 440 ± 47 | 119 ± 8 | .22 ± .06 |
| hGH Period | | 20.8 ± 2.0 | 3.97 ± .41** | 529 ± 102 | 96 ± 9* | .41 ± .07 |

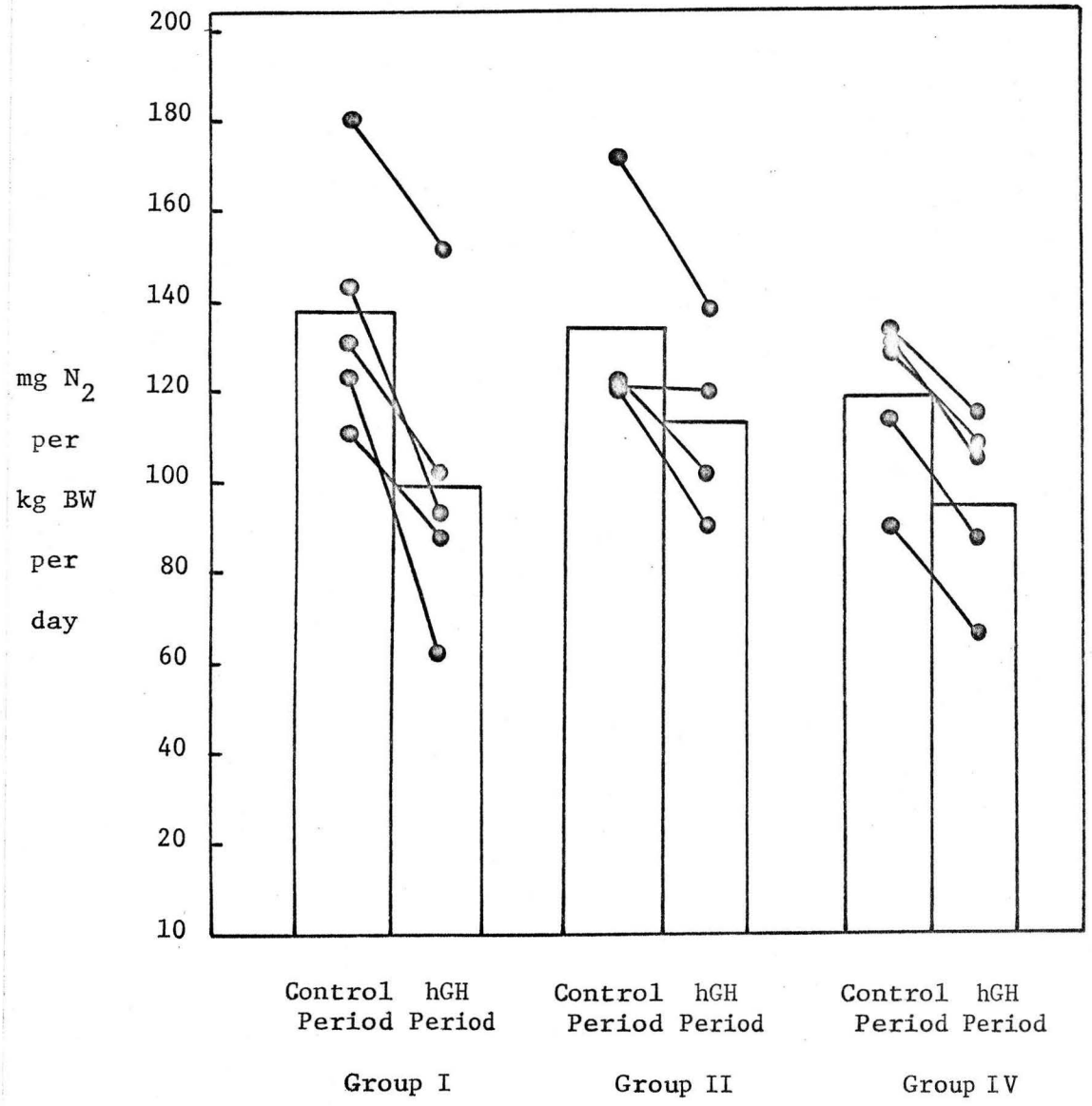
* - hGH period statistically different from control period ($P < 0.05$).

** - hGH period statistically different from control period ($P < 0.01$).

statistically different from the control period for any of the experimental groups.

Hydroxyproline: creatinine ratio. The increased hydroxyproline: creatinine ratio was statistically greater ($P < 0.01$) for group I, 0.97 ug:mg, than that of group II, 0.06 ug:mg/ kg BW/ day, or group IV, .19 ug:mg. The hydroxyproline: creatinine ratio of the hGH period did not increase significantly over the control period for any of the groups.

Nitrogen. A decrease in urinary nitrogen excretion which represents nitrogen retention was observed in all subjects (Appendix E). The greatest decrease in nitrogen excretion was observed in the hypopituitary subjects which was statistically different ($P < 0.05$) from the older subjects. In spite of a smaller mean decrease in nitrogen excretion in the younger men than the subjects in group IV, the difference between group I and II was not statistically different. This discrepancy was apparently related to the wider variability in nitrogen excretion in group II. There was no statistical change between nitrogen excretion of the control period and the hGH administration period for the young men; however, the change was statistically different for the hypopituitary subjects and for the older subjects ($P < 0.05$). The change in urinary nitrogen excretion as a result of hGH is presented in Figure 2.




Bars represent group means.

Figure 2

Individual and Mean Urinary Nitrogen Excretion
Before and After hGH Administration

# CHAPTER V

## DISCUSSION

Our study represents an attempt to answer several basic questions concerning hGH metabolism in relation to the aging process:

(1) Does pituitary response to provocative stimuli known to elicit hGH release decline with advancing age?

(2) Are nocturnal peaks associated with the advent of sleep, diminished as a result of advancing age?

(3) Do 24 hour integrated hGH concentrations decrease with advancing age?

(4) If pituitary hGH release decreases with age such that the availability of metabolically active hGH becomes limited, do the effects of hGH administration in older individuals approach those observed in hypopituitary subjects with hGH deficiency?

Before addressing any possible answers to these questions, such comments must be prefaced by acknowledging some limitations of our study with regard to the small number of observations and the wide variability in some data within experimental groups. We attempted to offset these restrictions by selecting subjects without evidence of cardiovascular, liver or kidney disease who were in a good state of health and who were close to their desired body weight.

The results of our provocative tests suggest that not only do circulating levels of hGH decline with advancing age, but that the various mechanisms of release associated with these tests decline at varying rates with respect to age. On the basis of our results, it would appear that the mechanism of insulin-stimulated hGH release remains intact to a

greater degree than that of exercise of L-DOPA stimulation (Table 3). The failure of nerve cells to replace themselves may provide a partial explanation for the decline in hGH response to L-DOPA. The decline in exercise stimulation of hGH release may result from decreased activity since Sidney et al. (86) achieved significant responses to exercise stimulated hGH release in both elderly men and women, following a seven week training program, who failed to release hGH in response to the stress of exercise prior to their conditioning. If habitual physical activity is essential for the optimum maintenance of the mechanism for exercise-stimulated hGH release, then we can account for the lack of response to exercise in some subjects. The individuals in our study who did respond to this provocative stimulus could best be distinguished from the other subjects by the presence of a fairly regular activity program as part of their life style. The tendency toward increasing adiposity with age may also account for the failure of some of our subjects to respond to the exercise stimulus since a person could be over-fat and still be within 10% of their desired body weight.

As a result of examining hGH responses to a battery of provocative tests, we have been able to present possible explanations for the nature of conflicting reports by others that hGH metabolism either does or does not decline with age. The examination of elderly individuals responses to only a single provocative test of hGH stimulation was the only means of evaluation in most of these studies. Conclusions drawn in previous experiments with regard to this question were dependent upon responses to specific stimuli.

Sidney et al. (86) showed an absence of any significant response to

exercise in their elderly subjects prior to a graduated conditioning program. Dudl et al. (79) showed a mean maxumum peak to arginine stimulation of 4 ng hGH/ ml in 8 elderly individuals 60 - 80 years of age in comparison to a mean maximum peak response of 12 ng hGH/ ml in 7 individuals 20 - 40 years of age. Blichert-Toft (74) observed a mean peak response of 13.7 ng hGH/ ml in 43 individuals 17 - 57 years old and 13.4 ng hGH/ ml in 77 individuals 68 - 90 years of age in response to arginine stimulation. Buckler (84) observed a failure of only 3 of 18 young men under age 25 to significantly respond to the oral ingestion of Bovril with an increase in hGH concentration to at least 10 ng/ ml or greater whereas 18 of 19 men over age 30 failed to give a significant response to Bovril stimulation. Cartlidge et al. (80) showed 8 elderly individuals 80 - 92 years of age suffering from various pathological conditions such as osteoarthritis, gangrene of the toe and urinary incontinence had peak responses to insulin stimulation ranging from 27 to greater than 50 ng/ ml. Kalk et al. (81) found the mean peak response of 26 $\pm$ 4 ng/ ml to insulin stimulation in 8 elderly males 63 - 99 years old was similar to a mean peak response of 37 $\pm$ 6 ng/ ml in 18 males whose mean age was 32.7 years. The responses of our subjects to insulin parallel those of Kalk et al. (81) in that in spite of the absence of statistical differences between young and old subjects, the mean responses of the elderly were much lower than those of the younger subjects. Although adipose tissue mass was not estimated in either study, Kalk et al. (81) observed that when their elderly subjects were divided into obese and non-obese groups on the basis of ideal body weight, the 14 obese subjects had a statistically lower ($P<.025$) fasting

hGH than the 16 non-obese subjects. There was no statistical difference in the rise in hGH between the obese and non-obese subjects; however, three of the four elderly subjects who failed to respond to insulin stimulation were obese. Obesity was defined as greater than 12% over the ideal body weight for age, height and sex.

Sleep associated peaks of hGH appeared to decline with advancing age. In spite of the lack of more statistically convincing data, not only was the magnitude of mean peak hGH levels greater in the young men, but the duration of the rise and fall of nycthemeral hGH peaks was greater in all of the young men than the duration observed for any subjects of the two older aged groups. Martin (47) suggests that advanced age may diminish SWS-induced elevations of hGH presumably as a result of decreased or absent REM (rapid eye movement) periods of sleep. Furthermore, Martin reports that an elevation in free fatty acids and obesity are among several factors that decrease SWS-induced hGH release (47). Thus, one begins to speculate whether or not the increase in body fat characteristic of the aging process, leads to a change in the neuro-humoral mechanism of SWS-induced stimulation of hGH release such that the pituitary becomes less responsive to this stimulus. Again the question of sufficient vigorous activity becomes pertinent: Is the accumulation of fat with advancing age a result of the lack of an appropriate level of physical activity? One would also like to consider whether or not the mechanisms of SWS-induced and exercise-induced hGH release operate by the same pathway? If both mechanisms should operate by a common pathway, could exercise enhance SWS-induced hGH release by acting as a priming agent?

Other studies have reported a decline in the presence of nycthemeral peaks of hGH in older individuals. Finkelstein et al. (49) failed to observe any nocturnal peaks in four of five 24 hour trials in 3 adults over age 50. Carlson et al. (50) reported four of six normal individuals about 50 years old failed to show a hGH peak during sleep. Blicert-Toft (74) observed a mean maximum nocturnal hGH peak of 11.1 ng/ ml in 12 subjects 20 - 41 years of age and 6.4 ng/ ml for 5 subjects 71 - 90 years old.

HGH secretory episodes during daytime also appear to diminish with age. Blichert-Toft (74) found the daytime peak hGH concentration in the 21 - 40 year old group to range from 2.2 to 30.7 ng/ ml in comparison to a range of 1.6 to 10.4 ng/ ml for the 71 - 90 year old group for their peak hGH concentration. Daytime hGH peaks ranged from less than 2 ng/ ml to about 15 ng/ ml in our young men. Daytime peaks exceeded 5 ng/ ml in the fourth young man and in 8 of the 9 older individuals could be related to a degree of body fatness. Not only should the questions concerning exercise and body composition need to be considered in relation to the decreased hGH levels during daytime periods, but the modifying role of diet should also be examined in this regard. Erickson et al. (85) observed slightly higher daytime hGH responses to a standard meal in 13 year old boys, 11.4 ng/ ml, than they observed in 23 year old men, 8.7 ng/ ml.

The 24 hour integrated hGH concentrations declined with advancing age and approached those concentrations observed in the hypopituitary subjects. The mean 24 hour integrated hGH concentration was greater in the hypopituitary during the hGH administration period than any of the

other groups in spite of the administration of the hormone on a per unit body mass basis. There was, however, no statistical difference among the four groups with regard to their integrated concentrations during GH administration. These observations, coupled with the fact that the young men had a statistically greater ($P < 0.05$) mean 24 hour integrated concentration than either of the older groups or the hypopituitary subjects, suggests that the metabolic utilization of hGH may be decreased in the hypopituitary subjects as well as the older normal individuals. The 24 hour integrated hGH concentrations during the hGH administration period were 150% greater or more than the concentrations observed for the control period for all subjects in groups I, III or IV. The 24 hour ICGH declined or remained the same in three of the four young men suggesting more rapid mobilization, utilization and breakdown of the injected hormone in this group than in the other groups. The increased 24 hour ICGH in some individuals following hGH administration may also be related to some alteration in the feedback mechanism of hGH release, however, one would need to account for the difference between the older individuals whose pituitary content is comparable to that of younger individuals (5) and the hypopituitary subjects who presumably have few hGH pituitary secreting cells.

Our results may be compared to mean 24 hour integrated hGH concentrations of $5.6 \pm 3.6$ ng/ ml in 17 males 7 - 16 years of age and $1.8 \pm 1.0$ ng/ ml in 16 males 30 - 50 years of age reported by Thompson et al. (69). One study of a partially hGH deficient subject, SA, suggests the 24 hour integrated concentration of such individuals may be of clinical importance since his integrated concentration was less than 1 ng/ ml in

spite of marginal, but nevertheless adequate hGH response to exercise and L-DOPA stimulation.

Experimental studies by Salans et al. (54) indicate that growth in adults is achieved primarily by increased cell size rather than increased cell number. The hyperplasia of obesity was found to correlate extremely well with the hyperinsulinemia which is also characteristic of the obese condition in other studies by Salans (54). They hypothesized that the hyperinsulinemia may result from a failure of the adipose cell to increase the number of receptor sites for insulin as the cell increases in size. If this hypothesis is true, then the elevated insulin levels and presumably subsequent elevations in blood glucose would both act to inhibit the release mechanisms of GH. Exercise and nutrition may both have their correlates here in that they could operate to promote the utilization of the triglyceride and free fatty acid stores of adipose cells which would prevent adipose hyperplasia. One might consider the increased life expectancy of laboratory rodents following caloric restriction (2,3) as indirect support for such a hypothesis.

Although the analysis of our growth hormone data was limited in terms of the small number of observations and wide variability within some of the groups, we felt that the short-comings of our experiment were corrected to some degree by examining the responses of healthy individuals to several provocative stimuli of hGH release and by examining GH secretion by the constant withdrawal technique from which the 24 hour integrated concentrations were determined. The determination of 24 hour integrated concentrations provides a means of monitoring hormones and

other substances whose concentrations in the blood are subject to rapid changes and wide variability (90). Thus, one is able to obtain a more representative picture of fluctuating metabolic processes without the added trauma of drawing multiple samples. In addition, the true secretory rate of hormones such as hGH can be determined without the danger of using radio-actively-labelled compounds such as $^{125}I$ or $^{131}I$-hGH used in the studies by Taylor et al. (71) and McGillivray et al. (72).

Metabolic responses to hGH administration

The metabolic responses of the older subjects to 0.168 U hGH/ kg $BW^{0.75}$/ day were more like those of the younger men than those of the hypopituitary subjects in terms of quantitative urine concentrations per kg BW during both the control and hGH administration periods and in terms of the mean change observed for these groups. Although these findings were different than expected, the mean responses of the older subjects were consistently intermediate between the hypopituitary subjects and the young men's for urinary creatinine, calcium, hydroxyproline, hydroxyproline: creatinine ratio and nitrogen excretion. The hypopituitary subjects consistently showed the greatest mean change in excretion while the young men consistently showed the least mean change. The older subjects were similar to the hypopituitary subjects in that the urinary excretions of calcium and nitrogen during the hGH period were statistically different from the control period when compared to each other. In contrast, the mean urinary excretions of creatinine, calcium, hydroxyproline and nitrogen during the hGH period were not statistically different from any of the mean excretion rates observed during the control period for the young men.

Because of the short-term nature of this study, we did not expect to observe any statistically different change in urinary creatinine during the hGH administration period for any of the groups in our study. The changes in urinary creatinine were complemented by parallel changes in body weight in each respective group. The greatest mean change in creatinine excretion was observed in the hypopituitary subjects, 1.43 mg/ kg BW/ day which was indicative of more rapid assimilation of muscle tissue as represented by the greatest increase in nitrogen retention in this group. The anabolic effects of hGH produced their greatest response in the hypopituitary subjects for creatinine and the other urinary excretory products examined in this study.

Since creatinine has been used as a means of assessing relative muscle mass, the muscle mass per kg BW would appear to be the least in the hypopituitary subjects and the greatest in the young men. On this basis, the muscle mass of the older individuals per kg BW was more like that of the hypopituitary subjects than the young men. Assuming the amount of body fat to be inversely related to muscle mass as represented by creatinine/ kg BW, then the hypopituitary subjects exhibited the greatest percent body fat per kg BW. The percent body fat per kg BW of the older individuals would then be closer to that of the hypopituitary subjects than the young men in our study. Reports by Prader et al. (38) and Tanner and Whitehouse (39) showed that adipose tissue mass was preserved in children with isolated growth hormone deficiency who subsequently showed a decrease in subcutaneous fat when placed on hGH therapy. Although experimental evidence was not available from our study to support the hypothesis that body fat accumulation with advancing age may be a

factor in diminished hGH secretion of the elderly, these extrapolations support the need to examine the relationships of specific tissue receptors on feedback mechanisms of hormone release. Experimental studies in rats and dogs showed GH administration decreased adipose tissue mass in contrast to the anabolic effects of GH on most other tissues (36,37).

The increased response in urinary calcium excretion following hGH administration was statistically greater in the hypopituitary subjects than either the young men or the older subjects. The mean urinary calcium excretion of the hGH period was, however, statistically greater than the mean urinary calcium excretion of the control period for both the hypopituitary subjects and the older subjects but not the young men. The increased calcium storage associated with GH administration (8) may be important not only in promoting skeletal growth and development in hypopituitary subjects, but suggests the possibility of a therapeutic agent for osteoporosis, especially if a physiologically active fragment of GH can be synthesized. If diet or exercise could be used as a means of maintaining higher hGH concentrations with advancing age, they may represent preventive measures for osteoporosis.

Studies by Prader et al. (38) and Wright et al. (26) observed increased urinary calcium excretion in hypopituitary subjects (26,38) and in control subjects without hGH deficiency (38). Prader et al. (38) did not find statistically greater calcium excretion in either group of their 24 subjects who received 2 mg Raben hGH/ $m^2$/ day for a 5 day period which proceeded a 5 day control period.

Several reports indicate that urinary hydroxyproline excretion provides a good means of assessing growth since hydroxyproline excretion is

the highest during childhood and declines with age (101 - 103). Hydroxyproline excretion also represents a means of assessing the aging process since hydroxyproline is found in significant amounts only in collagen and because increases in the collagen content of animal and human subjects have been found to occur with advancing age (66,101,102,104). Although collagen is metabolically active tissue, the rate of conversion from proline to hydroxyproline declines with age resulting in a decrease in the heat-soluble or labile content of collagen. Urinary hydroxyproline increased in all subjects following hGH administration, however, the mean hydroxyproline excretion of the hGH period was not statistically different from the control period. The increase in hydroxyproline excretion of the hypopituitary subjects following hGH administration was statistically different from the increased excretion of the other two groups. Table VI is presented as a means of comparing our data with that of Jasin et al. (99) whose measurements are expressed in terms of mg/ day and mg/ $m^2$/ day.

Jasin et al. (99) found that 24 hour urinary hydroxyproline excretion rates were not statistically different between normal control children and normal control adults, however, statistical differences were observed between these groups when the 24 hour excretion rates were expressed per $m^2$ body surface area. Daily hydroxyproline excretion ranged from 14 - 54 mg/ day in 12 normal adults whose mean excretion was 32.9 mg/ day, 18 - 43 mg/ day for 7 normal children 0 - 1 year old with a mean of 33 mg/ day, and 15 - 150 mg/ day with a mean of 49 in twenty-one children 1 - 10 years old (99). Mean hydroxyproline excretions were 18, 102 and 66 mg/ $m^2$/ day for the adults, 0 - 1 year old children and 1 - 10

year old children respectively (99). The range varied from 9 - 31, 48 - 130 and 42 - 145 mg/ $m^2$/ day for the same groups (99). All subjects were on low hydroxyproline diets at the time of these determinations.

Jasin et al. (99), August et al. (101), and Teller et al. (102) found hGH administration results in increased urinary hydroxyproline excretion in hypopituitary children. August et al. (101) reported hGH also increased the total heat-soluble or labile hydroxyproline content of skin in 5 of 7 hypopituitary children. Teller et al. (102) were able to distinguish differences in growth retardation in children with dwarfism of endocrine origin from primordial dwarfs of non-endocrine origin on the basis of lower hydroxyproline excretion expressed in mg/ $m^2$/ day.

Changes in the total hydroxyproline: creatinine ratio (THP:CR) paralleled the observations made for hydroxyproline in our subjects. The THP:CR expressed as mg hydroxyproline per g creatinine (Table VII) may be compared to the data of Wharton et al. (103) who showed a range of 20 - 100 THP:CR for 26 boys 11 - 14 years old, and with Williams and Windsor (104) who showed a range of 14 - 65 THP:CR for 35 elderly subjects 60 - 100 years old with a mean THP:CR of 31. Wright et al.(26) reported 5 mg hGH/ day increased the percentage THP:CR 23 - 26% in 15 hypopituitary subjects. The percent increase in the THP:CR following hGH administration in our subjects ranged from less than 5% to about 60%.

Williams and Windsor (104) suggested that the THP:CR may represent a better means of evaluating changes in collagen metabolism in the elderly since the collection of 24 hour urines in older subjects is

TABLE VI

Urinary Hydroxyproline Excretion
(mg/ day)

| Group: Subject | Control Period | GH Period |
|---|---|---|
| I. Hypopituitary | | |
| RP | 22.1 | 38.7 |
| EF | 40.9 | 60.4 |
| MF | 32.0 | 44.8 |
| WC | 103.4 | 120.5 |
| JD | 24.6 | 33.5 |
| II. 23 - 29 year old men | | |
| TB | 26.0 | 35.2 |
| DS | 22.3 | 26.2 |
| JB | 32.7 | 36.8 |
| WH | 27.2 | 31.8 |
| IV. 61 - 69 year old subjects | | |
| HW | 36.7 | 44.7 |
| JW | 24.0 | 28.8 |
| JJ | 32.6 | 20.7 |
| FB | 29.2 | 36.6 |
| WB | 26.7 | 33.2 |

TABLE VII

Hydroxyproline: Creatinine Ratio

| Group: Subject | Control Period | GH Period |
|---|---|---|
| I. Hypopituitary | | |
| RP | 79 | 114 |
| EF | 37 | 47 |
| MF | 55 | 88 |
| WC | 130 | 136 |
| JD | 129 | 157 |
| II. 23 - 29 year old males | | |
| TB | 17 | 24 |
| DS | 12 | 17 |
| JB | 17 | 20 |
| WH | 13 | 14 |
| IV. 61 - 69 year old subjects | | |
| HW | 40 | 47 |
| JW | 17 | 19 |
| JJ | 21 | 24 |
| FB | 20 | 24 |
| WB | 17 | 20 |

often difficult and because creatinine excretion in the elderly is often half that observed in younger subjects. Wharton et al. (103) report that the THP:CR has been used to measure changes in growth rate since the total hydroxyproline (THP) over a 24 hour period has been found to be related to growth and because the THP:CR in random 24 hour urine collections behaves similarly. The circumstantial evidence relating THP:CR to THP is based on the following observations: THP decreases throughout childhood to maturity with a small temporary rise during adolescence; hGH administration to hypopituitary dwarfs significantly increases THP; and because changes in height velocities in boys are paralleled by comparable changes in THP (103). THP:CR determinations in 24 hour urines were observed to compensate for differences in body size in normal subjects of the same age thus reducing the range of variability. Wharton et al. (103) showed acceleration and deceleration of height and weight velocities correlated very well with corresponding changes in THP:CR data of young boys over a 6 - 12 month period.

The hypopituitary subjects showed the greatest mean decrease in urinary nitrogen excretion which was statistically different from the decrease in nitrogen retention of the older individuals but not the young men. The absence of a statistical difference between the hypopituitary subjects and the young men was not expected since the young men's mean response was of less magnitude than the older subjects. These discrepancies are probably related to the wide variability of response and few number of observations within each group. The mean daily urinary nitrogen excretion of the hypopituitary subjects and the

older subjects during the hGH administration period was statistically decreased from the mean values observed during the control period for each of these groups respectively. There was no statistical difference between the mean daily nitrogen excretion of the hGH period and control period for the young men.

The metabolic response of urinary nitrogen excretion to hGH administration has been reported by Prader et al. (105), Wright et al. (26) and Zafar et al. (106). Prader et al. (105) reported nitrogen retention increased 70 - 200 mg nitrogen per day in hypopituitary subjects and 20 - 60 mg nitrogen per day in control subjects receiving 2 mg hGH (Raben) per day which represented increased nitrogen retention of 25 - 52% and 12 - 38% in the two groups respectively. The range in nitrogen excretion decreased from 3 - 50 mg/ kg BW/ day to 1 - 38 mg/ kg BW/ day in 5 hypopituitary subjects receiving 2 U hGH/day three times a week (106). The change in nitrogen excretion following hGH administration ranged from 600 to 3200 mg/ day in 15 hypopituitary subjects receiving 5 mg hGH/ day reported by Wright et al. (26). Rudman et al. (92) reported a mean decrease of 800 mg nitrogen per kg $BW^{0.75}$ $day^{-1}$ for normal children, 1500 mg nitrogen per kg $BW^{0.75}day^{-1}$ for hypopituitary children and 300 mg nitrogen per kg $BW^{0.75}day^{-1}$ in normal adults.

These results may be compared to a range in decreased nitrogen excretion of 400 - 2200 mg/ day in the hypopituitary subjects, 80 - 2000 mg/ day in the young men and 1100 - 1800 mg/ day in the older subjects in our study. Our results differ from those of Prader et al. (105) and Rudman et al. (92) in that our normal subjects exhibited greater decreases in urinary nitrogen excretion than normal control subjects in

their studies. Our hypopituitary subjects appeared to be more responsive to hGH administration than those in the study by Prader et al. (105); however, not all of our hypopituitary subjects appeared to be as responsive to hGH therapy as the hypopituitary subjects in the studies by Wright et al. (26) and Rudman et al. (92). Variations between these studies are probably related to a number of factors such as differences in the amount and source of hGH administered, dietary nitrogen and individual variation.

We have been unable to explain the wide variation in nitrogen retention within the young men in group II; however, several factors may account for the variation in the hypopituitary subjects. The effects of prior diet may account for the lack of a greater decrease in nitrogen excretion in the hypopituitary subjects, RP and JD, whose diet histories (Appendix B) indicated that their protein intake was marginal or inadequate. Both subjects retained fifty percent of the dietary nitrogen consumed during the control period in comparison to a 5 - 30% retention in both the other hypopituitary subjects and the control subjects. It is unlikely that a hypopituitary subject with failure to grow would retain 50% of the dietary nitrogen consumed unless as indicated in these subjects their previous diet was inadequate or marginal in meeting their protein needs. If the diets of these subjects were marginal in meeting protein requirements, physicians should evaluate diet histories of hypopituitary subjects in order to recommend changes that would help assure optimum responses to hGH therapy. Zachmann et al. (107) in evaluating their observations of the effects of hGH administration in both hypopituitary and normal subjects stated that although exogenous hGH

exhibited a more marked metabolic effect in GH deficient subjects than in normal subjects, there was too much overlap between the two groups to be diagnostically useful in an individual case. On the basis of a limited number of observations, these preliminary data would support such an observation.

## CHAPTER VI

## SUMMARY AND CONCLUSIONS

The purpose of these studies was to examine the question of whether or not hGH metabolism declines with advancing age. Responses of 22 subjects to the hGH provocative tests of exercise (87), L-DOPA (88) and insulin (89), peaks in hGH concentrations during sleep and, 24 hour integrated concentrations (90) during a control period and following the administration of 0.168 U hGH/ kg $BW^{0.75}$/ day were examined. Urinary excretion of creatinine, calcium, hydroxyproline and nitrogen before and after hGH administration were also examined.

Subjects studied were divided into the following groups: (I) 6 hypopituitary subjects 9 - 29 years old; (II) 4 men 23 - 29 years old; (III) 6 men 35 - 51 years old and (IV) 6 subjects 61 - 69 years old. The metabolic balance studies consisted of a 3 day adjustment period and a 5 day control period followed by a 5 day hGH administration period during which the subjects were fed a constant low, hydroxyproline diet.

HGH levels were determined by the double antibody radioimmunoassay procedure of Schalch and Parker (94). Urinary creatinine (95), nitrogen (96) and hydroxyproline (97) were determined by Technicon AutoAnalyzer II procedures. Urinary calcium concentrations were determined spectrophotometrically (98). Statistical analyses were determined by the unpaired student t test.

The young men in the study consistently had the highest mean response to the provocative stimulation tests for hGH release. Their mean values were 22 ng/ ml following exercise stimulation, 23 ng/ ml following

oral ingestion of 250 - 500 mg L-DOPA and 30 ng/ ml post 0.1 U insulin/ kg BW intravenously. The greatest mean peak hGH level 21 ng/ ml, associated with sleep was observed in the young men. The young men's 24 hour I CGH, 3.5 ng/ ml, was the highest concentration observed for any of the groups determined during the control period. The effects of hGH on I CGH were of the least magnitude in the young men who had a mean I CGH of 3.7 ng/ ml during this period.

In contrast to the young men, the hypopituitary subjects exhibited the least response to the hGH provocative tests: less than 2 ng hGH/ ml for all subjects in response to all tests. The 24 hour I CGH was less than 2 ng/ ml for all of the hypopituitary subjects and for all of the subjects in the two older groups. These subjects responded with significant elevations in their 24 hour I CGH to hGH administration. Mean values were 5.2 ng/ ml for the hypopituitary subjects and 3.7 ng/ ml for the 61 - 69 year old subjects. An I CGH of 4.3 ng/ ml was reported for FM, a 48 year old male, during the hGH period. Mean hGH release to the exercise and insulin provocative tests was higher in the middle-aged men than the elderly subjects. Mean hGH concentrations were 4 and 29 ng/ ml in response to exercise and insulin stimulation in the 35 - 51 year old men and 3 and 15 ng/ml for the 61 - 69 year old subjects in response to the same tests respectively. The elderly subjects showed a statistically higher response of 11 ng/ ml than the middle-aged men's mean response of 4.9 ng/ ml to L-DOPA stimulation.

In the young men the mean daily urinary excretion during hGH administration of creatinine, calcium, hydroxyproline and nitrogen were not statistically different from the control period excretion rates. Calcium

excretion increased significantly from 2.91 to 6.31 mg/ kg BW/ day in the hypopituitary subjects and from 2.91 to 3.97 mg/ kg BW/ day for the elderly subjects following hGH administration. Nitrogen excretion decreased significantly from 137 to 99 mg/ kg BW/ day in the hypopituitary subjects and from 119 to 96 mg/ kg BW/ day in the elderly subjects following hGH administration. Increased creatinine excretion and hydroxyproline excretion following hGH administration were not significantly different from the control period for either group I or IV.

The mean change in creatinine excretion was not statistically different among the three groups. The mean change in calcium, +3.40 mg/ kg BW/day, hydroxyproline, +495 ug/ kg BW/ day, and nitrogen -38.6 mg/ kg BW/ day in the hypopituitary subjects was statistically different from the mean change in calcium, +1.05 mg/ kg BW/ day, hydroxyproline, + 89 ug/ kg BW/ day,and nitrogen, -24.5 mg/ kg BW/ day, for the elderly and for calcium, +0.78 mg/ kg BW/ day and hydroxyproline, + 83 ug/ kg BW/ day, for the young men. The mean change in nitrogen excretion, -20.8 mg/ kg BW/ day, in the young men was not statistically different from either the hypopituitary subjects or the elderly subjects.

Although the magnitude of response to hGH administration with regard to changes in urinary excretion of creatinine, calcium, hydroxyproline, and nitrogen of the older individuals was not statistically different from that of the young men, the mean daily excretion of both calcium and nitrogen during the hGH period were statistically different from the control period for the older subjects but not for the young men. These differences may suggest a decline in hGH

metabolism with advancing age as a result of decreased circulating hGH, decreased response to hGH stimuli, fewer receptor sites at the tissue level for active hormone, or because of decreased hGH dependent-stimulation of somatomedin synthesis. Radioreceptor assays of hGH and 24 hour integrated concentrations of somatomedin need to be determined to further evaluate whether or not there is a physiological decline in hGH metabolism as a result of aging. The statistically different rates of excretion of calcium and nitrogen during the hGH administration period for the elderly in association with a statistical decrease in the circulating concentration of hGH in these subjects may indicate the development of a type of functional hypopituitarism as a consequence of aging.

In conclusion, on the basis of these data, hGH metabolism declines with advancing age. The release of hGH to provocative stimuli such as exercise, L-DOPA and insulin becomes diminished in older individuals. Peak elevations of hGH concentrations are small or absent in the elderly. Twenty-four-hour integrated concentrations decrease and approach those levels determined for hypopituitary subjects. In response to hGH administration, the changes in urinary excretion of creatinine, calcium, hydroxyproline and nitrogen in older, normal individuals is intermediate between the responses of younger, normal subjects and hypopituitary subjects. HGH administration is effective in significantly increasing urinary calcium excretion and in significantly promoting increased nitrogen retention in both hypopituitary and older subjects. On the basis of these observations, we suggest further investigations

be conducted to determine if the decline in hGH metabolism with age is etiologically related to physiological declines associated with other systems.

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## Appendix A

### Protocol: Growth Hormone Changes with Age[1]

Four treatment groups of male subjects will be admitted for the study of hormonal changes with advancing age. The subjects will be placed into the following treatment groups:

I. 4 males with hypopituitarism.

II. 4 males 25 ± 5 years of age who weigh ± 10% of their ideal weight for their height and age.

III. 4 males 50 ± 10 years of age who weigh ± 10% of their ideal weight for their height and age.

IV. 4 males 65 ± 5 years of age who weigh ± 10% of their ideal weight for their height and age.

Tables adapted from Metropolitan Life Insurance Company, Statistical Bulletin 40: 3(Nov. - Dec.) 1959 will be used to determine the percent of ideal weight of each individual.

Prior to admission each subject will be instructed by the Clinical Research Center dietitian to keep a 3 day diary of all food intake. The diaries will be used (1) to estimate the nutrient intake of the individuals in each group; (2) to evaluate food preferences and thus provide the research dietitian with information to be utilized in formulating appetizing meals. The dietary information will be analyzed by a computer program (FOODS) at Ohio State University which is available through our computer center. Two nutritional evaluation forms from NIH will also be

[1] This protocol was presented for approval by the human experimentation committee and clinical research committee of the University of Virginia Hospital, Charlottesville, Virginia.

used.

Eight to ten days prior to admission, the research dietitian will present to be followed a seven-day constant low hydroxyproline diet to each subject. During the 13 day study period, the patients will be fed a constant isocaloric, low-hydroxyproline diet with the protein and caloric levels based on the current RDA specifications. Food fed to all patients from the same shipment lots.

Standing orders:

1. Up ad lib except as specifically noted in protocol.
2. Weigh daily in shorts and hospital gown before breakfast and after voiding. Nurses are to instruct the subjects to urinate before each bowel movement.
3. Unless otherwise specified, meals are at 0800, 1200 and 1700.
4. Subjects will be asked to be in bed by 1200 p.m. and nurses will be instructed to monitor sleeping periods.
5. Begin iron supplementation according to physician's orders.

Day 0:

1. Admit in a.m.
2. History, physical examination including height and weight.
3. Subjects to be placed on a low hydroxyproline (collagen free) constant diet during the entire experimental period.
4. Routine CRC differential, urinalysis and SMA 6 and 12.
5. EKG, Chest x-ray.

Day 1: Begin 24 hour urine collection for determination of 24 hour urinary gonadotropins, nitrogen, calcium, creatinine and hydroxyproline excretion. The bladder is to be emptied at 0800. 5ml of glacial acetic acid will be added to each urine collection. The distilled water bedpan technique is not to be used and the preservative added at the beginning of the collection period. Collections will be measured for total urine volume and 200 ml aliquots stored frozen until analyzed.

2. L-DOPA - insulin test administered 0800.
3. Withhold breakfast until after the completion of the L-DOPA - insulin test.

Day 2: Restart 24 hour urine collection at 0800.

2. Administer exercise stimulation test at 0800.
3. Withhold breakfast until after the completion of the exercise test.

Day 3: End 3 day adjustment period; first day control period

Restart 24 hour urine collection 0800.

Day 4: Second day control period -

Restart 24 hour urine collection 0800.

Day 5: Third day control period -

Restart 24 hour urine collection 0800.

Day 6: Fourth day control period -

Restart 24 hour urine collection 0800

Day 7: Fifth day control period -

1. 0800: Begin 24 hour integrated constant withdrawal for hGH, prolactin, testosterone, gonadotropins and somatomedin.

2. Restart 24 hour urine collection 0800.

Day 8: First day hGH administration period

1. Restart 24 hour urine collection 0800.
2. Inject hGH (0.168 U hGH/ kg $BW^{0.75}$/ day) 0800.

Day 9: Second day hGH administration period

1. Restart 24 hour urine collection 0800.
2. Inject hGH 0800.

Day 10: Third day hGH administration period

1. Restart 24 hour urine collection 0800.
2. Inject hGH 0800.

Day 11: Fourth day hGH administration

1. Restart 24 hour urine collection 0800.
2. Inject hGH 0800.

Day 12: Fifth day hGH administration period

1. Restart 24 hour urine collection 0800.
2. Inject hGH 0800.
3. 0800:Begin 24 hour integrated constant withdrawal for hGH prolactin, testosterone, gonadotropins and somatomedin.

Appendix B

Diet History Evaluation

| RDA (1974): | Energy (kcal) | Protein (g) | Calcium (mg) | Iron (mg) | Vitamin A (IU) | Vitamin C (mg) |
|---|---|---|---|---|---|---|
| Children: | | | | | | |
| 1-3 years old | 1300 | 23 | 800 | 15 | 2000 | 40 |
| 4-6 years old | 1800 | 30 | 800 | 10 | 2500 | 40 |
| 7-10 years old | 2400 | 36 | 800 | 10 | 2800 | 40 |
| Males: | | | | | | |
| 23-50 years old | 2700 | 56 | 800 | 10 | 5000 | 45 |
| 51+ years old | 2400 | 56 | 800 | 10 | 5000 | 45 |
| Group: Subject (age) | | | | | | |
| I. Hypopituitary (CA/BA) | | | | | | |
| RP (15/ 7) | 1286- | 36 | 663- | 9- | 5036 | 24- |
| AJ (13/ 6) | 1389- | 50 | 578- | 8- | 7085 | 97 |
| MF (18/11) | 2663 | 100 | 1199 | 16 | 3236 | 38 |
| WC (11/10) | 3689 | 112 | 2512 | 18 | 5156 | 101 |
| EF (29) | 1979- | 54- | 462- | 12 | 2349- | 200 |
| JD ( 9/ 8) | 1801- | 43 | 564- | 11 | 2758 | 97 |
| II. 23-29 year old males | | | | | | |
| TB (25) | 1831- | 66 | 724 | 9- | 3874- | 171 |
| DS (29) | 2580- | 104 | 890 | 15 | 2245- | 149 |
| JB (23) | 2478- | 115 | 772 | 19 | 7460 | 103 |
| WH (25) | 2372- | 73 | 571- | 12 | 2115- | 70 |
| IV. 61-69 year old subjects | | | | | | |
| HW (61) | 936- | 57 | 149- | 10 | 1367- | 5- |
| FB (66) | 1932- | 65 | 568- | 13 | 3071- | 27- |
| WB (61) | 2130- | 85 | 657- | 13 | 5406 | 18- |
| JW (66) | 1653- | 76 | 351- | 23 | 1569- | 10- |
| JJ (69) | 2528 | 72 | 772- | 12 | 3725- | 118 |
| GA (67) | 1473- | 48- | 182- | 9- | 2776- | 92 |

(- indicates daily average of 3 day diet diary was inadequate)

## Appendix C

## Sample Diet Pattern

DS: 29 year old male
53.1 g protein
2282 calories

| | Breakfast | | Lunch | | Dinner | |
|---|---|---|---|---|---|---|
| **Day I:** | | | | | | |
| | orange juice | 150 ml | vegetable soup | 240 ml | grapefruit j. | 150 ml |
| | scrambled egg | 50 g | grilled cheese | | filet mignon | 60 g |
| | toast | 50 g | cheese | 1 oz | b. potato | 150 g |
| | jelly | 10 g | bread | 50 g | g. beans | 100 g |
| | margarine | 5 g | margarine | 5 g | mushrooms | 10 g |
| | | | pear | 100 g | lettuce | 50 g |
| | | | lettuce | 20 g | T.I. dressing | 15 g |
| | | | cottage cheese | 16 g | rolls | 120 g |
| | | | lemon bar | 50 g | spice cake w/ caramel icg. | 60 g |
| | 100 g sugar or equivalent (10 pieces hard candy) | | | | | |
| | 2 g sodium | | | | | |
| **Day II:** | | | | | | |
| | corn flakes | 25 g | hamburger | 60 g | tomato j. | 100 ml |
| | orange juice | 150 ml | bun | 50 g | cheese souffle | 100 g |
| | toast | 50 g | french fries | 100 g | broccoli | 100 g |
| | margarine | 5 g | coleslaw | 100 g | carrots | 100 g |
| | jelly | 10 g | tart dressing | 10 g | lettuce | 100 g |
| | milk | 40 ml | fruit cup | 100 g | T.I. dressing | 10 g |
| | | | | | rolls | 120 g |
| | | | | | pound cake | 30 g |
| | 100 g sugar or equivalent (10 pieces hard candy) | | | | | |
| | 2 g sodium | | | | | |

Appendix D

Individual hGH response to provocative testing; sleep peaks; and 24 hour integrated concentrations (ICGH): ng hGH/ ml

| Group | Exercise Peak | L-DOPA Peak | Insulin Peak | Sleep Peak | Control Period ICGH | hGH Period ICGH |
|---|---|---|---|---|---|---|
| I. Hypopituitary | | | | | | |
| RP | < 2 | < 2 | < 2 | | < 2 | 4.6 |
| AJ | < 2 | < 2 | < 2 | | < 2 | 5.8 |
| EF | < 2 | < 2 | < 2 | | < 2 | 7.1 |
| MF | < 2 | < 2 | < 2 | | < 2 | 3.4 |
| WC | < 2 | < 2 | < 2 | | < 2 | |
| JD | < 2 | < 2 | < 2 | | < 2 | |
| II. 23-29 year old males | | | | | | |
| TB | 40 | 40 | 18 | 11 | < 2 | < 2 |
| DS | 40 | 23 | 29 | 36 | 3.7 | 6.3 |
| JB | 4.4 | 18 | 17 | 27 | 5.2 | 3.2 |
| WH | < 2 | 9.3 | 54 | 7.5 | 3.2 | 4.0 |
| III. 35-51 year old males | | | | | | |
| FM | 3.1 | < 2 | 40 | < 2 | < 2 | 4.3 |
| RB | 5.2 | 5.9 | 17 | 10.5 | < 2 | |
| MA | 3.7 | 6.9 | | < 2 | < 2 | |
| RD | | | | | | |
| JH | | | | | | |
| MV | | | | | | |
| IV. 61-69 year old subjects | | | | | | |
| HW | < 2 | < 2 | 10 | < 2 | < 2 | 3.2 |
| FB | 2.2 | 18 | 9.6 | 4.6 | < 2 | |
| WB | < 2 | 8.5 | 11 | < 2 | < 2 | |
| JW | < 2 | 3.1 | 42 | 8.2 | < 2 | 4.7 |
| JJ | 7.7 | 9.7 | 8.4 | < 2 | < 2 | 3.2 |
| GA | < 2 | 8.3 | 10 | < 2 | < 2 | 3.8 |

Appendix E

Individual Urinary Excretion

| | Creatinine (mg/kg BW/day) | | Calcium (mg/kg BW/day) | | Hydroxyproline (ug/kg BW/day) | | Nitrogen (mg/kg BW/day) | |
|---|---|---|---|---|---|---|---|---|
| | Control | hGH | Control | hGH | Control | hGH | Control | hGH |
| I. Hypopituitary | | | | | | | | |
| RP | 11.9 | 14.6 | 1.72 | 4.76 | 948 | 1660 | 110 | 87 |
| EF | 21.8 | 24.9 | 3.02 | 6.40 | 795 | 1175 | 131 | 102 |
| MF | 16.9 | 14.6 | 4.47 | 9.48 | 921 | 1290 | 123 | 61 |
| WC | 16.8 | 18.7 | 2.99 | 5.40 | 2191 | 2553 | 143 | 94 |
| JD | 14.0 | 15.7 | 2.35 | 5.52 | 1809 | 2463 | 180 | 151 |
| MEAN | 16.3 | 17.7 | 2.91 | 6.31 | 1333 | 1828 | 137 | 99 |
| II. 23-29 year old males | | | | | | | | |
| TB | 22.9 | 22.4 | 3.42 | 4.99 | 396 | 535 | 120 | 90 |
| DS | 30.7 | 27.7 | 3.41 | 3.41 | 397 | 467 | 171 | 138 |
| JB | 27.0 | 26.3 | 3.34 | 3.91 | 473 | 533 | 119 | 120 |
| WH | 26.9 | 29.7 | 2.61 | 3.29 | 349 | 409 | 122 | 102 |
| MEAN | 26.9 | 26.5 | 3.20 | 3.90 | 404 | 486 | 133 | 113 |
| FM, 48 year old male | 25.4 | 25.8 | 4.76 | 5.76 | 414 | 578 | 116 | 84 |
| IV. 61-69 year old subjects | | | | | | | | |
| FB | 26.2 | 27.3 | 2.08 | 2.82 | 524 | 657 | 133 | 115 |
| WB | 21.4 | 21.7 | 4.13 | 4.58 | 356 | 442 | 90 | 66 |
| JW | 19.1 | 20.9 | 2.72 | 3.82 | 326 | 392 | 128 | 108 |
| JJ | 20.6 | 19.5 | 2.21 | 3.47 | 426 | 462 | 113 | 87 |
| HW | 14.3 | 14.7 | 3.44 | 5.14 | 567 | 692 | 131 | 104 |
| MEAN | 21.2 | 21.7 | 3.22 | 4.27 | 436 | 537 | 119 | 94 |

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GROWTH HORMONE METABOLISM IN HYPOPITUITARY SUBJECTS AND YOUNG, MIDDLE-AGED AND ELDERLY NORMAL SUBJECTS

by

Terry Lee Bazzarre

(ABSTRACT)

The purpose of these studies was to determine if human growth hormone (hGH) metabolism declines with advancing age. Twenty-two subjects' responses to the hGH provocative tests of exercise, L-DOPA, and insulin, hGH peak concentrations during sleep, and 24-hour integrated concentrations (ICGH) during a control period and following intramuscular administration of 0.168 U hGH/ kg $BW^{3/4}$/day were examined. Metabolic responses of 17 of these subjects were examined before and after hGH by measuring urinary excretion of creatinine, calcium, hydroxyproline and nitrogen.

Subjects were divided into the following groups: (I) 6 hypopituitary subjects 9 - 29 years old; (II) 4 men 23-29 years old; (III) 6 middle-aged men 35 - 51 years old; and (IV) 6 elderly subjects 61 - 69 years old. Metabolic balance studies consisted of a 3 day adjustment period followed by a 5 day control period and a 5 day hGH administration period during which subjects were fed a constant, low hydroxyproline diet.

No hypopituitary subject exhibited a hGH concentration above 2 ng/ ml at any time except following hGH administration, their mean ICGH increased from less than 2 ng/ ml to 5.2 ng/ ml. Group II had the greatest mean hGH concentrations - 22 ng/ ml following exercise, 23 ng/ ml post L-DOPA, 30 ng/ ml post insulin, 21 ng/ ml during sleep and 3.5 ng/ ml control period 24-hour ICGH - to all tests except the ICGH, 3.7 ng/ ml,

following hGH administration. ICGH increased from less than 2 ng/ ml for all subjects in groups III and IV to 4.3 ng/ ml for one subject in group III and to a mean of 3.7 ng/ ml for group IV. Mean hGH concentrations of 4 and 3 ng/ ml following exercise, 5 and 11 ng/ ml post L-DOPA and 29 and 15 ng/ ml post insulin for groups III and IV respectively were intermediate between groups I and II. HGH concentrations above 5 ng/ ml were observed in only one subject in group III or IV during sleep.

Mean daily creatinine, calcium, hydroxyproline and nitrogen excretion for group II and for creatinine and hydroxyproline excretion for groups I and IV were not statistically different during hGH administration from the control period. Calcium excretion increased significantly from 2.91 to 6.31 mg/ kg BW/ day in group I and from 2.91 to 3.97 mg/ kg BW/ day in group IV following hGH administration. Nitrogen excretion decreased significantly from 137 to 99 ng/ kg BW/ day in group I and from 119 to 96 mg/ kg BW/ day in group IV following hGH administration.

These differences suggest a decline in hGH metabolism with age resulting from decreased circulating hGH, decreased response to hGH stimuli, fewer receptor sites at the tissue level for active hormone, or because of decreased hGH dependent-stimulation of somatomedin synthesis. Statistically different rates of calcium and nitrogen excretion during hGH administration associated with a statistical decrease in circulating hGH in the elderly suggests the development of a type of functional hypopituitarism as a consequence of aging.