ACCSCIENCE PUBLISHING

Global Translational Medicine

ORIGINAL RESEARCH ARTICLE

Thyroid morphology and functional alterations in male and female rats with diet-induced visceral obesity

Tatyana A. Mityukova*, Anastasia A. Basalai*, Tatyana E. Kuznetsova, Olga Y. Poluliakh, and Mikita S. Kastsiuchenka

Department of Laboratory of Biomedical Technologies and Medical Rehabilitation, Institute of Physiology, National Academy of Sciences of Belarus, Minsk, Republic of Belarus

Abstract

With obesity reaching epidemic proportions worldwide, its impact on thyroid function is gaining increasing attention. Epidemiological studies show an association between obesity, hypothyroidism, and circulating thyroid antibodies but experimental research is needed to investigate the mechanisms underlying these associations. This study aimed to investigate thyroid function indicators in male and female rats subjected to a high-calorie diet for 16 weeks. We assessed mass-metric indices, blood biochemical markers, thyroid morphometry, and tissue concentrations of triglycerides, malonic dialdehyde (MDA), and thyroperoxidase (TPO) activity. The results revealed biochemical features of metabolic syndrome, including elevated thyroxine (T4) levels in peripheral blood. Morphological analysis indicated steatosis and thyroid hypofunction, with increased triglyceride accumulation, decreased TPO activity, and lower MDA levels in the thyroid tissue. These findings suggest that visceral obesity in male and female rats promotes early signs of thyroid dysfunction, potentially leading to hypothyroidism.

Keywords: High-calorie diet; Visceral obesity; Rats; Thyroid function; Thyroid hormones; Morphological changes

***Corresponding authors:**
Tatyana A. Mityukova (mityukovat@gmail.com)
Anastasia A. Basalai (anastasiya.basalay@gmail.com)

Citation: Mityukova TA, Basalai AA, Kuznetsova TE, Poluliakh OY, Kastsiuchenka MS. Thyroid morphology and functional alterations in male and female rats with diet-induced visceral obesity. *Global Transl Med*. 2025;4(2):86-95. doi: 10.36922/GTM025080020

Received: February 22, 2025

Revised: March 28, 2025

Accepted: April 1, 2025

Published online: April 16, 2025

1. Introduction

The link between obesity and endocrine dysfunction, including thyroid pathology, has been well established in many studies. As obesity reaches epidemic proportions worldwide, this relationship has gained increasing clinical relevance.[1] Thyroid hormones are known to play an important role in the regulation of metabolism. Elevated blood levels of thyroxine (T4) and triiodothyronine (T3) increase resting energy expenditure (REE), reducing fat accumulation. These hormones increase REE through sodium-potassium adenosine triphosphatase activity and activate the adrenergic nervous system to enhance thermogenesis, particularly in cold conditions.[2] This process, called "adaptive thermogenesis," occurs in brown adipose tissue through the thyroid hormone receptors TRα and TRβ.[2]

Obese individuals usually exhibit slightly elevated thyroid-stimulating hormone (TSH) levels, which correlate positively with body mass index (BMI).[3] A characteristic

hormonal pattern in obesity includes reduced free T4 (fT4) levels and moderately elevated levels of total or free T3 (fT3) levels. In addition, the fT3/fT4 ratio has been positively associated with waist circumference and BMI in obese patients.[3] The mechanisms underlying these changes remain controversial.

Leptin, an adipose tissue hormone, plays an important role in the regulation of the hypothalamic-pituitary-thyroid axis. It activates this system in response to excess caloric intake. This adaptive response to overnutrition results in a moderate increase in blood levels of thyrotropin-releasing hormone (TRH) and TSH. This is accompanied by a slight increase in circulating thyroid hormone levels within or slightly above the upper limit of normal.[2]

One theory explaining the relationship between obesity and thyroid function is increased deiodinase activity, leading to enhanced conversion of T4 to T3.[2] This may serve as a compensatory mechanism to counteract fat accumulation by increasing energy expenditure.[2] Another hypothesis suggests that obesity-induced reductions in TSH receptor expression in the thyroid and T4/T3 receptor expression in adipocytes drive compensatory increases in TSH and fT3 secretion.[2] In addition, leptin influences thyroid function by stimulating the central conversion of prothyroliberin to TRH and, consequently, the synthesis of TSH. Leptin also increases the activity of deiodinases. Pro-inflammatory cytokines secreted by adipose tissue – such as tumor necrosis factor-alpha, interleukin-1 (IL)1, and IL-6 – may also contribute to thyroid dysfunction by inhibiting sodium/iodide symporter Na+/I- symporter (NIS) mRNA expression and iodide uptake.[2]

Epidemiological studies suggest that the risk of obesity is associated with elevated TSH levels and reduced concentrations of the free T4 and T3 hormones.[4,5] These data suggest that reduced levels of circulating thyroid hormones are a predictor of obesity. Hypothyroidism and thyroperoxidase antibodies (TPO-Ab) are highly prevalent in people with excess fat mass.[6] A meta-analysis of 22 studies showed that obesity is associated with an increased risk of clinical and subclinical hypothyroidism.[6] Symptoms of hypothyroidism, including reduced physical activity, fatigue, sleepiness, memory loss, cold intolerance, weight gain, and constipation, often develop gradually. Another meta-analysis found that obesity was significantly associated with Hashimoto's thyroiditis, but not with Graves' disease. Weight gain correlated with TPO-Ab positivity but not with thyroglobulin antibodies (TG-Ab).[6] However, some studies have reported associations between subclinical hypothyroidism and both TPO-Ab and TG-Ab. A survey of 2,505 subjects showed that 11.5% of the subjects (289/2505) were obese, of whom 165 (57%) had subclinical hypothyroidism. Thyroid autoantibodies (TPO-Ab and TG-Ab) were found in 17.64% of the obese subjects.[7] Thus, a large body of data in the literature highlights the role of autoimmune processes in obesity-related thyroid glands. However, alternative mechanisms influencing thyroid hormone synthesis remain to be fully elucidated and warrant further investigation.

The aim of this study was to investigate indices of thyroid functional activity in diet-induced visceral obesity in male and female rats.

2. Materials and methods

2.1. Animals and diets

This study was conducted on 2-month-old sexually mature male and female Wistar rats. These rats were bred in-house in a certified vivarium at the Institute of Physiology of the National Academy of Sciences of Belarus. The rats were kept under controlled conditions (12/12 h light/dark cycle, 22 ± 2°C temperature, and 60 – 65% humidity). The male (n = 27) and female (n = 27) Wistar rats were randomly divided into two experimental groups: control and high-calorie diet (HCD). The control group, consisting of 13 male and 14 female rats, received a standard diet (StD; the normal diet at the vivarium), while the HCD group, comprising 14 male and 13 female rats, was given an HCD for 16 weeks.

The HCD consisted of StD supplemented with animal fats (lard), consisting of 45% of daily caloric intake, along with a 10% fructose solution provided *ad libitum* in place of water.[8] The caloric intake was 150 kcal/day for StD and 228 kcal/day for HCD.

This study was approved by the Bioethics Committee of the Institute of Physiology, National Academy of Sciences of Belarus (Protocol No.1, January 22, 2021; Protocol No.2, February 2, 2022) and conducted in accordance with the guidelines set forth by the European Convention for the Protection of Vertebrate Animals (ETS No. 123).

Euthanasia was performed through decapitation under sodium thiopental anesthesia. Female rats were euthanized during the diestrus phase of the estrous cycle, determined by the type of cells present in vaginal swabs.[9]

Body weight of the rats was measured using a weighing scale (Saturn ST-KS7230, China). After euthanasia, the visceral fat was collected and weighed on a laboratory weighing scale (Scout Pro, China). For male rats, the visceral fat mass included paranephral and epididymal fat deposits, while for female rats, it included paranephral and periovarian fat deposits. The mass coefficient (MC) of the visceral fat was calculated using the following formula: (Visceral fat mass/body weight) ×100%.

2.2. Morphological analysis

At necropsy, the thyroid gland was extracted from the animals for morphologic examination. Thyroid tissue fragments were subjected to rapid freezing in an HM 525 cryostat (MICROM International GmbH, Germany). Cryostat sections (7 μm) were stained with hematoxylin-eosin for structural analysis. Microstructure examination, morphometry, and microphotography were performed using a light microscope (Altami LUM-1, Altami LLC, Russia) equipped with a digital camera (1300D EOS Body, Canon, Japan).

Images were analyzed using ImageJ software (National Institutes of Health, USA). For thyroid follicles counted in ten fields of view at ×400 magnification, we measured follicle area (in μm^2), inner follicle diameter (in μm), and follicular epithelium height (in μm). The colloid accumulation index (CAI) was calculated as the ratio of the inner follicle diameter to twice the follicular epithelium height.

2.3. Biochemical and hormonal parameters

Biochemical parameters in blood serum and thyroid homogenates (diluted 1:80 in 0.05 M PBS) were determined using a BS-200 biochemical automatic analyzer (Shenzhen Mindray Bio-Medical Electronics, China) and commercial kits (Production Unitary Enterprise "Diasens," Belarus). Quality control was performed using control sera (Randox Laboratories, UK). Malonic dialdehyde (MDA) levels in thyroid homogenates were determined using the spectrophotometric method by reaction with thiobarbituric acid.[10] Thyroid peroxidase (TPO) activity was estimated based on iodide oxidation reaction in the presence of hydrogen peroxide, producing molecular iodine and I_3^- (periodide ion).[11] The periodide concentration, determined spectrophotometrically at 353 nm, was used to estimate TPO activity in thyroid tissue.[12] The triiodothyronine (T3) and thyroxine (T4) levels in blood serum were determined by enzyme immunoassay using commercial kits (Xema Co., Russia).

2.4. Statistical analysis

Statistical analysis was performed using Statistica 10.0 (Tibco, USA). Normality was assessed with the Shapiro–Wilk test. Parametric variables were expressed as mean ± standard deviation and analyzed with Student's t-test. Non-parametric variables were expressed as 25th percentiles, median, and 75th percentiles and analyzed using the Mann–Whitney U-test. Correlation analysis was performed using the Pearson correlation coefficient. A $p<0.05$ was considered statistically significant for all tests.

3. Results

3.1. Mass-metric parameters

At the start of the experiment, male and female rats (2 months old) weighed approximately 220 – 230 g. After 16 weeks, the mean body weight of males in the control group was 433.46 ± 48.38 g, while those in the HCD group reached 471.14 ± 94.11 g. The mean body weight of females in the control group was 310.93 ± 25.43 g, while that of the HCD group was 316.31 ± 72.07 g. No significant differences in final body weight were observed between the control and HCD groups of the same sex.

Male rats in the control group had visceral adipose tissue that weighed 7.99 ± 2.64 g and had an MC of 1.82 ± 0.49%. In the HCD group, these values increased significantly to 22.99 g ± 11.97 g ($p<0.001$) and 4.64 ± 1.67% ($p<0.0001$), respectively (Figure 1A and B). In female rats, visceral fat mass was 8.86 ± 4.86 g in the control group (MC: 2.78 ± 1.31%) and significantly higher than in the HCD group (17.22 ± 9.07 g, $p=0.006$; MC: 5.15 ± 2.11%; $p=0.002$) (Figure 1A and B). These data indicate the development of visceral obesity in rats of both sexes following HCD administration.

3.2. Morphological analysis of the thyroid gland

The examination of thyroid gland preparations from control male and female rats revealed a well-developed follicular structure with thin connective tissue septa separating gland lobules (Figure 2A and C). The follicles were round or angular, lined with single-layer cuboidal epithelium, and larger at the gland periphery compared to the center. The follicular cavities contained colloid, including thyroglobulin. Thyrocytes had a cubic shape, with nuclei located in the basal part of the cell (Figure 2B and D). Their cytoplasm appeared oxyphilic, and the cells were tightly adherent. Interfollicular islets, consisting of poorly differentiated thyrocytes, were observed between follicles. These features indicate that the thyroid glands of rats maintained on a balanced diet were within physiological norms.

In the HCD group, male rats maintained the thyroid gland's lobular structure (Figure 3A), but follicles enlarged with colloid accumulation (Figure 3A). The walls of follicles were thinned (Figure 3B) and thyrocytes appeared flattened, revealing pyknotic nuclei surrounded by a thin rim of cytoplasm. The number of interfollicular islets was insignificant, and moderate diffuse leukocytic infiltration was observed. Large lipid inclusions were frequently visualized near blood vessels (Figure 3A and B). Morphometric analysis relative to the control males showed a statistically significant increase

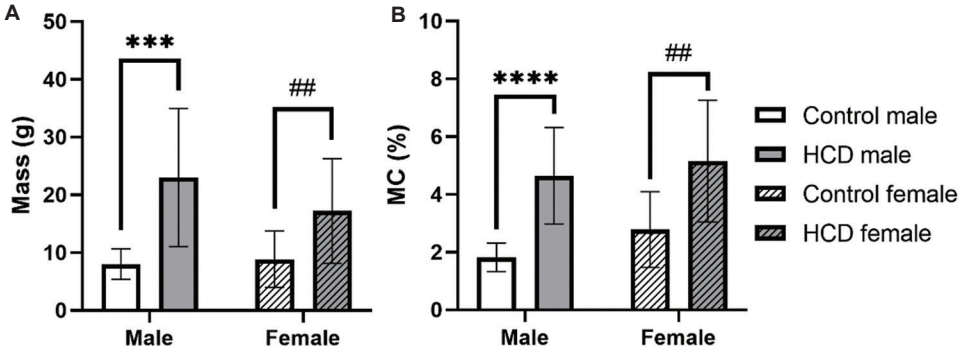

Figure 1. Mass-metric indices of visceral adipose tissue in experimental animals. Mass (A) and MC (B) of visceral adipose tissue in male and female rats. Data are presented as mean ± standard deviation. Statistically significant differences: ***p<0.001, ****p<0.0001 between male HCD and male control groups; ##p<0.01 between female HCD and female control groups.
Abbreviations: HCD: High-calorie diet; MC: Mass coefficient.

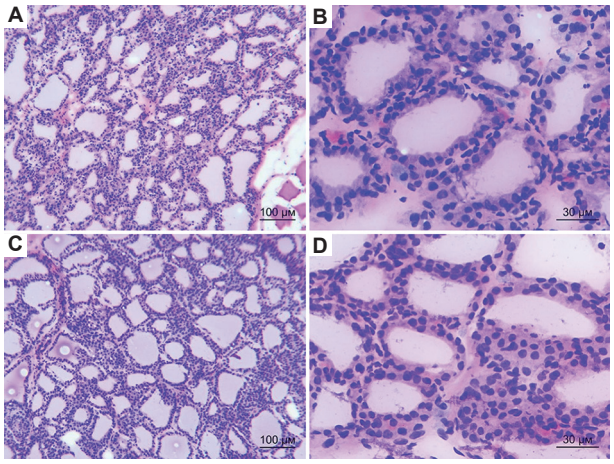

Figure 2. Histological structure of hematoxylin-eosin-stained thyroid glands of control male rats (A and B) and female (C and D) rats. Magnification: A and C: ×100; B and D: ×400.

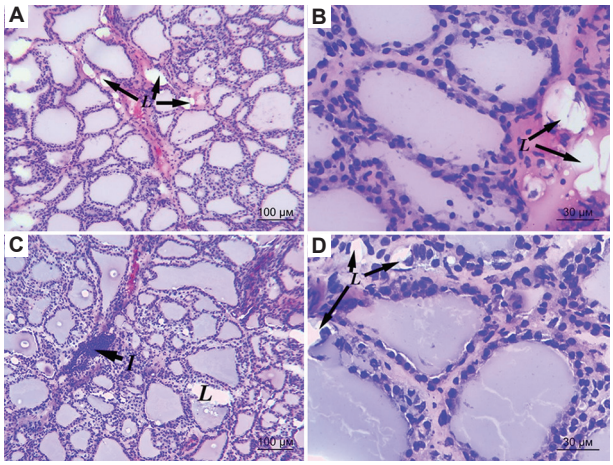

Figure 3. Histological structure of hematoxylin-eosin-stained thyroid glands in HCD male (A and B) and female (C and D) rats. Magnification: A and C: ×100; B and D: ×400.
Abbreviations: HCD: High-calorie diet; I: Inflammatory infiltration; L: Lipid inclusions.

in follicle area (50.7%, p<0.0001), follicle inner diameter (14.0%, p=0.043), and CAI (67.9%, p<0.0001). The height of follicular epithelium was reduced by 37.0% (p<0.0001) (Table 1). These histological and morphometric changes are characteristic of thyroid hypofunction.

In the HCD female rats, the follicular structure of the thyroid was also preserved, with follicles enlarged, colloid accumulation, and flattened thyrocytes (Figure 3C). Moderate diffuse inflammatory infiltration was also evident, with large foci of lymphocytic infiltrate in some regions (Figure 3C). Large lipid inclusions were observed in connective tissue septa and perivascular spaces (Figure 3C and D). Morphometric analysis relative to the control revealed a statistically significant increase in follicular area (73.3%, p<0.0001), follicle inner diameter (36.6%, p<0.0001), and CAI (1.9-fold, p<0.0001). The height of follicular epithelium was reduced by 24.0% (p<0.0001) (Table 1). Similar to HCD male rats, these histological and morphometric alterations suggest thyroid hypofunction.

3.3. Biochemical and hormonal parameters of blood serum and thyroid gland

Biochemical analysis of blood serum from male rats in the HCD group revealed changes associated with liver dysfunction (Table 2). Compared to the control group, a statistically significant change in four liver function markers was observed: urea concentration (−36.3%, p<0.0001), aspartate aminotransferase activity (−12.2%, p=0.019), alkaline phosphatase activity (+80.8%, p=0.009), and total bilirubin level (+71.4%, p=0.007). The HCD female showed similar statistically significant alterations in urea concentration (−42.4%, p<0.0001), alanine aminotransferase activity (−33.3%, p=0.002), alkaline

Table 1. Morphometric indices of thyroid tissue in experimental animals

Index	Male rats		Female rats	
	Control (*n*=13)	HCD (*n*=14)	Control (*n*=14)	HCD (*n*=13)
Follicle area (μm²)	3694.67 (2629.37, 5529.83)	5566.28 (4039.65, 7748.07)****	2513.43 (1737.55, 3932.96)	4355.28 (2677.50, 6265.74)####
Inner follicle diameter (μm)	44.30 (33.27, 56.00)	50.51 (36.27, 65.20)*	39.84 (32.62, 51.49)	54.41 (41.15, 74.41)####
Follicle epithelium height (μm)	7.52 (6.47, 8.32)	4.74 (4.28, 5.27)****	6.63 (6.15, 7.18)	5.04 (4.65, 5.53)####
Colloid accumulation index	3.08 (2.34, 4.05)	5.17 (3.69, 7.07)****	2.95 (2.54, 3.83)	5.58 (4.27, 7.25)####

Note: Data are presented as median (25th percentile, 75th percentile). Statistically significant differences: *p<0.05, ****p<0.0001 between male HCD and male control groups; ####p<0.0001 between female HCD and female control groups.
Abbreviation: HCD: High-calorie diet.

Table 2. Biochemical indices of serum in experimental animals

Index	Male rats		Female rats	
	Control (*n*=13)	HCD (*n*=14)	Control (*n*=14)	HCD (*n*=13)
Total bilirubin (μmol/L)	1.40 (1.20, 1.60)	2.40 (1.60, 3.00)**	2.30 (2.10, 2.90)	3.60 (2.40, 4.20)#
Aspartate aminotransferase (U/L)	196.00 (181.00, 217.00)	172.00 (146.00, 189.00)*	162.00 (144.00, 185.00)	159.00 (129.00, 164.00)
Alanine aminotransferase (U/L)	65.00 (62.00, 74.00)	57.50 (51.00, 68.00)	54.00 (43.00, 67.00)	36.00 (33.00, 41.00)##
Alkaline phosphatase (U/L)	373.00 (333.00, 423.00)	674.50 (371.00, 850.00)**	276.50 (219.00, 392.00)	496.00 (365.00, 661.00)###
Urea (mmol/L)	6.83 (6.45, 8.40)	4.35 (3.45, 4.77)****	6.35 (5.83, 6.73)	3.66 (3.37, 4.21)####
Glucose (mmol/L)	5.81 (5.61, 7.37)	7.54 (7.10, 8.21)**	6.88 (6.07, 7.21)	7.80 (7.24, 8.03)#
Alpha amylase (U/L)	1508 (1426, 1712)	1940 (1788, 2124)***	1556 (1418, 1748)	1758 (1580, 1860)#
Cholesterol (mmol/L)	1.43 (1.23, 1.61)	1.88 (1.50, 1.95)**	1.62 (1.47, 1.77)	1.67 (1.36, 1.90)
Triglycerides (mmol/L)	0.88 (0.63, 1.18)	1.18 (0.94, 2.21)*	1.37 (0.84, 1.80)	0.96 (0.66, 2.47)

Note: Data are presented as median (25th percentile, 75th percentile). Statistically significant differences: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 between male HCD and male control groups; #p<0.05, ##p<0.01, ###p<0.001, and ####p<0.0001.
Abbreviation: HCD: High-calorie diet.

phosphatase activity (+79.4%, p<0.001), and total bilirubin level (+56.5%, p=0.037).

Rats with HCD-induced visceral obesity also showed abnormalities in carbohydrate metabolism and pancreatic function. In relation to control animals, HCD rats exhibited a statistically significant increase in serum glucose concentration (male: 29.8%, p=0.003; female: 13.4%, p=0.047) and alpha-amylase activity (male: 28.6%, p<0.001; female: 13.0%, p=0.036) (Table 2).

In male rats with HCD-induced visceral obesity, a statistically significant increase in serum cholesterol concentration (31.5%, p=0.003), serum triglycerides (61.8%, p=0.029) (Table 2), and thyroid tissue triglycerides (2.5-fold, p=0.020) were observed compared to the control (Figure 4). Notably, these lipid disturbances were absent in female rats.

A statistically significant decrease in TPO activity in the thyroid tissue was observed in the HCD group compared to the controls: by 53.0% (p=0.018) in male rats and by 26.5% (p=0.017) in female rats (Figure 5A). Similarly, MDA content in thyroid tissue was lower in the HCD group

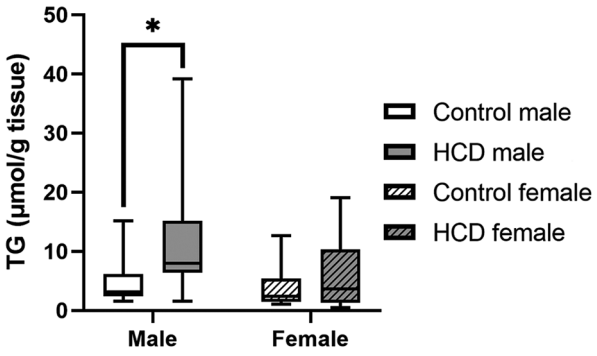

Figure 4. TG content in the thyroid tissue of experimental animals. Data are presented in boxplots showing the 25th percentile, median, and 75th percentile. *p<0.05 indicates a statistically significant difference between male HCD and male control groups.
Abbreviations: HCD: High-calorie diet; TG: Triglyceride.

(male: 30.4%, p=0.005; female: 36.0%, p=0.020) compared to the corresponding controls (Figure 5B). Correlation analysis showed a moderate positive correlation between TPO activity and MDA levels in male rats (r=0.54, p=0.014) but not in female rats.

After 16 weeks of HCD, a statistically significant increase in serum T4 levels by 34.2% (p=0.002) and 29.5% (p=0.020) was observed in male and female rats, respectively, compared with the corresponding control groups (Figure 6A). No differences in serum T3 levels were observed between the experimental groups (Figure 6B).

4. Discussion

Our findings demonstrate that 16 weeks of HCD consumption resulted in a significant increase in visceral fat mass in both male and female rats, with male rats exhibiting an almost threefold increase and females a twofold increase compared to their respective controls. With increasing visceral fat mass, triglyceride levels in both serum and thyroid tissues increased in male but not in female rats. Analysis of serum biochemical parameters further indicated liver and pancreatic dysfunction in HCD-fed rats, consistent with our previous morphological studies, which confirmed fatty liver degeneration following prolonged HCD consumption.[13] These findings align with established models of diet-induced visceral obesity[8] and are comparable to those of Shao *et al.*,[14] who observed similar metabolic and hepatic alterations in rats fed a high-fat diet for 24 – 30 weeks.

Shao *et al.*[14] also found an enlarged thyroid with lower echotexture and relatively heterogeneous features in ultrasound imaging of rats fed a high-fat diet. The authors showed that rats fed a high-fat diet exhibited focal colloid goiter, flattened follicular epithelium, and distended follicles, which are consistent with our data. Electron microscopy revealed dilated endoplasmic reticulum and twisted nuclei, as well as fewer microvilli and secretory vesicles, all of which indicate thyroid hypofunction.[14] Steatosis and reduced thyroid function have also been reported in diet-induced obese mice and genetically obese ob/ob and db/db mouse models.[15]

Our evaluation of TPO activity revealed a more than twofold reduction in HCD males and a 1.4-fold reduction in HCD females. Shao *et al.* further investigated the molecular mechanisms of thyroid hormone synthesis and showed that a high-fat diet downregulated the expression of thyroid transcription factor 1 and NIS, both of which

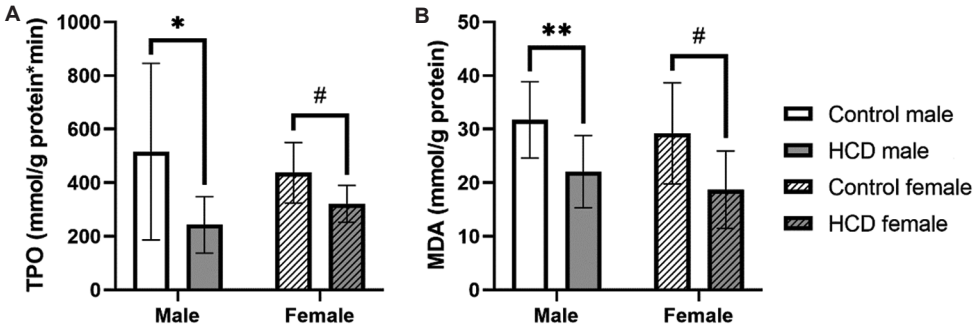

Figure 5. TPO activity and MDA content in the thyroid tissue of experimental animals. TPO activity (A) and MDA content (B) in male and female rats. Data are presented as mean ± standard deviation. Statistically significant differences: *p<0.05, **p<0.01 between male HCD and male control groups; #p<0.05 between female HCD and female control groups.
Abbreviations: HCD: High-calorie diet; TPO: Thyroperoxidase; MDA: Malonic dialdehyde.

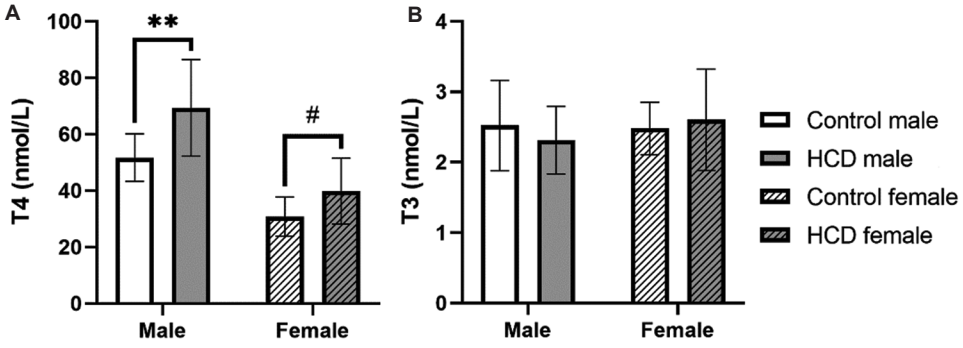

Figure 6. Thyroid hormone levels in the serum of experimental animals. Serum levels of T4 (A) and T3 (B) in male and female rats. Data are presented as mean ± standard deviation. Statistically significant differences: **p<0.01 between male HCD and male control groups; #p<0.05 between female HCD and female control groups.
Abbreviations: HCD: High-calorie diet; T4: Thyroxine; T3: Triiodothyronine.

are essential for thyroid hormone synthesis.[14] The authors suggested that the decrease in NIS activity could be due to lipotoxicity. The decrease in TPO activity in obesity may also be attributable to proinflammatory IL, including IL-6.[2] Our previous studies revealed that IL-6 levels in thyroid tissue were significantly reduced almost twofold in HCD-fed rats compared to the control. However, upon switching to StD, IL-6 levels began to normalize, with the most substantial recovery observed in rats subjected to a combined intervention of StD and moderate treadmill exercise ("HCD/StD + running").[16] IL-6 is known to play a pleiotropic role in activating intracellular signaling pathways, including phosphatidylinositol 3-kinase, AMP-activated protein kinase, and Janus kinase pathways, all of which are necessary for the regulation of intracellular metabolism.[17] This suggests that IL-6 reduction may contribute to metabolic suppression in the thyroid gland, while dietary and physical interventions can restore metabolic function. Interestingly, our data indicate that exercise alone (even without dietary changes) led to partial normalization of IL-6 levels, suggesting that physical activity may directly stimulate energy metabolism within the thyroid gland, potentially mitigating HCD-induced hypofunction.

Our findings indicate that the decrease in TPO activity in thyroid tissue occurs against a background of reduced MDA levels, a fact that deserves special attention. Generally, obesity is associated with increased lipid peroxidation, leading to elevated MDA levels in various tissues. We previously demonstrated this phenomenon in muscle tissue homogenates of rats subjected to a 16-week HCD.[18] However, in the thyroid gland, we observed the opposite trend—a decrease in MDA levels despite visceral obesity. This paradox can be explained by the specificity of TPO, a hemin peroxidase. Only the oxidized form of this enzyme can oxidize the substrate.[19] Efficient thyroid hormone synthesis depends on the adequate production of hydrogen peroxide (H_2O_2), which serves as an oxidant in the synthesis of T4 and T3. H_2O_2 is produced by a member of the NADPH oxidase (NOX) family—double oxidase 2 (DUOX2). Studies indicate that a deficient reactive oxygen species production, due to insufficient NOX/DUOX activity, can lead to thyroid hypofunction in rodent models.[20] In this study, the observed reduction in MDA content in thyroid tissue and the positive correlation between TPO activity and MDA levels in males suggest a potential decrease in H_2O_2 production at the apical membrane of thyrocytes. However, the precise mechanisms underlying reduced TPO activity in obesity remain poorly understood.

Analysis of thyroid status parameters revealed a statistically significant increase in blood T4 levels in rats receiving HCD. Previously, we demonstrated that 16 weeks of HCD led to increased serum TSH levels and heightened 5′-deiodinase type 1 (DI-1) activity in the liver.[21] Since DI-1 catalyzes the conversion of T4 to the active hormone T3, these findings suggest an adaptive response to prolonged overnutrition, aimed at increasing metabolic activity under excessive caloric intake.[22]

Notably, thyroid follicles store large amounts of thyroid hormones,[23] which can be released into the blood circulation under the influence of TSH, despite the inhibition of T4 and T3 synthesis. However, a decrease in TPO activity, along with morphological signs of thyroid hypofunction, indicates an incipient trend towards hypothyroidism. A prolonged high-fat diet may eventually lead to overt thyroid dysfunction, as demonstrated by Shao *et al.*, who reported decreased T4 levels and persistently high TSH levels in rats fed a high-fat diet for 24 – 30 weeks.[14] This reduction in T4 was accompanied by downregulation of thyroid hormone synthesis-related proteins in thyroid tissue, suggesting a progressive decline in thyroid function. Similarly, studies in genetically obese mouse models reveal pronounced thyroid dysfunction with reduced circulating thyroid hormone levels.[15] Thyroid hypofunction has also been reported in obese rats with streptozotocin-induced type 2 diabetes (T2DM).[24] Patients with T2DM often manifest hypothyroidism.[25] Insulin resistance has been shown to play a crucial role in both T2DM and thyroid dysfunction.[26]

Several molecular mechanisms underlie the interplay between insulin resistance and thyroid dysfunction. Insulin resistance in subclinical hypothyroidism is linked to decreased insulin-stimulated glucose transport, likely caused by impaired translocation of glucose transporter type 2. The Thr92Ala polymorphism in the gene encoding DI-2 reduces T3 activation, leading to intrathyroidal deiodination defects and contributing to insulin resistance.[27]

According to Shpakov,[28] altered adenylate cyclase signaling is the most important mechanism linking thyroid disorders to T2DM; in T2DM, there is reduced sensitivity of the thyrocyte adenylate cyclase signaling system in thyrocytes to TSH, along with decreased thyroid hormone receptor expression in peripheral tissues and dysregulated deiodinase activity. A decreased activity of DI-2, a deiodinase that converts T4 into the active form of T3, is associated with insulin resistance. A decreased activity of DI-3, a deiodinase that catalyzes T3 inactivation in pancreatic β-cells, suppresses insulin secretion and leads to insulin deficiency.[28] Together, these findings highlight the bidirectional relationship between thyroid dysfunction and insulin resistance, where thyroid hormone imbalances

contribute to metabolic dysregulation, and insulin resistance exacerbates thyroid dysfunction.

From a clinical point of view, these findings underscore the long-term risk of developing hypothyroidism in obesity. Given the strong link between obesity, insulin resistance, and thyroid dysfunction, overweight individuals should undergo regular thyroid function assessments, including an ultrasound examination of the thyroid gland and determination of the thyroid hormonal status.

5. Conclusion

Our findings confirm the adaptive changes in the thyroid function in response to 16 weeks of HCD consumption in rats. While serum T4 increased, there was a concurrent trend toward thyroid hypofunction, as evidenced by a decrease in TPO activity. Morphological studies of the thyroid gland further support this emerging hypothyroid state, revealing lipid inclusions, enlarged follicles with colloid accumulation, and flattened thyrocytes in both male and female rats.

Acknowledgments

None.

Funding

This work was supported by the State Program for Scientific Research of the National Academy of Sciences of Belarus (No.: 4.1.1.5).

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Tatyana A. Mityukova
Formal analysis: All authors
Investigation: Anastasia A. Basalai, Tatyana E. Kuznetsova, Olga Y. Poluliakh, Mikita S. Kastsiuchenka
Methodology: Anastasia A. Basalai, Tatyana E. Kuznetsova, Olga Y. Poluliakh, Mikita S. Kastsiuchenka
Writing – original draft: Tatyana A. Mityukova, Anastasia A. Basalai, Tatyana E. Kuznetsova
Writing – review & editing: All authors

Ethics approval and consent to participate

This study was approved by the Bioethics Committee of the Institute of Physiology, National Academy of Sciences of Belarus (Protocols No.1 on January 22, 2021; Protocols No.2 on February 2, 2022) and conducted in accordance with the guidelines set forth by the European Convention for the Protection of Vertebrate Animals (ETS No. 123).

Consent for publication

Not applicable.

Availability of data

The datasets used and/or analyzed are available from the Institute of Physiology of the National Academy of Sciences of Belarus, upon reasonable and justifiable request in accordance with the rules and procedures of the institute (https://physiology.by/or biblio@fizio.bas-net.by).

References

1. Okunogbe A, Nugent R, Spencer G, Powis J, Ralston J, Wilding J. Economic impacts of overweight and obesity: Current and future estimates for 161 countries. *BMJ Glob Health*. 2022;7(9):e009773.

   doi: 10.1136/bmjgh-2022-009773

2. Reinehr T. Obesity and thyroid function. *Mol Cell Endocrinol*. 2010;316(2):165-171.

   doi: 10.1016/j.mce.2009.06.005

3. Sanyal D, Raychaudhuri M. Hypothyroidism and obesity: An intriguing link. *Indian J Endocrinol Metab*. 2016;20(4):554-557.

   doi: 10.4103/2230-8210.183454

4. Du FM, Kuang HY, Duan BH, Liu DN, Yu XY. Associations between thyroid hormones within the euthyroid range and indices of obesity in obese Chinese women of reproductive age. *Metab Syndr Relat Disord*. 2019;17(8):416-422.

   doi: 10.1089/met.2019.0036

5. Alevizaki M, Saltiki K, Voidonikola P, Mantzou E, Papamichael C, Stamatelopoulos K. Free thyroxine is an independent predictor of subcutaneous fat in euthyroid individuals. *Eur J Endocrinol*. 2009;161(3):459-465.

   doi: 10.1530/EJE-09-0441

6. Song RH, Wang B, Yao QM, Li Q, Jia X, Zhang JA. The impact of obesity on thyroid autoimmunity and dysfunction: A systematic review and meta-analysis. *Front Immunol*. 2019;10:2349.

   doi: 10.3389/fimmu.2019.02349

7. Yan Y, Xu M, Wu M, *et al*. Obesity is associated with subclinical hypothyroidism in the presence of thyroid autoantibodies: A cross-sectional study. *BMC Endocr Disord*. 2022;22(1):94.

   doi: 10.1186/s12902-022-00981-0

8. Gancheva S, Zhelyazkova-Savova M, Galunska B, Chervenkov T. Experimental models of metabolic syndrome in rats. *Scr Sci Med*. 2015;47(2):14-21.

   doi: 10.14748/ssm.v47i2.1145

9. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the

estrous cycle phases of rats: Some helpful considerations. *Braz J Biol*. 2002;62(4A):609-614.

doi: 10.1590/s1519-69842002000400008

10. Khoubnasabjafari M, Ansarin K, Jouyban A. Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. *Bioimpacts*. 2015;5(3):123-127.

doi: 10.15171/bi.2015.20

11. Alexander NM. A spectrophotometric assay for iodide oxidation by thyroid peroxidase. *Anal Biochem*. 1962;4: 341-345.

doi: 10.1016/0003-2697(62)90097-0

12. Митюкова ТА, Чудиловская ЕН, Мигалевич АС. Определение активности тиреопероксидазы в ткани щитовидной железы (экспериментальное исследование). Лабораторная диагностика. *Восточная Европа*. (Mityukova TA, Chudilovskaya EH, Mihalevich AC. Determination of thyroid peroxidase activity in the thyroid tissue of rats [experimental study]. Laboratory diagnostics. *East Eur*). 2020;9(3):285-293. [In Russ].

doi: 10.34883/PI.2020.9.3.009

13. Басалай АА, Кузнецова ТЕ, Митюкова ТА, *и др*. Морфофункциональное состояние печени крыс-самцов линии Вистар при диет-индуцированном ожирении и его коррекции. Известия Национальной Академии Наук Беларуси. *Серия Медицинских Наук*. (Basalai AA, Kuznetsova TE, Mityukova TA, *et al.* Morphofunctional state of the liver of male Wistar rats during diet-induced obesity and its correction. *Proc Natl Acad Sci Belarus Med Ser*). 2022;19(3):308-320. [In Russ].

doi: 10.29235/1814-6023-2022-19-3-308-320

14. Shao SS, Zhao YF, Song YF, *et al*. Dietary high-fat lard intake induces thyroid dysfunction and abnormal morphology in rats. *Acta Pharmacol Sin*. 2014;35(11):1411-1420.

doi: 10.1038/aps.2014.82

15. Lee MH, Lee JU, Joung KH, *et al*. Thyroid dysfunction associated with follicular cell steatosis in obese male mice and humans. *Endocrinology*. 2015;156(3):1181-1193.

doi: 10.1210/en.2014-1670

16. Митюкова ТА, Кузнецова ТЕ, Басалай АА, *и др*. Морфологические и функциональные характеристики щитовидной железы при диет-индуцированном ожирении и его коррекции у крыс самцов Вистар. *Патологическая Физиология и Экспериментальная Терапия*. (Mityukova TA, Kuznetsova TE, Basalai AA, *et al*. Morphological and functional characteristics of the thyroid gland in diet-induced obesity and its correction in male Wistar rats. *Pathol Physiol Exp Ther*). 2023;67(4):47-55. [In Russ].

doi: 10.25557/0031-2991.2023.04.47-55

17. Kistner TM, Pedersen BK, Lieberman DE. Interleukin 6 as an energy allocator in muscle tissue. *Nat Metab*. 2022;4(2): 170-179.

doi: 10.1038/s42255-022-00538-4

18. Mityukova TA, Basalai AA, Chudilovskaya KN, Poluliakh OE, Shcherbakov YV, Kastiuchenka MS. Decrease in muscle mass in diet-induced visceral obesity in male wistar rats: Relationship with hormonal and metabolic parameters. *J Evol Biochem Physiol*. 2023;59(4):1277-1286.

doi: 10.1134/S0022093023040208

19. Carvalho DP, Dupuy C. Thyroid hormone biosynthesis and release. *Mol Cell Endocrinol*. 2017;458:6-15.

doi: 10.1016/j.mce.2017.01.038

20. Szanto I, Pusztaszeri M, Mavromati M. H_2O_2 metabolism in normal thyroid cells and in thyroid tumorigenesis: Focus on NADPH oxidases. *Antioxidants* (*Basel*). 2019;8(5):126.

doi: 10.3390/antiox8050126

21. Митюкова ТА, Чудиловская ЕН, Басалай АА. Активность 5'-дейодиназы йодотиронина I типа в печени у крыс, получающих высококалорийную диету (экспериментальное исследование). Лабораторная диагностика. *Восточная Европа*. (Mityukova TA, Chudilovskaya EH, Basalai AA. Activity of type I iodothyronine 5'-deiodinase in the liver in rats receiving a high-calorie diet [experimental study]. laboratory diagnostics. *East Eur*). 2022;11(1):60-68. [In Russ].

doi: 10.34883/PI.2022.11.1.016

22. Gereben B, Zeöld A, Dentice M, Salvatore D, Bianco AC. Activation and inactivation of thyroid hormone by deiodinases: Local action with general consequences. *Cell Mol Life Sci*. 2008;65(4):570-590.

doi: 10.1007/s00018-007-7396-0

23. Chawla S, Jena S. The anatomy and physiology of laboratory rat. In: *Essentials of Laboratory Animal Science: Principles and Practices*. Vol. 1. Singapore: Springer; 2021. p. 187-209.

doi: 10.1007/978-981-16-0987-9_9

24. Rizk FH, Barhoma RAE, El-Saka MH, *et al*. Exercise training and spexin ameliorate thyroid changes in obese type 2 diabetic rats: The possible interlaying mechanisms. *Am J Physiol Endocrinol Metab*. 2024;327(3):E313-E327.

doi: 10.1152/ajpendo.00213.2024

25. Biondi B, Kahaly GJ, Robertson RP. Thyroid dysfunction and diabetes mellitus: Two closely associated disorders. *Endocr Rev*. 2019;40(3):789-824.

doi: 10.1210/er.2018-00163

26. Mohammed Hussein SM, AbdElmageed RM. The relationship between type 2 diabetes mellitus and related

thyroid diseases. *Cureus*. 2021;13(12):e20697.

doi: 10.7759/cureus.20697

27. Butler PW, Smith SM, Linderman JD, *et al*. The Thr92Ala 5' type 2 deiodinase gene polymorphism is associated with a delayed triiodothyronine secretion in response to the thyrotropin-releasing hormone-stimulation test: A pharmacogenomic study. *Thyroid*. 2010; 20(12):1407-1412.

doi: 10.1089/thy.2010.0244

28. Shpakov AO. Molecular mechanisms of the relationship between thyroid dysfunctions and diabetes mellitus. *J Evol Biochem Physiol*. 2018;54:257-266.

doi: 10.1134/S0022093018040014