Probable Protective Effect of Exogenous Administration of Growth Hormone on Age-induced Hypercholesterolemia in Rats.

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Abstract

Background: Aging is an inevitable biological process accompanied by many metabolic changes such as hypercholesterolemia that increased risk of cardiovascular disease in elderly. It was reported that growth hormone GH substitution contributes to dyslipidemia improvement by modulating expression of LDL R. To our knowledge, no clarified distinguished mechanisms have been published yet. Objectives: To test the hypothesis that GH would improve age induced hypercholesterolemia by over expression of LDL-R m RNA . Also, to distinguish its effects on the inflammatory response and the oxidative state that may clarify the mediating mechanisms. Material& method: thirty male albino rats were divided into three groups (10 rats each): adult control, aging group, GH aged treated groups. At the end of experiment, retro-orbital blood samples were taken for estimation, lipid parameters and serum amyloid a then liver were removed for measurement of tissue catalase and LDL-R mRNA real time. Results: The present study revealed that aging worsened lipid profile. Fortunately, GH substitution leads to lipid profile improvement. These metabolic changes might assert to oxidative state and inflammatory response improvement accompanying apparent LDL-R mRNA real time. **Conclusion**: GH supplementation can positively modulate the lipid profile in aging through modifying effect on the inflammatory response, anti-oxidant state and over expression of hepatic LDL-R . The clinical inferences of these results require further investigations.

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Introduction

Aging has been defined as the series of the deteriorative changes occurring during the adult period of life [1]. Aging is characterized by the loss of homeostasis allover human body [2],that leads to changes in the biochemical composition of tissues [3,4], and increased susceptibility and vulnerability to diseases[5].

Aging without disease is important to improve personal QOL (quality of life) and for of social and economic development. This requires development of a better understanding of senescence [6].

A number of metabolic changes occur with normal aging in both animals and humans [7]. Among these, phenomena such as reduced physical activity, decreased oxygen consumption, redistribution of body tissues with a relative increase in adipose over muscle mass, decreased insulin sensitivity, and increased blood pressure may all contribute to the hypercholesterolemia known to occur with age [8,9,10]

Cholesterol metabolism is profoundly modified during normal aging , and in humans plasma LDL-cholesterol increases by about 40% from 20 to 60 yr of age. The physiological mechanism for this age-dependent change in plasma cholesterol is still unclear.[11]

The physiological mechanisms for this age-related dyslipidemia are incompletely characterized. Some evidence demonstrates that the causes of age-related disruption of lipid homeostasis include the gradual decline in fractional clearance of LDL with increasing age, the progressively reduced ability to remove cholesterol through conversion to bile acids, and the decreased activity of the rate-limiting enzyme in bile acid biosynthesis, cholesterol 7α-hydroxylase (C7αOH) [12].

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyzes the decomposition of hydrogen peroxide to water and oxygen [13]. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) [14]

Relationship between aging and oxidative stress has been discussed for several decades. The free radical theory of aging, which is widely accepted, proposes that oxidative stress plays a key role in the aging process [15].

Oxidative stress occurs when there is an excess of pro-oxidants over antioxidants and it has been implicated in several diseases. Catalase is involved in hydrogen peroxide catabolism and is important in defense against oxidative stress. In summary, inherited catalase deficiency is associated with clinical features, pathologic laboratory test results, age and oxidative stress related disorders. Rather than considering it a benign condition, it should be considered as a complicating condition for aging and oxidative stress [16].

Acute-phase serum amyloid A proteins (A-SAAs) are secreted during the acute phase of inflammation. These proteins have several roles, including the transport of cholesterol to the liver for secretion into the bile, the recruitment of immune cells to inflammatory sites, and the induction of enzymes that degrade extracellular matrix. A-SAAs are implicated in several chronic inflammatory diseases [17]

In the present study, we aimed to evaluate the protective effects of growth hormone therapy in aging with emphasis on lipid metabolism

dysfunction by age trying to elucidate the mechanism(s) by which growth hormone may execute its protective effect and the probable action of catalase and SAA-A effect.

Materials and Methods

Animals and Experimental Design

The study procedures were conducted in adherence to the Guiding Principles in the Use and Care of Animals published by the National Institutes of Health (NIH Publication No 85–23, Revised 1996). Animal care and use was approved by the University Experimentation Ethics Committee. Animals were kept for 10 days prior to the start of the study to allow proper acclimatization. Male Wister albino rats about 6 and 22 months old were used . The animals were housed at 20-24°C with a 12-h light, 12-h dark cycle and they were provided with standard laboratory rat chow and tap water freely available.

Rats were divided into three groups (n = 10) as follows; Adult Control : Rats in this group were about 6 months old and received standard rodent diet. Aging: Rats in this group were about 22 months old and received standard rodent diet. Aged treated GH : Rats in this group were 22 months old , received standard rodent diet and s.c. injected with GH (Somatropin 4 IU SEDICO Pharmaceutical Co-Egypt) twice daily at a dose 1 mg/kg [18] for 6 weeks. Untreated animals were injected with saline twice daily for 6 weeks.

At the end of the experiment (after 6 weeks), animals were fasted overnight and blood samples were collected via cardiac puncture after rats being anaesthetized. Blood samples were left for clotting

for 10 min and centrifuged at 4000 rpm for another 10 min to isolate the serum and kept at -20^0C for further analysis of Lipid profile and Serum Amyloid A. Lastly, rats of all groups were sacrificed by decapitation and the livers were excised and kept at -80^0C for estimation of catalase and PCR of LDL receptors mRNA.

Serum Biochemical Analysis

Lipid profile

Total cholesterol (mg/dl) and HDL-C (mg/dl) levels were determined following their hydrolysis and oxidation to yield colored quinoneimine derivatives using test reagent kits (Biodiagnostic, Egypt). Triglycerides (TGs) level (mg/dl) was estimated by a reagent kit (EMAPOL, Poland), in which TGs were hydrolyzed with lipoprotein lipase to form glycerol, which forms a complex with H2O2 giving a colored derivative. The obtained levels of total cholesterol, HDL and TGs were then used to calculate the serum level of LDL-C as that described by Friedewald et al.,[19]

LDL = Total Cholesterol - (HDL + Triglycerides/5).

Catalase assay

BY Colorimetric Method **[20].**

Serum Amyloid A

3 ml of venous blood in a plain va container tube , incubated for 15 min , then centrifuge at 3000 rpm for 10 min. Then serum was separated into aliquots for measurement of Serum Amyloid A. Quantitative detection of Serum Amyloid A was carried out using Human Serum Amyloid A ELISA Kits (Sigma- Aldrich) according to manufacture protocol.

Quantitative assay of LDL receptor mRNA expression using reverse transcription polymerase chain reaction technique (Figure 1 , 2).

Tissue samples were prepared for total RNA isolation using Qiagen RN easy plus Universal kit from USA according to manufacturer instructions.
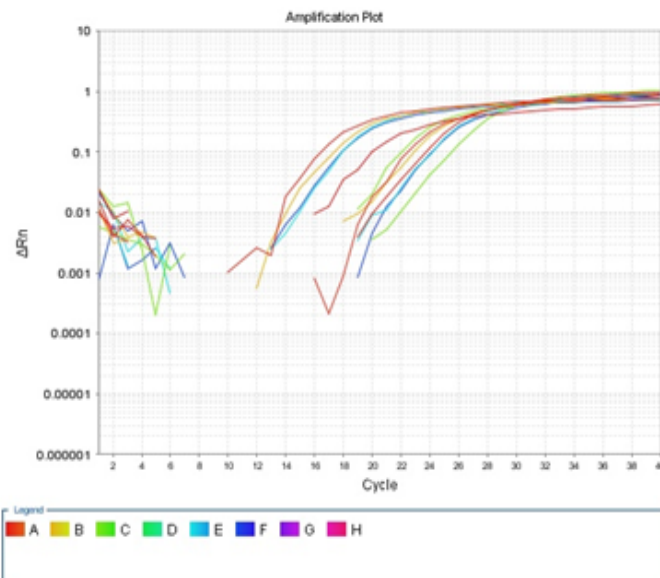

Figure 1 : Amplification plot of LDL receptors mRNA gene expression in liver tissue in studied groups. A typical qPCR amplification plot (normalized florescence signal (Δ Rn) versus cycle number with threshold line to show accumulation of LDL- receptor over duration of real time PCR assay. Amplification curve of LDL-receptor expressed in liver tissue in studied groups, control using SYBR green 12 steps qPCR .
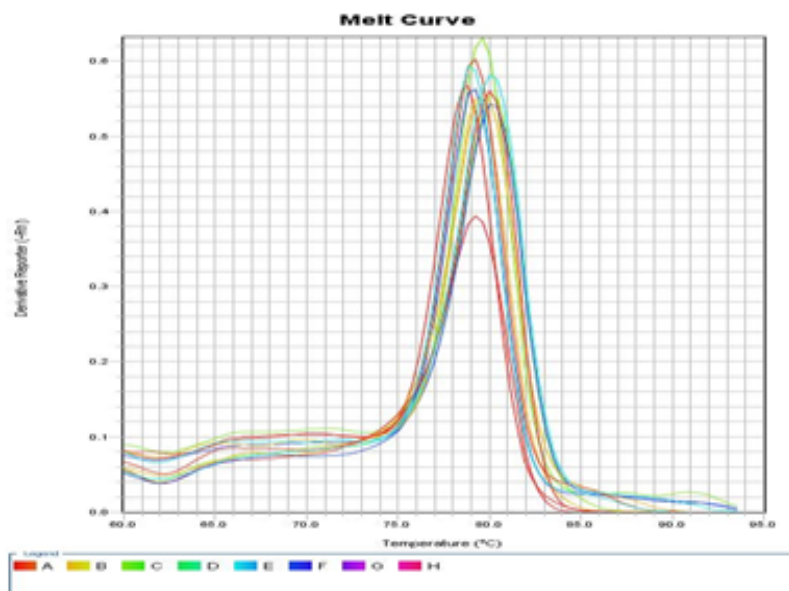
·Figure 2 : Representative melting curve from the assay performed in laboratory representing real time PCR LDL-receptor gene expression in liver tissue in studied groups .This was achieved by incrementally increasing temperature and monitoring the fluorescence as a function of temperature. When the temperature is high enough to denaturant dsDNA, large drop of fluorescence are recorded because the florophore molecule is released .The software of each qPCR instrument calculated the annealing temperature (Tm) from melting curve data by plotting the negative first derivative vs temperature.

Statistical analysis:

The data were tabulated and analyzed by SPSS (statistical package for the social science software) using statistical package version 20 on IBM compatible computer. Quantitative data were expressed as mean $\pm$ standard error of mean (X$\pm$ S.E.M).Data from control and test groups were compared using one way ANOVA, followed by Turkey post Hoc test, Probability value of less than 0.05 was considered as statistically significant (P<0.05)

Results:

In the present study age induced hypercholesterolemia was successfully established in list results of lipid profile & PCR

LDL-R mRNA was significantly increased in aged treated group when compared to the aging - non treated group (0.81+ 0.2 Vs 0.3+ 0.1........ P < 0.05). There was no significant difference in LDL-R m-RNA between aged treated group, adult control group (0.81+ 0.2 vs 0.8+ 0.12 l...P ≥ 0.05). (figure 3).

Serum amyloid-a level was significantly decreased in aged treated group when compared to the aging -non treated group (0.13+ 0.01 Vs 0.22+ 0.01 .... P < 0.05). There was no significant difference in serum amyloid –a level between aging treated group, adult control group (0.13+ 0.01vs 0.15 + 0.01.... P ≥ 0.05). (figure 4).

Serum LDL level was significantly decreased in aged treated group when compared to the aging - non treated group (41+ 6.5Vs. 75.9+0.4 mg/dl P < 0.05). There was no significant difference in serum LDL level between aging treated group, adult control group (41+ 6.5vs 29.7+ 4.1...P ≥ 0.05). (figure 5).

Serum cholesterol level was significantly decreased in aged treated group when compared to the aging -non treated group (93.8+ 2.1Vs 106.8+2.6 mg/dl P < 0.05). There was no significant difference in serum cholesterol level between aging treated group, adult control group (93.8+ 2.1vs 85.1+1.8 mg/dl .P ≥ 0.05). (figure 5).

Serum HDL level was significantly increased in aged treated group when compared to the aging - non treated group (50.1+ 3.3Vs 17.9+1.7 mg/dl P < 0.05). There was no significant difference in serum HDL level between aging treated group, adult control group (50.1+ 3.3vs 74.3+4.2mg/dl .P ≥ 0.05). (figure 5).

There was no significant difference in TG level among aged treated group, adult control and aging-non treated groups (69.6 + 3.1vs 63.8+ 5.8, 94.6+ 4.5 mg/dl .P ≥ 0.05). (figure 5).

Catalase level was significantly increased in aged treated group when compared to the aging -non treated group (49.3 + 3 Vs. 29.7+ 4.1 .... P < 0.05). There was no significant difference in serum catalase level between aging treated group, adult control group (49.3 + 3 vs 82.2+ 10 ...P ≥ 0.05) . (figure 6).
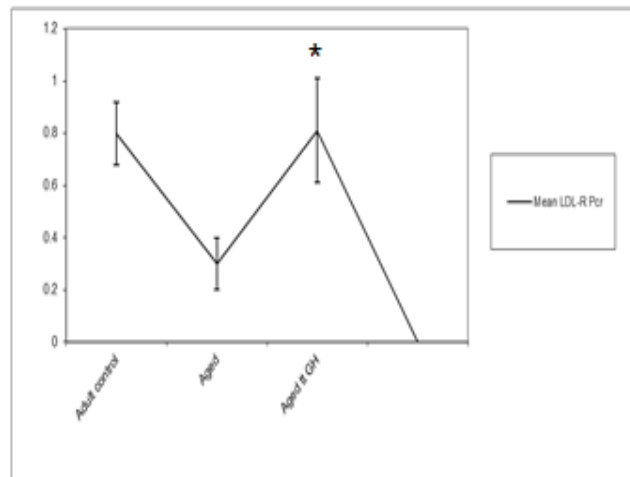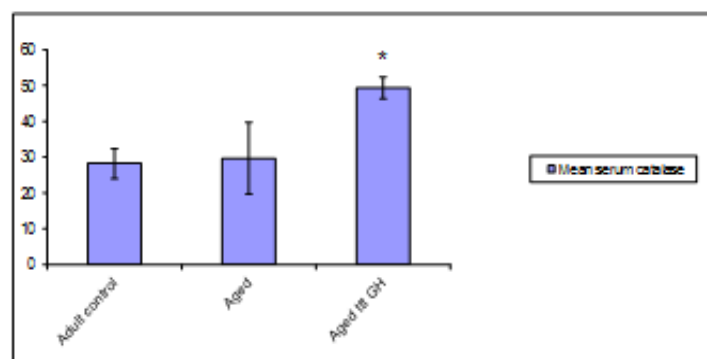
- Figure 3 : PCR real time histogram of LDL –receptors mRNA gene expression in liver tissue of adult control , aged non treated and aged treated with GH groups.
- Data were expressed as mean ±S.E. (n=10). One way ANOVA: *$p<0.05$, vs. aged non treated ; #$p<0.05$, vs. adult control group.
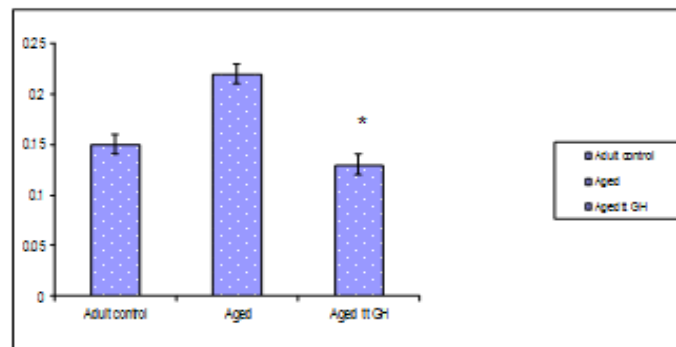
- Figure 4 : Serum amyloid-A level among adult control , aged non treated and aged treated with GH groups.
- Data were expressed as mean ±S.E. (n=10). One way ANOVA: *$p<0.05$, vs. aged non treated ; #$p<0.05$, vs. adult control group.
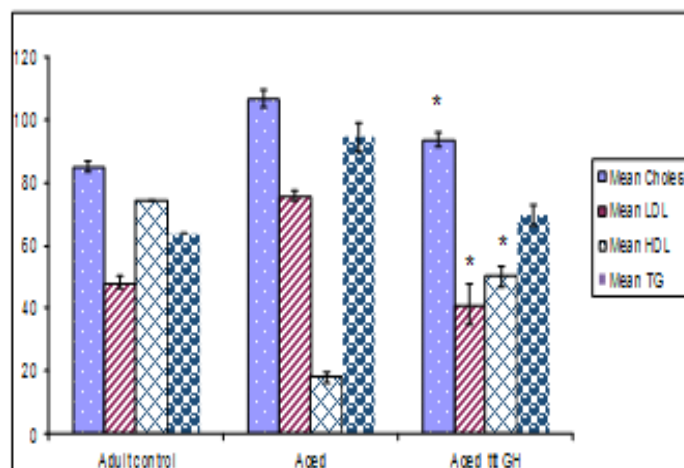
·Figure 5 : Serum LDL , Cholesterol ,HDL and Triglyceride levels among adult control , aged non treated and aged treated with GH groups.

·Data were expressed as mean ±S.E. (n=10). One way ANOVA: *$p<0.05$, vs. aged non treated ; #$p<0.05$, vs. adult control group.

- Figure 6 : Serum catalase level among adult control , aged non treated and aged treated with GH groups.
- Data were expressed as mean ±S.E. (n=10). One way ANOVA: *p<0.05, vs. aged non treated ; #p<0.05, vs. adult control group.

Discussion

The results of present study strongly support the concept that GH has an important role in the physiological regulation of cholesterol which, significantly increased during aging and the LDL-R mRNA level was significantly diminished; suggesting that during aging hypercholesterolemia is caused by reduced hepatic LDL-R gene expression. We showed that GH supplementation of aged rats resulted in a normalization of LDL-R mRNA and this indicates that LDL-R activity is stimulated by GH. The present data confirm and extend previous studies of cholesterol metabolism in the aging rat.[21,22,23].

In the aged rats with growth hormone deficiency, there was a trend to a more unfavourable lipid profile with a higher total cholesterol, atherogenic LDL particle, triglycrides and lower HDL- C . Fortunately there was noticeable improvement seen in the growth hormone treated group, with a significant decrease in the total cholesterol, atherogenic LDL particle (P < 0·0001). Higher HDL-C, unaffected triglycrides when compared to adult control group .

This is in agreement with Liu et al., [24] and Corpas et al. [25], who stated that GH exerts many important effects in the regulation of lipid metabolism. In general, GH increases the flux of energy in the lipid transport system by stimulating lipolysis in adipose tissue, fatty acid assimilation and triglyceride production in the liver, VLDL secretion and catabolism, LDL clearance, and cholesterol excretion. Some of these effects are only observed in GH deficiency where they can be counterbalanced by GH supplementation, whereas supporting the concept that GH is an important hormone in the complex physiological plasma LDL flux in humans' regulation [26]. Refusal of this result by Goldstein and Brown, 2009 increased production of LDL-R rate was not affected by the supplementation of GH and explain that by decreased membrane fluidity[27]. The stiffer membranes might interfere with internalization or recycling of the LDL-R. In such a situation, even a normal amount of LDL-R can result in a less efficient catabolism .

The results of the present study clarified a significant increase of the acute phase protein serum amyloid A (SAA) in aged group [28].

It was reported that, although fluctuations with time for various individuals may occur as a reflection of subclinical infection, inflammation or even malnutrition, no age-related increase in plasma SAA concentrations was observed in healthy subjects [29] . Previous study showed that the variability of A-SAA levels was higher in older subjects [30].

Interestingly, recent proteomic studies provided convincing evidence that chronic inflammation alters the protein composition of HDL thereby generating dysfunctional or even pro-atherogenic forms of HDL by enriching pro-inflammatory proteins such as serum amyloid A (SAA) and complement C3 content in HDL. Therefore, increased SAA and complement C3 content of HDL observed in HDL of elderly subjects suggest that aging-induced alterations in the composition of HDL may play critical roles in the inflammatory response and lipid metabolism [31].

The intimate relationship between cytokines and SAA synthesis and release as well as the SAA/HDL/cholesterol relationships have implicated the role of SAA proteins in atherosclerosis [32]. It was shown that HDL containing SAA bound to proteoglycans from vascular endothelium leading to lipoprotein retention within the vessel wall [33]. Also , it was found that SAA could induce a change in the phenotype of vascular smooth muscle cells from a quiescent and contractile form to a synthetic, proliferative form and that the latter could be important in development and propagation of atherosclerosis [34]

Lastly , the results of the present research showed that the level of catalase in tissue homogenate was significantly decreased with aging.

Previous published reports described age-related changes in the activities of antioxidant enzymes . It was reported that activity of CAT either declined or did not change during aging in livers of male rats[35].

Results reported in a previous study demonstrate that, among a set of age-modified proteins in the liver mitochondrial extract with aging, catalase was the only antioxidant enzyme that was strongly glycated, constitutively present and located in the matrix, as demonstrated by immunogold labeling. So, catalase protein being more susceptible to glycation stress it is appeared to be age-damaged [36].

Insulin-like growth factor I (IGF-I) is a polypeptide hormone produced mainly by the liver in response to the endocrine GH stimulus. It is partly responsible for systemic GH activities plus its own properties as anabolic, antioxidant, anti-inflammatory and cytoprotective [37].

Interestingly, IGF-I is somehow related to all diverse theories of aging [38].Its antioxidant capability was shown by restoring mitochondrial dysfunction (being the main source of endogenous free radicals) during aging and improving antioxidant enzymes activities (superoxide dismutase, catalase and glutathione peroxidase) and parameters of oxidative damage (MDA and PCC) [39, 40].

In conclusion

We showed here that aging worsened lipid profile by significant increase of serum cholesterol and LDL and significant decrease of HDL cholesterol.

This was suggested to be due to significantly diminished hepatic LDL-R mRNA level. Also , reduced GH with aging together with the significant increased acute phase protein serum amyloid a and diminished antioxidant catalase may played an important role in mediating this atherogenic lipid profile. GH could be considered a therapeutic target as its supplementation for aged rats resulted in a normalization of LDL-R mRNA and this together with normalized serum amyloid a and catalase activity had an important improving effect on lipid profile. The present data confirm and extend previous studies of cholesterol metabolism in the aging rat.

Aknoledgement

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