

## HISTOLOGICAL STRUCTURE OF THE THYROID GLAND IN APOLIPOPROTEIN E DEFICIENT FEMALE MICE AFTER LEVOTHYROXINE APPLICATION

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### Abstract

The aim of this study was to determine the histological characteristics of the thyroid gland in ApoE KO<sup>-/-</sup> mice following the application of levothyroxine (l-thyroxine).

A total of 12 female ApoE KO<sup>-/-</sup> mice were divided into two groups. A control group of mice received distilled water and the experimental group received l-thyroxine dissolved in drinking water at a daily dose of 2 µg/ml, over 12 weeks. The paraffin sections were processed with the usual haematoxylin-eosin technique of staining.

Qualitative histological analysis demonstrated: the presence of large distended follicles in the peripheral areas of the gland; fulfillment of the lumen of follicles with an ample amount of colloid; complete absence of resorptive vacuoles in the colloid; a flattened follicular epithelium. Morphometric assessment showed a significant increase in the diameters of follicles in the peripheral areas of the gland and a significant decrease in the height of the follicular epithelium ( $p < 0,001$ ).

Our results demonstrated that l-thyroxine causes characteristic morphological changes in the structure of the thyroid gland in the direction of the occurrence of hyperthyroidism.

**Key words:** thyroid gland, ApoE KO<sup>-/-</sup> female mice, morphometric analysis, l-thyroxine.

### Introduction

The thyroid gland is an endocrine gland, producing hormones which play a role in the basal metabolism of the body. Thyroid hormone increases the metabolic rate of cells of all tissues in the body [1]. This means that the thyroid gland has a leading role in the metabolism of the lipoproteins in the body.

When the gland is in a normal physiological status histo-morphologically it is presented with round or oval follicles. The largest follicles are located in the peripheral zone and the follicles of smaller sizes are in the central zone of the gland. The walls of the follicles are lined with cuboidal thyroid epithelium in one layer, which has fluctuations in height depending on the functional status of the gland [8]. The lumen of the follicles is filled with an eosi-

nophilic, homogenous colloid, containing resorptive vacuoles [9, 13]. When the thyroid gland is in a hyperthyroid status, there are morphological changes in its structure: changes in order to resize follicles, the occurrence of follicles with an irregular shape, a changes in the height and shape of the thyroid epithelium and the amount of colloid filling the follicles [5].

Thyrocytes secrete hormones into the lumen of the follicles, stored as colloid and this presents a depot for these hormones. Thyroxine is released from thyroglobulin by proteolysis and secreted into the blood. Thyroxine is peripherally deiodinated to form triiodothyronine (T<sub>3</sub>) which exerts a broad spectrum of stimulatory effects on cell metabolism. Levothyroxine (l-thyroxine) is a synthetically prepared levo-isomer of thyroxine, typically used to treat hypothyroidism [4].

L-thyroxine is a synthetic form of the thyroid hormone thyroxine and is often used for the treatment of hypothyroidism and thyroid hormone deficiency. It has the ability to lower the thyroid-stimulating hormone (TSH), a hormone that is considered goitre-inducing [10, 11].

Morphological examination of the thyroid gland is extremely important because changes in the structure of the gland indicate its functional status, or they may indicate a medically induced state of hypothyroidism or hyperthyroidism (Serakides *et al.*) [3].

The aim of this study was to determine histo-morphological changes in the structure of the thyroid gland after l-thyroxin treatment in dislipidemic apolipoprotein E deficient female mice (ApoE KO<sup>-/-</sup>).

In order to investigate the influence of thyroid status on the metabolism of lipo-proteins, we used exactly these mice because they have a specific genetic base, C57BL/6, which gives spontaneously developed atherosclerosis. The establishment of this animal model allows testing requirements of the occurrence of atherosclerosis and its relation to thyroid function.

This paper is part of a larger study dedicated to the emergence and development of atherosclerosis associated with impaired function of the thyroid gland.

Therefore, the aim of the study was to determine the effect of l-thyroxine on the histological structure of the thyroid gland in female ApoE KO<sup>-/-</sup> mice.

### Material and methods

The experiment included 12 dislipidemic apolipoprotein E deficient female mice (ApoE KO<sup>-/-</sup>). 5 week old animals were divided into two groups. Each group was represented with 6 animals: the first one, the control group, received distilled water, the second one, the experimental group, received L-thyroxine diluted in the drinking water at a daily dose of 2 µg/ml. The drug was administered for 12 weeks in an attempt to obtain an iatrogenic status of thyroid dysfunction in the animals (hyperthyroidism).

The animals were acclimatised to a room temperature of 18–22°C, submitted to a regimen of 12 hours of light and 12 hours of darkness, supplied with water and food *ad libitum*.

All the procedures concerning manipulation with the animals were in accordance with the legal legislatives for experimental work with laboratory animals.

After the treatment period of 12 weeks, the animals were sacrificed using ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.), as is described in the literature [16, 18]. One type of anaesthetic is ketamine, used mainly in animal research. Ketamine acts as a non-competitive antagonist of N-methyl-D-aspartate (NMDA), one of the glutamate receptors. A consequence is a reduction of the action of glutamate, which is essential for neuronal survival. Ketamine can also be combined with xylazine, a painkiller and muscle relaxant. Xylazine is an agonist of alpha 2 adrenergic receptors located presynaptically. These receptors reduce noradrenaline release by inhibiting the influx of calcium in the neuron, with a consequent hypotensive and tranquilizing effect [17]. In one study it has been observed that the ketamine/xylazine combination markedly suppresses cardiac function, characterized by reductions in heart rate [18]. The animals were not sacrificed with ether because of its hepatotoxic effect and disturbance of the biochemical processes in the blood [19]. Also, animals were not sacrificed by bleeding, because we need the blood for use in additional analyses for further investigations.

Thyroid glands were extirpated and fixed in buffered formalin, processed to the routine technique of paraffin inclusion [6] and stained according to the haematoxylin-eosin method [7]. Both histological (qualitative) and morphometric (quantitative) analyses were done. In order to measure the diameters of the follicles and the height of the thyroid epithelium in the peripheral and the central zone of the thyroid gland, we assessed the sections of the middle part of the gland. For the morphometric analysis we used an eyepiece (enlargement 40 and 100 ×), with a built-in micrometer ruler with 100 length divisions, using light microscope, model Olympus CX21FS1. Diameters of the follicles were measured on 10 randomly selected follicles in the peripheral and 10 follicles in the central zone of the gland. The height of thyroid epithelium was determined in the same follicles.

Statistical elaboration of the morphometric results was performed using the Statistica software program, version 7.0, defining the

means and standard deviation of each morphometric parameter. The differences between the groups were tested using the Student-Newman-Kewls test (t-test) and the correlation was set using Pearson's coefficient (p) with a level of significance  $p < 0.05$ .

## Results

### *Qualitative assessment*

In the control group of animals, thyroid glands presented with round shaped or oval follicles of different size. Larger follicles were located in the peripheral zone and those with a smaller dimension were noted at the central part of the gland. Their wall presented with a low cuboidal thyroid epithelium in one layer. The cells had a smooth apical surface which was in direct contact with the colloid. The lumen of the follicles was filled with homogenic, eosinophilic colloid. In the peripheral parts of the colloid rare and small resorptive vacuoles were noticed. The interstitial connective tissue of the gland presented with thin fibrous septa between the follicles (see Fig. 1).

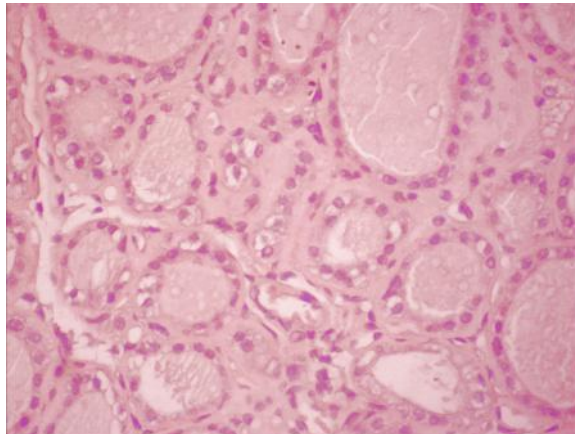


Figure 1 – Thyroid gland in ApoE KO<sup>-</sup> female mice – control group. Round and oval follicles, lined with cuboidal thyroid epithelium and filled with vacuolized colloid. H&E (10 × 40)

In the experimental group of animals, thyroid glands presented with follicles of various sizes with a dominance of large and distended follicles in the peripheral zone of the gland. Contrary to that, the dimensions of the follicles were lowered in the central zone of the gland. The thyroid epithelium was flattened and squamous in the follicles of the two zones of the gland. Cell nuclei were also flattened and hyperchromatic. Follicles contained a large qu-

antity of homogenous, eosinophilic colloid. There were no resorptive vacuoles inside the colloid. The interstitium of the gland presented with scanty connective tissue between the follicles, when compared with the control group (see Fig. 2, 3).

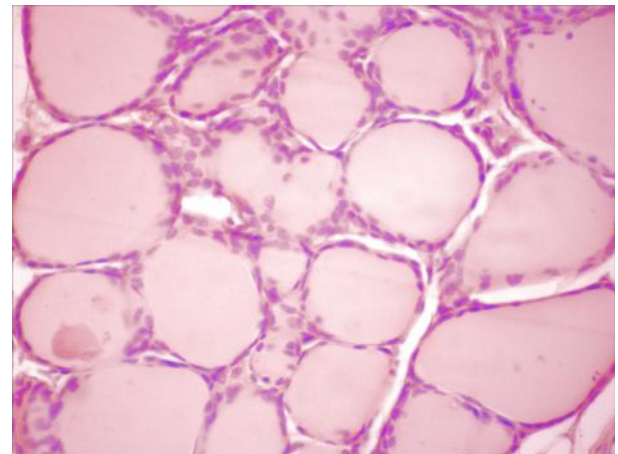


Figure 2 – Thyroid gland in ApoE KO<sup>-</sup> female mice – experimental group. Distended follicles with flattened epithelium, filled with colloid. H&E (10 × 40)

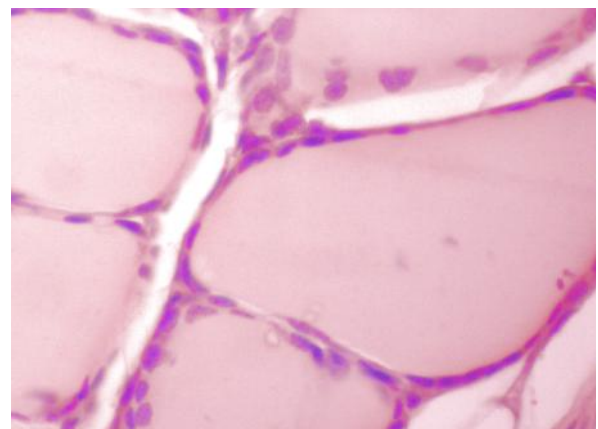


Figure 3 – Thyroid gland in ApoE KO<sup>-</sup> female mice – experimental group. Flattened thyroid epithelium in distended follicles filled with colloid, H&E (10 × 100)

### *Quantitative assessment*

#### Size of the follicles in the thyroid gland

In the control group of animals the diameter of the follicles depends on their localization in the gland. Peripheral follicles have a greater diameter when compared with central follicles in the gland. The comparison of the mean values of follicular diameter in the peripheral zone of the gland between the control and treated group of mice has shown an insignificant increasing of this parameter: from  $108.95 \pm 20.47 \mu\text{m}$  as was found in the control group

to  $125.05 \pm 43.73 \mu\text{m}$  after l-thyroxine administration ( $p = 0.102$ ). Contrary to that, the diameter of the central follicles was significantly

decreased from  $68.45 \pm 17.57 \mu\text{m}$  (control group) to  $52.20 \pm 8.80 \mu\text{m}$  (group treated with l-thyroxine),  $p < 0.001$  (see Table 1).

Table 1

*Size of follicles in thyroid gland in female ApoE KO<sup>-/-</sup> mice*

Diameter ( $\mu\text{m}$ )	Control group ( $\bar{X}$ ; $\sigma$ )	Experimental group ( $\bar{X}$ ; $\sigma$ )	t-test	p
Peripheral follicles	$108.95 \pm 20.47$	$125.05 \pm 43.73$	-1.66	0.102
Central follicles	$68.45 \pm 17.57$	$52.20 \pm 8.80$	4.13	0.001

$p < 0.05$  (significant level)

*Height of the epithelium in the follicles*

Morphometric assessment showed statistical significance in the decreased height of the follicular epithelium, in both peripheral and central follicles. The height of the thyroid epithelium in the peripheral follicles was  $9.9 \pm 2.10 \mu\text{m}$  in the control group of mice and was

significantly decreased to  $4.95 \pm 1.16 \mu\text{m}$  after l-thyroxine treatment. The height of the thyroid epithelium of the central follicles significantly decreased from  $10.3 \pm 1.95 \mu\text{m}$  in the control group to  $4.10 \pm 1.22 \mu\text{m}$  after l-thyroxine application ( $p < 0.001$ ); see Table 2.

Table 2

*Height of follicular epithelium in female ApoE KO<sup>-/-</sup> mice*

Height of epithelium ( $\mu\text{m}$ )	Control group ( $\bar{X}$ ; $\sigma$ )	Experimental group ( $\bar{X}$ ; $\sigma$ )	t-test	p
Peripheral follicles	$9.9 \pm 2.10$	$4.95 \pm 1.16$	10.29	0.0000
Central follicles	$10.3 \pm 1.95$	$4.10 \pm 1.22$	13.44	0.0000

$p < 0.05$  (significant level)

## Discussion

The normal histological structure of the thyroid gland in the athyreotic condition is presented with round shaped or oval follicles of different size. Larger follicles are located in the peripheral zone and those of smaller dimension are noted at the central part of the gland. Their wall is lined by one layer of cubical or low columnar epithelium. Cell nuclei are round and euchromatic. Cell cytoplasm is filled with colloid resorptive droplets in the apical part of the cell. These cells produce thyroxine and triiodothyronine. Follicles are filled with colloid, a depot for these hormones, which contains small resorptive vacuoles. There is another type of cell on the basal lamina of the follicles, parafollicular cells, and these produce the hormone calcitonine [9].

Among the population of Macedonia the presence of dysfunction of the thyroid gland is often observed [14,15]. The occurrence of hy-

pothyroidism (a state in which the thyroid gland does not produce a sufficient amount of the thyroid hormones thyroxine and triiodothyronine) is especially common. This condition of the body must be treated by drug (hormone) therapy. On the other hand, drug therapy may lead to structural changes in the tissues of the thyroid gland.

In our study we analysed the histological structure of the thyroid gland according to certain qualitative and quantitative parameters. Qualitative assessment of the gland in the experimental group of animals showed changes in the histological structure of the thyroid gland. Changes were noticed in the size and the shape of the follicles. They were large and distended in both peripheral and central parts of the gland. The epithelium lining the follicles was flattened. Cell nuclei were hyperchromatic. The lumen of follicles was filled with a lot of colloid and there were no resorptive vacuoles in

the colloid. Similar findings have been presented by other authors, Ferreira *et al.* [5]. Qualitative analysis of the morphological changes of the thyroid gland in our study indicated a medically induced state of hyperthyroidism. Quantitative assessment of the gland was based on quantitative measurement of the size of the follicles in the peripheral and central parts of the gland and height of the follicular epithelium. Morphometric analysis of the height of the follicular epithelium showed statistically significant differences when compared with the control group. In the control group of female ApoE KO<sup>-/-</sup> mice the height of the epithelium in the peripheral follicles was  $9.9 \pm 2.10 \mu\text{m}$  and in the group of animals treated with l-thyroxine this value was  $4.95 \pm 1.16 \mu\text{m}$ . Quantitative morphometric analysis confirmed the qualitative analysis. In the study made on female mice by Ferreira *et al.*, the values for the height of the follicular epithelium was  $3.7 \pm 0.28 \mu\text{m}$  in the euthyroid group and  $2.31 \pm 0.22 \mu\text{m}$  in the hyperthyroid group [5]. The results of the histological analysis of our study were very similar to the results obtained by Ferreira *et al.* Shalaby NM. demonstrated thyroid glands with variable changes in albino rats injected with amiodarone for 3 months: a few samples were more or less similar to the control ones, while others showed a dilated lumen engorged with colloid and lined with flat squamous cells [12].

The results of this study are similar to the results found in the literature concerning the morphological changes seen in the thyroid gland, in terms of matching hyperthyroidism. This paper makes a contribution to the research because it is set with dislipidemic apolipoprotein E deficient female mice (ApoE KO<sup>-/-</sup>), a new animal model that allows examination of the relationship of atherosclerosis with the state of hyperthyroidism.

Morphological assessment of the thyroid gland in female ApoE KO<sup>-/-</sup> mice treated with l-thyroxine confirmed histological changes in the parenchima of the gland in addition to hyperthyroidism.

### Conclusion

The results obtained in this investigation have shown that 12 weeks application of l-thyroxine at a daily dose of  $2 \mu\text{g/ml}$  provoked distended and enhanced follicles in the peripheral

parts of the gland, filled with lots of colloid without resorptive vacuoles and flattened epithelium in the follicles of both parts of the gland, its peripheral and central zone.

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ApoE KO<sup>-/-</sup>

12

ApoE KO<sup>-/-</sup>

2 µg/ml,

12

( $p < 0,001$ ).

, ApoE KO<sup>-/-</sup>