CHANGES DETECTED IN THE ORGANS OF QUAILS WITH EXPERIMENTALLY INDUCED HYPOTHYROIDISM

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Deneysel Hipotiroidizm Oluşturulan Bildircinlarda Organlarda Saptanan Değişimler

Özet: Çalışmada deneysel hipotiroidizm oluşturulan erkek bildircinlarda gelişim durumlarını ve testisler başta olmak üzere organlarda meydana gelen değişimleri incelenmiştir.

Bu amaçla 4 haftalık yaşta 30 tane erkek bildircin kullanılmıştır. Bu bildircin 16'sı deney, 14'ü kontrol olmak üzere iki gruba ayrılmıştır. Deneme grubundaki bildircinlara 3 hafta süreyle yem içerisinde %0.2 dozunda propil thiouracil verilirken, kontrol grubundaki her bildircin yemde beslenmiştir. Üçüncü hafta her iki gruptaki bildircinlere kan alınarak, serumda T4 düzeyleri tespit edilmiş ve 3. hafta sonunda eter ile ötenazi edilen her iki gruptakibildircinlerin nekropsileri yapılmıştır.

Deneysel grubundaki bildircinlerin kalplarında hafif büyüme olduğu, karaciğerlerinin sarıltı renkte olduğu ve testislerinin belirgin olarak küçülmuş olduğu saptanmıştır. Tiroidlerin ise normallerine ulaştığı 2-3 katı kadar büyüdüğü görülmüştür.


Her iki gruptaki bildircinlarda vücut ağırlığında artışların istatistiksel açıdan önemli olduğu tespit edilmiştir. Testislerdeki ağırlık azalmının ve kandaki T4 miktarındaki azalmanın P<0.01 düzeyinde önemli olduğu saptanmıştır.

Summary: In this study, changes in the organs, especially in the testicles and the growth states of male quails with experimentally induced hypothyroidism were examined.

For this purpose, 30 four-week-old male quails were used, 16 of these were separated as the experimental group and the remaining 14 were used as controls. The quails in the control group were fed with normal quail feed while the quails in the experimental group were given 0.2% propyl thiouracil by feed for a period of 21 days. Blood samples were collected from both groups on the 21st day of the experiment, and serum T4 levels were determined. Necropsy was performed on the quails of both groups which were euthanised with ether at the end of the 21st day.

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In the experimental group, a slight enlargement of the hearts and a yellowish in color of the livers and an obvious atrophy of the testicles were observed. Also the thyroid glands were found to be swollen 2-3 times larger than their normal size.

Microscopically, the spaces between some heart muscle fibres were dilated and granulation occurred in the cell cytoplasm. Moreover, tubuli of the testicles were atrophied. In the thyroid it was observed that the follicle epithelia were hypertrophied thus the growing cells caused the obstruction of the follicle luminae.

The increase in body weights was determined to be statistically insignificant. The weight loss in the testicles and $T_4$ levels in blood were determined to be significant as $P<0.01$.

**Introduction**

Hypothyroidism is a systemic endocrine disorder caused by the lack of the thyroid hormones. This disorder is seen in all animal species ranging from fish to mammals (4,17,19). Generally, the function of the hormones secreted by the thyroid glands is to keep the body tissues' oxidative metabolism at normal levels. These hormones are essential for normal growth. As these increase the basal metabolism, they are directly related to the live-weight increase and it is declared that a decrease in body weight occurs at different species of animals (4,19) in the case of hypothyroidism.

The secretion of the thyroid hormones is maintained by the complex interactions of the thyroid, hypopysis and hypothalamus. If any disorder destroys these interactions of these organs, the normal secretion of hormones ($T_3$, $T_4$) is also destroyed (3,4,17,19,26).

While hypothyroidism appears as a normal result of a disorder in these organs due to several reasons, it can be formed by the antithyroidal agents experimentally, as well (2,4,5,8,10).

In the case of hypothyroidism, the basal metabolic rate decreases 30–45%. Related to this, the animal can not grow up and remains small (4,19). It is declared that the death rate increases and body weight decreases in the female chicks of which thyroids are removed and which are given thiouracil (8, 14, 23).

In the case of hypothyroidism, it is reported that a decrease in libido, sexual insufficiency, decrease in sperma formation occurs in males (1,4,19,21,24).

The heart is one of the organs in which important disorders occur in hypothyroidism. Thus, in different types of animals, bradycardia, cardiac arrhythmia and cardiomyopathy occurs in the heart (4,5,6,15,18,19,22).

It is well known that thyroid gland hormones $T_3$, $T_4$ have a significant effect on lipid metabolism in all mammals and avian species. The increase of these hormones causes a decrease in cholesterol, phospholipid and triglyceride levels in blood. On the contrary, it is proved that in the case of hypothyroidism, while the level of these hormones in blood decreases, the levels of phospholipid, triglyceride and cholesterol in
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blood, tissue and especially in liver increase significantly in both human and some animal species (2,4,6,9,17,20,27,28).

As a result of studies carried out by giving antithyroid drug to dogs and avian species, it is declared that changes occur in the structure of organs and growth which can be macroscopically determined is observed in the thyroid (1,2,5,8,10,11,16,23).

In this study, growing state of male quails in which hypothyroidism is induced by antithyroid drug supplemented in the food and pathological changes in their organs, especially in the testicles, were aimed to be examined.

**Material and Method**

In the study, as the changes in the testicles were aimed to be examined, 30 four-week-old male Japanese quails (*Coturnix coturnix japonica*) were used. These were divided into two groups, 14 as control, and 16 as experimental. While the control group were fed with the normal quail feed of Erisler Food Industry for 3 weeks, the birds in the experimental group were fed with 0.2% g propylthiouracil (PTU) in order to induce hypothyroidism for the same period. Water and food were given ad libitum to both groups.

Blood samples were taken from V. cutanea ulnaris into anticoagulant tubes (heparinized) from the control and experimental groups after 3 weeks and sera were separated by centrifuging the blood at 3500 Rpm for 15 minutes. The sera were kept at -20 °C till they were used to determine T₄ levels. The T₄ levels were determined by the RIA method (25).

On the last day of the study, after the blood samples were taken from the both groups, the quails were euthanised with ether. After their body weights were recorded, necropsies were performed. The testicles’ weights were also determined during the necropsies. Later on, appropriate size of pieces from all of the organs were taken for fixation and put into Bouins’ solution. The organ pieces which were kept in the fixation solution for 4 days were routinely processed and embedded into paraffin blocks (12). 5-7 μ sections prepared from these blocks were stained with Hematoxylin - Eosin (H.E.) and examined under light microscope. Furthermore, to visualize the fat globules, 10-12 μ thick sections were cut by cryostat from liver pieces in the fixation solution and these were stained with Sudan III method for fat (13).

In order to be able to make a quantitative analysis of the atrophy of the testicles of the quails in the experimental group, the testicle slides prepared from both of the experimental and the trial group of quails were examined by a micrometric ocular. In each slide, the diameter of 10 different seminiferous tubules in 10 different microscopical areas were measured in 10 X magnification. The result was divided into 100, in order to calculate the mean size of a tubule.
The mean of all data such as the body weights and T₄ levels, standart error of the mean and the significance controls of the differences between the groups were determined by the “t test” (Macintosh Classic Stat View Program).

Findings

Macroscopical Findings:

At the necropy, the livers were yellowish in colour and fragile consistency and bleeding occurred on some parts of the lobes in all quails in the experimental group. It was also determined that the thyroids of all quails were enlarged two times as big as their normal size and appeared to be hyperemic. Moreover, the testicles were smaller in size and lighter in weight, compared to the control group. In the hearts, the veins were observed to be congested and the apex of the hearts were rounded. It was also observed that the kidneys were extremely hyperemic.

Microscopical Findings:

In the thyroid, it was observed that the sizes of the follicles which the organ consists of were different from each other, the veins seemed to be congested. And diffuse hemorrhagic areas were determined within the interfollicular space. Simple cuboidal epithelial cells lining the follicles were seen to be extremely hypertrophied, and there were also hyperplastic areas (Fig. 1). It was observed that in the follicles in which hypertrophy was more evident, the extremely growing cells obstructed the lumen. In the follicles in which hyperplasia was seen, multiplying cells made some projections to the luminae (Fig. 2).

In all the slides examined, it was observed that follicle luminae became narrower because of the events mentioned above in cells. Moreover, the amount of colloid in the follicles decreased and in some follicles, the luminae were obstructed completely and there was no colloid left in them. In some areas of the sections, it was found out that some of the follicles, especially the ones which were on the periphery of the organs had different sized vacuoles which were located in the colloid (Fig. 3). No obvious change was detected in the thyroid glands of the quails in the control group (Fig. 4).

In all of the liver slides that belong to the experimental group of quails, it was observed that generally the larger veins and sinusoidal spaces were filled with erythrocytes. Furthermore, within all these spaces, dense vacuolizations occurred in parenchymal cell cytoplasm. The size of these vacuoles were varying, while the cytoplasm were observed to be empty in some cells. In some parts by the lysis of the cell wall, spaces as large as the size of 2 or 3 cells were formed (Fig. 5). Sudan III fat staining application on these sections revealed that these vacuoles were fat droplets (Fig. 6).
Furthermore, it was observed that some of the nuclei of the cells were dislocated to the periphery of the cells by the fat globules in these cells and some of nuclei were entirely lysed.

In some parts of the heart, edematous fluid and many erythrocytes were observed among the muscle fibers, the interfibrillar spaces were widened. While some muscle cell cytoplasms became granular in structure, some others turned out to be compact structures. It was also found that some cell fibers fragmented and the integrity of the muscle fibers was degenerated (Fig. 7). In the kidneys, the larger veins both in the cortex and medulla were congested and lots of erythrocyte groups were formed in the intertubular spaces.

In the testicles, seminiferous tubules were generally smaller in size and in all tubuli, spermatogonii and spermatoids were present at different developmental stages (Fig. 8). The mean of the diameters of the seminiferous tubules of the control group were found to be 132.5 µ while this was 87.29 µ in the quails of the experimental group.

The statistical calculations related with T₄ levels the body and the testicles' weights of the experimental and the control group of quails were analysed with the student "t" test and all the results were shown at (Table 1).

The T₄ levels in blood sera were observed to be decreased in the experimental group of quails compared to the control group. The decrease was found to be significant at a level of P<0.01 (Table 1).

No statistically significant difference was observed in the body weights of both the control and the experimental group of quails.

Furthermore, a significant decrease was observed in the experimental group quails' testicles weights compared to the control group. This was found to be significant at a level of P<0.01 (Table 1).

### Table 1

<table>
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* P < 0.01
Figure 1. Hypertrophy and hyperplasia in follicular cells of thyroid of a quail in the experimental group 200X.H.E.

Figure 2. Colloid decrease and shrinkage in follicle lumen in a quail in the experimental group 200X.H.E.
Figure 3. Peripheral vacuolisations in some follicles of thyroid. 400X.H.E.

Figure 4. The thyroid gland of a quail in the control group.
Figure 5. Vacuolisations at different sizes in liver parenchymal cells. 200X. H.E.

Figure 6. Lipid globules in parenchymal cells of the liver. Sudan III 200X. H.E.
Figure 7. Granulation and coagulation in sarcoplasms. 600X.H.E.

Figure 8. Atrophy in seminiferous tubules 100X.H.E.
Discussion and Results

It is declared that the body weights decrease in domestic avian species in the case of experimental hypothyroidism induced with methimazole and propyl thiouracil (3,8,14,23,29) and in lamas a result of spontaneous hypothyroidism of lamas (26). Our finding concerning the body weight decrease in quails in the hypothyroidism group (Table 1) compared to the control group, is in accordance with the relevant literatures (3,8,14,23,26,29). The decrease of the body weights in the hypothyroidism group may be a result of the affected growth hormone. Besides, Yam et al. (29) claim that thyroid hormones have a strong correlation with the growth hormones. Therefore, the decrease of the thyroid hormones might have caused the body weight decrease by limiting the growth hormone.

Physiologically the hormone (T₃ - T₄) secretion from the thyroid gland takes place as a result of a cooperation between the hypophysis, hypothalamus and thyroid. In case of a decrease in thyroid hormones (hypothyroidism), TRH secretion, which is responsible for thyrotropin secretion from hypothalamus, is stimulated. This hormone arrives the hypophysis via portal circulation and stimulates the adenohypophysis to secrete TSH. The secreted TSH arrives the thyroid and stimulates the growing epithelial cells surrounding the follicles to grow and secrete the hormones, T₃ and T₄. This continues till sufficient amount of hormone is secreted. After secretion of sufficient amount of hormone, the size of the cells return to their previous size (4, 5, 19). Iodine is essential for hormone synthesis. The iodine in blood is kept by the thyroid gland epithelial cells and as a result of a subsequent chemical mechanisms, T₃ - T₄ hormones are formed (4,17,19). In the control group of our study, the mean levels of T₄ determined as 0.76 ± 0.01 μg/dl were within the limits declared in literatures (23). Hypothyroidism in the experimental groups were induced by feeding with an antithyroidal agent, propyl-thiouracil, for a period of 21 days and T₄ levels in this group were observed to be decreased to a level of 0.018 ± 0.01 μg/dl (Table 1). Propyl-thiouracil ceases the T₃ - T₄ synthesis by preventing thyroid glandular epithelial cells from using the iodine in blood. Induction of hypothyroidism was determined by with the significant decrease of T₄ level in blood serum (4, 19). This decrease is statistically significant at a level of P < 0.01. In our study, we observed that at all the quails in the experimental group, the size of both thyroids became two or three times larger than their normal size. By microscopical examination, it was observed that the cuboid epithelial cells which form the follicles were extremely hypertrophied and they were about to invade the follicle luminae. Therefore, the luminae of most of the follicles were narrowed and the amount of colloid decreased significantly, even in the luminae of some follicles there existed no trace of colloid at all. Moreover, in some of the follicular cells, some hyperplasia occured and in some of them vacuolisations were formed in the colloid left in luminae, especially on the peripheral parts. In some of the literature this is declared as a sign of iodine organization disorder (2,4,19). We suggest that the mechanism of these macroscopical and microscopical changes in the thyroid according to the physiologic process explained above, take place as follows: According to the physiological mechanisms, thyroid is stimulated for hormone production as a result of a decrease in hormone levels in blood. The cells become larger but as a result of the antithyroidal drug's (PTU) deiodination
effect, the hormone production does not occur. Therefore, while the follicular epithelial cells grow as their production capacity increases as a result of physiological stimulation, still on the other hand \( T_3 - T_4 \) are not produced because of the deiodinisation effect of the drug, thus \( T_3 - T_4 \) levels in blood decrease and hypothyroidism occurs.

As it was stated by the other authors (2,11,16,17,22), microscopical changes such as atrophy in follicles of the thyroid, interfollicular inflammation cellular infiltration and changes such as fibrosis which occur in 90% of primary hypothyroidism cases of dogs were not observed in our study. On the other hand, it is declared that thyroids grow in different proportions and hypertrophy and hyperplasia occur in follicular epithelial cells microscopically in animals fed with food known as guatrogenic, or in the cases induced by antithyroidal drugs (5,8,10,14,23). It our study, It was seen that the determined changes in the thyroid are in agreement with these findings.

It is stated that in the case of hypothyroidism, a significant increase occurs in cholesterol and triglyceride levels in blood. As a result of this, fatty change takes place in the liver (2, 4, 6, 9, 19, 20, 22, 27, 28) both in human and in different animal species. In our study, cholesterol and triglyceride values were not determined. However, we determined that parenchymal cells were filled with vacuoles and some cells cytoplasms turned into larger vacuoles as a result of lysis. We observed the typical microscopical findings of a fatty change (fatty degeneration, fat metamorphosis) in the nuclei of the liver parenchymal cells; their diffuse and dense appearance are important findings of a dense fat accumulation in the liver. Moreover, fat accumulation is revealed in the cytoplasms of the cells of these types of liver, via fat staining method. It is also declared that in spontaneous and experimental hypothyroidism cases of dogs, a fatty change occurs in the liver (2,6,22).

Heart is one of the important organs which is affected from hypothyroidism both in human and animal species. As a result of the studies carried out (4,5,6,18,19) on this subject on human and animals, it has been stated that a decrease occurs in heart pulse, pulse volume, blood collection rate and cardiac arrhythmias also occur. The thyroid hormones have chronotrophic and inothrophic effects on heart. In the case of hypothyroidism, this effect turns into negative direction and a decrease occurs in heart muscle contraction force. In an experimental study carried out on chickens (14), it is declared that in the case of hypothyroidism, a decrease occurs in functional capacity of the fast-phasic muscles. On the other hand no change has been observed in slow tonic muscles. No study could be found on histopathological findings in heart in avian hypothyroidism cases.

There is a very limited number of studies (6,18,22) on this subject in other animals. As a result of these studies, in hypothyroidism cases generally interstitial oedema of the heart, myofibrillar swelling, a corruption in the linear order and fibrosis were observed. It was determined that the findings we obtained at the end of the study such as oedema, granular formations in sarcoplasms and coagulation, disintegration in the myofibrils are identical with the results of the similar studies (6,18,22).
We suggest that congestion in the abdominal organs following the occurrence of hypothyroidism in these animals, is a result of the decrease in the contraction force of the heart muscle fibres or in other words, heart insufficiency which occurs as a consequence of a decrease in the levels of the thyroid hormones in blood.

It is known that sexual activity (libido) decreases in humans in the case of hypothyroidism (4, 19). It is declared that atrophy of testicles occur in dogs with hypothyroidism (2). Moreover, it is declared that after the experimental hypothyroidism is induced in 2-5 month-old male goats (16, 24), a significant decrease occurs in the testicles’ weights. But a while after the cessation of antithyroidal drug administration, the testicles recover to their normal size compared with the control group of animals. In a study on poultry (7), it is declared that after hypothyroidism is induced by removing the thyroids, the sperma production decreases in adult male animals but no difference is observed at the young ones.

It is declared that after experimental induction of hypothyroidism by methimazole in broiler breeder hens, the size and the weight of the testicles appear to be 50% less than the control group (1). It is also declared that in the case of hypothyroidism, degeneration occurs in the sertoli cell structure of rats (21). In our study, it was observed that the quails given antithyroidal drug had a significant decrease in the weight and the size of the testicles, compared to the control group. By the help of the statistical methods it was declared that the decrease of the testicles’ weight was significant at a level of P<0.01.

It is declared that in hypothyroidism cases of dogs and goats (2, 24) tubular atrophy occurs microscopically in the testicles. Moreover, it is declared that germinal epithelial cells remain in one row, spermatogenesis ceases completely and no more sertoli cells can be seen in the tubules of the goat testicles (24). In our study, no other findings could be achieved except tubular atrophy (the mean of the diameters of seminiferous tubules of the quails in the experimental group were 87.29 μ while it was 132.5 μ in the control group) and some disorders in spermatogenesis in the experimental group. We suggest that this depends on the insufficient dosage and short administration period of the antithyroidal drug.

**Literatures**


