Histological changes induced by exogenous human growth hormone in the thymus of male albino mice.

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Abstract:

It is well documented that hormones of the pituitary gland affect lymphoid organs both structurally and physiologically. This experiment has been conducted to practically confirm the effect of Growth Hormone (a major pituitary hormone) on the histological changes that occur in the thymus (a primary lymphoid organ) in relation to age-related and steroid-induced atrophy.

Male albino mice of 8, 28 and 41 weeks of age were used. Each age group was subdivided into 4 subgroups, two of which as controls while the other two received human Growth Hormone for one and two week periods. The young mice were given high dose Dexamethasone to induce thymic atrophy before starting Growth Hormone treatment. Growth Hormone was given as a single daily subcutaneous injection in the morning.

Statistical analysis of the results and histological examination of the GH-treated groups revealed that GH was effective in restoring histological structure and possibly the function of the thymus following steroid-induced and age-related atrophy. This was evident by the finding of deceleration of fatty infiltration of thymic tissue and maintenance of the clear thymic architecture, increased thymic weight/ body weight ratio and increased numbers of thymocytes and epitheliocytes in cortical and medullary zones.

Keywords: Growth Hormone, Thymus, Age-related / Drug-induced thymic atrophy, Immunity.

JBMS 2006; Volume 6: 65-77

Introduction:

The neuroendocrine and immune systems communicate bidirectionally through cytokines, peptide hormones and neurotransmitters and in this process the receptors can be also shared. In addition to the endocrine actions of GH, this hormone also appears to be involved in the autocrine/paracrine regulatory actions in various immune cells and lymphoid tissues including the thymus [(1)].

GH is considered to be a cytokine because its receptor (GHR) is a member of the haematpoietin /cytokine receptor family. [(2)]

It's been proved that, in addition to the pituitary source; GH can be produced (and recognized) by lymphoid tissues. GH gene expression was detected in the lymphocytes and epithelial / reticular cells thymus of rats [(3)] and humans [(4)].

Expression of GH receptors by human thymic epithelial cells [(5)] & thymocytes [(6)] has been demonstrated in several studies.

Exogenous GH, acting directly and via its active mediators [the insulin-like growth factors (IGF's)], enhances thymic microenvironmental cell-derived secretory products such as cytokines and thymic hormones and increases thymocyte & epitheliocyte proliferation [(7)].

Therapeutic trials of GH treatment in HIV-infected adults resulted in marked increase in thymic mass & circulating naïve CD4 T-cells in all patients during GH therapy, suggesting an enhancement of thymopoiesis [(8)].

Aim of the study: The study tried to histologically confirm that GH is effective in rejuvenating thymic architecture (and hence immune function) following age-related or steroid-induced thymic atrophy.

Materials & Methods:

48 male albino mice were used & were divided into three age groups, 16 mice each:

- Group-A: *(Young age group)* were 8 weeks of age.
- Group-B: *(Middle age group)* were 28 weeks of age.
- Group-C: *(old age group)* were 41 weeks of age.

The animals were kept in the animal house with constant controllable temperature (18-22 °C) and a light schedule of 12-12 hour's light/dark cycle. They were fed a control diet and were allowed to become acclimated before starting the experiment.

Each age group was subdivided into 4 subgroups (4 mice each). All young mice were given high dose Dexamethasone (25mg/kg, single daily intramuscular dose) for 5 days before starting GH treatment to induce thymic atrophy. Afterwards two subgroups were used as controls while the other two were given GH (6.2 mg/kg, single daily subcutaneous dose) for one and two week periods, respectively.

In the middle-aged and old mice GH was given in a similar time schedule but without previous steroid therapy and in different doses (4.2 mg/kg for middle-aged mice, 3.4 mg/kg for old mice).

The control mice were provided with the same type of block of food and injected with 0.9% normal saline. Each control group was sacrificed at the same time of the sacrifice of its corresponding treated group.

Body weight was recorded at the beginning and at the end of the experimental periods. The thymus was removed while the heart was still beating. The gland was separated as far from the surrounding connective tissues and fat as possible by naked eye dissection. After weighing the gland, both lobes were taken and used for standard paraffin section. The thymic volume was estimated by dividing thymic weight by 1.1 which represents the tissue density of the thymus [9]. The tissue was prepared for routine paraffin sections which were dyed with H&E according to Bancroft & Stevens (1987) [10] & examined under a light microscope.

The number of epithelial cells and lymphocytes per mm^2 were calculated by counting their nuclei in the section using Zeiss Integrating Micrometer disc-Turret and an Objective Micrometer Slide and Lens according to the following formula: [11]

$$\mathbf{Ai = Pi} \quad \sqrt{\frac{3}{2}}\, \mathbf{Z^2}$$

Where **Ai** represents the part of total section area belonging to the tissue component **i** (cortex or medulla). **Pi** is the number of points falling on the nuclei of that part and **Z** is the equivalent in mm of the distance between two points on the integrating system.

For each animal, one slide of thymic tissue was used. In each slide, three sections were selected randomly. From each section, ten areas were selected in random: five from the cortex and five from the medulla. The mean of the cell count for every five areas and for all sections in the slide was then calculated and used as the mean of cell counts for the animal.

Cell numbers per mm^3 (numerical density) were calculated according to the following equation: [12]

$$\mathbf{Nv} = \frac{\mathbf{Na}}{\mathbf{(D + t)}}$$

Where **Nv** represents the number of nuclei per mm^3, **Na** is the number of their profiles per mm^2 (as calculated from the first equation), **D** is the mean equivalent diameter of the cell nucleus and **t** is the section thickness (in mm).

Slides were examined under a light microscope and four histological categories were looked for: (Changes in vascularity, changes in adipose tissue, general thymic architectural changes and changes in cellular morphology & presence or absence of Hassall's corpuscles).

Results:

There was an increase in body weight of all treated animals in comparison to their controls being statistically significant for those animals which were killed after the first week of treatment but not for those killed after the second week of treatment (Table 1). Thymic weight and volume (Table 2) increased significantly after the first week of GH treatment for the young and middle aged animals but not for old mice. In the animals which were killed after the second week of GH treatment, the increase in thymic weight and volume was highly significant for all age groups. The thymus weight / body weight ratio was calculated to determine whether GH-induced thymic growth was part of its general growth-promoting effect on the body as a whole or was an isolated effect (Table 3). The ratio changes were statistically significant for the treated young mice after both the first and second weeks of GH treatment. In middle aged and old mice the ratio change was statistically significant for the group that received GH for two weeks. Cortical thymocyte counts increased significantly in all age groups and throughout both weeks of treatment (Figure 1). Cortical epitheliocyte counts increased significantly in young mice during both periods of GH treatment while in middle aged animals the increase was significant after the second week of treatment only, while in the old mice it was not significant after neither weeks of treatment (Figure 2).

Medullary thymocyte count increased with statistical significance after the first week of GH treatment in the young and middle aged groups but not in old animals. Following the second week of the experiment the count increased with high statistical significance in all age groups (Figure 3). Medullary epitheliocyte counts increased significantly during both periods of treatment in young and middle aged groups but not in old animals (Figure 4).

Histological examination revealed the following findings:

- The gland as a whole appeared to be more vascular in treated animals when compared to the controls (Figure 5, 6).
- In all treated groups the gross amount of thymic tissue to adipose tissue mass appeared greater than in their corresponding controls with preservation of thymic architecture and limitation of adipose tissue infiltration (Figure 7, 8).
- In the treated groups, the general known architecture of the thymic lobule composed of an outer dark cortex and an inner pale medulla was more obvious with increasing cortical density, clearer corticomedullary junctions and relatively thicker trabeculae with abundant amount of connective tissue (Figure 9, 10).
- Thymocytes and thymic epithelial cells did not show any specific changes in regard to their size,

staining or general morphology. Macrophages were seen mainly near the trabeculae and around the blood vessels and were of normal morphology. Large sized epithelioid cells were observed in the young steroid-only groups (Figure 11). They were mainly seen in the cortex near the connective tissue septa and had an average diameter of 36-41 µm. Apoptotic and pyknotic cells appeared as small cells with very dark small nuclei surrounded by a thin rim of cytoplasm.

- The cells were more observed in the control groups than in the treated groups and were most abundant in the steroid-only young group after the second week of the experiment as clusters of atrophying thymic tissue (Figure 8).
- Hassall's corpuscles were observed in young animals following the second week of treatment (Figure 12) & in middle-aged mice following the second week of GH treatment (Figure 13).

Table 1: The effects of exogenous GH on the body weights (in grams) of different age groups of mice after 1 and 2 weeks of treatment.

GROUP	*DURATION*	*BODY WEIGHT (grams)*					
		TREATED			*CONTROL*		
		1st day weight	**Last day weight**	**difference**	**1st day weight**	**Last day weight**	**difference**
A	1 week	15.25±0.96	21.75±1.7	6.5±1	16.25±0.5	18±0.82	1.75±0.96 ***
	2 weeks	18±0.82	23.25±0.96	5.25±0.5	17±1.16	21.75±1.26	4.75±1.5 NS
B	1 week	22.75±0.96	26.75±1.25	4±1.41	23.25±0.96	25±0.82	1.75±0.5 *
	2 weeks	24.5±0.58	28.25±0.5	3.75±0.96	22.5±1.29	24±0.82	1.5±1.73 NS
C	1 week	30.5±1.30	34±0.82	3.5±0.58	30.5±1.29	31.25±0.5	0.75±0.96 *
	2 weeks	30.75±1.5	33.75±0.96	3±2.16	29.5±1.29	31±1.83	1.5±0.58 NS

- Results are expressed in Mean ± Standard Deviation of 4 mice.
- Markers: ***** = Statistically significant ($P < 0.05$), ******* = Statistically Highly significant ($P < 0.001$), **NS** = statistically not significant.

Table 2: The effects of exogenous GH on the thymus weight (in mg) and volume (in mm^3) of different age groups of mice after 1 and 2 weeks of treatment.

GROUP	*DURATION*	*THYMUS WEIGHT (mg) AND VOLUME (mm^3)*					
		TREATED		*CONTROL*		*DIFFERENCE*	
		Thymus weight	**Thymus volume**	**Thymus weight**	**Thymus volume**	**Thymus weight**	**Thymus volume**
A	1 week	44.77±4.76 *	40.7±4.33 *	25.34±4.01	23.04±3.56	19.43±6.31	17.66±5.74
	2 weeks	34.78±3.24***	31.62±2.94***	22.28±1.24	20.25±1.13	12.51±2.66	11.37±2.41
B	1 week	31.79±0.58 *	28.89±0.53 *	25.78±2.12	23.44±1.93	6±2.27	5.46±2.06
	2 weeks	34.27±0.69***	31.15±0.62***	23.23±0.99	21.12±0.90	11.03±0.79	10.04±.072
C	1 week	25.74±0.89 NS	23.35±0.74 NS	24.5±0.96	22.27±0.87	1.24±1.3	1.13±1.18
	2 weeks	32.82±2.25***	29.84±2.04***	19.13±2.7	17.39±2.45	13.69±2.26	11.69±3.37

- Results are expressed in Mean ± Standard Deviation of 4 mice.
- Markers: ***** = Statistically significant ($P < 0.05$), ******* = Statistically Highly significant ($P < 0.001$), **NS** = statistically not significant.

Table 3: The effects of exogenous GH on the thymus weight / body weight ratio of different age groups of mice after 1 and 2 weeks of treatment.

GROUP	*DURATION*	*THYMUS WEIGHT / BODY WEIGHT RATIO*	
		TREATED	**CONTROL**
A	1 week	0.2055±0.00804 *	0.1406±0.0196
	2 weeks	0.1497±0.0140 *	0.1026±0.00712
B	1 week	0.1190±0.0055 NS	0.1034±0.0115
	2 weeks	0.1212±0.00072 ***	0.0968±0.00517
C	1 week	0.0757±0.00022 NS	0.0784±0.00034
	2 weeks	0.0973±0.0075 ***	0.0616±0.0071

- Results are expressed in Mean ± Standard Deviation of 4 mice.
- Markers: ***** = Statistically significant ($P < 0.05$), ******* = Statistically Highly significant ($P < 0.001$), **NS** = statistically not significant.

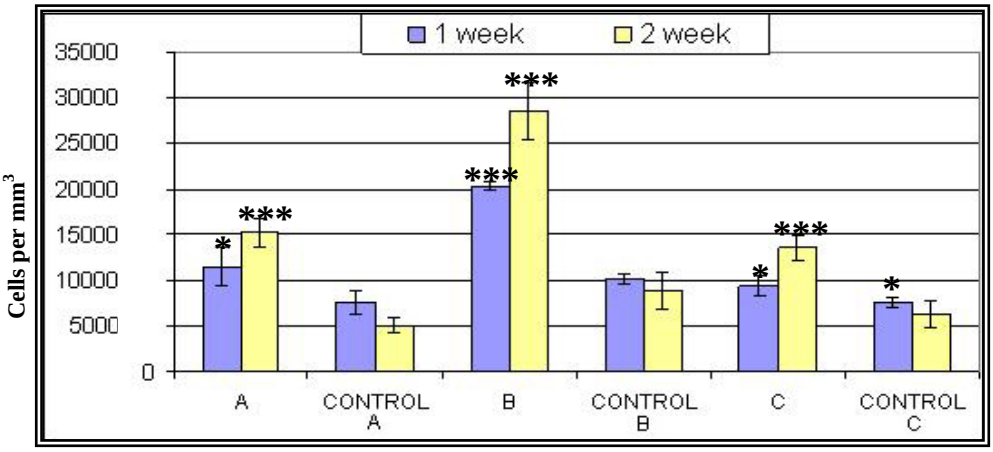

Figure 1: Effect of exogenous GH on cortical thymocyte counts (Data presented as mean ± standard deviation, ***P<0.001 {highly significant},*P<0.05 {significant}, NS = not significant).

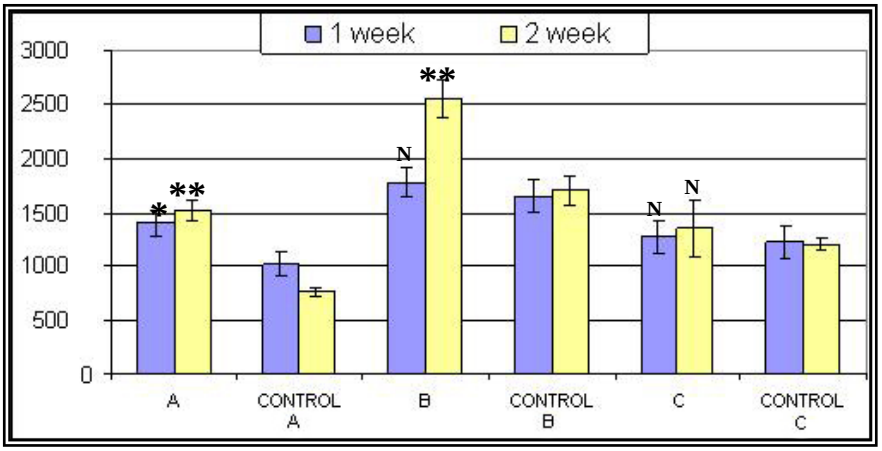

Figure 2: Effect of exogenous GH on cortical epitheliocyte counts (Data presented as mean± standard deviation, ***P<0.001 {highly significant},*P<0.05 {significant}, NS= not significant).

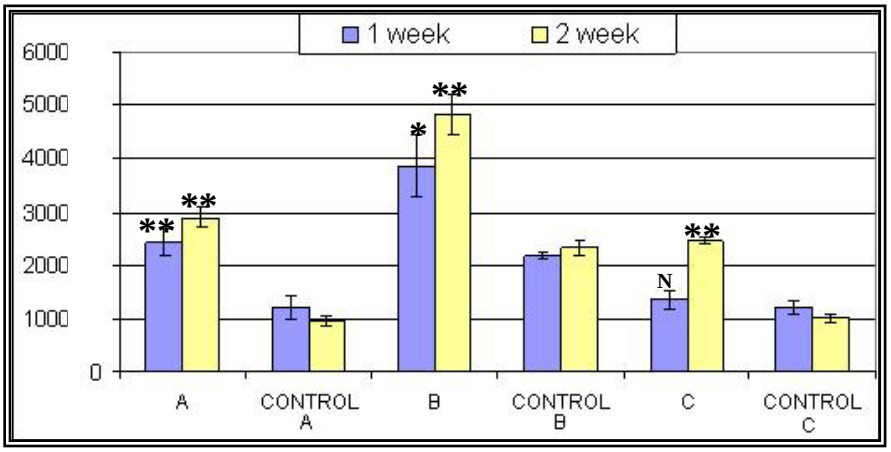

Figure 3: Effect of exogenous GH on medullary thymocyte counts (Data presented as mean±standard deviation, ***P<0.001 {highly significant},*P<0.05 {significant}, NS= not significant).

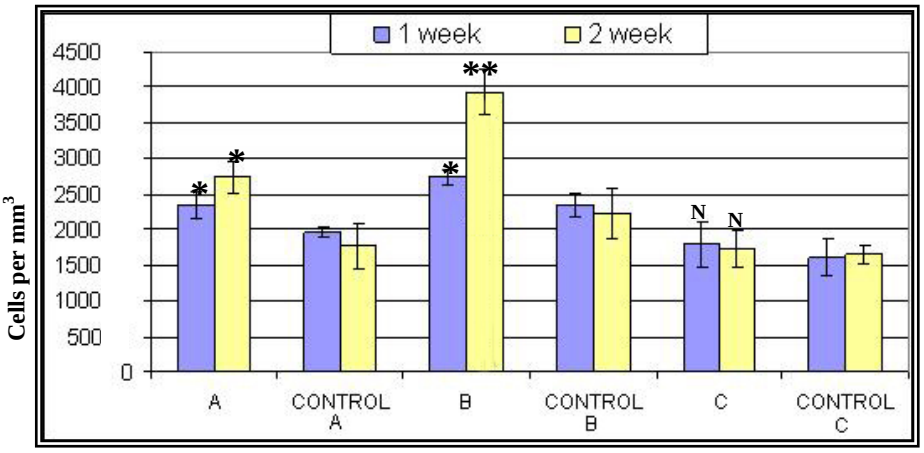

Figure 4: Effect of exogenous GH on medullary epitheliocyte counts (Data presented as mean±standard deviation, ***P<0.001 {highly significant},*P<0.05 {significant}, NS= not significant).

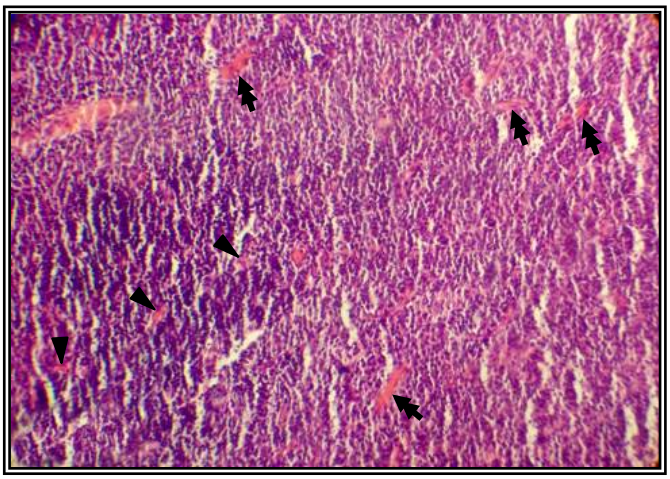

Figure 5: Cortical capillary (arrow heads) and medullary venular (double arrows) vascularity in middle-aged mice treated with GH for 2 weeks. H&E stain, x200.

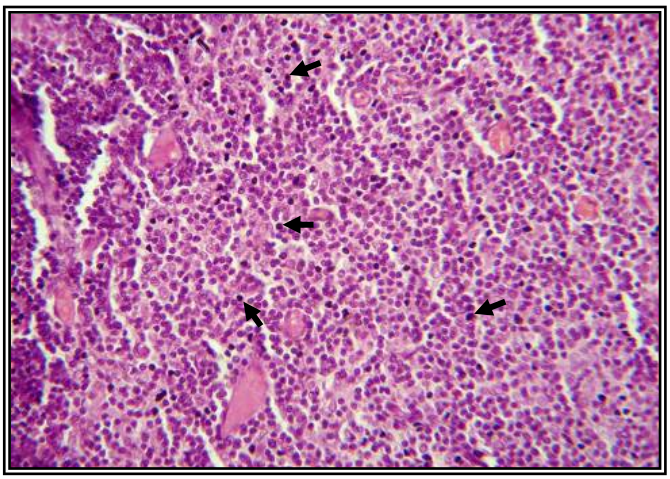

Figure 6: Thymic vascularity and cellularity in middle aged control mice at the end of the 2 week experiment. Also shown are sparsely located pyknotic and apoptotic cells (arrows). H&E stain, x400.

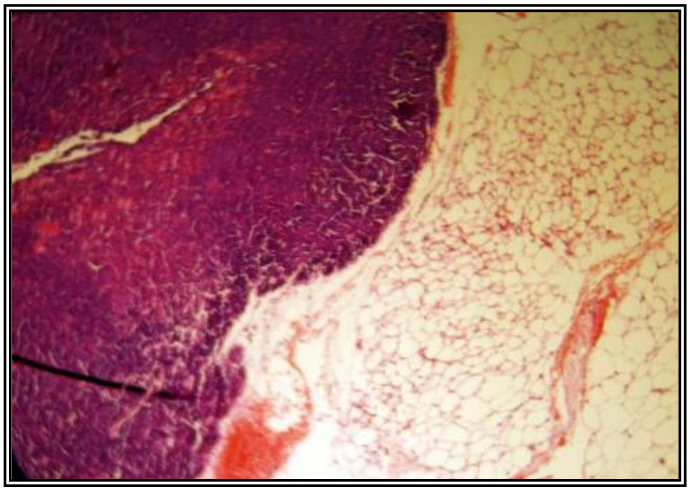

Figure 7: Preserved thymic architecture with clear septa and cortical & medullary areas in young mice treated with GH for 2 weeks. Fatty accumulation is not as extensive as in the control groups. H&E stain, x100.

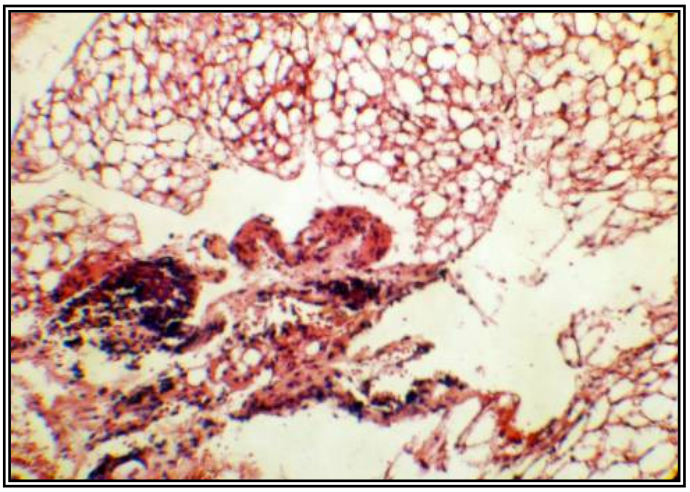

Figure 8: Extensive fatty infiltration and thymic atrophy in young control mice at the end of the 2 weeks experiment. Regular thymic architecture is lost and replaced by clusters of apoptotic and pyknotic cells. H&E stain, x100.

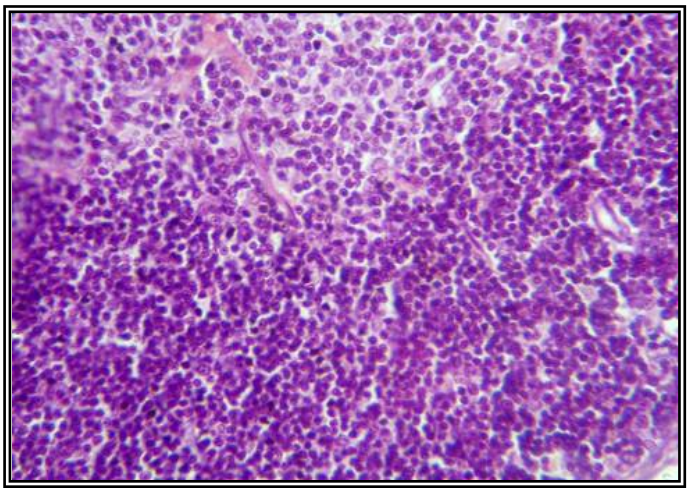

Figure 9: Clearly appreciated thymic zones in middle aged mice treated with GH for 1 week. H&E stain, x400.

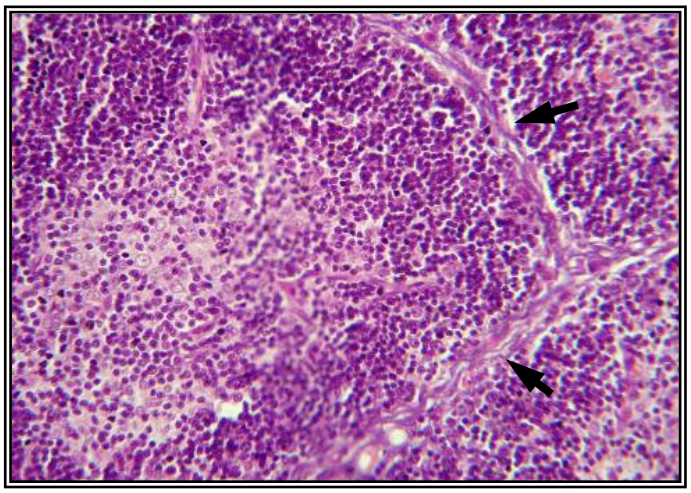

Figure 10: Sharply demarcated corticomedullary zones and thick trabeculae (arrows) with abundant amount of connective tissue in middle aged mice treated with GH for 2 weeks, H&E stain, x400.

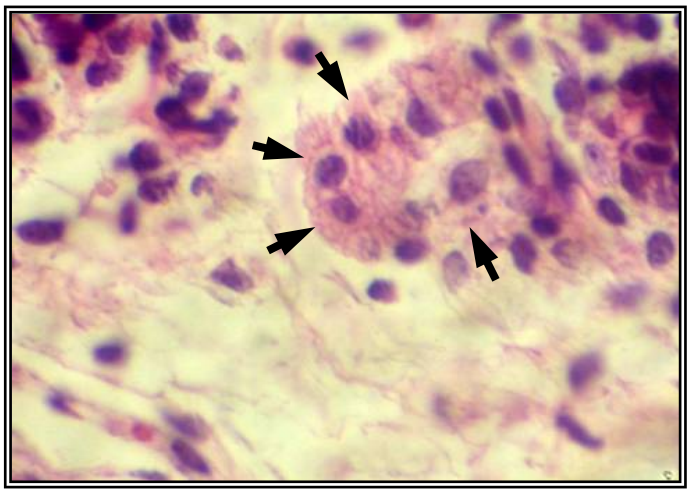

Figure 11: A cluster of large epithelioid cells (arrows) in young control mice following the first week of the experiment. H&E stain, x1000

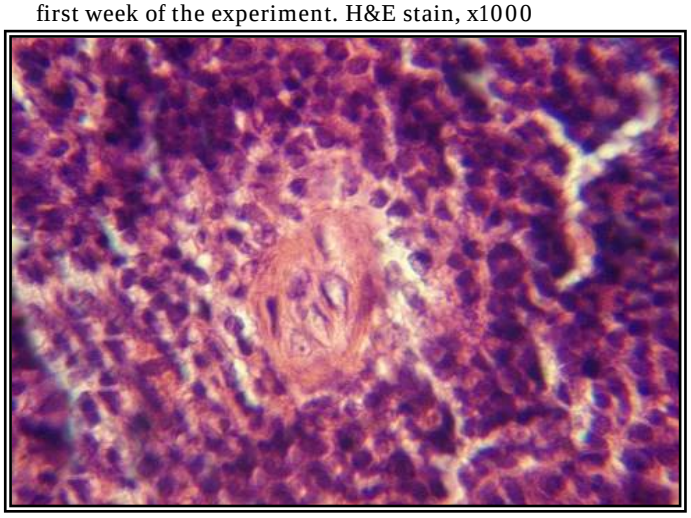

Figure 12: A medium sized Hassall's corpuscle near the corticomedullary junction in young mice treated with GH for 2 weeks. H&E stain, x1000.

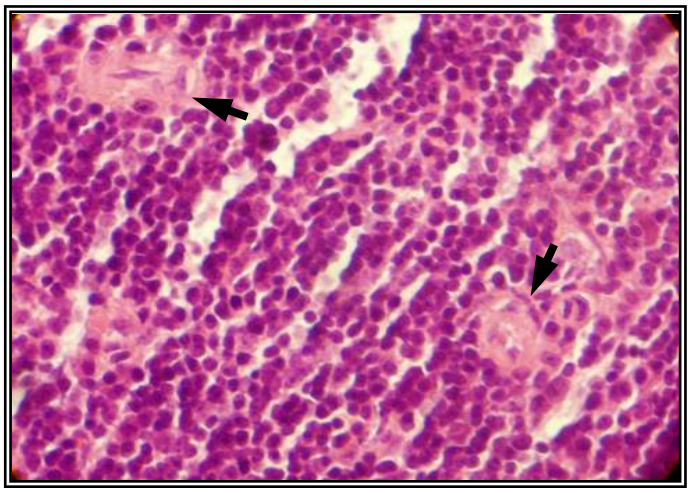

Figure 13: Two newly formed Hassall's corpuscles (arrows) in the medulla of middle-aged mice treated with GH for 2 weeks. H&E stain, x600.

Discussion:

Significant body weight changes being limited to the first week of GH treatment may be associated with the stress-related decrease in dietary intake in control groups during the first week and the development of toleration to the metabolic effects of GH in the treated groups during the second week. Significant increases in thymic weight and volume are chiefly related to changes in thymic cellularity and vascularity as GH & IGF's are known to function at 2 levels: induction of T-cell formation in the bone marrow, which is mainly an action of IGF-I [(13)] & increasing thymic vascularity and allowing more thymocyte progenitors (from the bone marrow) to extravasate through the vascular endothelium of the postcapillary venules to enter the organ and seed the tissue [(14)]. As thymus growth continued rapidly exceeding the general visceral growth and at the same time the whole body growth ceased to increase significantly during the second week, a lag was created allowing the thymus to gain more percentage of the whole body mass and hence the thymus weight/ body weight ration increased. It's likely that GH, through IGF-I action, has promoted angiogenesis in the thymus via activating vascular endothelial growth factor (VEGF), in response to increased thymic cellularity and metabolism, thus resulting in more vascular glands.

It appears that the limitation of adipose tissue in our study is the combined result of the negative effect of GH on adipose tissue growth (by decreasing pre-adipocyte differentiation [(15)] & inhibiting the expression of markers of terminal differentiation [(16)] and its positive replenishing effect on lymphoid tissue. Increases in cellular counts and maintenance of normal thymic architecture are mostly related to the documented effects of GH in enhancing

thymic epithelial cell proliferation [17], acting as a cytokine modulating thymocyte counts and differentiation [2] and enhancing thymic microenvironmental cell-derived secretory products including chemokines and thymic hormones [18].

The appearance of epithelioid cells and multinuclear giant cells in the control young mice may be related to the increased thymocyte apoptosis secondary to high steroid dose creating excessive cellular debris which adequately stimulated macrophages to transform into more effective forms to help clear out the thymus of that debris. The presence of apoptotic and pyknotic cells in the control groups is primarily the result of the effect of steroids, exogenously introduced to young mice in high doses (hence the highest concentration of apoptotic and pyknotic cells) and endogenously increased with age in middle-aged and old mice.

Although Hassall's corpuscles are of unknown clear function, it is possible that they serve as graveyards for aged thymic cells. This possibility is supported in our experiment by the fact that the corpuscles appeared only in the young and middle-aged GH-treated mice, the two groups which witnessed a significant increase in thymic epithelial cell counts alongside the increase thymocyte counts.

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