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Histomorphological features of the thyroid gland in rats of different ages with alimentary-induced obesity

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Abstract

The aim of the study was to compare histomorphological disorders of the thyroid gland in rats of different ages with alimentary-induced obesity. The study was carried out on male Wistar rats, aged six and 21 months. Alimentary obesity in animals was modelled by keeping them on a high-calorie diet (580 kcal 100 g^{-1}) for 12 weeks, with an excess content of fats and carbohydrates. Control animals were given a standard diet (330 kcal 100 g^{-1}). Histological preparations were made from thyroid tissue. Histomorphometry was carried out using the computer program "Image J". It was revealed that a 3-month high-calorie diet led to the appearance of pronounced histomorphological signs of thyroid hypofunction in rats. Intensive colloid resorption and a decrease in colloid area occurred in the gland. Devastated follicles were common. Thyrocytes acquired a prismatic shape. There was hyperplasia of the thyroid gland, which led to an increase in its size. The intensity of morphological disorders of the thyroid gland depended on the degree of obesity and had a distinct age-dependent character. In young rats, these changes were more pronounced than in older animals.

Key words: alimentary obesity, rats, thyroid gland.
Abbreviations: AO, alimentary obesity; CT, connective tissue.

Introduction

In recent decades, the prevalence of obesity has increased rapidly, reaching pandemic proportions. This problem is becoming a heavy social and economic burden for modern society. The causes of obesity, in the first place, are the features of lifestyle and malnutrition, mainly excess caloric content of food with a predominance of fats and carbohydrates in the diet. International attention to the problem of obesity has led to a sharp increase in the number of studies on the possible relationship between obesity and other diseases of various organs and systems of the body (Grundy 2004; Han, Lean 2016; Yanko et al. 2021). Obesity is accompanied by a violation of all types of metabolism and decrease in the function of most organs, including endocrine glands (Zakharova et al. 2013).

In the scientific literature, there is still no definite opinion on the nature of changes in the functional activity of the thyroid gland in obesity (Sanyal, Raychaudhuri 2016). To date, there is a particular lack of research regarding the study of structural changes in the thyroid gland in humans or animals with obesity. The relationship between dietary obesity and thyroid function is complex. Overeating, which leads to weight gain and obesity, seems to activate the hypothalamic-pituitary-thyroid axis, which leads to changes in the functional state of the gland (Laurberg et al. 2012). It has been suggested that obesity, an inflammatory condition, and lipotoxicity may lead to thyroid dysfunction (Song et al. 2019; Walczak, Sieminska 2021). Despite the prevailing idea that reduced thyroid function plays a significant role in the pathogenesis of obesity, there is still no definite opinion in the literature about the nature of the functional activity of this organ in obesity (Demidova, Galieva 2007).

The thyroid gland, like other organs, reacts differently to the same adverse effects in the process of ontogenesis (Ajish, Jayakumar 2012). The age-related susceptibility of this organ to the adverse effects of obesity is no exception (Ghergherehchi, Hazhir 2015). At the same time, there is no unanimous opinion about the features of the effect of alimentary obesity (AO) on the histomorphological changes in the thyroid gland in animals of different ages. Therefore, we studied the intensity of age-related structural changes in the thyroid gland in relation to AO in rats of different age groups.

The aim of the study was to compare the histomorphological disorders of the thyroid gland in rats of different ages, in relation to AO.

Materials and methods

Forty-eight Wistar male rats aged three months (weighing 250 ± 10 g) and 18 months (weighing 450 ± 10 g) were taken for the experiment. There were 12 rats in each group. AO was modelled by feeding animals on a high-calorie diet. Experimental rats were kept on a diet with excess fat (45%) and carbohydrates (31%) for 12 weeks, Each rat received 6 g of a specially prepared granular feed (70% standard mixed feed with the addition of 30% lard) containing 6.8 g lard, 3.6 g white crackers, and 3.6 g sunflower seeds, which provided a total of 116 kcal. Experimental animals received food ad libitum under daily control of the completeness of its consumption. In one day, instead of water, experimental rats received a 10% fructose solution. Control animals received a standard diet. The rats of the control group received 20 g of balanced feed daily, with a calorie content of 66 kcal (Yanko et al. 2022).

The presence of AO in rats was determined at the end of the experiment by measuring the mass of visceral fat and its ratio to body weight. Visceral fat was isolated by dissection method. Rats were terminated at the age of six and 21 months by decapitation under ether anaesthesia. The studies were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg 1986). The study protocol (No. 5 dated 11.31.2019) was also approved by the biomedical ethics committee of the Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine.

Tissue samples were taken from the thyroid gland for morphological and morphometric studies. Histological preparations were made according to the standard method: they were fixed in Bouin's fluid, dehydrated in alcohol of increasing concentration (from 70 to 96%) and dioxane. The obtained samples were embedded in paraffin. Paraffin sections 6-μm thick were made on a sledge microtome. The resulting sections were stained with Behmer's hematoxylin and eosin. The Van Gieson staining method was used to visualize connective tissue (Nikishin 2008). Micropreparations were photographed using a digital camera using a "Eclipse E100" microscope (Nikon, Japan). Morphometry on digital images of micropreparations was carried out using the "Image J" computer program.

In total, 96 histological preparations of the thyroid gland were processed and on each, 210 measurements were made: cross-sectional area of follicles (20), colloid (20), follicular epithelium (20), external (20) and internal (20) diameter of follicle and height of follicular epithelium (20). The mean number of thyrocytes in the follicles (20) was determined. The follicular-colloidal index (the ratio of area of the follicular epithelium to area of the colloid), the stereological resorption index (4/h, where h is the mean length of chords of line segments per colloid), and the colloid accumulation index (ratio of the average internal diameter to the double height of the epithelium) were determined. Using the method of point morphometric grids, we determined the relative area of connective and parenchymal tissue of the gland (10) and calculated the stromal-parenchymal index (ratio of the relative area of stroma to relative area of parenchyma of the gland). The width of layers of interlobar (20), interlobular (20) and interfollicular (20) connective tissue was measured (Nikishin 2008; Yanko, Levashov 2021).

The obtained data were statistically analyzed using "Statistica 6.0 for Windows" software (StatSoft, USA) and the "Excel 2010" program (Microsoft, USA). The normality of data distribution was checked using the Pearson test. When the distribution was normal, the Student's *t*-test was used to determine significant differences between control and experimental groups. Differences were considered significant at $p < 0.05$.

Results

The mean mass of visceral fat in both 6- and 21-month-old rats on a high-calorie diet was significantly higher by 145 and 58%, respectively, compared with control rats of the same age. The ratio of visceral fat mass to body weight (visceral adiposity index) in experimental animals was also significantly higher than the control values, by 122% (6 months) and 56% (21 months) (Table 1). Changes in these parameters indicated the presence of a pronounced AO in experimental rats. The development of obesity was more intense in young animals. A statistically significant ($p < 0.05$) inverse correlation was found between age and total visceral fat mass and visceral obesity index ($r = -0.757$ and -0.838, respectively) in rats with AO. This suggests that young animals on a high-calorie diet are significantly more likely to develop alimentary obesity than older animals. In control animals, there was no statistically significant correlation between age and indicators of visceral obesity.

An increase of thyroid gland mass was observed in rats fed a high-calorie diet. The mass of the thyroid gland in 6- and 21-month-old experimental rats was significantly higher (by 71 and 67%, respectively), compared with the control (Table 1).

Some histomorphometric differences in the structure of the thyroid gland of control rats of different ages were observed. In rats aged 21 months, compared with rats aged 6 months, a the area of follicles and follicular epithelium was smaller and the colloid area was somewhat larger. The height of thyrocytes in old rats, compared with young animals, was significantly lower (by 15%). Also, in 21-month-old rats, a lower follicular-colloidal index (by 23%, $p < 0.05$) and stereological resorption index (by 9%), and higher colloid accumulation index (by 30%, $p < 0.05$) was observed compared with the younger rats (Table 2). The above indicated a decrease in thyroid function with age, which is consistent with the general biological pattern.

Table 1. Weight of thyroid gland and visceral fat (n = 12, M ± m). *p < 0.05 – significance of differences compared with the control; ^p < 0.05 – significance of differences compared with the control of 6-month-old rats

Indicators	6-month-old rats		21-month-old rats	
	Control	Obesity	Control	Obesity
Mass of visceral fat (g)	19.0 ± 1.1	46.6 ± 1.7*	23. 9± 1.6^	37.7 ± 1.0*
The ratio of visceral fat mass / body weight	0.046 ± 0.005	0.102 ± 0.01*	0.052 ± 0.006^	0.081 ± 0.006*
Thyroid mass (mg)	24 ± 1	41 ± 2*	18 ± 1^	30 ± 2*

The thyroid gland in rats of the control group had a characteristic lobular structure and consisted of round and oval follicles. On the periphery, the follicles were larger compared to the central part of the gland, often irregular in shape with stretched walls formed by a flattened epithelium. The wall of the follicles was formed by cubic thyrocytes located on the basement membrane. An oxyphilic colloid of moderate density was located in the cavity of the follicles. Intensive colloid resorption and a decrease in its area occurred in the thyroid gland of rats with AO. The follicles were small, but were more numerous. Empty follicles were often observed, which indicated depletion of the gland and its hypofunction, and inhibition of colloid accumulation. Thyrocytes increased in size, often acquired a prismatic shape (Fig. 1 & 2).

Histomorphometric examination showed that in the thyroid gland of young rats with AO, the area of follicles was significantly lower (by 39%), colloid by 59% and follicular epithelium by 28%, compared with the control. These parameters changed less significantly in old experimental rats. The area of follicles tended to decrease, the area of colloid significantly decreased by 34%, and the area of follicular epithelium, in contrast, increased by 18% (Table 2).

The external and internal diameters of the thyroid follicles in 6-month-old rats with AO were significantly smaller (by 21 and 59%, respectively), compared to the control. However, in experimental rats aged 21 months, only the internal diameter of the follicles was significantly smaller (by 27%) (Table 2).

The follicular epithelium is formed by thyrocytes, which make up the main part of the thyroid parenchyma. Thyrocytes of experimental rats had a prismatic and cubic shape. The height of the cells did not change in young experimental animals. However, in old rats it significantly increased (by 37%) compared with the control. The average number of thyrocytes in the follicle of 6-month-old rats was 13% lower (p < 0.05), and in 21-month-old rats 8% lower (Table 2).

The stereological resorption index characterizes the dynamics of accumulation and removal of the colloid. We found a significant increase in this index in rats with AO histomorphometry, by 50% (6 months) and 38% (21 months), compared with the control. The follicular-

Table 2. Morphometric parameters of the thyroid gland (n = 12, M ± m). *p < 0.05 – significance of differences compared with the control; ^p < 0.05 – significance of differences compared with the control of 6-month-old rats

Indicators		6-month-old rats		21-month-old rats	
		Control	Obesity	Control	Obesity
Parenchyma					
The relative area (%)		80.7 ± 1.6	79.2 ± 1.1	76.8 ± 1.7	80.1 ± 2.1
Area (μm^2)	follicle	2675 ± 75	1630 ± 61*	2419 ± 78	2331 ± 60
	colloid	945 ± 56	392 ± 22*	1001 ± 68	661 ± 25*
	follicular epithelium	1730 ± 80	1238 ± 69*	1418 ± 51^	1670 ± 79*
Follicle diameter (μm)	external	52.0 ± 1.2	41.3 ± 1.1*	51.4 ± 2.1	49.4 ± 1.9
	internal	29.6 ± 1.4	19.8 ± 1.4*	32.4 ± 1.4	23.5 ± 1.3*
The height of follicular epithelium (μm)		11.2 ± 0.3	10.8 ± 0.8	9.5 ± 0.8^	13.0 ± 0.5*
Follicular colloid index		1.83 ± 0.12	3.16 ± 0.80*	1.41 ± 0.17^	2.53 ± 0.35*
Colloid accumulation index		1.32 ± 0.09	0.92 ± 0.09*	1.71 ± 0.09^	0.90 ± 0.04*
Stereological resorption index		0.135 ± 0.002	0.202 ± 0.010*	0.123 ± 0.006	0.170 ± 0.005*
The number of thyrocytes in the follicle (n)		21.8 ± 0.6	18.9±0.3*	20.6 ± 0.8	18.9 ± 0.2
Connective tissue					
The relative area (%)		19.3 ± 1.0	20.8 ± 0.9	23.2 ± 0.9^	19.9 ± 0.8
Stromal-parenchymal index		0.24 ± 0.02	0.27 ± 0.03	0.30 ± 0.01^	0.25 ± 0.03
The width of the interlayers of the connective tissue (μm)	interlobar	22.3 ± 0.5	23.5 ± 0.9	19.6 ±0.5^	26.6 ± 0.7*
	interlobular	8.0 ± 0.3	13.2 ± 0.2*	9.7 ± 0.5^	8.3 ± 0.2*
	interfollicular	1.42 ± 0.04	1.62 ± 0.06*	1.86 ± 0.12^	1.65 ± 0.08

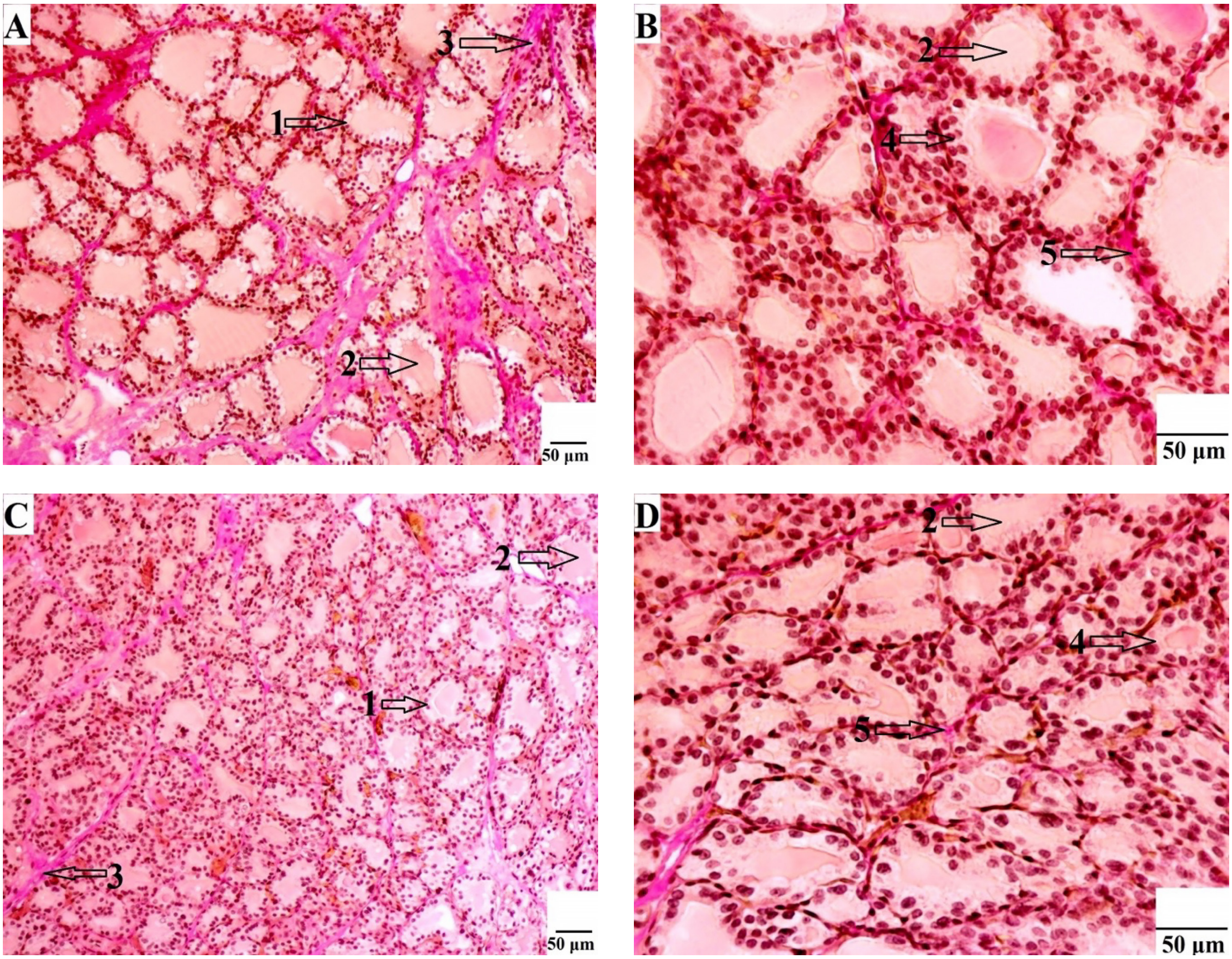

Fig. 1. Micrographs of the thyroid gland of a 6-month-old control rat (A, × 200, B, × 400) and a rat with alimentary obesity (C, × 200, D, × 400). 1, follicle; 2, colloid; 3, interlobular connective tissue; 4, follicular epithelium; 5, interfollicular connective tissue. Van Gieson stain.

colloidal index was significantly higher (by 73% in young rats and by 79% in old animals). The colloid accumulation index, in contrast, was lower in both 6-month-old and 21-month-old rats – by 30 and 47%, respectively (Table 2).

A clear trend towards an increase in the number of interfollicular islets in AO rats, regardless of age, was found (Fig. 1 & 2).

Correlation analysis showed a high negative correlation between the mass of visceral fat in rats with AO and histomorphological parameters of the thyroid gland – colloid area, internal diameter of the follicle and colloid accumulation index (respectively $r = -0.727$; -0.718 and -0.747) and a positive correlation with follicular-colloidal index ($r = 0.819$). No statistically significant correlation between the above indicators was found in rats of the control group.

A clear trend towards an increase in the relative area of the CT and stromal-parenchymal index was shown for 6-month-old rats on a high-calorie diet. A significant increase in the width of the interlobular and interfollicular CT layers by 65 and 14%, respectively, was also observed in these rats. The relative area of the CT and the stromal-parenchymal index had a slightly pronounced tendency to decrease in old rats with AO compared with the control. The width of the interlobar CT layers in these rats was significantly larger (by 36%), and the width of the interlobular CT was 14% smaller compared to the control. (Table 2).

Discussion

The results of our studies indicate that a high-calorie diet of rats for 3 months leads to the development of clear signs of AO. The mass of visceral fat and its ratio with body weight increased significantly. An increase in the mass of the thyroid gland in experimental animals indicated its hyperplasia, which occurred due to an increase in the number of cells. As is known, one of the main reasons for the development of thyroid hyperplasia is insufficient hormonal secretion, leading to active stimulation of the

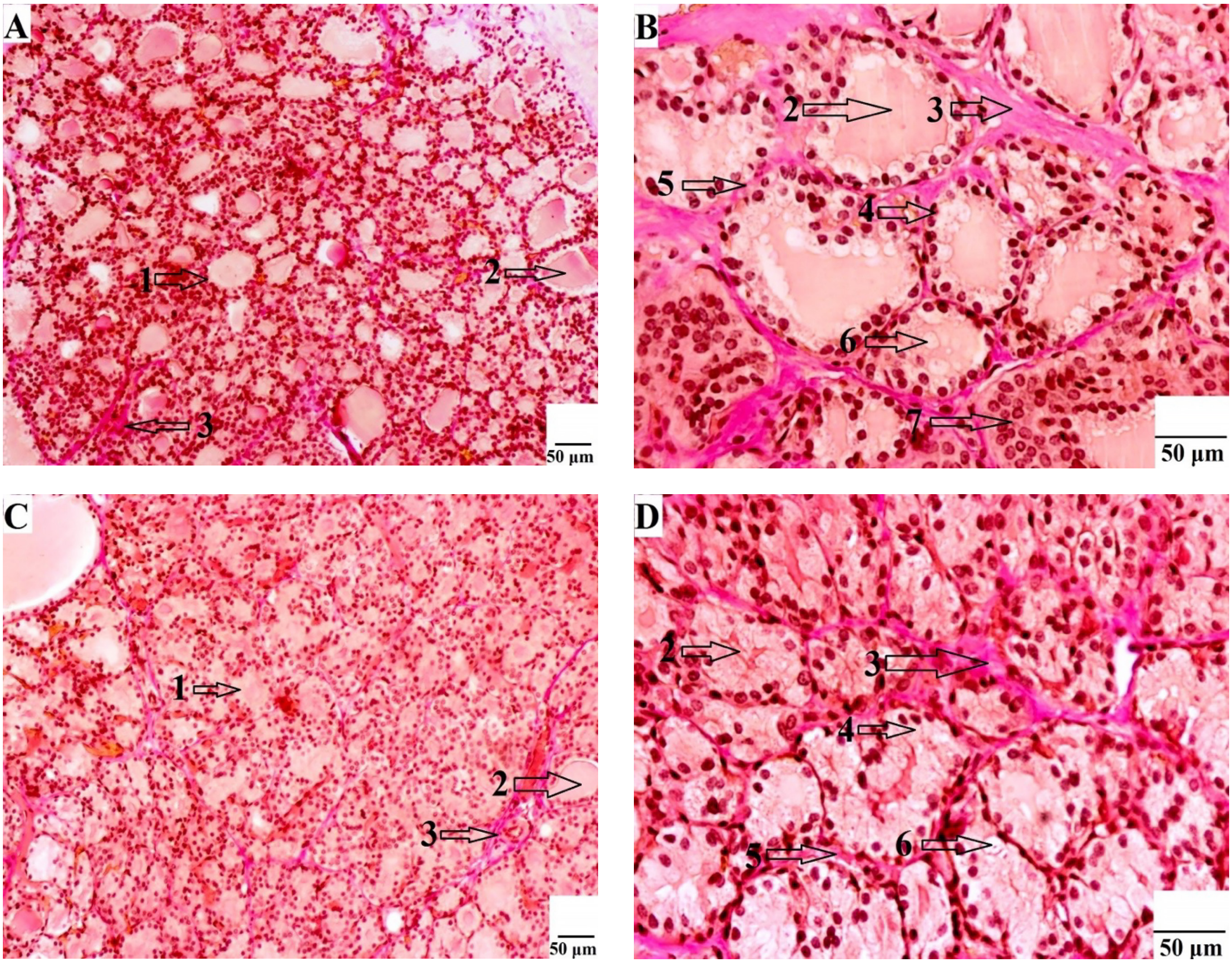

Fig. 2. Micrographs of the thyroid gland of a 21-month-old control rat (A, × 200, B, × 400) and a rat with alimentary obesity (C, × 200, D, × 400). 1, follicle; 2, colloid; 3, interlobular connective tissue; 4, follicular epithelium; 5, interfollicular connective tissue; 6, resorption vacuole; 7, interfollicular islets. Van Gieson stain.

gland with a subsequent increase in its size (Sheu et al. 2003).

It was found that in rats of different ages consuming a high-calorie diet, unidirectional changes in the structure of the thyroid gland were observed. However, in younger animals, changes in the gland were more pronounced. A decrease in the size of follicles and their colloid, the internal diameter of the follicles, the colloid accumulation index and the number of thyrocytes in the follicles, as well as an increase in the follicular-colloidal index and stereological resorption index were observed in the thyroid gland of experimental rats. Such changes in histomorphological parameters indicate devastation of the follicles from the colloid and inhibition of its accumulation. The results of correlation analysis confirmed a close relationship between the degree of obesity and the severity of histomorphological disorders of the thyroid gland in animals.

Thyrocytes line not only the wall of the follicle, but can be located in the middle (intrafollicular epithelium) or outside the follicles (interfollicular islets). Interfollicular islets are accumulation of thyrocytes without a cavity, which also produce small amounts of thyroid hormones. These islets are activated when the functional load on the gland increases. Thyrocytes begin to produce colloid, and the islets turn into normal follicles (Khan, Farhana 2021). A clearly pronounced trend towards an increase in the number of interfollicular islets formed by different types of thyrocytes was observed in experimental animals (Fig. 1 & 2). This was most likely due to the compensatory mechanisms of the gland (due to insufficient hormonal secretion), which manifested itself in thyrocyte hyperplasia.

In recent decades, scientists have tried to determine whether thyroid dysfunction is a cause or a consequence of excessive accumulation of adipose tissue in the body. The answer, however, is still unclear. The relationship between the hypothalamic-pituitary-thyroid axis and obesity is complex and involves various interactions. Thyroid hormones independently regulate adipose tissue mass and function, but adipose tissue, through adipokine production, also influences gland activity (Walczak 2021).

A previous study (El-Sayed, Ibrahim 2020) showed that after three months of feeding female rats on a high-fat diet, follicles of various sizes appeared in their thyroid gland, with a preponderance towards large follicles (macro-follicles). There was an excess amount of colloid in the follicles, the height of thyrocytes decreased, the cytoplasm of cells was vacuolated, and there was a violation of the membrane. Mast cell infiltration was identified in interfollicular CT (El-Sayed, Ibrahim 2020).

The structure of the CT of the thyroid gland includes a capsule and stroma. In the stroma, interlobar, interlobular and interfollicular CT are distinguished. Interlobar CT surrounds the thyroid gland from the outside. Interlobular CT occupies a much smaller area compared to interlobar CT. It consists of thin bundles of collagen fibers intertwined in different directions. Interfollicular CT consists of reticular and collagen fibers that are woven into the follicular membranes, connecting the follicles with each other (Nikishin 2008).

In another study, after 6 months of keeping rats on a high-fat diet, an increase in the concentration of triglycerides in both serum and in the thyroid gland, as well as a decrease in the concentration of thyroxine in the serum, was found, in parallel with an increase in the concentration of thyroid-stimulating hormone (Shao et al. 2014). The thyroid gland increased in size. In the thyroid gland of rats receiving a high-fat diet, the cavity of follicles increased and thyrocytes flattened, the cisterns of the endoplasmic reticulum expanded, and the number of microvillus and secretory vesicles decreased. In addition, in the thyroid gland of these rats, the concentration of proteins associated with the synthesis of gland hormones significantly decreased (Shao et al. 2014). It is possible that excessive obesity may lead to an increase in interfollicular fat depot or steatosis in thyroid follicular cells. Thus, steatosis and ultrastructural changes, including stretching of the endoplasmic reticulum and mitochondrial disorders in thyroid follicular cells, have been consistently observed in mice with AO (Min et al. 2015). Thus, the analysis of the literature once again confirms the ambiguity of existing data on the structural state of the thyroid gland in animals with AO. This may be due to the use of different models of alimentary-induced obesity, the duration of the experiments, the age and sex of the animals, etc.

As the results of our studies showed, a 3-month period of male Wistar rats on high-calorie diet led to the appearance of pronounced histomorphological signs of thyroid hypofunction. The severity of histomorphological disorders of the thyroid gland had a distinct age-dependent character and depended on the degree of obesity of the animals. In young rats, these changes were more pronounced than in old rats. The age dependence of the negative impact of obesity on the thyroid gland must be taken into account not only in the correction of clinically pronounced disorders of the gland function, but also at the preclinical stages of the development of obesity. The results obtained are not only of theoretical importance, but are also of interest for practical medicine in addressing treatment and prevention of thyroid diseases in patients of different age with AO. In the future, we plan to investigate the role of tryptophan amino acid metabolism products in the correction of hypothyroidism caused by alimentary obesity.

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