

Cross-Sectional Analysis of Pituitary Gland Zones Exposed to Thyroid Modulators

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ABSTRACT

Background: The pituitary gland takes part in coordinating the hypothalamic-pituitary-thyroid (HPT) network. This study aimed to examine the effects of hypothyroidism and hyperthyroidism on the structure of the pars distalis, pars intermedia, and pars nervosa regions of the pituitary gland.

Methods: In this in vivo study, 30 male Wistar rats that were in normal health were assigned to the control (untreated), hypothyroid (propylthiouracil-treated), and hyperthyroid (levothyroxine-treated) conditions for 4 weeks using a random sampling technique in an Animal Research Center. The study was conducted for 3 months from February 2024 to May 2024 at LUMHS and the Asian Institute of Medical Sciences. OpenEpi 3.0.0 was used to calculate the sample size. After performing euthanasia, the pituitary glands were taken out, weighed, and sliced for analysis with H&E staining. SPSS version 26.0 was used for statistical analysis.

Results: Rats with hypothyroidism had larger pituitary glands (11.5 ± 1.3 mg, $p < 0.01$), whereas hyperthyroid rats had shrunken pituitary glands (8.2 ± 0.9 mg, $p < 0.05$) than the controls. A histological examination revealed there were too many (hyperplasia) somatotrophs and thyrotrophs in the pars distalis of hypothyroid animals.

Conclusion: Changes in thyroid hormone levels caused the pituitary gland to reorganize by changing the number of endocrine cells and the stability of the tissue. These findings pointed out that the structure of the pituitary was easily affected by thyroid hormones and emphasized the need for balance among the endocrine glands.

Keywords: Pituitary Gland, Thyroid, Hypothyroidism, Hyperthyroidism, Endocrine Cells, Rats, in vivo.

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INTRODUCTION

The pituitary gland plays a key role in regulating several bodily processes by secreting trophic hormones, due to which it is often called as master gland as well¹. It coordinates its activity through the hypothalamus and works closely with peripheral glands, primarily with the thyroid, for forming the hypothalamic pituitary thyroid (HPT) axis². Pituitary function can be significantly impacted by the change in thyroid hormone levels, but the effect of these changes on the structure of the pituitary gland is not well understood³.

Thyroid hormones, mainly triiodothyronine (T3) and thyroxine (T4), provide feedback to the anterior pituitary, primarily influencing thyrotrophs, and indirectly affecting other hormone-producing cells in the gland⁴. In hypothyroidism (a deficiency of thyroid hormones), the reduced negative feedback leads to an increase in pituitary cell numbers⁵. Conversely, in hyperthyroidism (an excess of thyroid hormones), these cells can shrink or become damaged⁶.

While studies regularly find that changes in thyroid hormones can interfere with hormone regulation, not many have looked into how such changes, on a small scale, affect the pars distalis, pars intermedia, and pars nervosa of the pituitary⁷. Looking at these structural changes is necessary to understand the effects of thyroid problems on the body. The use of animal models allows scientists to observe the changes step by step within a living body to identify both reversible and progressively damaging histological patterns^{8,9}. Moreover, the gain of such knowledge may assist doctors and clinicians in diagnosing and treating thyroid-related illnesses and pituitary gland disorders.

The study aimed to analyze the histological appearance of the pituitary glands of rats after exposure to hypothyroidism and hyperthyroidism via medication. The goal was to find structural indicators of thyroid disease by examining changes in the gland's regions, which can be proven important for endocrine pathology and morphophysiological science.

METHODS

In this *in vivo* study, 30 male Wistar rats that were in normal health were assigned to the control (untreated), hypothyroid (propylthiouracil-treated), and hyperthyroid (levothyroxine-treated) conditions for 4 weeks using a random sampling technique in an Animal Research Center. The study was conducted for 3 months from February 2024 to May 2024 at LUMHS and the Asian Institute of Medical Sciences (ref/EC/9303/24). OpenEpi 3.0.0 was used for the calculation of sample size.

This study involved 30 Wistar rats, all healthy adult males who weighed between 200–250 grams.

The laboratory housing the animals provided a light-dark cycle, controlled the temperature, and there was free access to water and food for all the animals. The rats were divided into 10 animals per group after seven days: The Control rat group, the Hypothyroid rat group, and the Hyperthyroid rat group. The groups were assigned using a random sampling technique. OpenEpi 3.0.0 was used for the calculation of sample size using a power of 80% and 0.05 alpha¹⁰.

Only healthy adult male Wistar rats that were not more than 12 weeks old and weighed up to 200–250 grams were included in the study. Rats that showed signs of illness, abnormal weight, or any deformities were excluded. Animals that failed to consume the treatments, died during the experiment, or had damaged pituitary tissues post-euthanasia were also excluded. This ensured that the uniformity and integrity of the histological evaluation were preserved across groups.

The Hypothyroid group was given 0.05% propylthiouracil (PTU) in the drinking water for 4 weeks to cause hypothyroidism. L-thyroxine was given to the Hyperthyroid group at 0.4 mg/kg body weight daily by mouth gavage for the same period. The Control group was given no form of treatment. At the close of the experiment, all animals were given high doses of ketamine (100 mg/kg) and xylazine (10 mg/kg) injected into the abdomen to put them to sleep. All pituitary glands were weighed on a digital balance.

Each gland was placed in 10% neutral-buffered formalin for 24–48 hours and got processed for paraffin embedding. Hematoxylin and eosin (H&E) staining was performed after obtaining 5 µm thick serial sagittal sections. Examinations were done using a light microscope. Cellular density, blood supply, vacuoles, scar formations and zonal arrangement of the pars distalis, pars intermedia and pars nervosa were recorded for evaluation of changes. Effort was made to identify acidophilic, basophilic and chromophobe cells and to look for hyperplasia, atrophy or edema.

SPSS version 25 was utilized to perform statistical analysis. Quantitative data (e.g., gland weights) were presented as the mean ± standard deviation (SD) and the differences among groups were compared using one-way ANOVA at a significance level of $p < 0.05$.

RESULTS

Histoanatomical changes in the pituitary gland were studied in rats that received thyroid modulators in a

controlled setting. It was statistically proven that gland weight, microscopic structures, and some types of endocrine cell populations differed between hypothyroid and hyperthyroid groups versus controls.

Table 1: Experimental Groups and Animal Demographics

Group	Treatment	No. of Rats	Avg. Age (Weeks)	Avg. Body Weight (g)	Pituitary Weight (mg)	p-value
Control	No treatment	10	12 ± 1	210 ± 15	9.8 ± 1.1	-
Hypothyroid	Propylthiouracil	10	12 ± 1	195 ± 20	11.5 ± 1.3	p<0.01
Hyperthyroid	Levothyroxine	10	12 ± 1	230 ± 18	8.2 ± 0.9	p<0.05

Data analyzed using descriptive statistics mean ± standard deviation, one-way ANOVA; p < 0.01 and p < 0.05 considered significant. PTU – Propylthiouracil; T4 – Levothyroxine.

In **Table 1**, rats with hypothyroidism weighed less, but their pituitary glands expanded far more compared to the others. On the other hand, hyperthyroid rats were heavier, yet the pituitary gland was smaller, which suggested atrophy in this organ. All groups started with about the same age and health. The pituitary weight was significantly higher in hypothyroid mice (11.5 ± 1.3 mg, p < 0.01) and significantly lower in the hyperthyroid group (8.2 ± 0.9 mg, p < 0.05).

Table 2: Histoanatomical Changes in Pituitary Zones (Microscopic Findings)

Zone	Control	Hypothyroid	Hyperthyroid
Pars Distalis	Normal acidophils, basophils	↑ Acidophils, hyperplasia, vacuolation	↓ Basophils, atrophy, cellular thinning
Pars Intermedia	Normal compact chromophores	Disorganized melanotrophs, thickened	Cell loss, reduced pigmentation
Pars Nervosa	Normal pituicytes & axons	Edema, swollen fibers, mild gliosis	Fibrosis, rare axonal tracts, degeneration

Tissues were stained with H&E and observed under light microscopy. H&E – Hematoxylin and Eosin

In **Table 2**, it is clear that the pars distalis underwent overgrowth and became filled with cavities in the hypothyroid group; and the hyperthyroid group, on the other hand, had atrophic cells and lacked many of them. Hypothyroidism led to clumped melanotrophs in the pars intermedia, and exposure to hyperthyroidism resulted in less pigment. Animal models with hypothyroidism had edematous axons and gliosis in the par’s nervosa, while the pars nervosa of hyperthyroid animals displayed fibrosis and fewer nerve fibers. The control group did not have any changes to its tissue structure in any part.

Table 3: Endocrine Cell-Specific Alterations in Pituitary Gland

Pituitary Cell Type	Control Appearance	Hypothyroid Group	Hyperthyroid Group
Somatotrophs	Moderate, rounded	↑ Hyperplasia, hypertrophied	↓ Sparse, flattened, shrunken cells
Thyrotrophs	Scattered, regular	↑ Dense clusters, activated appearance	↓ Very few, degenerating morphology
Corticotrophs	Evenly distributed	Mild increase, hypertrophy	↓ Moderate loss, shrinkage
Gonadotrophs	Clear cytoplasm, low frequency	Unchanged	Slight ↓ in number
Lactotrophs	Normal distribution	↑ Proliferation	↓ Vacuolated, fewer cells

Evaluation based on H&E staining and light microscopy; changes assessed qualitatively by cell morphology.

As seen in **Table 3**, the reaction of endocrine-specific cells to thyroid state changes was different from each other. The number of somatotrophs and thyrotrophs grew greatly in the hypothyroid group, suggesting the pituitary was working overtime. On the other hand, hyperthyroid animals had many fewer and flatter somatotrophs and a significant decrease in thyrotroph cells. Hyper or underactive thyroids led to slight growth or shrinkage, respectively, in the corticotroph cells of the hypothalamus. Levels of gonadotrophs were the same, while in hypothyroidism, the number of lactotrophs increased, and they exhibited degeneration with vacuoles in hyperthyroidism.

Figure 1: Illustrates the thyroid hormone-dependent histoanatomical plasticity of the pituitary gland, correlating systemic thyroid states with regional structural responses. A schematic representation of the anterior (pars distalis), intermediate (pars intermedia), and posterior (pars nervosa) pituitary zones in a control group (left column), hypothyroid group (middle column), and hyperthyroid group (right column). In the control group, all regions maintain normal cellular architecture. The pars distalis shows uniform basophilic and acidophilic cells, the pars intermedia is compact with melanotrophs, and the pars nervosa displays clear axonal tracts without pathology. In the hypothyroid group, green arrows indicate hyperplastic changes in the pars distalis, with increased cell density reflecting compensatory upregulation of pituitary function. The pars intermedia and nervosa demonstrate edematous changes and structural swelling, indicating endocrine feedback stress. In the hyperthyroid group, red arrows point to cellular atrophy within the pars distalis, suggesting suppressed trophic stimulation. Green arrows highlight degenerative changes in the intermediate zone, while blue arrows mark fibrotic alterations in the pars nervosa, demonstrating irreversible tissue remodeling.

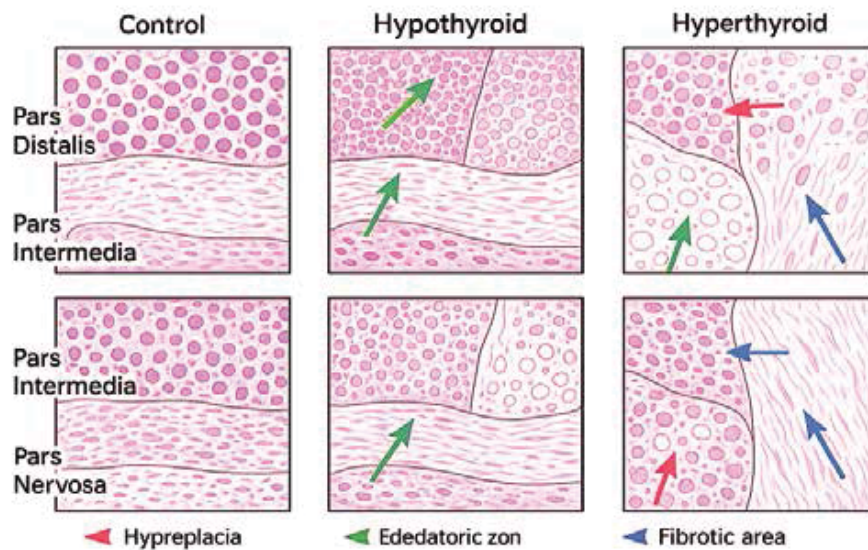


Figure 1: Comparative Histoanatomical Illustration of Pituitary Gland Zones Under Control, Hypothyroid, and Hyperthyroid Conditions. Red Arrow: Hyperplasia (in Hypothyroid) / Atrophy (in Hyperthyroid); Green Arrow: Edematous/Degenerative Zones; Blue Arrow: Fibrotic Tissue Areas in Pars Nervosa.

From the study's findings, it is clear that hypothyroidism caused hyperplasia and gland enlargement, while hyperthyroidism led to atrophy and fibrotic degeneration, which can be proved significant for further study on this matter.

DISCUSSION

The investigation examined changes in the pituitary regions, such as pars distalis, pars intermedia, and pars nervosa, as a result of induced hypothyroidism and hyperthyroidism. The results from the structure of the pituitary gland confirmed that it was highly adaptable and flexible to changes, but it could also undergo degeneration due to altered thyroid hormone levels. In animals with hypothyroidism, the pituitary glands were enlarged, and there was noticeable tissue thickening within the pars distalis. This phenomenon most usually occurs when negative feedback from thyroid hormones is lowered, and TRH and TSH levels are raised¹¹. TRH is seen to stimulate lactotrophs, which may explain the observed rise in acidophilic cells¹².

Several previous studies have shown that the pituitary gland expands when thyroid suppression remains for a long period of time^{13,14}. Hyperplasia is a result of the thyroid gland's forced production of more TSH; chronic hypothyroidism can cause the gland to look aberrant, mimicking changes seen in neoplastic diseases, thus stressing its relevance in clinical treatment¹⁵.

On the contrary, the pituitary weighed much less in hyperthyroid rats, and the pars distalis seemed shrunken. This might arise from high thyroid hormone levels suppressing the pituitary and hypothalamus's TRH and TSH output^{16,17}.

By examining these tissues, both acidophils and basophils were observed to be diminished, and the pattern of zonal regions appeared tightened with reduced cellular activity. These results are in line with the earlier endocrine research that showed how low hormonal demand typically results in gland shrinkage^{18,19}. Though the pars intermedia has not been the subject of much research, it still showed interesting changes. In hypothyroidism, there was increased cellular disorganization and a rise in melanotrophs, which may result from altered signaling pathways that regulate pro-opiomelanocortin (POMC)²⁰. In this study, the pars intermedia looked thinner and pale, which suggested possible tissue damage.

The pars nervosa also clearly showed morphological changes. This portion of the organ is commonly considered as neurosecretory²¹. Enlargement of axons and increased glial activity were seen in hypothyroid glands, indicating stress within the neuroendocrine system²². In hyperthyroid tissue slices, reduced myelin thickness, degenerating axons, and signs of fibrosis were observed, possibly reflecting oxidative stress triggered by excessive thyroid hormones²³. These effects might be the result of altered hypothalamic signaling and broader metabolic changes, even though the posterior

pituitary does not play a direct part in controlling thyroid hormone levels²⁴.

Generally, these points suggested that the pituitary gland's internal layout was easily influenced by thyroid state²⁵. These histological findings were related to the clinical disturbances in hormone function. The model helped in showing that it was important for the thyroid and pituitary to be in balance, as shown by the microscopic evidence. Suggesting a diagnosis can be difficult by solely relying on the results of light microscopes, and sometimes demands immunohistochemistry to provide more evidence. Further studies should add hormone analysis, investigation of genes, and models that examine the progress and reversal of alterations.

Ultimately, the thyroid hormone results in specific structural changes in the pituitary. Enlargement and extra cell growth occur in glands during hypothyroidism, but hyperthyroidism leads to shrinkage and scar formation. By using these insights, pituitary adaptation can be understood, which may help with diagnoses in diseases that show pituitary enlargement or contraction due to changes in the pituitary lobe.

CONCLUSION

The study pointed towards the major histoanatomical alterations in the pituitary gland caused by changes in thyroid hormone levels. In hypothyroidism, the weight of the glands was raised, the pars distalis showed hyperplasia of acidophils and basophils, and both pars intermedia and pars nervosa showed loss of structure. On the other hand, hyperthyroidism caused shrinkage of the pituitary glands, fewer cell production, and the formation of fibrous tissue, suggesting that the endocrine activity was lowered.

This proved that changes in thyroid hormones could bring alterations to the pituitary gland, which was capable of adjusting its structure. Being aware of these different changes was necessary for learning about thyroid-related pituitary disorders and helped with differentiating between gland overgrowth and insufficient function. The study gave a foundation for the ongoing investigations into the pituitary-thyroid interactions and proposed that early histological review could help detect and interpret endocrine disorders that might be missed by other means.

LIST OF ABBREVIATIONS

PTU – Propylthiouracil
H&E – Hematoxylin and Eosin
TRH – Thyrotropin-Releasing Hormone
TSH – Thyroid-Stimulating Hormone
T3 – Triiodothyronine
T4 – Thyroxine

POMC – Pro-opiomelanocortin
SPSS – Statistical Package for the Social Sciences
ANOVA – Analysis of Variance

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CONFLICT OF INTEREST

None

ETHICAL APPROVAL

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AUTHORS' CONTRIBUTIONS

All contributed equally as per ICMJE.

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