Exercise Induced Growth Hormone And The Effect Of Age And Oxidative Stress Parameters In Iraqi Subjects.

Hedef D. El-Yassin, * Ph.D. Post Doctorate

Summary:

Background: *Exercise is a potent stimulus for growth hormone (GH). Age and oxidative stress are associated with a diminution of GH secretion.*
Aim: *The aim is to explore the changes that occur in such parameters with exercise in an attempt to determine which one is more important for growth hormone production in order to set a milestone for overcoming such a cause in the future. This is one of many steps in trying to improve the life quality and style in elderly subjects.*
Subjects and methods: *Sixty healthy Iraqi subjects were enrolled in this study. Divided into three groups according to age, group I (age range 20-29), group II (age range 30-39), and group III (age range 40-49). They were asked to exercise according to modified Bruce protocol. Blood samples were taken from each subject pre and post exercise for biochemical tests. The test included were GH, creatin kinase, uric acid, malonedialdehyde (MDA) , lipid profile (triglycerides, cholesterol, high density lipoprotein HDL). Low density lipoprotein LDL was calculated.*
Results: *The same trend of having high triglyceride level had been shown in all groups in males and females before and after exercise and decrease after that. However the level is lower in the age group 20-29 and increases with increasing age. Same can be said about cholesterol. But HDL has a plateau pattern in all three groups but it decreases with increasing age. LDL on the other hand shows a significant decrease after exercise in both males and females in the small age group. Such a behavior can also be observed in the two other age groups but with less significancy. The behavior of MDA and CK is the same in all age groups with a significant increase after exercise.*
One characteristic important finding is that the level of growth hormone in the females shows a significant increase after exercise in all age groups when compared with males although its level is less in the higher age group than in the lower. Mean serum levels of uric acid showed an elevation after exercise from its basic level before exercise. A significant difference was found between group I and group III.
Conclusion: *In conclusion regular exercise increases the secretion of growth hormone which in turns leads to an increase in physical fitness thus delays aging process despite the oxidative stress process that occur during these events. These conclusions have important clinical implications if we are to prevent the frailty and morbidity associated with old age.*

Introduction:

Growth hormone which is an anabolic polypeptide, is secreted by the anterior pituitary gland. Secretion is usually increased gradually during childhood reaches a peak level during puberty and then declines during adult life [1]. The progressive fall in growth hormone secretion is usually associated with somatic changes that occur as part of the aging process [2, 3]. The mechanisms behind this fall in secretion are still unclear.

Exercise is the most potent physiological stimulator for the secretion of growth hormone[4] It increases within a few minutes of onset of exercise and remains elevated for up to 30 minutes during recovery[5]. A maximal response is usually achieved at 70% of the maximal oxygen uptake (VO_2 max). Many factors can affect the magnitude of this rise, among them hypoxia, change in body temperature and availability of energy substrate [6, 7].

Chemically, growth hormone is somewhat similar to insulin although it is secreted in short pulses during the first hours of sleep and after exercise [8]. As it remains for only few minutes in circulation, it is extremely difficult to measure it in blood stream. However, the body binds most of the growth hormone in the liver and converts some of it into somatomedine–C, another protein hormone also called insulin-like growth factor-1. somatomedine–C which remains in the blood stream for 24 hours[9].

Obesity is associated with a blunted response to all stimuli of growth hormone and that significant weight loss is accompanied by the restoration of normal growth hormone reaction. Severity of the secretory defect is proportional to the degree of obesity [10].

* *Department of Physiological Chemistry College of Medicine, University of Baghdad Bab al-Muadum, Baghdad, Iraq*

In this study growth hormone response to maximal exercise test was examined together with lipid peroxidation and uric acid as an anti oxidant in young and older Iraqies. The aim was to explore the changes that occur in these parameters according to age in an attempt to determine whether aging or oxidative stress were the more important determinants of growth hormone production so as to set a milestone in overcoming such a cause in the future.

Subjects and methods

Subjects

Sixty healthy Iraqi subjects were enrolled in this study. They were categorized according to their age, into three groups. Group I included subjects of age range 20-29, while group II included subjects of age range between 30-39, and the last group (group III) was consisted of subjects with age range 40-49 years old.

All subjects were volunteers selected mainly from students of the college of medicine university of Baghdad (post and undergraduate) and other volunteers. They were thoroughly examined by a physician to exclude any unhealthy subjects. All were asked to have a good night sleep and not to do any physical activity prior to the experiment.

Anthropometry

Height was measured to the nearest centimeter using a measuring tape while weight was measured using (Berkel weight measuring device) BMI was calculated from height and weight using the formula wt (kg/ht(m^2)[11].

Exercises protocol

The Bruce (modified) protocol [12] for treadmill testing was applied in this study as shown in the following table:

Table (1): Modified Bruce Protocol

Stage	Speed km/h	Grade %	Time/min
1	2.7	10	3
2	4	12	3
3	5.4	14	3
4	6.7	16	3
5	8	18	3

Blood samples

Venous blood samples were collected from all subjects 5ml. aspirated prior to the exercise test and similar amount after the exercise. Blood samples were withdrawn by vein puncture from each subject using disposable plastic syringe. The blood was allowed to coagulate at room temperature and then centrifuged for 10 minutes at 2500rpm at 4°C. The serum was stored at -20°C until analysis. The biochemical parameters studied included serum growth hormone level, serum albumin, uric acid, creatine kinase, malonedialdehyde (MDA) and lipid profile (cholesterol, triglycerides, high density lipoprotein HDL, low density lipoprotein LDL and very low density lipoprotein VLDL).

Biochemical assay

Human growth hormone measurement Serum growth hormone level was measured by enzyme immunoassay. The optical density was read at 450 nm. Kits were provided by BioCheck Inc.

Uric acid measurement

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide, measured at 510nm at 37°C.Kits was supplied by Biomaghraeb.

Creatine kinase (CK) measurement

The activity of CK in serum was determined by the increase in absorbance at 340 nm pH 6.6 at 30°C.

Lipid profile

Measurement of Triglycerides (TG)

Triglycerides in the serum were determined by the enzymatic method. Quinoneimine is a red-colored dye that can be measured colorimetrically at 500nm. Kits were supplied by BioMérieux Company -France.

Measurement of Total Cholesterol (TC)

Serum TC was determined by enzymatic method. The quinoneimine dye (chromogen) production is directly proportional to the concentration of total cholesterol in the original sample, at 500nm. Kits used were from BioMérieux Company –France.

Measurement of High Density Lipoprotein Cholesterol (HDL-C)

Serum HDL-C was determined by precipitation with phosphotungstate-$MgCl_2$ solution followed by enzymatic method, for determination of cholesterol in the supernatant. Kits were supplied by BioMérieux Company –France.

Estimation of Very Low Density Lipoproteins Cholesterol (VLDL-C) and Low Density Lipoproteins Cholesterol (LDL-C)

Serum level of LDL-C was calculated by Friedwald formula [13]. Friedwald, Levy and Friedrickson in 1972 had postulated a formula to calculate LDL-C value, which was based on the assumption that VLDL-C is present in serum at a concentration equal to one fifth of the triglyceride concentration.

Therefore:

$$\text{LDL-C} = \text{TC} - [\text{HDL-C} + \text{VLDL}]$$

$$\text{LDL-C} = \text{TC} - [\text{HDL-C} + \text{TG}/5]$$

The formula is only valid at serum triglyceride concentration of less than 400mg/100ml.

Statistical analysis:

All statistical analysis have been made by applying the excel programm 2002 (10.2614.2625), t-test was applied to estmate the significancy. P value was taken to the closest ($P<0.05$).

Results

Figure (1) represents the GH level in all three groups male and females' pre and post exercise. Although GH secretion is induced after exercise, this induction declines with increasing age, as can be observed in figure (2).

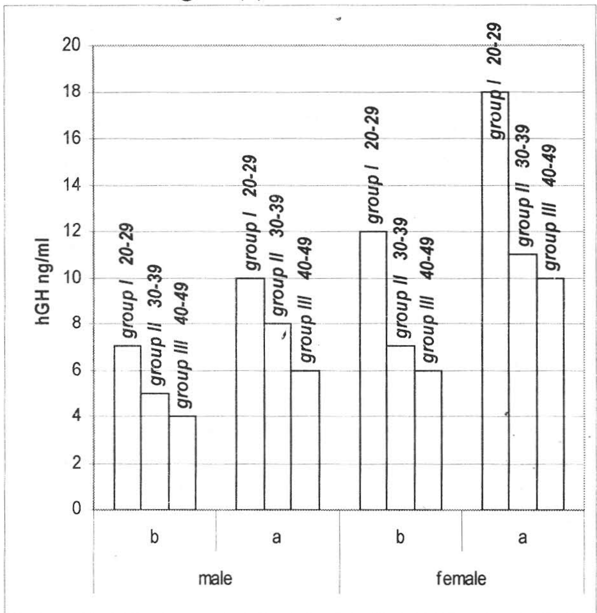


Figure (1): Serum GH level in all three groups male and females' pre (b) and post (a) exercise.

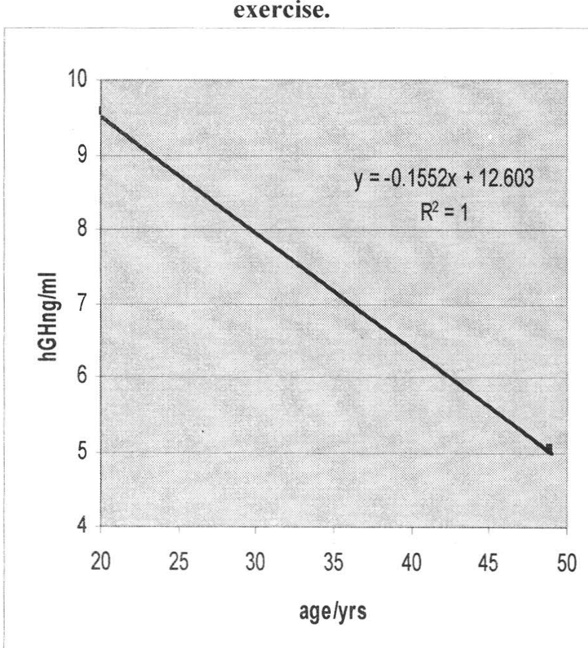


Figure (2): Correlation of GH with age.

Ongoing oxidative stress induced by maximal exercise was analyzed by measurements of antioxidants which are the defense mechanisms against such stress.

Mean serum levels of uric acid are shown in figure (3). As shown there is an increase in mean uric acid level after the exercise from its level before exercise. A significant difference was found between group I and group II.

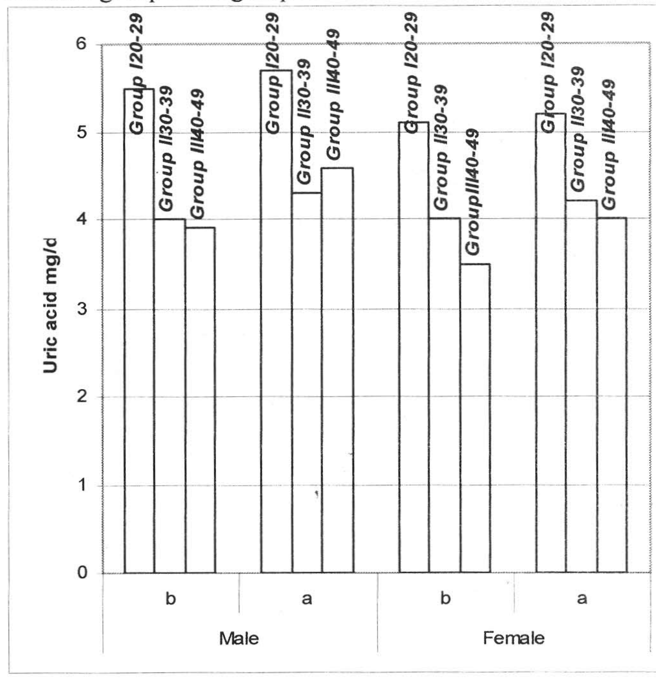


Figure (3): Serum uric acid level in all three groups male and females' pre (b) and post (a) exercise.

Membrane integrity is thought to be compromised by oxidative stress and is evaluated through the measurement of creatine kinase. Results are shown in figure (4)

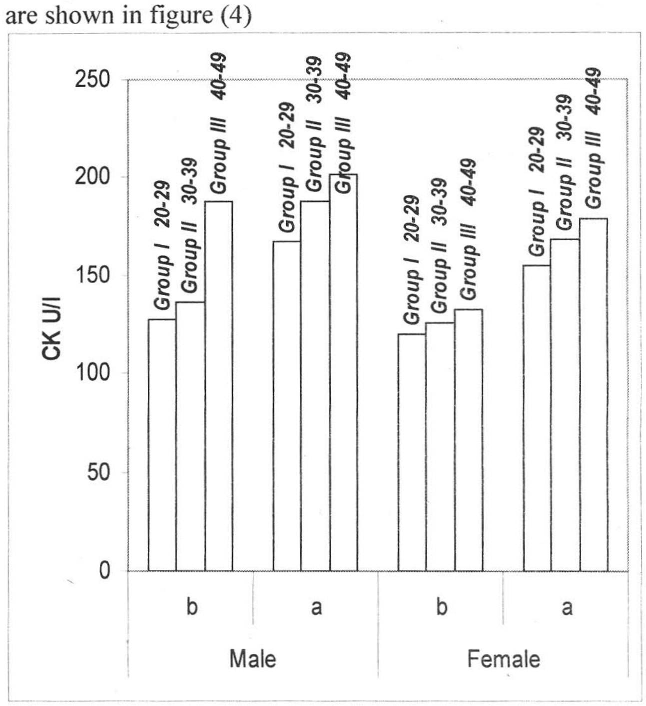


Figure (4): Serum CK level in all three groups male and females' pre (b) and post (a) exercise.

Mean value of triglyceride levels (TG) decreased significantly in all age groups after exercise as shown in figure (5). The pre and post exercise vales showed a linear positive correlation with increasing age being in female less than in male.

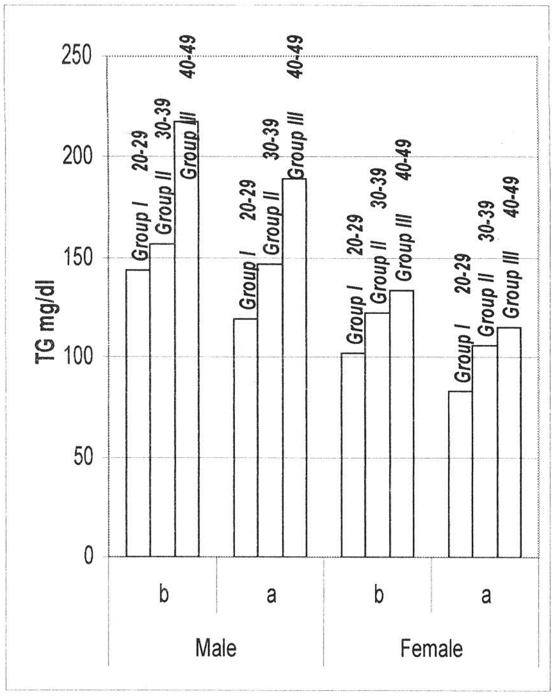


Figure (5): Serum TG level in all three groups male and females' pre (b) and post (a) exercise.

It was found that mean total cholesterol concentration values decreased after exercise in all age groups as shown in figure (6).

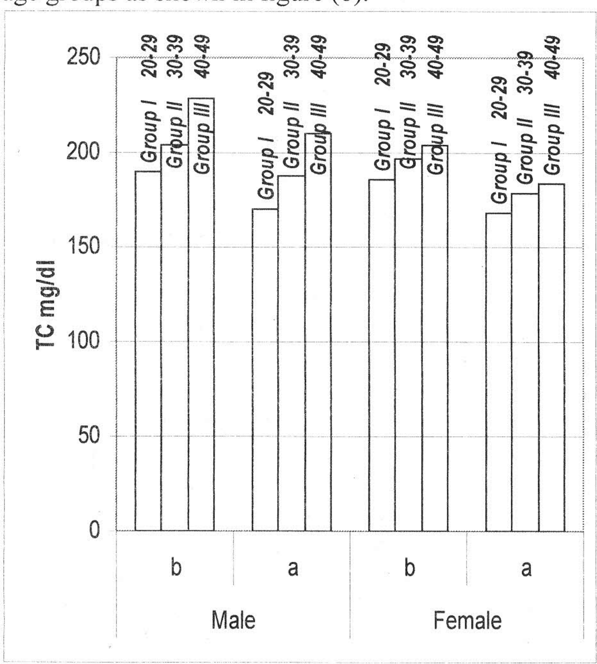
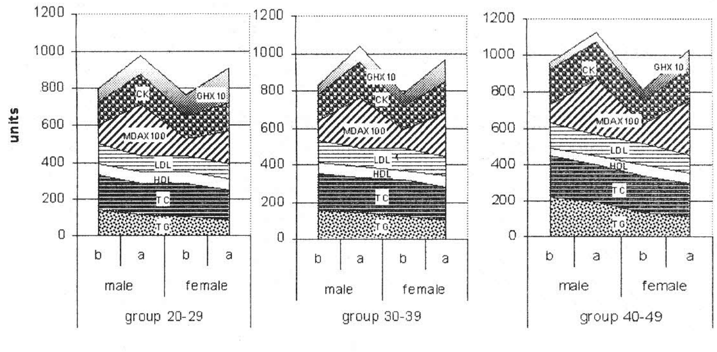


Figure (6): Serum TC level in all three groups male and females' pre (b) and post (a) exercise.

The studied parameters could all be compared as shown in figure (7).

Figure (7): Comparison of all the studied parameters in the three groups, pre (b) and post (a) exercise.



This figure compares the values of all the biochemical parameters studied in this work (in form of area under the curve) in all three groups before and after exercise. The figue shows the same trend of having high triglyceride level in all groups in males and females before and after exercise and decrease after that. However the level is lower in the age group 20-29 (group I) and increases with increasing age. Same can be said about the cholesterol. But looking at the HDL area in the figure one can postulate that it has a plateau pattern in all three groups but it decreases with increasing age.

LDL on the other hand shows a significant decrease after exercise in both males and females in the small age group. Such a behavior can also be observed in the two age groups but with less significancy. Although the area under the curve is small in the small age group and increases with increasing age.

The behavior of MDA and CK is the same in all age groups with a significant increase after exercise. The area under the curve increases with increasing age.

One characteristic an important finding is that growth hormone in the female shows a significant increase after exercise in all age groups when compared with males although the actual area under the curve is less in the higher age group than in the lower.

Discussion

Human growth hormone is manufactured by the pituitary gland in the brain. This vitally important hormone is essential to natural cell repair and growth [14].

For reasons unknown to medical science, after the age of 35 the body continues to manufacture GH but releases less of it. Its decline is more rapid between adolescence and age 40-45 years old. The decline continues at a slower rate in later life [15]. As weight increases GH secretion decreases independent of age. However it has been shown that GH production declines significantly with age and this variable is more important in determining the response than adiposity [6].

In this study we compared the effect of exercise on the induction of GH and antioxidants in different age groups.

As shown in figures (1), (2) and (7) the of elevation of GH after exercise declines with increasing age. This matches the idea that GH declines with age. Numerous studies have shown associations between increasing age and declining GH secretion [16] .In a recent study concerning this matter it was confirmed that increasing age was associated with a decline in VO_2 max. This is a surrogate marker for fitness and therefore supports the hypothesis that the decline in GH production with age is associated with a decline in physical fitness.

Furthermore it remains unclear whether the decline in GH reflects the effects aging itself or the influences of age-associated changes in lifestyle.

Uric acid is one of the most important secondary antioxidant chosen to be studied in this work. As shown in figure (3) and (7) there was a significant increase in mean uric acid concentration post exercise in all age groups being in females less than in males. Stress caused by exercise enhances the catabolism of ATP to hypoxanthine by virtue of the enzyme xanthine oxidase, this is further converted to uric acid in the process. Free radicals including the toxic superoxide and hydroxyl radicals are produced and cause muscle damage. At the same time uric acid that accumulates in cells leaks or secrets out of the cells causing a significant increase in plasma level [17].

In figures (4) and (7) there is a positive linear correlation between CK level and age i.e. CK level increased with increasing age. Older adults experience greater muscle damage following exercise than younger subjects being in female less than in male, this is may be due in part to the smaller muscle mass and lower VO_2max. Similar findings were reported by Manfredi TG et al.[18]

Mean value of triglyceride levels (TG) decreased significantly after exercise in all age groups as shown in figures (5) and (7), due to intramyocellular triglyceride hydrolysis during exercise. The pre and post exercise values showed a linear positive correlation with increasing age being in female less than in male.

Similar findings have been reported, this may be attributed to age related hyperlipidemia due to decreased rate of removal from the plasma.

Mean value of total cholesterol levels (TC) decreased in all age groups after exercise as shown in figures (6) and (7).

In figure (7) there is a decrease in lipid profile after exercise when compared to that before exercise in all age groups. This trend is typical for short-term lipid modifications that occur after a single session of aerobic exercise. This might be due to increase in lecithin cholesterol acyltransferase[19,20]. It also be due to the fact that exercise induces modification in hormonal factors promoting lipid mobilization during exercise, plasma catecholamines, growth hormone, cortisol levels increased while insulin decrease[21]

Figure (7) also shows an increase in mean MDA concentration values significantly after exercise in all age groups. After short time resistance exercise there becomes an increase in mobilization of fat-soluble antioxidants despite mobilization of antioxidants, oxidative stress occurs during submaximal exercise which was indicated by increased MDA concentration [22].

Although the behavior of lipid profile and lipid peroxiadtion is the same in all age groups. But it is clear that the extent of variation in these parameters is different between these groups. As shown in figures (5, 6 and 7) the levels of triglycerides and cholesterol are higher in group III than in group I and II. Age related hyperlipidemia could be explained by either increased lipid production and /or decreased removal from the plasma. DeSchrijver (1990) proposed that a change in cholesterol fraction carried by different lipoprotein fractions could explain such age related hypercholesterimia. Alterations in metabolism in these lipoprotein macromolecules are accompanied by a decrease in rate of lipoprotein cholesterol estrification [23].

While successive increase in MDA formation with increasing age (figure 7) provides evidence for accumulation of an oxidatively damaged lipid components with age [24].

Figure (7) points out a very important finding in which GH levels are increased post exercise in females more than in males in all age groups studied. This is most likely due to estradiol. Estrogen is thought to increase growth hormone secretion by increasing sensitivity to pulsatile secretion of growth hormone –releasing hormone [25].

In conclusion regular exercise increase the secretion of growth hormone which in turns leads to increase in physical fitness thus delays aging process despite the oxidative stress process that occur during these events. These conclusions have important clinical implications if we are to prevent the frailty and morbidity associated with old age.

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