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Impact of Oral Collagen-A® Supplementation on Thyroid Functions in Mature Male Rats

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Abstract

The thyroid gland is severely affected in cases of disruption of the normal proportions of both proteins and minerals. The study aimed to reveal the effect of giving collagen as a supplement on thyroid function and some trace elements in cases of hypo- and hyperthyroidism in mature male rats. 36 rats were treated orally for 28 days, divided into 6 equal groups: control (G1) was given normal saline, group (G2) was given collagen-α®, the hypothyroid group (G3) given propylthiouracil, Hypothyroidism + Collagen-α® (G4) group, Hyperthyroidism (G5) levothyroxin, and Hyperthyroidism + Collagen-α group® (G6). Results show that in the hypothyroidism group, levels of (TSH, rT3, and phosphorus) were increased while both (T3 and T4) levels were significantly decreased. In the hyperthyroidism group, the levels of (TSH) decreased while (T3 and T4) levels significantly increased. Significant increases in TSH and decreases in T3 and T4 were observed in hypothyroidism + collagen groups; the opposite results occurred in hyperthyroidism + collagen groups. The levels of TBG and TPO significantly decreased in hypothyroidism, but increased in hyperthyroidism. The differences in these hormones were similar in both the hypo+ Collagen and hyper+ Collagen groups. In the case of hypothyroidism, there was a significant decrease in the calcium and calcium levels. Opposite results occur in hyperthyroidism. There were no differences significantly in zinc levels in treated groups. In conclusion, Collagen caused different effects in the treatment groups. In hypothyroidism, it caused a significant decrease in both calcium and zinc and an increase in phosphorus levels in the blood serum of adult rats; in hyperthyroidism, the opposite results occur.

Keywords: Thyroid, Hypo-Hyperthyroidism, Collagen, Trace elements.

Introduction

Scientists have classified the thyroid gland as one of the most important endocrine glands; its primary function lies in making, storing, and disseminating the basic thyroid hormones, which are thyroid stimulating hormone (TSH), thyroxin (T4) and triiodothyronine (T3) (1). Both (T4) and (T3) are considered endothermic animals. It is a critical regulator of basal metabolic rate and energy expenditure (2).

Collagen, which is a protein substance (amino acid chains), is considered one of the most quantitative types of protein in animals, as it is a type of structural protein that forms the basic building block in the cells and tissues of the body, especially skin, tendons, ligaments, and bones (3).

Collagen is considered one of the substances necessary for the health of cells, tissues, and organs in the body because it contains fatty acids, vitamins, and other nutrients necessary for a normal, healthy life (4). Collagen and its supplements are natural sources of many nutrients important for life, such as amino acids, so taking them with thyroid medications may not cause a health problem. However, it should be noted that this is not always true (5). Many patients use collagen supplements to treat various diseases, including thyroid disorders Collagen supplements may interfere with thyroid medications and disrupt the body's physiology due to their potential to mimic the active hormone triiodothyronine (T3), a common medication in thyroid disorders (6). To perform vital functions to the fullest extent, the body and its organs, especially the thyroid gland, need balanced nutrients and essential trace elements (7). Trace elements are essential for human survival and many physiological processes, including those of the thyroid gland, where the concentration of many trace elements is higher than that of other tissues (8). The thyroid affects trace element metabolism, and the level of trace elements also affects normal thyroid metabolism and function. Change in trace element concentration will affect the endocrine and other body systems, causing thyroid dysfunction, including hyperthyroidism and hypothyroidism (7).

The basic trace elements are very important because they play a key role in many biological processes. For example, they affect how enzymes and hormones work by changing how they are secreted and how they connect with different tissues. On the other hand, hormones play the opposite role, affecting the metabolism of basic elements, including secretion and transport (9).

Due to the lack of previous research dealing with the effect of collagen therapy on the thyroid and some trace elements like (calcium, zinc, and phosphate) disturbances in particular, this research aimed to determine whether there is an effect or not of collagen supplements in cases of hypo- and hyperthyroidism in a model of mature male rats.

Materials and Methods

Laboratory animals and experimental design: The Guide for the Care and Use of Laboratory Animals issued by the National

Institute of Health (10) was used to conduct this experiment, and approval was obtained from the Scientific Research Ethics Committee at the College of Veterinary Medicine at the University of Mosul, UM.VET.2023.031.

The research was structured as a speculative comparative study, achieved at the Physiology Department of Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq. It will cover the 1st of March 2024 to 30 April 2024. 36 Wister albino male rats (Rattus norvegicus) at age (3) months and weighting (210±5g) taken from the Animal House at the College of Veterinary Medicine at the University of Mosul.

They were separated individually into clean, standard rat cages. Wood chips were spread as bedding. Typical conditions were provided by (22 ± 2 °C), humidity (50 ± 10%), a 12-h light/dark cycle, and animals were left ad libitum. To obtain healthy models, the rats were left to adapt for a week before the experiments. In addition, reliance was placed on the National Research Council (11) in the standard nutrition for all experimental groups, which consisted of (60% carbohydrates, 26% crude protein, 5% fat, 5% crude fiber, 2% vitamin mixture, and 2% mineral mixture).

Animals were divided into 6 equal groups (n=6) and duration was for 28 days as follows:

1. Control group (G1): given orally 1 ml/ animal of normal saline by gavage needle (Pioneer Company, Iraq).

2. Collagen -α® group (G2): were administered orally (1 ml/kg Bw) of collagen-α® by using a gavage needle, an the collagen ampulla 10 ml contained 10ml of collagen peptide (5g), rosehip extract (0.5g) and 60 mg of Vitamin-C (60mg), dosage (1ml/kg BW) (12).

3. Hypothyroidism group (G3): induced hypothyroidism by giving Propylthiouracil (PTU, by Takeda group, Turkey, each tablet contains 50 mg of Propylthiouracil), (1mg/kg Bw) orally by gavage needle, (13).

4. Hypothyroidism (1mg/kg Bw) + Collagen -α® (1 ml/animal) orally by gavage needle groups (G4).

5. Hyperthyroidism group (G5): induced hyperthyroidism by giving Levothyroxine (anthrax25µg ®, Mark, Germany, each tablet contains 25µg of levothyroxine) (400 µg/kg Bw) a fresh suspension prepared every day and administered orally by gavage needle (14).

6. Hyperthyroidism (400 µg/kg Bw) + Collagen -α® (1 ml/animal) orally by gavage needle, groups (G6).

Blood collection for hormones and elements analysis: Using ether anesthesia and capillary tubes for microhematocrit, we collected blood samples from the posterior orbital plexus (15) in the traditional manner to obtain thyroid hormone concentrations. We collected approximately 2 cm3 of blood

in regular gelatinous tubes. The tubes were centrifuged at a rate of 3000 rounds per minute for 15 minutes. Serum samples were removed, transferred to Eppendorf tubes, and stored at −70 °C until thyroid hormone analysis.

Total concentrations of serum were measured using commercially available enzyme immunoassay test kits (Roche kit) by using the Roche Elecsys 2010 analyzers (Roche Diagnostics, Mannheim, Germany) (16).

To determine serum calcium zinc and phosphorus levels, assay kits manufactured by Solarbio (Life Sciences) were used. Methods and calculations were followed according to the manufacturer's protocols.

Data analysis by statistics: This included using the SPSS (ver. 22) software program for analytical estimations where information was shared as mean ± basic inconsistency (SD). The evaluation consisted of ANOVA for the contrasting team, which is indicated by post-hoc examinations for particular contrasts. Relevance was evaluated at $p< 0.05$ (17).

Results

The study analyzed hormonal agent levels in various groups (tables 1, 2), revealing significant differences between the control and speculative groups. There were big differences in the levels of thyroid-stimulating hormone (TSH), T3, T4, free T3, free T4, reverse T3, thyroxine-binding globulin (TBG), and anti-thyroid peroxidase (TPO). This showed that hypothyroidism, hyperthyroidism, and collagen replacement therapy all had a big effect.

Table (1) Measurement of the overall TSH, T3, and T4 concentrations also shed light on the pattern of the total pools of these hormones and on hormone availability to target tissues. Compared with the control group, the hypothyroidism group, TSH levels increased whileTSH levels increased while T3 and T4 levels decreased significantly ($P < 0.05$). In the hyperthyroidism group, the TSH level decreased when T3 - T4 levelsincreased significantly ($P < 0.05$). Significant increases in TSH and decreases in T3 with T4 were observed in hypothyroidism + collagen groups; the opposite results occurred in hyperthyroidism + collagen groups.

Table (2) showed significant differences in the levels of rT3, TBG, and TPO between the groups, and compared with the "control group," rT3 levels increased significantly ($P < 0.05$) in the hypothyroidism group, while they decreased in the hyperthyroidism group.TBG and TPO levels decreased significantly ($P < 0.05$) in hypothyroidism, but increased in hyperthyroidism, and the differences in those hormones in both the hypo+ Collagen and hyper+ collagen groups were similar.

Table-1: Variations in serum hormone levels of (TSH, T3 and T4) across different experimental groups in the study.

Hormone profile Groups	TSH "ng/ml"	T3" nmol/l"	"T4 nmol/l"
Control group (G1) 1ml orally	6.91 ± 2.0 b	1.86 ± 0.10 a	96.14 ± 2.41 a
Collagen -α® group (G2) 1ml orally	6.28 ± 9.2 b	1.92 ± 1.80 a	99.23 ± 1.80 a
Hypothyroidism group (G3) 1mg/kg BW orally	10.55 ± 3.2 a	0.89 ± 0.92 c	51.81 ± 0.54 c
Hypothyroidism + Collagen -α® groups (G4) orally	9.71 ± 3.3 a	0.98 ± 2.10 c	69.67 ± 0.30 c
Hyperthyroidism group (G5) 400 µg/kg BW orally	3.91 ± 7.2 c	1.40 ± 0.99 b	95.13 ± 0.44 b
Hyperthyroidism + Collagen -α® groups (G6) orally	4.78 ± 6.1 c	1.38 ± 3.10 b	90.55 ± 0.72 b

Different letters in groups indicate a significant difference (P < 0.05).

Table-2: Variations in hormone levels of (rT3, TBG, and TPO) across different experimental groups in the study.

Hormone profile Groups	rT3 ng/ml	TBG (nm/l)	TPO (pg/ml)
Control group (G1) 1ml orally	0.10 ± 0.1 b	5.76 ± 0.6 b	412.1 ± 0.1 b
Collagen -α® group (G2) 1ml orally	0.12 ± 1.2 b	5.73 ± 0.3 b	491.6 ± 0.1 b
Hypothyroidism group (G3) 1mg/kg Bw orally	0.05 ± 0.6 c	9.52 ± 1.1 a	611.6 ± 1.9 a
Hypothyroidism + Collagen -α® groups (G4) orally	0.07 ± 4.1 c	8.19 ± 3.5 a	501.9 ± 2.1 a
Hyperthyroidism group (G5) 400 µg/kg Bw orally	1.01 ± 5.2 a	3.27 ± 5.1 c	387.1 ± 0.1 c
Hyperthyroidism + Collagen -α® groups (G6) orally	1.02 ± 1.9 a	3.81 ± 9.0 c	355.5 ± 2.3 c

Different letters in groups indicate a significant difference (P < 0.05).

Table (3) demonstrates the calcium, zinc and phosphorus serum levels. From the results we obtained in the case of hypothyroidism, there was a significant decrease in the calcium level. In contrast, phosphorus level was higher than the control group (p value < 0.05). Opposite results occur in hyperthyroidism. No significant differences in zinc levels between groups compared with the control one.

Table-3: Variations in minerals levels of (Calcium, Zinc, Magnesium, and Phosphorus) across different experimental groups in the study.

Hormone profile Groups	Calcium (mg/dl)	Zinc (mg/dl)	Phosphorus (mg/dl)
Control group (G1) 1ml orally	9.95 ± 1.12 b	89.30 ± 1.88 a	6.47 ± 1.98 b
Collagen -α® group (G2) 1ml orally	9.31 ± 2.10 b	90.21 ± 2.98 a	7.27 ± 4.92 b
Hypothyroidism group (G3) 1mg/kg Bw orally	5.99 ± 0.90 c	89.40 ± 3.54 a	9.55 ± 5.70 a
Hypothyroidism + Collagen -α® groups (G4) orally	6.22 ± 8.22 c	91.11 ± 0.23 a	10.45 ± 3.27 a
Hyperthyroidism group (G5) 400 µg/kg Bw orally	13.12 ± 0.28 a	95.77 ± 0.82 a	4.99 ± 2.77 c
Hyperthyroidism + Collagen -α® groups (G6) orally	13.54 ± 0.74 a	94.75 ± 0.66 a	5.11 ± 1.70 c

Different letters in groups indicate a significant difference (P < 0.05).

Discussion

First of all, the hypothyroid group's observed elevation in thyroid-stimulating hormonal agent (TSH) degrees, compared to the control group, is a traditional sign of thyroid hormonal agent shortage. This is better corroborated by the equivalent reduction in T3 as well as T4 degrees (18). On the other hand, the hyperthyroid group revealed lowered TSH degrees accompanied by enhanced T3 and T4 degrees, suggesting that there is too much thyroid hormonal agent manufacturing (19). These searches correspond with the recognized physiological feedback to hypo- and hyperthyroid problems together with confirming our speculative version. Collagen's role in endocrine health and hormone production is critical, although not as widely discussed as its beauty benefits. The collagen treatment alone showed significant differences between the groups and the control group concerning concentration of TSH, T3, and T4, which indicates an

effective connection between collagen treatment and thyroid function. This agrees with (20), who says that thyroid dysfunction leads to accelerated collagen breakdown compared to normal thyroid function. Collagen supports healthy thyroid function and replenishes cortisol (the stress hormone). Theoretically, hyperthyroidism is likely to be accompanied by an increase in the metabolism of both soluble and insoluble Collagen. In contrast, hypothyroidism is accompanied by a decrease in the rates of collagen metabolism (21). At the same time, in contrast, hypothyroidism is accompanied by a decrease in the rates of collagen metabolism (21). At the same time (22) demonstrates there are no effects of protein nutrition in thyroid gland functions especially in both hypo-and hyperthyroidism conditions. However, some of this is considered something that has not yet been explained and requires further studies. We believe that the discrepancy in the results we obtained is due to the varied differences based on the collagen source and duration of supplementation (23). Another study found that nutrition containing a high percentage of protein increases the T3 hormone (24). The (25) found that, where low-protein diets have been observed to increase T3 levels and decrease free T3, T4, and TSH blood levels. According to (26), T3 does not increase as protein decreases because of increased T3 binding affinity by thyroid hormone transport proteins due to unbalanced consumption of macronutrients.

rT3 values, which could echo changes in thyroid hormone metabolism under various physiological and pathological circumstances, were highly distinctive throughout the groups. The hypothyroidism group had higher rT3 values, while the hyperthyroidism group had decreased rT3 values. These results align with the findings of (27), which suggest that the hypothyroidism group exhibited higher rT3 values due to increased thyroid hormone metabolism, while the hyperthyroidism group showed decreased rT3 values, suggesting a faster transformation from T4 to T3, rather than rT3.

Thyroid-binding globulin (TBG) is made in the liver and is a protein that can reversibly bind to the hormones (T3) and (T4) and carry them in the bloodstream. Thyroid peroxidase (TPO) is a heme-containing enzyme located in the apical membrane of thyroid gland follicular cells. It performs an important biological function by catalyzing the two reactions required for thyroid hormone synthesis, iodination of tyrosyl residues in thyroglobulin, and subsequent oxidative coupling to produce thyroxine (T4) and triiodothyronine (T3). The hypothyroid group had a high level of TBG as well as TPO. This agrees with (28), which may have implications for thyroid hormone distribution and consequent effects on various tissues. Hormonal differences in experimental groups reveal the delicate balance of activity and the potential for disruption resulting from an unbalanced hormonal situation.

Regarding the Collagen-treated groups, the results showed significant increases in both TBG and TPO levels in the hypothyroid groups, and vice versa in the hyperthyroidism group. These results align with reference (29), demonstrating that consuming a low-calorie protein diet

influences TBG and TPO concentrations. This is attributed to the re-expression of TBG, which typically disappears during maturation, leading to an increase in its plasma level. One of the most dangerous factors that affect thyroid function is a diet that contains protein and essential trace elements (30). Any imbalance in these substances in the body can cause severe disturbances in the activity of the thyroid gland and its hormones (31). For example, in the case of fasting and low levels of protein in the body, a significant decrease in the TSH version of the pituitary gland and T3 level in plasma was observed in mice fed a protein-free diet compared to mice fed a control diet (32).

There is a close relationship between thyroid hormones and the balance of essential elements in the body (33). In the case of hypothyroidism, there is an imbalance in the proportion of essential minerals, such as calcium, due to a significant decrease in levels. (34). At the same time, there was a significant increase in the phosphorus level in the case of hypothyroidism (35). This is consistent with our study. Suneel (36) demonstrated that the increased filtration and secretion of calcium through the kidneys in hypothyroidism, due to increased cell secretions, was responsible for the rate of decrease in calcium in blood serum. According to Bouillon and Moor (37), hypothyroidism causes a decrease in parathyroid hormone (PTH), and this causes a significant decrease in calcium and an increase in phosphate reabsorption, which causes a significant increase in phosphate blood serum (38).

In contrast, hyperthyroidism causes the opposite effect, with increased phosphate secretion from the renal tubules and decreased phosphate levels in the blood serum due to low renal excretion of zinc and low calcium reabsorption (39).

The use of collagen as an oral treatment caused an increase in bone mass in treated rats. This indicates calcium deposition and its low level in the blood serum of treated animals. This shows the benefit of collagen therapy in osteoporosis (40).

Conclusion

We conclude from this study that the cases of hypothyroidism caused a decrease in the concentration of calcium and zinc and an increase in phosphorus levels in the blood serum of adult rats. In the case of hyperthyroidism, the opposite results occur. The differences in results between other studies may be due to differences in nutrition and absorption of trace elements in the kidneys and digestive system, as well as the complex metabolism of these elements with pathological conditions and hormonal disturbances, especially in thyroid diseases. However, given the variations in essential trace element status among thyroid cases, we advise further research on the basic trace elements in thyroid gland disorders to lessen the harm that metabolic disorders can cause.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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تأثير مكملات الكولاجين الفموية على وظيفة الغدة الدرقية في ذكور الجرذان البالغة

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الخلاصة

تتأثر الغدة الدرقية بشدة في حالات اختلال النسب الطبيعية لكل من البروتينات والمعادن. هدفت هذه الدراسة إلى معرفة تأثير إعطاء الكولاجين كمكمل غذائي على وظيفة الغدة الدرقية وبعض العناصر النزرة في حالات قصور وفرط نشاط الغدة الدرقية في ذكور الفئران الناضجة. تم علاج 36 فأرًا عن طريق الفم لمدة 28 يومًا، تم تقسيمهم إلى 6 مجموعات متساوية، أعطيت المجموعة الضابطة (G1) محلول ملحي عادي، أعطيت المجموعة (G2) كولاجين-α®، أعطيت مجموعة قصور الغدة الدرقية (G3) بروبيل ثيوراسيل، مجموعة قصور الغدة الدرقية + كولاجين-α® (G4)، مجموعة فرط نشاط الغدة الدرقية (G5) ليفوثيروكسين، ومجموعة فرط نشاط الغدة الدرقية + كولاجين-α® (G6). أظهرت النتائج أن. أظهرت النتائج أنه في مجموعة قصور الغدة الدرقية، ارتفعت مستويات TSH وrT3 والفوسفور بينما انخفضت مستويات T3 وT4 بشكل ملحوظ، وفي مجموعة فرط نشاط الغدة الدرقية، انخفض مستوى TSH بينما ارتفعت مستويات T3 وT4 بشكل ملحوظ، . كانت هناك زيادات كبيرة في TSH وانخفاض في T3 وT4 في مجموعات قصور الغدة الدرقية + الكولاجين، ؛ تحدث النتائج المعاكسة في مجموعات فرط نشاط الغدة الدرقية + الكولاجين. انخفضت مستويات TBG وTPO بشكل ملحوظ في قصور الغدة الدرقية، ولكنها زادت في فرط نشاط الغدة الدرقية، وكانت الاختلافات في تلك الهرمونات في كل من مجموعتي نقص الكولاجين وفرط الكولاجين متشابهة، . في حالة قصور الغدة الدرقية، كان هناك انخفاض كبير في مستوى الكالسيوم، تحدث نتائج معاكسة في فرط نشاط الغدة الدرقية. لم تكن هناك فروق كبيرة في مستوى الزنك بين المجموعات المعالجة. الاستنتاج، تسبب الكولاجين في تأثيرات مختلفة في مجموعات العلاج، ؛ في حالة قصور الغدة الدرقية يسبب انخفاض مستويات الكالسيوم والزنك وزيادة مستويات الفسفور في مصل الدم لدى الفئران البالغة، وفي حالة فرط نشاط الغدة الدرقية تحدث النتائج العكسية.

لكلمات المفتاحية: الغدة الدرقية، قصور أو فرط نشاط الغدة الدرقية، الكولاجين، العناصر النزرة.