Original Article

Effect of aging on growth hormone-leptin axis in normal and obese healthy subjects

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Summary:

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Background: Growth hormone is a hormone responsible for the normal body growth and development by stimulation protein production in muscle cells and energy release for breakdown of fat. On the other hand leptin is a newly discovered hormone that is mainly synthesized in adipose tissues it decreases food intake by causing satiety and promoting energy combustion . Both aging and obesity are associated with a reduction in growth hormone secretion. In the mean time obese humans have increased circulating leptin.

Objective: The aim of this paper is to shed light on the contribution of these two hormones in the mechanism of aging process in an attempt of improving this process for a better life at old ages.

Subjects and methods: Two hundred and seventy healthy subjects aged 25-64 years old participated in this study. The subjects' were divided into four groups according to their ages and to three groups according to their weights. Urine analysis from each subject was carried out to exclude diabetes and renal failure. Sera from blood samples were used to carry out certain biochemical parameters and hormone (growth hormone and leptin).

Results: The results obtained show a decrease in the level of growth hormone with progression of age. In the mean time there is an increase in the level of serum leptin with the advancement of age. Aging is usually associated with adiposity. Increasing fat with age is probably multifactorial one potential mechanism for that is reduced leptin transport across blood-brain barrier..

Conclusion: The increase in leptin level which was observed in elderly age group and obese group suggest that the associated decrease in growth hormone serum level is related to obesity in general and in particular to the aging process.

Keywords: growth hormone, leptin, obesity, aging.

Introduction:

Growth hormone GH or somatotropin is a hormone responsible for the normal body growth and development by stimulation protein production in muscle cells and energy release from breakdown of fat. Several hormones play important roles in human growth. Growth hormone is a protein made up of 191 amino acid and secreted from the anterior pituitary gland which coordinates normal growth and development. Human growth is characterized by two spurts one at birth ant the other at puberty [(1)]. Growth hormone plays an important role at both of these times. Normal individuals have measurable levels of growth hormone throughout life. Yet level of growth hormone fluctuates during the day and is affected by eating and exercise [(2)]. Most of the growth hormone is released at night. Peak spikes of growth hormone release occurs around 10. p.m., midnight and 2 a.m. the logic behind this night time is that most of growth hormone effects are mediated by other hormones. As a result the effects of growth hormone are spread out more evenly during the day [(2)].

On the other hand leptin is a newly discovered hormone that is mainly synthesized

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in adipose tissue and is enclosed by *ob* gene[3], decreases food intake by causing satiety and promoting energy combustion[4]. The effect of leptin on body composition appears to be mediated by specific receptors in the hypothalamus [5]. Leptin shows a variety of effects in the hypothalamus, it reduces appetite and induces weight loss and thermogenesis [6]. Also it has peripheral sights of action; it stimulates vascular smooth muscle proliferation and migration[7]. A study showed that serum leptin levels are associated with sympathetic activity independent of the amount of fat, however it did not include obese subject[8]. Both aging and obesity are associated with a reduction in growth hormone secretion. This progressive fall in growth hormone secretion is associated with somatic changes that occur as part of the aging process[9]. Changes in body weight are immediately reflected in the pattern of both spontaneous and stimulated growth hormone secretion fact that is evident in situations of low body weight due to mal nutrition which are associated with enhanced growth hormone secretion[10]. Adipose tissue is the main source of circulating leptin, but leptin is also expressed in non-adiposite tissue sites such as placenta[11] , mammary epithelium[12] , skeletal muscle[13] and in particular the stomach[14].

The initial view of leptin has been extended to a wider neuroendocrine perspective, being involved in the regulation of a variety of functions including metabolism, neuroendocrine and immune function and development. All of which are related to energy balance and acting both through central and peripheral mechanisms.

Obese humans have increased circulating levels of leptin[15] and given the association with the sympathetic nervous system this might contribute to the pathogenesis of hypertension in obesity.

Several groups reported that leptin regulates growth hormone secretion in humans[16], rodent[17], sheep[18] and pigs[19].

This paper has been designed to shed some light on the important hormonal axis involved in the mechanism of aging process in an attempt to set a milestone in the process of improving the quality of later life for as many people as possible

Subjects and methods

Two hundred and seventy healthy subjects aged (25-64 years) were selected from individuals attending the out-patient clinic of the Medical City Teaching Hospital. Only 120 subjects satisfied the full requirements of the study.

The subjects were divided into four groups according to their ages, and to three groups according to their weights in order to achieve the mechanism of aging process.

The following tests were carried out for each subject.

1. Urine analysis: a random specimen of urine was taken from each subject involved in the study to test for protein, glucose and pH by dipstick to exclude diabetics and kidney failure subjects.
2. Blood samples: about 10 ml of blood were withdrawn by vein puncture from each subject in the morning after 12 hours fasting. Each blood sample was immediately centrifuged at 300rpm for 15 minutes in order to provide about 3mls of serum to cover the entire test required. The sera were aspirated and divided into two aliquots in plastic tubes and stored. The first aliquot stored at -20°C for assaying growth hormone and leptin. The remaining aliquot stored at +2°C for the following tests:

a. Glucose, urea, creatinin and albumin. These tests were carried out in order to exclude those suffering from diabetes or renal failure.

b. Lipid profile test included total cholesterol (TC), triglycerides (TG), high density lipoprotein(HDL).

Serum glucose and creatinine were determined using kits supplied by Randox laboratories Ltd. The kit used for urea measurement was from Atlas medical.

Albumin on the other hand was measured spctrophotometrically using a kit supplied from Giesse diagnostic-Italy.

Measurement of TC, HDLC and LDL were done by kits from Biomaghrib-France. the enzymatic method was adopted for the measurement of TG using a kit from Teco diagnostics.

Measurements of human growth hormone: the enzyme-linked immunoassay method

(ELISA) was employed using a kit from Biochek HGH enzyme immunoassay.

Leptin (sandwich) enzyme immunoassay kit provided material for the quantitative determination of leptin in serum.

Body mass index (BMI) was calculated at weight (kg) divided by the square of height (m^2) this measurement was performed according to the standardized procedures[20].

All subjects were weighed by the same scale and bare footed. Height was measured using measuring tape.

Person correlation coefficient (p) was used to test the relationship between two parameters.

t-test was used to compare the means of the parameters between the groups.

Results:

Tables 1 and 2 show mean±SD of studied parameters of age and weight groups.

Table 1: Mean±SD of studied parameters in different age groups

	Gr1(25-34y) n=33	Gr2 (35-44y) n=32	Gr3 (45-54y) n=30	G4 (55-64y) n=25
Age (years)	28.5±3.1	40.8±2.3	48.5±2.2	60±3.1
BMI	27.4±5.1	32.9±4.4	33.3±4.9	29.2±5
F.B.S.(mg/dl)	96.2±17.6	112.9±9.8	108±9.9	111±5.3
Urea (mg/dl)	21±3.2	23.6±3.7	28.6±4.7	31.14±6
Creatinin(mg/dl)	0.9±0.16	1.0±0.1	0.97±0.09	1.0±0.1
Albumin(mg/dl)	3.9±0.3	3.7±0.7	3.8±0.3	3.9±0.4
Cholesterol(mg/dl)	123.9±29.1	145.6±33	168.8±1.8	170.8±28.3
TG(mg/dl)	96.5±21.7	120.3±17.2	121.2±10.6	129.61±6.0
HDL(mg/dl)	44.9±3.8	42.8±3.8	41.7±3.1	41.2±3.1
LDL(mg/dl)	32.5±20.5	53.4±16.7	55.2±10.2	62.3±14.6
VLDL(mg/dl)	19.3±4.3	24.1±3.4	24.2±2.1	25.9±3.2

Table 2: Mean±SD of studied parameters in different weight groups

	Group1 (20-25) n=17	Group2 (26-29) n=29	Group3 (30-) n=74
Age (years)	34.9±14.3	42.9±11.2	45.5±10.3
BMI	22.5±2.4	28.07±1.2	34.9±4.6
F.B.S.(mg/dl)	84.2±11.8	98.6±11.3	114.7±8.9
Urea (mg/dl)	22±5.2	23.6±5.4	27.4±5.1
Creatinin(mg/dl)	0.89±0.12	0.93±0.12	1.0±0.12
Albumin(mg/dl)	3.7±0.34	3.94±0.31	3.9±0.36
Cholesterol(mg/dl)	123.2±19.6	132.3±23.86	163.8±32.6
TG(mg/dl)	94.7±15.8	101.7±14.5	126.3±16.88
HDL(mg/dl)	46.05±1.85	45.1±2.8	41.1±3.37

LDL(mg/dl)	18.9±3.17	20.3±2.9	25.3±2.27
VLDL(mg/dl)	29.7±12.9	36.3±12.9	59.9±15.88

Tables 3 and 4 show mean±SD of serum growth hormone and serum leptin concentrations and risk factor cholesterol/HDL in different age and weight groups.

Table 3: Mean±SD of serum GH, leptin and risk factor of cardiovascular disease in different age groups

	Gr1 (25-34y) n=33	Gr2 (35-44y) n=32	Gr3 (45-54y) n=30	G4 (55-64y) n=25
Age (years)	28.5±3.1	40.8±2.3	48.5±2.2	60±3.1
BMI	27.4±5.1	32.9±4.4	33.3±4.9	29.2±5
GH (ng/ml)	6.9±0.5	5.9±0.49	3.9±0.53	3.0±0.65
Leptin (ng/ml)	19.2±7.9	23.5±7.6	24±7.5	24.0±8.0
Cholesterol/HDL	3.46±1.2	3.49±1.1	4.04±0.62	4.22±0.91

Table 4: Mean±SD of serum GH, leptin and risk factor of cardiovascular disease in different weight groups

	Group1 (20-25) n=17	Group2 (26-29) n=29	Group3 (30-) n=74
Age (years)	34.9±14.3	42.9±11.2	45.5±10.3
BMI	22.5±2.4	28.07±1.2	34.9±4.6
GH (ng/ml)	6.0±1.7	5.4±1.5	4.8±1.6
Leptin (ng/ml)	12.8±6.4	13.0±6.9	21.5±9.5
Cholesterol/HDL	2.7±0.52	3.0±0.64	4.05±1.03

The comparison between age groups show a significant increase ($p<0.05$) in BMI of group aged between (45-54) years old when compared with aging group between (25-34) years old in addition the significant difference ($p<0.05$) between age group (45-54) years old and (55-64) years old while no significant difference can be seen between other aging groups when they are compared with each other.

In these tables it can be seen that growth hormone level decreases with age but there is a significant decrease ($p<0.05$) in age group (45-54) years old and (55-64) years old when compared with age group (25-34) years old.

Also the results show an increase in leptin level when age is increased.

The risk factor cholesterol/HDL of the cardiovascular disease seen to be increased with aging process.

Table 5 shows the correlation between different weight groups and some studied parameters

Table 5: Correlation between different weight groups and some studied parameters

	Group1 (20-25) n=17	Group2 (26-29) n=29	Group3 (30-) n=74
GH (ng/ml)	-0.31 n.s.	-0.063 n.s.	-0.207*
Leptin (ng/ml)	0.20 n.s.	0.136 n.s.	0.22*
Cholesterol/HDL	0.063 n.s.	0.155 n.s.	0.58**

*significantly correlated
**highly significantly correlated
Table 6 shows the percentile decline of growth hormone level in different weight groups

Table 6: percentile decline of growth hormone level in different age groups

Aging groups (years old)	GH percentile decline	Incidence of BMI
(25-34) and (35-44)	1%	Overweight and obese weight group
(35-44) and (45-54)	2%	obese weight group
(45-54) and (55-64)	1%	Overweight and obese weight group
(25-34) and (45-54)	3%	Overweight and obese weight group
(25-34) and (55-64)	4%	Overweight group
(35-44) and (55-64)	3%	Overweight and obese weight group

In this table:

No percentage difference of GH level decline for each 10 years different, facing Overweight and obese weight group.

Percentage differences of GH level decline is more for each 20 years different facing Overweight and obese weight group.

Percentage differences of GH level decline is more for each 30 years different facing Overweight group.

Table 7 shows the percentile decline of growth hormone level in different weight groups

Table 7: percentile decline of growth hormone level in different weight groups

BMI	GH percentile decline	age difference
Lean and overweight group	0.6%	8 years
Overweight and obese group	0.6%	3 years
Lean and obese group	1.2%	11 years

In this table:

No difference between percentage declines of GH facing 8 years difference, the same result facing 3 years difference

Percentage difference of GH level decline in obes group was 1.2% which is more than overweight and lean group facing 11 years

Discussion

Growth hormone and leptin are two hormones that seem to have interrelated functions.

Growth hormone is a hormone responsible for normal body growth and development by stimulation protein production and energy release from breakdown of fat.

On the other hand leptin, the newly discovered hormone is synthesized exclusively in the adipose tissue and is enclosed by *ob* gene, decreases food intake by causing satiety and promote energy composition.

In this study a decrease in the level of human growth hormone with progression of age is noted. This decrease could be due either to a loss of growth hormone receptors or the known decrease of growth hormone secretion with age which is related to the increase in somatostatin hormone concentration[21] .

Meanwhile it was observed an increasing level of serum leptin hormone with the advancement of age. As aging is usually associated with increase adiposity in human and animals[22] . Increasing body fat with age is probably multifactorial. It has been postulated that changes in body composition with age could partially be the result of insensitivity of the *ob* gene product leptin[23] .

The mechanism underlying this insensitivity to leptin is not known. However one potential mechanism is reduced leptin transport across blood brain barrier[24].

This is supported by previous observations indicating that aging is associated with a host of structural and physiological changes of the blood brain barrier[25] , keeping in mind that one of the potential determinants of blood brain barrier of leptin is the plasma leptin binding properties. However although increasing plasma leptin levels with age correlate with increased body weight and adiposity, the rate of change in leptin level with age is higher than the rate of change in body adiposity.

At the same time although some authors consider obesity as an independent risk factor for cardiovascular disease, many others believe that increased body fat contributes to the other risk factors that ultimately lead to heart diseases (table 3). In this table there is a highly significant increase in cholesterol /HDL in progression of age. This risk factor is most closely associated with high serum leptin level and with low HDL-cholesterol levels and hypertension[8,26].

Table 4 shows a highly significant increase in leptin hormone level accompanied by a highly significant decrease in growth hormone concentration with increasing weight. The common form of obesity in humans are associated with high levels of circulating leptin and probably with reduced leptin transport through the blood brain barrier and /or reduced action at the hypothalamic receptor level[24].

As leptin had been shown to be an important mediator of the functioning of the somatotroph axis[16] a logical conclusion is that leptin may well be the signal to the human hypothalamus through which excess adipose mass inhibits growth hormone secretion.

This working hypothesis is coherent with the reports published on leptin values and growth hormone secretion in some disease states and experimental models[27] and with stepwise regression analyses indicating that leptin has a significant negative effect on growth hormone secretion[28].

However this hypothesis has not been rigorously addressed until now, as in humans it is not possible to dissociate obesity from leptin levels as they are severely elevated in obesity and correlate with both the percentage of body fat and BMI[29].

Furthermore, changes in some somatotrophs activity normally precede the variations in leptin levels.

The decline in growth hormone with age appears to be independent of body composition, therefore the related fall in growth hormone secretion, or somatopause, may be causal in determining the changes in body composition with age. This statement in agreement with numerous studies that had shown association between increasing age and declining growth hormone secretion[28]. Therefore these data support the hypothesis that growth hormone production regulates

body composition and age-related changes in body composition are a consequence of a fall in the growth hormone concentration with age.

The presence of a positive correlation of risk factor with age, can be explained by numerous factors, the most important factor is the change of metabolic hormones (growth hormone, cortisol and thyroid hormones) as well as sex hormones especially estradiol hormone.[30]

Leptin on the other hand is expressed in the adiposites; both its expression and its secretion are highly correlated with body fat and adiposite size. This inability of such elevated leptin levels to alter the obese state of subjects may be related to "leptin resistance" which is the inability of leptin to enter the cerebrospinal fluid to reach the hypothalamus rejoin that regulate appetite , or it may simply reflect the large amount of tissue in the body[28].

In table 5 the correlation between studied hormones show that leptin concentration is modulated by weight gain and loss in adult humans this finding lead to the hypothesis of resistance to leptin activity at the level of the hypothalamus resulting in increased appetite and decreased energy expenditure despite leptin production in the adiposite in which its increasing level caused decrease in growth hormone concentration[25].

Finally the findings are in disagreement with those of Ahima. and his associates[31] who suggest that serum leptin concentration in humans gradually decline during aging. The aging –related reduction is higher in women than in men but it is independent from BMI and other hormones.

The inclusion of several hormones in a regression model shows that only testosterone in men and estradiol and dihydroxyethyl stebesterol (DHEAS) in women were independent contributors to severe leptin levels, possibly accounting for part of the leptin dimorphism[32].

None of the hormonal parameters studied abolished the negative correlation between leptin and age indicating that the age-related reduction in leptin is independent from other major endocrine changes.

In tables 6 and 7 obese group had 1.2% decline in GH level while for 20 and 30 years difference there was 3% and 4% decline in GH level respectivle. This percentage shows that GH level decline is affected by age rather than BMI.

Conclusion

In conclusion the increase in leptin hormone level which was observed in elderly age group and obese weight group suggest that the decrease in growth hormone concentration is related to obesity in general and to the aging process in particular, as there was no change in GH level beloww 10 years difference while a significant decline in level was obsereved above 10 years difference.

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