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Growth hormone secretory in healthy aged women and men of Tunisian population

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With aging, men and women experience multiple hormonal changes, and in particular the activity of the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis. This perturbation may be involved in aggravations of numerous abnormalities. In 64 healthy elderly, we determined the concentrations of GH in both sexes and its correlation with thyroid-stimulating hormone (TSH), the descriptive data, BMI, electrolytic assessment and some biochemical parameters. Collected data suggest that there was an age-dependent decrease in GH secretion. For TSH, there was a slight increase. The simple regression analysis revealed non-significant direct relation between these two hormones. We also found that BMI values were inversely related to the serum concentrations of these hormones. For the lipid metabolism, the positively correlated relation only exists between GH and total-cholesterol, on one hand, and between GH and LDL-cholesterol, on the other. For the other parameters such as glucose and triglycerides as well as HDL-cholesterol, this relation does not exist. For the BMI, this index shows a positively correlated relation with glucose and triglycerides. The electrolytic assessment shows that in men, phosphorus was positively related to GH. In women, calcium and sodium were positively related to GH. Aging was found to be associated with decreased morning serum GH levels and slightly increased TSH levels in healthy elderly Tunisian population. Reduction of the GH-IGF-I axis in the elderly has important clinical implications. Indeed, this deficiency in GH could contribute to the decline of various functions associated with normal aging.

Key words: Aging, growth hormone, thyroid-stimulating hormone, body mass index, triglycerides, HDL-cholesterol, LDL-cholesterol, electrolytic assessment.

INTRODUCTION

"Healthy" aging has been defined in several ways; Lee et al. (2004) define it as a decline in various body functions in the absence of any discernible disease process. The aging phenomenon is characterized by the alternation of

several periods such as the adrenopause, the menopause in women (Wise et al., 1996) and the somatopause (Lamberts et al., 1997). Each period is marked by changes on the levels of a number of hormones, resulting of a decline in the function of a number of endocrine glands (Lee et al., 2004). Growth hormone (GH) secretion undergoes dramatic changes during a life span (Corpas et al., 1993). Human aging is therefore associated with diminished activity of the hypothalamic-GH-insulin-like growth factor I (IGF-I) axis, a phenomenon referred to as the "somatopause" (Giustina and Veldhuis, 1998; Hoffman et al., 2004). The term "somatopause", a decline in somatic function, has been proposed to describe a range of age-related metabolic disorders. This range includes the reduction of muscular, osseous and adipose tissue masses, the change in body composi-

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Abbreviations: BMI, Body mass index; BM, bone marrow; BMD, bone mineral density; CV, coefficient of variation; FFAs, free fatty acids; GH, growth hormone; GHRH, GH-releasing hormone; GHRP, GH-releasing peptide; HDL, high-density lipoprotein; IGF-I, insulin-like growth factor I; LDL, low-density lipoprotein; SS, somatostatin; SD, standard Deviation; TSH, thyroid-stimulating hormone.

tion, including visceral obesity, insulin resistance and lipid profile disorders, etc. (Nass and Thorner, 2002). Although, normal aging is not typically associated with profound GH deficiency (Thorner et al., 1995), many people over 60 are GH deficient by young adult standards (Iranmanesh et al., 1991; Corpas et al., 1993).

GH is the most abundant anterior pituitary hormone that accounts for 4 - 10% of the net weight of the anterior pituitary in the human adult, amounting to about 5 - 10 mg per gland (Arce and Devesa, 2000). The GH is released in a pulsatile fashion from somatotrophs in the pituitary (Giustina and Veldhuis, 1998). The circulating levels of this hormone are relatively low before puberty. GH secretion reaches a peak around the time of the pubertal growth spurt and then declines steadily. However, sexual maturation and adolescence are accompanied with a period of high GH output and accelerated somatic growth after which GH secretion begins an inexorable decline towards senescence (Corpas et al., 1993). After 40 years of age, GH production decreases gradually at a rate of approximately 14% per decade (Lamberts et al., 1997).

Physiologically, GH has many effects on the body. First, GH is a potent stimulator of protein anabolism, directly or through the stimulation of IGF-I (Mauras, 2006). Due to this effect, GH induces an increase in the growth rate of long bones and skeletal muscles during childhood and teenage years (Ariznavarreta et al., 2003). Second, GH promotes loss of body fat and causes insulin resistance (Johansen et al., 2005), but its action on carbohydrates makes a diabetogenic. Thus, GH stimulates the insulin secretion (Liu et al., 2007). A recent report suggests that GH has a lipolytic domain, which resides in the carboxyl-terminus of the molecule (Liu et al., 2007). Generally, GH is the primary post-natal regulator of proportional growth, and a key metabolic regulatory hormone in humans (Woodhouse et al., 2006).

In elderly humans, mean daily attenuated GH levels accompanied with low plasma IGF-I concentrations (Iranmanesh et al., 1991; Corpas et al., 1993; Münzer et al., 2001), have been ascribed to hypothalamic somatostatin (SS) excess, GH-releasing hormone (GHRH) deficiency or both (Corpas et al., 1993). These two hypothalamic peptides, with opposite actions (Müller et al., 1999), have been firmly established as crucial for the regulation of pituitary somatotroph proliferation and the synthesis and secretion of GH (Giustina and Veldhuis, 1998). Moreover, these two specific neurohormones interact functionally at both hypothalamic and pituitary levels and their interactions are responsible for the pulsatile pattern of GH release in mammals, including humans (Müller et al., 1999). To date, aging related changes in the mechanisms controlling GH release remain to be clarified (Woller et al., 2002).

There are interactions between the somatotrophic system and the hypothalamic-pituitary-thyroid axis (Filipsson and Johannsson, 2007). Indeed, the somatopause is due

not only to decreases in GH and IGF-I with age (Martin et al., 1997), but also to the decline in the function of the thyroid gland in the elderly (Lee et al., 2004). The alteration of this function is assessed by a very sensitive and specific parameter, the thyrotropin or thyroid-stimulating hormone (TSH). This glycoprotein shows a variation with the passing of years (Corpas et al., 1993). In fact, the changes in the thyroid gland with aging are not constant and many factors can influence the thyroid activity.

To our knowledge, the interrelationships between GH and TSH secretion in the healthy elderly, and whether these relationships differ with sex or not, have not been elucidated. In the current study, we examined the interrelationships between diurnal GH and TSH release in healthy aged men and women. Then, we related GH concentrations to a number of GH-dependent parameters of body composition and lipid metabolism as well as to parameters of electrolytic assessment.

MATERIALS AND METHODS

Subjects

The study was approved by an Ethical Committee and a written informed consent was signed by each subject before participation. Sixty-four elderly volunteer subjects: 30 men and 34 women with a mean (\pm SD) age of 75.48 ± 7.97 (range; 65 - 96 year) were recruited to achieve the objective of this work.

This sample of Tunisian population was carefully chosen according to age and health criteria. First, a medical interrogation informed about some characteristics such as the age, the origin, etc. Medical history, physical examination, blood studies, biochemical testing and graded treadmill electrocardiogram proved that our subjects are healthy. All had normal biochemical indices of renal, hepatic, and hematological function. All data are recapitulated in Table 1.

These subjects are known to be active, and leading a normal life. None of the subjects had any signs of mental and cardiovascular disease; any history of clinically evident malignancy, obesity, depression, diabetes mellitus, untreated thyroid disease, pituitary or adrenal disease, or dyslipidemia. None used medication that could affect cardiovascular or metabolic function or interfere with the GH-insulin-like growth factor I (IGF-I) axis or the hypothalamic-pituitary-adrenal axis activities. None of the subjects consumed alcohol. In addition, none of the women had taken any estrogen or progestin for at least three months before the study and none ever smoked.

Standard anthropometric measures of height (cm) and weight (kg) were obtained. The body mass index (BMI) was calculated using the formula: weight (kilograms)/ height² (meters). This parameter range was (18.10 - 30.29 kg/m²) in all participants. BMI was used as an index of total adiposity. Blood pressure at rest was $120 \pm 20/80 \pm 10$ mmHg in all subjects.

Study participants were required to fast the night preceding the taking of blood samples. Venous blood samples were drawn in the morning, between 07.00 and 08.00; with a tolerance for 30 min. The samples were immediately chilled, centrifuged at $3000 \times g$ for 15 min at 4°C, and saved at - 80°C until analyzed.

Hormone assays

In the laboratory assay, all samples were tested in duplicate. Serum GH was measured by Biosource Hgh-EASIA kit (KAP1081) (Biosource Europe S.A. Nivelles Belgium). The BioSource hGH-

Table 1. Comparison of descriptive data (age, height and weight), BMI, endocrine measures, electrolytic assessment and biochemical parameters between men (n = 30) and women (n = 34).

Variable	Men (n = 30)		Women (n = 34)	
	Mean \pm SD	Range	Mean \pm SD	Range
Age (year)	76.07 \pm 7.80	65 - 92	74.97 \pm 8.21	65 - 96
Height (cm)	165.70 \pm 10.75*	145 - 190	157.14 \pm 11.85*	130 - 185
Weight (kg)	63.90 \pm 11.08	42 - 83	61.20 \pm 14.92	35 - 90
BMI (kg/m ²)	23.13 \pm 2.46*	18.66 - 28.38	25.11 \pm 3.86*	18.10 - 35.29
GH (μ g/l)	0.91 \pm 0.47*	0.20 - 1.98	0.64 \pm 0.33*	0.16 - 1.32
TSH (μ UI/ml)	2.67 \pm 1.58	0.64 - 5.90	2.87 \pm 1.61	0.61 - 5.97
Calcium (mmol/l)	2.26 \pm 0.21	1.80 - 2.48	2.26 \pm 0.22	1.81 - 2.9
Phosphorus (mmol/l)	1.12 \pm 0.66*	0.66 - 1.95	1.27 \pm 0.31*	1.01 - 2.59
Sodium (mmol/l)	144.74 \pm 4.38	134.9 - 151.10	144.46 \pm 4.87	134.80 - 151.70
Potassium (mmol/l)	4.18 \pm 0.46	3.34 - 4.98	4.11 \pm 0.40	3.32 - 4.89
Cholesterol (mmol/l)	4.35 \pm 0.99	2.70 - 6.60	4.61 \pm 0.79	3.10 - 6.30
HDL (mmol/l)	0.64 \pm 0.13	0.50 - 0.75	0.62 \pm 0.14	0.5 - 1.0
LDL (mmol/l)	3.46 \pm 0.94	2.00 - 5.60	3.63 \pm 0.79	2.12 - 5.33
Triglycerides (mmol/l)	1.02 \pm 0.41*	0.51 - 2.50	1.31 \pm 0.47*	0.53 - 1.88
Glucose (mmol/l)	3.91 \pm 0.75*	3.10 - 6.20	4.35 \pm 0.83*	3.10 - 6.20
Albumin (g/l)	39.30 \pm 3.84	29.80 - 44.80	38.82 \pm 4.50	26.3 - 46.5
Creatinine (μ mol/l)	82.76 \pm 17.08*	48 - 120	70.91 \pm 19.87*	42 - 106
Total Protein (g/l)	68.26 \pm 5.63	56 - 80	70.08 \pm 6.53	58 - 82

*P value < 0.05.

Data shown are mean \pm SD, with ranges.

EASIA is an "Enzyme Amplified Sensitivity Immunoassay" in solid phase carried out on microtiter plates. The assay sensitivity was 0.2 μ IU/ml, the mean intra-assay coefficient of variation (CV) was 3.7% between 1.9 \pm 0.1 μ IU/ml and 9.8% between 3.5 \pm 0.4 μ IU/ml. The inter-assay CV was 3.6% between 6.4 \pm 0.2 μ IU/ml.

Serum TSH was measured by the electrochemiluminescence immunoassay method "ECLIA" as intended for use on the Roche Elecsys 2010 and Modular Analytics E170 (Elecsys module) immunoassay analyzers. The interval of 0.005 - 100.0 μ IU/ml defined by the lower detection limit and the maximum of the master curve. The functional sensitivity is 0.014 μ IU/ml.

Other assays

Other biochemical analyses including glucose, total-cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, creatinine, albumin, phosphorus, calcium and total protein were measured on an automated COBAS INTEGRA 400 Plus. The sodium and the potassium were determined by an analyzer of ions Elite (Electra Medical).

Statistical analysis

All results were expressed as the mean \pm SD, with the range in parentheses. An unpaired Student's t test was used to analyze all the parameters (Statistica software, Stat Soft). Relationships between the various parameters were assessed by linear regression analyses using Pearson's correlation coefficient (r). Statistical significance was attained at P < 0.05.

RESULTS

Subjects' characteristics

The subject's characteristics of 64 healthy elderly men (n = 30) and women (n = 34) are shown in Table 1. All data are mean \pm SD. The mean elderly height was 161.16 \pm 12.06 cm (range; 130 - 190), and the mean elderly weight was 62.47 \pm 13.23 kg (range; 35 - 90). The mean ages and weights of men and women did not differ significantly. However, height (P = 0.003) was greater in men and BMI (P = 0.02) was greater in women. As shown in Table 1, men were heavier and taller than women and had lower BMIs.

Of the 30 men, 6 were smokers, but the 34 women had never smoked. The total-cholesterol levels and its fractions, HDL- and LDL-cholesterol, the calcium, the sodium, the potassium, albumin and the total protein concentrations did not differ according to gender. But, the phosphorus (P = 0.04), the triglycerides (P = 0.01), the glucose (P = 0.03) and the creatinine (P = 0.01) differed significantly with regard to sex. We found that the three first biochemical parameters were greater in women and the last parameter was greater in men. All biochemical parameter values were within the normal range.

For the endocrine measures, mean serum GH concentrations in this group of elderly subjects was 0.77 \pm 0.43

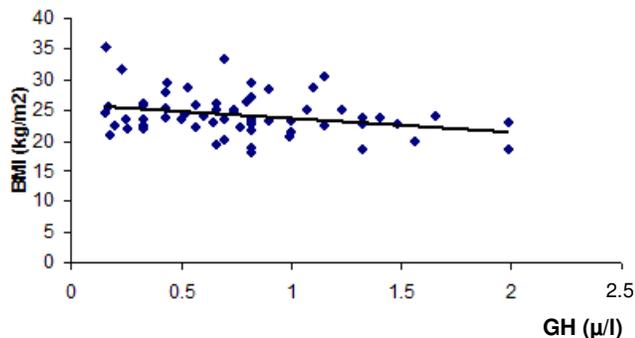


Figure 1. Relationship between serum GH concentrations ($\mu\text{g/l}$) and BMI (Kg/m^2) in 64 elderly subjects, 65 - 96 year old. BMI (kg/m^2) = $25.96 - 2.29 \text{ GH } (\mu\text{g/l})$.

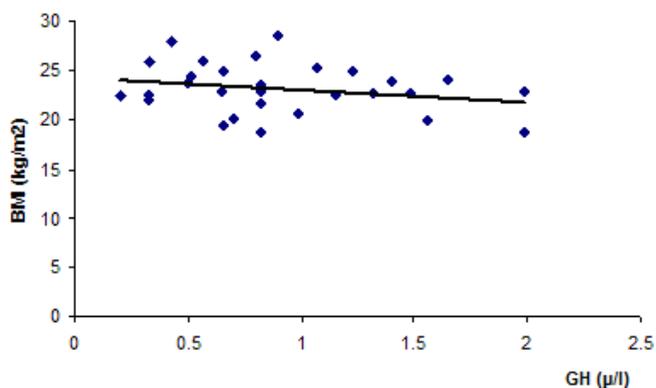


Figure 2. Relationship between serum GH concentrations ($\mu\text{g/l}$) and BMI (kg/m^2) in 34 elderly women, 65 - 96 year old. BMI (kg/m^2) = $26.73 - 2.50 \text{ GH } (\mu\text{g/l})$.

$\mu\text{g/l}$ (range; 0.16 - 1.98). The values of this hormone differed significantly between the two sexes ($P = 0.01$). Elderly women had lower serum GH concentrations, compared to the elderly men. The mean serum TSH concentrations was $2.78 \pm 1.60 \mu\text{UI/ml}$ (range; 0.61 - 6). This hormone did not differ between men and women.

GH and TSH status and body composition

Height: In women, serum GH concentrations were inversely related to height. In men, however, this hormone was positively related to height values.

Weight: For this anthropometric measurement, in both sexes, there was a negative relationship between serum GH concentrations and weight.

BMI: For all the group of elderly subjects, Figure 1 shows the relationship between serum GH concentrations ($\mu\text{g/l}$) and BMI values (Kg/m^2). This relation was significant and inversely proportional ($r = -0.29$, $P < 0.02$). Figures 2 and 3 illustrate the negative non-significant relationship of GH

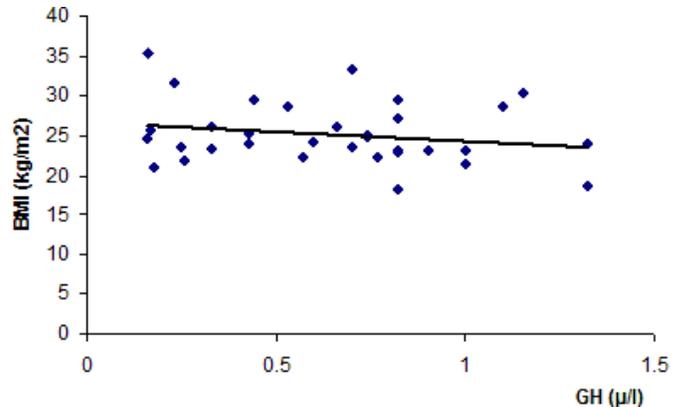


Figure 3. Relationship between serum GH concentrations ($\mu\text{g/l}$) and BMI (kg/m^2) in 30 elderly men, 65 - 92 year old. BMI (kg/m^2) = $24.33 - 1.31 \text{ GH } (\mu\text{g/l})$.

concentrations ($\mu\text{g/l}$) with BMI (Kg/m^2), in women ($r = -0.21$, $P = 0.22$) and men ($r = -0.25$, $P = 0.17$) respectively.

For TSH, in our population, in men and in women, BMI and TSH were respectively, inversely non-significantly related ($r = -0.1$, $P = 0.41$), ($r = -0.17$, $P = 0.34$) and ($r = -0.06$, $P = 0.72$).

GH, glucose, lipid metabolism, albumin and total protein

Table 2 shows a simple regression analysis of serum GH concentrations with fasting glucose, triglycerides and total-cholesterol and its parameters, HDL and LDL. According to the sex, at each group, we found positive non-significant relationships between GH and glucose concentrations, on the one hand, and GH and triglycerides concentrations, on the other hand. For the serum total-cholesterol concentration, we found a positive relationship between this biochemical parameter and the serum GH concentration, in men and in women. This relation was significantly correlated ($P = 0.04$), in the group of men. For the first fraction of total-cholesterol, HDL, we found that it was inversely related to GH concentrations in men. In women, this fraction was positively related to GH concentrations. However, the relation was non-significant in both sexes. The second cholesterol fraction, LDL, was positively related to GH concentrations in the men, where this relation was significantly correlated ($P = 0.03$). But women's LDL-cholesterol was negatively related to GH concentrations.

In men, the albumin concentrations were negatively non-significantly related to GH levels. The total protein concentrations were positively non-significantly related to this hormone. In women, the albumin and the total protein concentrations were both negatively non-significant related to GH.

Table 2. Simple linear regression (Pearson's correlation coefficient) of serum GH concentration with fasting glucose, triglycerides, cholesterol parameters, albumin and total protein.

Variable	Serum GH concentrations Men (n = 30)		Serum GH concentrations Women (n = 34)	
	r	p	r	p
Fasting glucose (mmol/l)	+0.01	0.93	+0.10	0.55
Triglycerides (mmol/l)	+0.04	0.83	+0.06	0.71
Total cholesterol (mmol/l)	+0.37	0.04	+0.13	0.45
HDL-cholesterol (mmol/l)	-0.001	0.99	+0.10	0.55
LDL-cholesterol (mmol/l)	+0.39	0.03	-0.0083	0.96
Albumin (g/l)	-0.24	0.19	-0.11	0.50
Total protein (g/l)	+0.07	0.70	-0.18	0.31

r: Pearson's correlation coefficient.
P < 0.05.

Table 3. Simple linear regression (Pearson's correlation coefficient) of serum GH concentration with electrolytic assessment parameters.

Variable	Serum GH concentrations Men (n = 30)		Serum GH concentrations Women (n = 34)	
	r	p	r	p
Calcium (mmol/l)	-0.10	0.57	+0.11	0.49
Phosphorus (mmol/l)	+0.06	0.72	-0.01	0.95
Sodium (mmol/l)	-0.009	0.96	+0.03	0.83
Potassium (mmol/l)	-0.09	0.63	-0.06	0.70

r: Pearson's correlation coefficient
P < 0.05

Table 4. Simple linear regression (Pearson's correlation coefficient) of BMI values (kg/m²) with fasting glucose, triglycerides and cholesterol parameters.

Variable	BMI values Men (n = 30)		BMI values Women (n = 34)		BMI values (n = 64)	
	r	p	r	p	r	p
Fasting glucose (mmol/l)	+0.38	0.03	+0.27	0.12	+0,36	0,0035
Triglycerides (mmol/l)	-0.07	0.68	+0.38	0.02	+0,29	0,017
Total cholesterol (mmol/l)	-0.12	0.50	+0.11	0.51	+0,055	0,66
HDL-cholesterol (mmol/l)	+0.06	0.75	-0.082	0.64	-0,06	0,64
LDL-cholesterol (mmol/l)	-0.13	0.47	+0.12	0.48	+0,048	0,70

r: Pearson's correlation coefficient.
P < 0.05.

GH and electrolytic assessment parameters

Table 3 shows a simple linear analysis of regression of the concentrations in serum GH with the electrolytic assessment variables including calcium, phosphorus, sodium and potassium. In men, calcium, sodium and potassium were negatively dependent with GH values, whereas phosphorus was positively dependent on this hormone. In women, calcium and sodium were positively

related to GH, whereas potassium and phosphorus showed the reverse.

BMI, glucose, and lipid metabolism

Table 4 illustrates a simple regression analysis of BMI values with fasting glucose, triglycerides, total-cholesterol and its fractions. This analysis shows that fasting serum

glucose was positively related to the BMI values in our population. This relation was significantly correlated in the entire group ($P = 0.0035$) and in the group of men ($P = 0.03$). For the triglycerides, this biochemical variable was inversely related to BMI values, in men. But, there was a positive significant relationship of this variable with BMI, in women ($P = 0.02$). For the total-cholesterol, there was a negative non-significant relationship with BMI values, in men. But a positive non-significant relationship was observed in the women.

The HDL-cholesterol levels were positively non-significantly related to BMI values, in men. But this fraction was negatively non-significantly related to this index, in the 34 women. The LDL-cholesterol levels were negatively non-significantly related to this index, in the 30 men. But, this fraction was positively non-significantly related to BMI values, in women.

Characteristics of GH and TSH

The GH concentrations decline with age and this decline is greater in women than in men. There were thus significant sex differences in GH values. The TSH concentrations increase slightly with age, in both sexes. Simple regression analyses revealed a direct non-significant relationship between GH concentrations and TSH concentrations ($r = 0.083$; $P = 0.51$), in both men and women.

DISCUSSION

The present study was performed to investigate whether in healthy elderly persons, in both sexes; the secretion of GH was modified. In fact, our GH data obtained in fasting Tunisian elderly subjects confirmed the reported decrease in GH secretion with age. According to sex, this reduction is larger among women compared to men. Moreover, this diminution was always described in healthy elderly men and women. Therefore, our findings are in agreement with the results of many studies realized in this domain (Lamberts et al., 1997; Hindmarsh et al., 1999; Gusenoff et al., 2001). In these studies, the researchers obtained their results while using various techniques such as sensitive chemiluminescent, immunofluorimetric and immunoradiometric assays in groups of volunteers with frequent sampling intervals. In addition, with advancing age, GH release decreases in men and women, particularly at night (Corpas et al., 1993). Our results concerning the GH assayed in our Tunisian population go in the same direction as these observations, but in the morning (08:00 am). However, Uberti et al. (1997) showed in their study that no substantial differences were observed in the basal plasma GH levels between young and elderly subjects. This finding is probably due to the small numbers of elderly men and women who were investigated.

Circadian GH secretion in elderly subjects is characterized by decreased GH burst frequency, amplitude, and secretory burst mass (Ho et al., 1987; Iranmanesh et al., 1991; Veldhuis et al., 1995). But to date, aging-related changes in the mechanisms controlling GH release remain to be clarified. Moreover, it is still unclear whether these modifications are primarily a consequence of aging, or induced by its epiphenomena (Woller et al., 2002). Among these epiphenomena, we can find the lower serum gonadal steroid milieu, the increased percentage of body fat and/or the reduced insulin sensitivity and the decreased physical fitness (De Boer et al., 1995), which are associated with advanced age (Veldhuis et al., 1995; Giustina and Veldhuis, 1998). Consequently, the neuroendocrine mechanisms of somatopause, which includes the decrease of GH, are uncertain. Some studies suggested that it is the pituitary itself that undergoes senescent changes (Takahashi et al., 1987). However, other data refute this explanation. First, there is no apparent decrease in the number of somatotrophs in the pituitaries with age (Shimokawa et al., 1996). Second, even though older rats have diminished responses to GHRH *in vivo*, the *in vitro* pituitary responsiveness to GHRH does not decline with age (Sonntag et al., 1983). Perhaps the strongest argument against a primary pituitary mechanism of somatopause is the ability of exogenous GHRH (Corpas et al., 1992) or GH-releasing peptide (GHRP) analogs (Chapman et al., 1996) to rejuvenate GH output and plasma IGF-I levels in elderly humans. Thus, the focus of investigations has shifted to potential alterations of the hypothalamic regulation of GH secretion (Russell-Aulet et al., 1999). Moreover, van Dam et al. (2000) have demonstrated in their study carried out on nine healthy elderly men that the decreased GH levels associated with the aging process are not the result of a decreased GH secretory reserve capacity.

Thus, it is not known why GH secretion declines with aging. Indeed, many possible mechanisms have been proposed to explain the attenuated circadian GH secretion in the elderly. These mechanisms can operate alone or in association. They include: (i) pituitary senescence translated by a decreased capacity or number of the GH-secreting cells of the anterior pituitary; (ii) reduced GHRH secretion or action; (iii) increased somatostatinergic inhibitory tone or secretion (Chapman et al., 1997; Giustina and Veldhuis, 1998; Kojima et al., 1999) and (iv) deficiency of ghrelin, a peptide of 28 amino acids that was recently found to be an endogenous ligand for the GH-releasing peptide (GHRP) receptor can represent another mechanism (Giustina and Veldhuis, 1998). However, it is essential to know the receptor parameters for the interpretation of some data. The number and the affinity of ligand receptors may be important determinants of hormonal response. Unfortunately, there is no information regarding the potential dynamics of GHRH receptors in aging (Russell-Aulet et al., 1999). The increased sensitivity to the negative feed-

back effects of IGF-I can also explain the reduction in the GH secretion. The IGF-I mediates the growth promoting and metabolic actions of GH (Chapman et al., 1997).

Reportedly, the cooperative interaction between stimulatory GHRH and inhibitory SS inputs is necessary to optimize GH release (Müller, 1987), and the amplitude of GH secretory bursts is dependent on the amount of GHRH impinging on the somatotrophs (Frohman et al., 1990). Nevertheless, SS may also have an important positive influence in modulating GH pulse amplitude (Tannenbaum and Ling, 1984). Especially, it has been suggested that age-related changes in GH secretion in man might be the result of an increase in hypothalamic inhibition by SS (Veldhuis et al., 2001). This situation can be associated or not with a defective pituitary responsiveness to GHRH (Corpas et al., 1992; Giustina and Veldhuis, 1998). Consequently, these two hypothalamic peptides have been firmly established as crucial for the regulation of pituitary somatotroph proliferation and the synthesis and secretion of GH (Giustina and Veldhuis, 1998). Nevertheless, the relative importance of those two hypothalamic regulators is not fully understood (Woller et al., 2002).

There is increasing evidence to suggest that a sexually dimorphic pattern of GH secretion is present in the human being (Jaffe et al., 1998; Hindmarsh et al., 1999; Orrego et al., 2001). Indeed, the neuroendocrine mechanisms of GH regulation of somatopause in the elderly appear to be sex-dependent (Orrego et al., 2001). In our population, the major difference between the two sexes at an interval of 65 to 96 year of age, in GH concentrations was achieved. We found that GH concentrations are greater in men compared to those in women. A sexually dimorphic variation in GH release in humans has been found by several investigators. Our findings are in agreement with that of Hindmarsh et al. (Hindmarsh et al., 1999) who reported a 50% higher daily GH secretion rate in men than in women. This analysis used an ultrasensitive chemiluminescence-based GH assay and a 20 min blood sampling for 24 h. The evidence that there is a sexually dimorphic pattern of GH secretion and GH concentration profiles is also demonstrated by various studies. We can find in the literature that the pattern of GH secretion differed significantly between the sexes, with women secreting GH in a more disordered manner than men (Pincus et al., 1996; Hindmarsh et al., 1999). So, it was noted that females exhibited greater statistical irregularity in their GH time series than males (Pincus et al., 1996). This was verified independently by a reduced spectral peak in women compared with men, indicating a reduced period of time during which regular GH oscillatory events took place. Furthermore, there was a significant difference in the periodicity of these regular events between the sexes, with a slower pulse pattern observed in women (Hindmarsh et al., 1999).

Other observations made in different populations (Jaffe et al., 1998; Engstrom et al., 1998) have shown that mean 24 h serum GH concentrations were greater in

females than in males. The major difference between the two sexes aged 59 - 73 year, lies less in the peak GH levels attained and more in an elevation of trough concentrations, with a 4-fold increase in trough GH concentration in females compared to that in males (Hindmarsh et al., 1999). Many biases can explain these divergences. The difference between the populations studied can be attributed to diet and/or age brackets. Moreover, the interpretation of data obtained by different methodologies can be influenced by the complexity of GH neuroregulation and the potential interactions between GHRH and SS.

In the elderly, some studies have shown no sex differences in GH secretion. Gusenoff et al. (2001) confirmed these findings. They found no significant sex differences in estimated GH secretory parameters, except for a slightly, but statistically greater total basal GH secretion in women. Moreover, these findings that mean and integrated GH secretion, as well as GH production rate, did not differ between elderly men and women, confirm the observation of Ho et al. (1987). This author shows that GH secretion is reduced to approximately similar levels in older men and postmenopausal women. However, our assessments differ by way of both assay and sampling paradigm.

The determinants of the activity of the somatotrophic axis, which have been studied extensively, are multiple. Beside GHRH, SS, putative GHRPs, and free fatty acids (FFAs) other factors such as catecholamines, dopamine, glucocorticoids, insulin, leptin, and sex steroids play a role at different levels leading to a complex regulation mechanism (Giustina and Veldhuis, 1998). It has been demonstrated that peak and trough GH concentrations have different associations beside insulin-like factor (IGF) axis, with body composition and metabolic parameters in adult males, 59 - 70 year of age (Hindmarsh et al., 1997). With aging, GH deficiency cause also a change in body composition resulting in an increase in total body fat (Clasey et al., 2001) especially abdominal visceral fat mass (De Boer et al., 1995; Clasey et al., 2001; Münzer et al., 2001), a decrease in lean body mass (De Boer et al., 1995; Clasey et al., 2001), a reduction in bone mineral content and density and a deranged metabolism of lipoproteins and carbohydrates (De Boer et al., 1995).

When we looked for the metabolic and catabolic/anabolic effects that GH exerts on peripheral tissues (Gusenoff et al., 2001), we found that GH deficiency in nonelderly adults is associated with increased total and intra-abdominal fat (Gertner, 1993). In the same context, in the elderly subjects, obesity, particularly central adiposity, and age are potential confounders of GH secretion because of their known influences on hormone axis (Gusenoff et al., 2001). Moreover, peripheral fat depots are well-known targets of GH action (Gevers et al., 2002). Theoretically, higher adiposity may result in heightened SS tone, which in turn, could conceivably potentiate the ability of GHRH antagonist to inhibit spon-

taneous GH secretion (Considine, 1997). The GH causes thus a higher relative adiposity and lower lean body mass in the elderly, especially in men.

In our population, the relationships of weight and height with morning GH concentrations were analyzed. We found that, in both sexes, the weight values are inversely proportional with GH concentrations. But for the height values, there is a difference between the two sexes. This anthropometric measurement is inversely proportional in women but not in men. Our data revealed also that the BMI is inversely proportional with the GH concentrations of our elderly, in both sexes. By extension, it has been proposed that reduced GH secretion may be a cause of the body composition changes that accompany aging. The latter and our current data are compatible with prior observations that aging and GH deficiency lead to similar effects on abdominal fat (Münzer et al., 2001). This suggests that the age-related decline in GH secretion could be explained by the increase in body fat mass. This suggestion is supported by the fact that, the peak and the trough GH concentrations appeared to relate inversely to BMI (Veldhuis et al., 1995; Hindmarsh et al., 1999; van Dam et al., 2000). Moreover, our finding is consistent with the reports of four studies: one open and three placebo-controlled, in which GH was administered to small numbers of older men and women and has favorable effects on body composition (Papadakis et al., 1996). Whether the changes in GH concentration with aging are a causative factor for the increase in body fat or a consequence of these changes has yet to be determined (Nass and Thorner, 2002).

However, the influence of obesity can be associated with increase in plasma FFAs concentrations which suppress GH secretion (van Dam et al., 2000). However, it was proposed that the higher circulating levels of FFAs are a cause of the reduced GH secretion associated with obesity (Lee et al., 1995). There is also a concept of a classic feedback loop between circulating FFAs and pituitary GH secretion (van Dam et al., 2000). Nevertheless, some studies suggest that differences in FFAs concentrations are not a cause of the aging-associated decline in GH secretion (Chapman et al., 1997). Russell-Aulet et al among others (Russell-Aulet et al., 1999) showed that the greater inhibition of GH by GHRH antagonist in the elderly could not be explained by the difference in body composition parameters.

Hindmarsh et al. (1997) has obtained results which are astonishing, considering a number of reports that demonstrate an inverse relationship between BMI and parameters of GH secretion, such as daily secretion rate (Iranmanesh et al., 1991) and integrated GH concentrations (Martin-Hernandez et al., 1996). They reported thus that there was no relationship between the peak or mean GH level and current height, weight and BMI of the individual. But, it should be noted that these data were obtained with a population of forty-five men aged from 59 to 69 year. The bias of a narrow age range allows the re-

moval of a potential confounding variable in the GH-BMI relationship (Veldhuis et al., 1995), namely age, which was a more important determinant of BMI than other factors.

The discordance may also relate in part to the heterogeneous population that has been studied in several reports. Particularly, in studies where a wide range in age of the population has been analyzed and where there is perhaps greater variance in BMI values than in this more focused study population. An alternative explanation is that the overall measure that BMI represents does not adequately represent the difference in body composition between sexes (Roemmich et al., 1998). So, different modes of assessing body composition revealed different influences on GH concentration. Moreover, a wide range of BMI can increase the variability in data significantly.

In elderly humans, feedback inhibition of GH release can be assured by IGF-I (Jaffe et al., 1998), and can be also carried out by glucose (Chapman et al., 1994) in males compared to females. In this context, several studies found that increased abdominal visceral fat is directly associated with higher fasting levels of glucose (Zamboni et al., 1997). In agreement with these results, our population shows that the glucose concentrations were directly related to BMI values, in both men and women. Moreover, these findings confirm a number of studies that show GH concentrations during oral glucose loading to be higher in the female population (Chapman et al., 1994; Pincus et al., 1996; Engstrom et al., 1998; Hindmarsh et al., 1999). All these observations serve to highlight the possibility that in females there is a degree of GH insensitivity (Hindmarsh et al., 1999). In our elderly women, glucose concentrations were directly related to the GH concentration. This relation is more remarkable than it is in men.

Furthermore, BMI can be compared with some other biochemical parameters such as serum total-cholesterol, its fractions and triglycerides levels. For these parameters, Zamboni et al. (1997) found, especially in postmenopausal women, that increased abdominal visceral fat is also directly related with cholesterol and triglycerides levels. In our studied group, the serum total cholesterol and triglycerides levels show a positive relation with the BMI values. Moreover, this relation was positively correlated, in the entire group, between BMI values and triglycerides levels. According to sex, the serum total-cholesterol and triglycerides levels go in different directions, in their relation with BMI values, in both sexes. So, we found that the concentrations of these parameters were directly related to BMI values in women, but they were inversely related to this index in men.

For the fractions of total-cholesterol, our data revealed that HDL-cholesterol values were inversely related with BMI in women, but in men we found the reverse. In our population, HDL-cholesterol was conversely related to the BMI and LDL-cholesterol was directly related to this index. These finding confirm the result of DiPietro et al.

(1999) who found that increased abdominal visceral fat was inversely related to HDL-cholesterol levels. In contrast to the profile of HDL, LDL shows direct relation with the BMI in women, and an inverse relation with this index in men. In some studies, cholesterol and, in particular, LDL-cholesterol values are elevated in GH insufficiency (Rosen et al., 1993). Other studies suggest that GH is involved in the regulation of lipoprotein metabolism, and GH deficiency is associated with elevated serum levels of total cholesterol and one of its fractions, the LDL-cholesterol (De Boer et al., 1994). In his study, Hindmarsh et al. (1997) was interested in peak and trough values of GH and found that neither peak nor trough levels were major determinants of total or LDL-cholesterol, which seemed to be determined simply by age and independent of GH.

We can also find the relations between serum GH concentration and these biochemical parameters. Indeed, the GH concentration is directly correlated with fasting total-cholesterol and with LDL-cholesterol.

In the light of these findings, the results of the present study strongly support the concept that GH has an important role in the physiological regulation of cholesterol and lipoprotein metabolism. Nevertheless, little is known about the influence of GH secretory patterns in man on metabolic parameters (Hindmarsh et al., 1997).

Reportedly, GH plays an important role not only in skeletal growth and development in young people, but also in regulating bone re-modeling throughout life (Johannsson et al., 1996). Indeed, GH stimulates longitudinal growth and bone formation (Andreassen and Oxlund, 2001), and GH deficiency is associated with a decreased bone mass (De Boer et al., 1995). GH could therefore act on the progenitors of adipocytes and osteoblasts, to affect their proliferation and differentiation (Gevers et al., 2002). A decrease in bone volume is often accompanied with an increase in bone marrow (BM) fat, for example, in old age, immobility and corticosteroid induced osteoporosis (Nuttall and Gimble, 2000). However, the relevance of these changes and the role of adipocytes in BM are far from clear. Most studies have focused on the osteoblast lineage; the regulation and function of the adipocyte lineage in this tissue has received much less attention (Gevers et al., 2002).

In our healthy aged men and women, we examined the relation between the GH concentrations and the serum values of the electrolytic assessment parameters. In men, this assessment shows that that calcium, sodium, and potassium concentrations were inversely related to GH concentrations. But, the phosphorus concentrations were directly associated with GH. In women, it shows that calcium and sodium levels were positively related to GH concentrations; and the phosphorus and potassium values were negatively associated with this hormone.

Our results are in agreement with some studies which interested themselves with the effect of GH on bone among old people. In addition, cross-sectional studies in

men and women aged 68 - 98 year show that increased age is significantly associated with decreased bone mineral density in the proximal femur and proximal radius (Hannan et al., 1992). The suggestion that changes in lean body mass, resulting in decreased muscle strength and decreased bone mineral density places the elderly at higher risk for fractures (Toogood et al., 1997; Nass and Thorner, 2002). Moreover, the age-related decline in GH secretion has been involved in the development of osteoporosis. GH-deficient subjects show a significant reduction in bone mineral density (BMD) with age which is well recognized (Toogood et al., 1997).

Our TSH data show a slight increase in the concentration of this hormone in our Tunisian population, in both men and women. The increase is greater in women than in men. Our results agree with previous studies which reported slightly increased TSH concentrations in elderly subjects (Erfurth et al., 1984). But, the results of some other studies are often discordant, for TSH secretion. These studies reported unchanged levels (Felicetta, 1988). Moreover, our findings are consistent with many observations which indicate that the resulting decrease in serum thyroid hormone level is accompanied by an increase in the secretion of pituitary thyrotrophin (TSH), giving an elevated serum TSH. This suggests that while there is a decline in thyroid gland function, the pituitary response remains intact (Lee et al., 2004). Other studies have reported significant changes in thyroid function, including a slight decline in serum TSH (Porretti et al., 2002).

The hypothalamic mechanism of the aging-related decrease in GH release remains speculative. It has been suggested that the aging of the somatotrophs is partly responsible (Van den Berg et al., 1996), and aging of the regulatory mechanism of GH release is also involved. In spite of this difficulty, GH is a pleiotropic hormone of increasing clinical importance, with uses ranging from the treatment of short stature in children to potential slowing of the aging process in the elderly (Murray and Shalet, 2000).

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