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INTERPLAY OF GROWTH HORMONE AND THYROID AXIS IN HYPO- AND HYPERTHYROIDISM: INSIGHTS FROM AN EXPERIMENTAL CASE-CONTROL STUDY ON THYROID HORMONES AND GENE EXPRESSION BY USING RT-PCR

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ABSTRACT

The study examines the complex relationship between growth hormone (GH) and thyroid hormones in hypo and hyperthyroid conditions in premature male rats, concentrating on the physiological impacts of hormonal alterations and genetic effects. It was done in an animal housing facility at the University of Mosul Faculty of Veterinary Medicine. A study of 50 male rats, 30 days old and weighing 160 ± 5g, was conducted over 21 days. The first control group (G1) received normal saline subcutaneously. The second group (G2) induced by growth hormone deficiency via sodium diethyldithiocarbamate (SDDT) subcutaneously, the third group (G3) were given a growth hormone drug (Norditropin) subcutaneously, while fourth group (G4) induced hypothyroidism by Propylthiouracil (PTU) orally, and the fifth group (G5) induced hyperthyroidism by (Levothyroxine) orally. This experiment showed that thyroid stimulating hormone (TSH) has a high significance value at $p>0.05$ in G4 and G2 compared to G1. However, Triiodothyronine (T3) and Thyroxin (T4) are significant in G3 and G5. Regarding Reverse Triiodothyronine (rT3) in G3 and G4 display highest level in contrast to the other groups. Thyroid-binding globulin (TBG) and Thyroid peroxidase antibodies (TPO) were greatest in G4, whereas growth hormone (GH) and IGF-1 were highest in G2 and G5. The gene expression results showed that GH and IGF-1are up-regulated in comparison to the rest of the groups by using a modified conventional PCR with the formula. In conclusion, one hormonal agent system may significantly affect other hormonal activities, stressing the need for a complete endocrine approach.

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INTRODUCTION

Growth hormonal agents (GH) and the thyroid axis play critical duties in endocrinology, standing for an intricate interplay necessary for various physiological procedures, consisting of the development of metabolic processes plus power policy (Colella *et al*., 2020). GH, mainly produced by the pituitary gland, is essential for growth and advancement, especially in youngsters and teenagers (Di Ciaula *et al*., 2021). It works in tandem with thyroid hormonal agents, which are crucial for controlling metabolic rate, body temperature level, and heart efficiency. The thyroid axis, including the thyroid gland as well as its hormonal agents, specifically thyroxin

(T4) together with triiodothyronine (T3), communicates very closely with GH, producing an equilibrium that is crucial for regular physiological performance (Sterle *et al*., 2023).

The research study of these hormonal agents in hypo- and hyperthyroid problems is exceptionally substantial because of their extensive influence on the body's general health wellness and advancement (Weare-Regales *et al*., 2022). Hypothyroidism, defined by an underactive thyroid gland generating inadequate thyroid hormonal agents, can cause stunted development and weight gain, along with a slower metabolic process (Basualto-Alarcón *et al*., 2021). Alternatively, hyperthyroidism, where the thyroid is overactive, can create sped-up development, weight loss well, and a raised metabolic rate. Recognizing how these problems impact GH and the thyroid axis is essential in creating reliable therapies for thyroid conditions and GH shortages (Gubbi *et al*., 2023).

Immature male rats have been created to discover these communications and speculative designs. Rats are selected for their physiological resemblances to human beings in endocrine research studies, making them outstanding versions of human conditions (Zhu *et al*., 2023). By generating hypo- as well as hyperthyroid states in these rats, scientists can very closely examine the modifications in GH as well as thyroid hormonal agent degrees together with their succeeding results on development, metabolic process, plus various other physiological specifications (Bendarska-Czerwińska *et al*., 2023). This design offers a beneficial understanding of the endocrine system's working under changed thyroid states, preparing for a far better understanding and administration of associated human problems (Torre *et al*., 2023).

The research intends to explore the ins and outs of hormone equilibriums, mainly concentrating on the influence of the development of hormonal agent (GH) substitute treatment on the development of hormonal agent shortage (Stucker *et al*., 2021). It looks to clarify precisely how much substitute treatments influence the body's GH degrees, possibly supplying brand-new understandings of dealing with GH shortages (Almira *et al*., 2004). In addition, the research discovers the variations in product thyroid hormonal agent focus under differing thyroid states - hypo- and hyperthyroidism- to better recognize their systemic effects. A vital facet of this examination is comprehending the professional importance of the thyroid axis's alterations throughout the GH substitute, which is crucial in taking care of problems entailing these hormonal agents. This extensive strategy underscores the goal of improving our understanding of endocrine shoes and their professional effects.

MATERIALS AND METHODS

Summary of the Experimental Case-Control Study Setup

The research was structured as a speculative case-control research study, achieved at the physiology Department of Basic Sciences, College of Dentistry / University of Mosul / Mosul, Iraq. Covering from 21st of February 2023 to the second of May 2023, the research study intended to examine the relation between the development of hormonal agents as well as the thyroid axis in hypo- as well as hyperthyroidism in immature male rats. This configuration supplied a regulated setting to properly analyze the hormone communications and also their effects.

Animal Model and Speculative Groups, along with Treatment Protocols used in the experiment

Ten healthy and pregnant female albino rats were taken from the Animal House at the College of Veterinary Medicine at the University of Mosul. Rats were separated individually into clean standard rat cages. Wood chips were spread as bedding until the pups were born. Typical conditions were provided by (22 ± 2 °C), humidity (50 ± 10%), a 12-h light/dark cycle and animals were left *ad libitum* (Sztainberg and Zoghbi,2016). All pregnant rats were monitored every morning until the time of delivery. Fifty immature male rats of one month's age, weighting about 160 ± 5g, will be used in this study, were divided into five equal groups (10 rats in each group) and treated for 21 days as follows:

1. Control group (G1): Immature rats (30 days old) were given normal saline (Pioneer Company, Iraq) for 21 days subcutaneously (S/C).
2. Growth hormone deficiency group (G2): Immature rats (30 days old) were induced growth hormone deficiency by using sodium diethyldithiocarbamate (SDDTC Sodium diethyldithiocarbamate is the organosulfur compound with the formula NaS2CN (C2H5)2. It is a pale yellow, water soluble salt), 100 mg/kg. BW for 21 days subcutaneously (S/C), (Saul *et al.*, 1993).
3. Growth hormone treatment group (G3): Immature rats (30 days old) were given human growth hormone (Norditropin (novo nordisk, Swiss mad, Somatotropin 10mg/1.5 ml. 200 µg/kg BW for 21 days subcutaneously (S/C), (Karin *et al.*, 1991).
4. Hypothyroidism group (G4): Immature rats (30 days old) were induced Hypothyroidism by giving Propylthiouracil (PTU, by Takeda group, Turkey, each tablet contains 50 mg of Propylthiouracil), (1mg/kg Bw) for 21 days as a fresh suspension was prepared every day and administered orally by gavage needle, (Cettour-Rose *et al.*, 2005).
5. Hyperthyroidism group (G5): Immature rats (30 days old) were induced hyperthyroidism by given Levothyroxine (anthrax25µg ®, Mark, Germany, each tablet contains 25µg of levothyroxine) 400 µg/kg Bw for 21 days a fresh suspension prepared every day and administered orally by gavage needle (Seong-Mo *et al.*, 2012).

Hormonal analysis during the Study

After the end of the experiment (21 days) and after a 12-hour fast, blood samples were collected from the retro-ocular eye vein, after which the serum was separated for hormonal analysis. 4 mL of blood samples were collected. The serum was split by centrifugation at 3000 rpm for 10 min at 4◦C. The serum was stored at −70◦C before the analysis process. Serum levels of (TSH), T3, T4, turn around T3 (rT3), (TBG), (TOP), and (GH) along with insulin-like development factor-1 (IGF-1), using individual ELISA kit (Shibayagi, Japan), (Subudhi, *et al.*, 2008), respectively. These dimensions consisted of degrees of importance in comprehending the physiological influences of the hormone changes caused by the rat designs.

Determination of GH and IGF-1 receptors expression:

Animals were slaughtered, and liver tissue was removed for gene expression. Throughout the research study, a variety of specifications were carefully gauged to monitor the animals and assess the therapies' results.

mRNA extraction was performed based on kit instructions (Gena Bioscience, Germany).

- Assessment of liver tissue IGF-1 and GH mRNA amount: The first step for determining gene expression through reverse transcription polymerase chain reaction (RT-PCR), quantification of the extracted RNA was measured using Qubit™ equipment (Qubit Fluorometer, Invitrogen, USA). For RNA quantification, a high sensitivity kit was used for the procedure. Prepared the working solution in a clean plastic tube: The final volume in each tube must be 200 µL. Sample volume was 1-20 µL added to the 180-199 µL working solution. Then mixed by vertexing 2–3 seconds. Incubated at room temperature for 2 minutes. All samples were measured using Qubit® Fluorimeter. The same quantification procedure used in RNA was used to quantify cDNA.
- Expression of IGF-1 and GH receptors in liver tissue: The RT-PCR kit was provided by Bioron GmbH, Germany. One RT-PCR Master Mix is proposed for all purposes that require Reverse Transcription of RNA into cDNA and a subsequent PCR amplification. Primers and sample RNA only were added to the Master mix.
- We used RT-PCR IGF-1 Forword/ Reverse primer sequences 5’ CTTTGCGGGGCTGAGCTGGT 3’, 5’ CTTCAGCGAGCAGTACA 3’, respectively (Li *et al*., 2004).
- We designed RT-PCR GH Forword/ Reverse primer sequences 5’GTGACATGTGCGATGGTACC 3’, 5’ TGCTCATTGGTGTAGAGGGG 3’, respectively (figure-1).

Figure Error! No text of specified style in document.**(1): Primer designed of GH as shown on national center for biotechnology information (NCBI) websites.**

- The engaged primers were synthesized by Eurofins Genomics company, Germany. Total volume of the assay was 20 µl (10 µl One RT-PCR Master Mix + 0.5 µl of each primer (Forword and Reverse) + 2 µl RNA template + 7 µl Nuclease-free water).
- According to the manufacturer's instructions, the following was optimal reaction condition: reverse transcription at 55 °C for 30 min, denaturation of RNA hybrid and inactivation of reverse transcriptase at 95 °C for 3 min. PCR for 45 cycles, denaturation at 92 for 10 s, annealing at 58 °C for 10 s, extension at 72 °C for 20 s, final extension at 72 C° for 7 min.
- RT-PCR was performed on the MiniAmp Plus TM Thermocycler PCR, USA. The quantification of genes expressions was determined according to Archer, 2017 and Rao, 2013 methods using different equations, (Archer, 2017; Rao *et al*., 2013).
- The PCR threshold standard curve was based on an exponential model of the initial phase of a PCR run where template replication efficiency is constant from cycle to cycle.
- Electrophoresis (Cleaver Scientific, England) was carried out on 2% agarose gel containing gel safe stain to separate charged DNA fragments according to their sizes. The RT-PCR bands were 184 bp IGF-1 cDNA and 240 bp GH cDNA.

Statistical Analysis

JMP Pro16.1 software (2021 SAS Institute Inc., North Carolina, USA) was used for descriptive and inferential statistics. The descriptive statistics included the mean and standard error. Data were analyzed using analysis of variance (ANOVA) to determine the effect of treatments on the animals. The means of the treatment groups selected from a normally distributed population were compared using Student's t-test and Duncan's multiple-range test. The significance level of the results was set at $P < 0.05$ (Ludbrook, 1997).

RESULTS AND DISCUSSION

Data presentation on hormone concentrations and their variations across different groups

The hormonal agent focuses information showed remarkable variants between the control group and the speculative group that dealt with various hormone manipulations. Product degrees of TSH, T3, T4, turn around T3 (rT3) as shown in Table (1). Table (2) explains the value of TBG, TPO and GH together with insulin-like development variable-1 (IGF-1) differed substantially amongst the groups, showing the extensive influence of hypo- plus hyperthyroid problems along with GH substitute treatment on these specifications. The thorough hormonal agent degree dimensions supplied essential understandings of the physiological modifications generated by the transformed thyroid plus GH states in the rat models.

The marked rise in TSH levels in the hypothyroid group, in contrast to the control group, is a classic sign of thyroid hormone deficiency. This is best confirmed by the equivalent decrease in T3 as well as T4 scores (Shankar *et al.*, 2021). On the

other hand, the hyperthyroidism group revealed lower TSH scores accompanied by enhanced T3 as well as T4 levels indicating too much thyroid hormone production. These searches are consistent with the recognized physiological reactions to hyperthyroidism problems as well as confirming our speculative version (González-Madrid *et al.,* 2022). This was explained by the fact that the TSH in the blood serum, this causes a natural physiological state of reducing the proportion of the hormones T3 and T4 to reduce the increased effect of these hormones and vice versa.

Table (1): Variations in hormonal levels of TSH, T3, T4, and rT3 (mean ± SE) across different groups of the study.

Groups	TSH (mU/L)	T3 (ng/dl)	T4 (µg/dl)	rT3 (pg/ml)
Control	31.52±0.38^{b}	113.51±0.36^{b}	24.85±1.00^{b}	174.9±13.02^{c}
GH deficiency	43.46±0.33^{a}	101.47±0.24^{c}	13.02±1.51^{c}	199.2±18.43^{b}
GH treatment	22.08±0.13^{c}	131.79±0.31^{a}	59.52±0.23^{a}	209.3±19.56^{a}
Hypothyroid	48.33±0.40^{a}	100.86±0.28^{c}	12.25±0.58^{c}	216.7±15.51^{a}
Hyperthyroid	21.88±0.15^{c}	135.61±0.30^{a}	62.92±2.91^{a}	155.8±17.22^{d}

Similar letters indicate no significant difference between the groups, while different letters indicate a significant difference between the groups ($P < 0.05$).

The variants in product degrees of TBG, and TPO throughout groups additionally lost light on the governing devices at play in thyroid pathophysiology (Begum, 2023). These variants can be a measure of adjustments in the transportation and also metabolic process of thyroid hormonal agents along with autoimmune reactions specifically (Bogusławska *et al*., 2022).

The GH treatment group revealed a boost in GH as well as IGF-1 degrees, which is anticipated to comply with GH supplements. This rise was related to modifications in thyroid hormonal agent degrees recommending a feasible communication between GH treatment as well as thyroid feature, a partnership that has been hinted at in previous research studies; however, it stays to be totally made clear (Shankar *et al*., 2021).

Remarkably, the GH Deficiency group revealed a various pattern of hormonal modifications, which is vital for comprehending the more comprehensive ramifications of GH in endocrine law (González-Madrid *et al*., 2022). The adjustments in T3, T4 as well as rT3 degrees in this team recommend an intricate connection between GH shortage as well as thyroid hormonal agent metabolic rate, which might have substantial effects on professional technique, particularly in the administration of development conditions (Romero-Márquez, 2021).

Table (2): Variations in hormonal levels of TBG, TPO, GH, and IGF-1 (mean ± SE) across different groups of the study.

Groups	TBG (µg/dl)	TPO (pg/ml)	GH (µg/l)	IGF-1 (ng/ml)
Control	29.0±1.21^{c}	366.8±16.51^{b}	35.6±1.57^{b}	28.3±1.19^{b}
GH deficiency	30.4±1.05^{c}	300.4±22.75^{c}	43.5±1.01^{a}	36.3±1.26^{a}
GH treatment	38.3±0.94^{b}	315.6±14.90^{c}	39.6±0.36^{b}	29.7±1.79^{b}
Hypothyroid	48.1±1.03^{a}	385.1±10.92^{a}	14.6±1.01^{c}	14.7±0.90^{c}
Hyperthyroid	20.2±1.39^{d}	259.3±24.52^{d}	41.2±1.08^{a}	31.9±1.78^{a}

Similar letters indicate no significant difference between the groups, while different letters indicate a significant difference between the groups ($P < 0.05$).

GH and IGF-1 receptors expression:

Regarding GH expression, our data revealed variances in the gene expression level of each group separately, as summarized in Figure (2). The growth hormone deficiency (GHD) group showed up-regulation (high concentration) of GH in contrast to the other groups (hyperthyroid, GH treatment, hypothyroid, and control). In contrast, the hyperthyroid group data presented a high quantification compared with different groups, excluding the (GHD). On the other hand, both the GH treatment and control groups were close to each other with low values in terms of concentration; however, their GH expression is below that of the GHD and hyperthyroid groups. Surprisingly, the control group was upregulated more than the hypothyroid, as the hypothyroid resulted in a low concentration compared to all other sets.

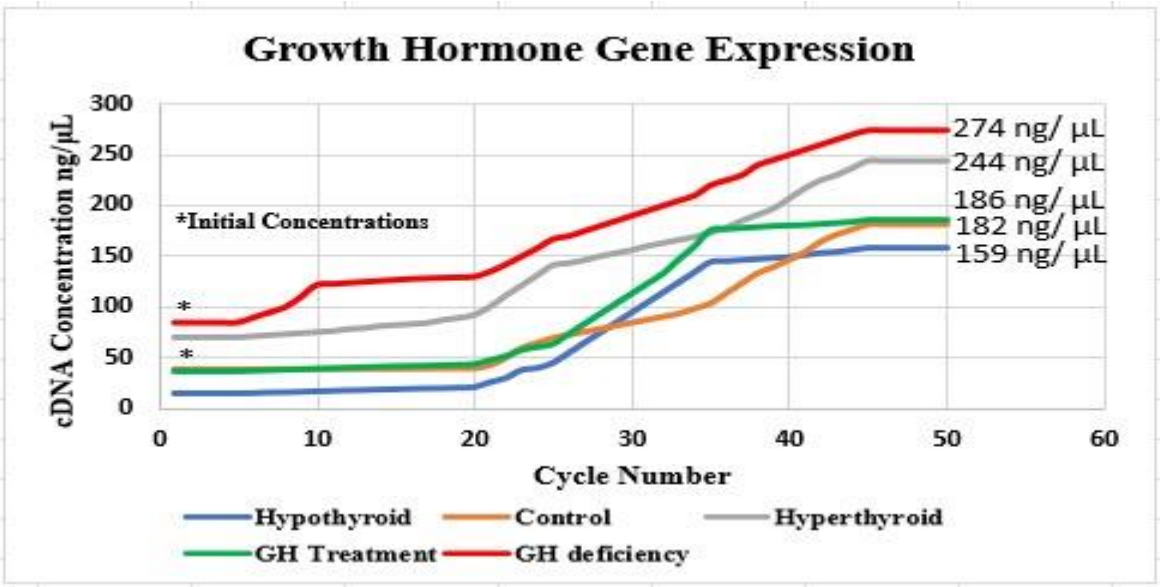

Figure (2): Represent the concentration of GH expression in different treatment.

Figure (2) illustrates the result of IGF-1 expressions, which display the same scenario as seen in Figure (2) as both hormones are mutually exclusive; accordingly, as expected, GHD treated group has a high concentration compared to all groups followed by hyperthyroid, it is noteworthy to mention that the GH in both GHD and hyperthyroid group have higher concentration compared to IGF-1 in same groups. Regarding GH, control, and hypothyroid, the IGF-1 expression is similar to that in GH, Figure (1). Nevertheless, hypothyroid revealed the lowest IGF-1 gene expression (137 ng/ul) than GH expression in Figure (1).

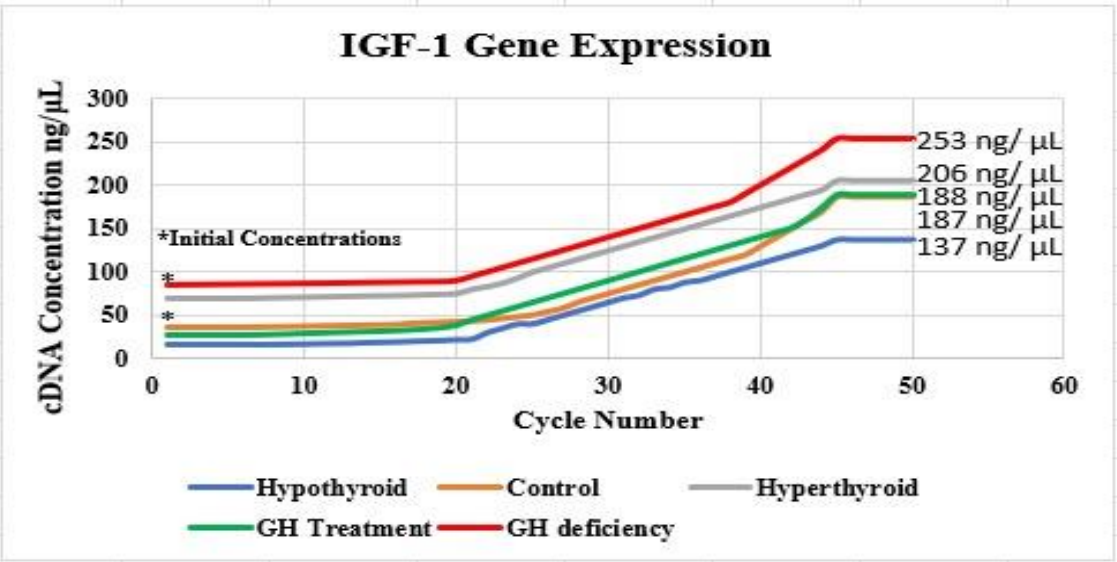

Figure (3): represent the concentration of IGF-1 expression in different treatments.

Figure (4) shows the resulting bands of both genes in an agar gel illustration. The first lane represents the ladder (m). Lanes 1- 4 are samples of the GH expression bands, which are 184 bp. Lane 5 represents a negative control for both genes. Lanes 6- 10 show bands of IGF-1 expression with 240 bp.

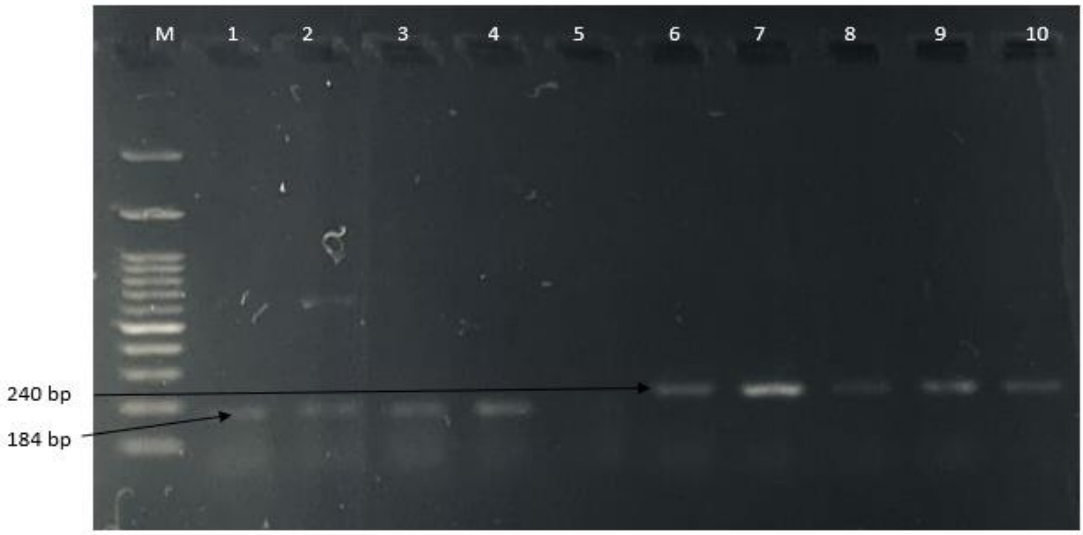

Figure (4): Positive IGF-1 gene located in lanes 1-4 (184 bp), positive GH gene (240 bp) located in lanes 6-10, Line 5 represents negative controls. Lane M: 100 bp DNA ladder (Addbio company, Korea).

The gene expression evaluation, as described in Figure (1,2 and 3) uses extensive understandings right into the molecular communications between development hormonal agents (GH) and thyroid hormonal agents. Our research shows substantial modifications in the expression of genetics related to these hormonal agents throughout various speculative groups highlighting the delicate plus complex partnership at the molecular degree (Scherk, 2020).

The outcomes revealed that GH therapy caused a higher guideline of GH and IGF-1 gene expression, which corresponds with the anticipated action to GH supplements. This rise in GH-related genetics expression, along with a small

reduction in Hypothyroid group, recommends a possible comment system where GH affects thyroid hormonal agent paths perhaps with the somatostatin signaling path (Raffa, 2021).

On the other hand, the hypothyroid group displayed a decline in GH and IGF-1 expression showing that minimized thyroid hormonal agent degrees could subdue GH genetics expression. This searching for is especially intriguing as it underscores the reliance of GH expression on thyroid hormonal agent degrees. In addition, the rise in gene expression in both GH deficiency and hypothyroid suggests the body's compensatory reaction to hypothyroidism, intending to improve thyroid hormonal agent manufacturing (Mukhtar, 2021).

The Hyperthyroid and GH deficiency group revealed a fascinating pattern with raised GH and IGF-1 expression. This pattern can be anticipatory of the hyper metabolic state generally observed in hyperthyroidism where the body's need for GH is enhanced for power metabolic procedures as well as development procedures (Choudhari, 2022).

The raised in gene expression in hyperthyroid and GH deficiency group offered higher GH as well as IGF-1 expression which lines up with the anticipated result of GH shortage, this might suggest an effort by the body to make up for the absence of GH with a boosted thyroid hormonal agent task (Alwan, 2023). These genetic expression modifications are important in comprehending the physiological effects of hormone communications in endocrine wellness. The information highlights the intricate and vibrant interaction between GH as well as thyroid hormonal agents exposing that alterations in one can substantially affect the expression patterns of various others (Aledari, 2023).

CONCLUSIONS

To conclude, our research highlights the crucial interdependence of GH and thyroid hormonal agents in controlling numerous physiological behaviors coupled with molecular procedures. The elaborate connection observed between these hormonal agents recommends that a comprehensive method is vital in treating endocrine conditions. Our research will lead to future research studies and possible targeted restorative treatments that consider the numerous natures of hormonal agent communications.

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CONFLICT OF INTEREST

The researchers declare that they do not have any competing tests and there is no conflict of interest.

التفاعل بين هرمون النمو ومحور الغدة الدرقية في قصور وفرط نشاط الغدة الدرقية: رؤى من دراسة تجريبية للتحكم في الحالات حول هرمونات الغدة الدرقية والتعبير الجيني

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الخلاصة

تتناول هذه الدراسة العلاقة المعقدة بين هرمون النمو (GH) وهرمونات الغدة الدرقية في حالات نقص وفرط نشاط الغدة الدرقية في ذكور الجرذان غير الناضجة، مع التركيز على التأثير الفسيولوجي للتغيرات الهرمونية والتأثيرات الوراثية. أجريت الدراسة في جامعة الموصل، كلية الطب البيطري، بيت الحيوانات المختبرية. تم اختيار 50 من ذكور الجرذان غير الناضجة، بعمر 30 يومًا ووزن 160 ± 5 جرام، على مدار 21 يومًا. تلقت المجموعة الأولى السيطرة (G1) محلول الملح الفسلجي الطبيعي تحت الجلد. المجموعة الثانية (G2) المستحثة بنقص هرمون النمو عن طريق ثنائي إيثيل ثيوكربامات الصوديوم (SDDT) تحت الجلد، المجموعة الثالثة (G3) أعطيت دواء هرمون النمو (نورديتروبين) تحت الجلد، في حين المجموعة الرابعة (G4) المحفزة لقصور الغدة الدرقية بواسطة بروبيل ثيوراسيل (PTU) عن طريق الفم والمجموعة الخامسة (G5) مستحثة بفرط نشاط الغدة الدرقية باستخدام (ليفوثيروكسين) عن طريق الفم. أظهرت هذه التجربة أن الهرمون المحفز للغدة الدرقية (TSH) له قيمة دلالة عالية عند $p>0.05$ في G4 وG2 مقارنة بـG1 . ومع ذلك، فإن ثلاثي يودوثيرونين (T3) وثيروكسين (T4) مهمان في G3 وG5. فيما يتعلق بعكس ثلاثي يودوثيرونين (rT3) في G3 وG4 يظهر أعلى مستوى على عكس المجموعات الأخرى. كان الجلوبيولين المرتبط بالغدة الدرقية (TBG) والأجسام المضادة لبيروكسيداز الغدة الدرقية (TPO) أكبر في G4، في حين كان هرمون النمو (GH) و−IGF 1 أعلى في G2 وG5. أظهرت نتائج التعبير الجيني أن GH و IGF−1 يتم تنظيمهما مقارنة ببقية المجموعات باستخدام PCR تقليدي معدل مع الصيغة. في الختام، قد يؤثر نظام هرموني واحد بشكل كبير على الأنشطة الهرمونية الأخرى، مما يؤكد الحاجة إلى نهج كامل للغدد الصماء.

الكلمات المفتاحية: التعبير الجيني، هرمون النمو، هرمونات الغدة الدرقية، قصور الدرقية، تسمم الدرقية.

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