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Comparative Analysis of Oxidative Metabolism in Liver in Different Experimental Models of Hypothyroidism: Low Iodine Diet and Anti-Thyroid drug (Methimazole)

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On the concept of oxidative stress in hypothyroidism, which still remains ambiguous and controversial, the article emphasizes the issue of the impact of the experimental conditions on the validity of the data obtained in different methods of modeling thyroid dysfunction. Experiments were conducted on 112 white nonlinear male rats. Thyroid hormones and biomarkers of oxidative metabolism in the liver tissue were determined in rats kept for 3 months on a low-iodine diet (LID) and in rats with methimazole (MMI)-induced hypothyroidism (2,5 mg/100 g of body weight for 3 weeks). In LID-rats (n=96) total serum T4 amounted 43, total T3 in liver tissue - 73% of the level found in euthyroid animal, p=0.0121 and p=0.0051, respectively), whereas in MMI-rats (n=96) both total and free serum T4 were 67% of control (p=0.0002 for both total and free T4). In LID-rats cytochrome oxidase (CcOX) activity in liver tissue was 68.5, concentration of malondialdehyde (MDA) - 58% of euthyroids (p value - 0.0202 and 0.0127, respectively), while protein carbonyls (PC) level was 116% of the control (p=0.0411). In MMI-rats liver malate dehydrogenase (MDH) activity decreased up to 70.9, but succinate dehydrogenase (SDH) activity and MDA concentration increased up to 163.6 and 154% of the level in euthyroid animals respectively (p?0.05). LID-model led to the more pronounced inhibition of thyroid function, than that the MMI-hypothyroidism model used. LID-model was accompanied by a decrease in the intensity of oxidative metabolism in liver tissue, whereas MMI-hypothyroidism - by activation of the succinate oxidation pathway and an increase in the concentration of secondary lipid peroxidation products in the liver of experimental animals. The results suggest that the conflicting data obtained from studies of oxidative metabolism in hypothyroidism, among other assumptions, may be due to the different approaches used by researchers to model thyroid dysfunction.

Keywords: Low-iodine diet; Methimazole-induced hypothyroidism; Oxidative metabolism.

About 2 billion people in the world live in regions with iodine deficiency. Iodine deficiency diseases prevail in the structure of both endocrine pathology and pathology in general[1], which makes thyroidology one of the most relevant areas of modern research. There are a large number of approaches to modeling thyroid pathology - surgical, dietary, chemical, immunological,

genetic, radioactive methods, etc[2]. The choice of an experimental model is determined by the purpose and objective of the study to obtain data that really correspond to clinical conditions. It is obvious that complete or partial thyroidectomy is the most appropriate method for studying postoperative hypothyroidism, which is not uncommon in clinical practice. Lines of laboratory animals with mutations, genetically engineered modifications of the key genes of the hypothalamic-pituitary-thyroid axis are ideal (if any) for studying genetically determined forms of thyroid dysfunction (5% in the structure of thyroid pathology)[2]. Diets with low iodine content are obviously preferable for studying the effectiveness of iodine supplementation and developing methods for the prevention of thyroid insufficiency. However, surgical models require certain skills, genetically modified animal lines are difficult to access, the development of low-iodine diets is a rather complicated and time-consuming procedure, radioactive methods require specially equipped laboratories. Simplicity and accessibility of reproduction often come to the fore when choosing an experimental model.

The rapid effect, accessibility, reproducibility of the result, water solubility, and relatively low costs have brought chemical methods of reproduction of hypothyroidism to the leading positions in experimental thyroidology. Administration of antithyroid drugs (thyrostatics), among which the most effective are thiourea derivatives (thioamides) – propylthiouracil (PTU) and methimazole (MMI) - without exaggeration are among the most common methods of modeling hypothyroidism. The mechanism of their action is based on the inhibition of thyroid peroxidase (TPO), as well as some isoenzymes of deiodinases (PTU) and some other mechanisms that are still being studied. [3-6]

The choice of the dose, mode of administration of thyrostatic drugs is crucial for obtaining results that most adequately reflect the changes occurring in organs and tissues in hypothyroidism. Numerous regimes for the administration of thyrostatics to experimental animals have been proposed[7]. We have previously shown, that daily intragastric administration to experimental animals (rats) of MMI in the dose 2.5 mg per 100 g of body weight (b.w.) for 3 months allows to achieve the persistent decrease in thyroid function, as evidenced by a decrease in the concentration of circulating thyroid hormones and hypothermia, and is accompanied by the least morphological and functional changes in animal organs and tissues[8]. At the same time, MMI in doses of 20 and 10 mg / 100 g of b. w. daily for 2 weeks, along with a persistent decrease in thyroid function, induces the development of tissue pathology of toxic genesis, not characteristic of hypothyroidism. The daily intake of 1 mg of MMI for 3 weeks was not sufficient to induce a hypothyroid state and persistent hypothermia.

However, none of the models excludes the extra thyroid effect of the administrated xenobiotic. There is a lot of evidences of extra-thyroid side effects of MMI and PTU[8-13]. In particular, it is reported that MMI causes damage to liver cells, changes in the redox environment and oxidative stress, unrelated to thyroid dysfunction[9]. The immunosuppressive effect of MMI, directly related to the induction of leukocyte apoptosis, has been shown[11]. It is known that the administration of both PTU and MMI is accompanied by an increase in serum calcitonin concentration due to reactive hyperplasia of thyroid C-cells[14]. In general, there are a sufficient number of reports on the side effects of methimazole, and there are evidences for the involvement of reactive oxygen species (ROS) in the mechanisms of toxicity of this anti-thyroid drug[15-19]. Given the ambiguous and conflicting data on oxidative stress in hypothyroidism[20], the question arises as to the contribution of different experimental conditions to the pathochemical picture obtained by researchers using different methods of inducing hypothyroidism.

In the spectrum of a wide range of methods used in modern thyroidology, attention is drawn to methods based on the creation of diets with low iodine content (for rodents, the iodine content is <0.02 μg/g)[7], as methods that most closely reproduce the changes that develop in the body under iodine and thyroid hormone deficiency. The aim of this study was to assess and juxtapose indicators oxidative metabolism in the liver tissue of rats fed with a low-iodine diet, and rats with MMI-induced hypothyroidism.

MATERIALS AND METHODS

Animals

The experiments were carried out on 112 nonlinear white male rats weighing 180-220 g in vivarium conditions with a feed and water *ad libitum* in compliance with Declaration of Helsinki, ethical standards and recommendations for humane treatment of laboratory animals (order of the Ministry of Healthcare of Russia N199 dated 1.04.2016 "On Approval of the Rules of Good Laboratory Practice").

Experimental design

Low iodine diet

The animals (n=56) were divided into 4 experimental groups of 14 rats each.

Group 1 (control, euthyroid) was fed with a standard vivarium diet (complete dry compound feed for laboratory animals «Chara», produced by «Assortiment-Agro» LLC, Russia).

Group 2 (LID) was fed with a special diet with a low iodine content [21].

Group 3 (LID+KI) was fed with a low-iodine diet[21] with the addition of potassium iodide in a dose that ensures the daily requirement of rodents for iodine, which is 2-3 μg per 100 g of body weight[7]. The purpose of introducing Group 3 (LID+KI) is to ensure that the changes observed in Group 2 (LID) are caused by iodine deficiency and not by other reasons.

Group 4 (LID+Iodine-Chitosan) was fed with a low-iodine diet[21], and for supplementation of iodine received "Iodine-Chitosan" complex[22], which was added to the feed at a dose that provided the daily iodine requirement.

The standard vivarium diet usually contains from 3 to 7 μg of iodine per day (based on the daily intake of 20 g of feed)[7]. To prepare a feed with a low iodine content, 6 kg of corn flour, 2.5 kg of wheat gluten, 1 kg of brewer's yeast, 0.15 kg of NaCl and 0.15 kg of $CaCO_3$ were thoroughly mixed according to the recipe described in the source [21] in the most detail. The animals were on the described feeding for 3 months.

On the second day after the last day of the experiment blood and tissue (liver) samples were taken after decapitation of animals under ether anesthesia.

MMI-induced hypothyroidism

Animals (n=56) were fed with a standard vivarium diet (complete dry compound feed for laboratory animals «Chara», produced by «Assortiment-Agro» LLC, Russia) with free access to water and divided into 4 experimental groups of 14 rats each.

Group 1 is a control (euthyroid).

Group 2 (MMI): rats received MMI solution intragastrically at the daily single dose 2,5 mg per 100 of b. w. for three weeks.

Group 3 (MMI + recovery period): rats received MMI solution intragastrically at the daily single dose 2,5 mg per 100 of body weight for three weeks. After reproducing the hypothyroidism model, starting from the 22nd day of the experiment, the animals for a month received a standard vivarium diet.

Group 4 (MMI + "RebA-iodine"): rats received MMI solution intragastrically at the daily single dose 2,5 mg per 100 of body weight for three weeks. After 21 days of MMI-administration animals received the iodine-enriched diet for a month: the iodine-polysaccharide complex on the base of steviol glycoside Rebaudioside A "RebA-iodine"[23] was added to the food at a dose providing the daily iodine requirement of rats for a month. Groups of animals were involved into the experiment in such a way that the reproduction of the hypothyroidism model in Group 2 coincided with the end of the experiment in Group 3 and Group 4.

On the second day after the last dose rats were anesthetized by ether, blood samples were obtained and liver dissected out.

Assessment of thyroid status

Thyroxine and triiodothyronine fractions (total, free T_4 and T_3) were determined in the blood serum, as well as the concentration of total T_3 in methanol extracts of liver tissue homogenate by the enzyme immunoassay using standard kits from AlcorBio (Russia).

Assessment of oxidative metabolism

Liver homogenate was prepared in phosphate buffer (pH=7.45) using a mechanical Potter's homogenizer (Teflon — glass). To remove partially destroyed cells and nuclei, the homogenates were centrifuged for 10 min at 1000 rpm. All procedures for the preparation of the homogenate and the isolation of subcellular fractions (differential centrifugation) were carried out at a temperature of 0 to +4 ° C.

Activity of cytochrome oxidase (EC 7.1.1.9) in the mitochondrial fraction of liver homogenate was determined by the rate of oxidation of dimethyl-p-phenyldiamine[24]. Succinate dehydrogenase (EC 1.3.5.1) activity was determined by ferricyanide method, malate dehydrogenase (EC 1.1.1.37) – by kinetic method by the rate of f NAD^+reduction[24].

Lipid peroxidation products (malondialdehyde) was determined on the base of interaction with thiobarbituric acid (TBA) to form a colored complex extracted with butanol.

The content of oxidatively modified proteins (protein carbonyls) was determined by the method of R.L. Levine modified by E.E. Dubinina[26-26]. The method is based on the reaction of carbonyl and imino groups of oxidized amino acid residues with 2,4–dinitrophenylhydrazine (2,4–DNPH) to form 2,4–dinitrophenylhydrazones of proteins determined spectrophotometrically at a wavelength of 370 nm.

Reagents and other materials

LID

1) Corn flour (Jinan Jinnuoakang Biotechnology Co. Ltd, China)

2) Wheat gluten (AGRANA Starke GmbH, Austria)

3) Brewer's yeast (Vito-House LLC, Russia)

4) "Iodine-Chitosan" (Research Center "Food technologies", Bashkir Institute of Technology and Management (Branch), Moscow State University of Technology and Management named after K. G. Razumovsky)

MMI-induced hypothyroidism

5) Complete dry compound feed for laboratory animals «Chara» («Assortiment-Agro» LLC, Russia)

6) Methimazole (2-mercapto-1-methylimidazole, MMI) (Innova Laboratories TD)

7) "RebA-iodine" (Research Center "Food technologies", Bashkir Institute of Technology and Management (Branch), Moscow State University of Technology and Management named after K. G. Razumovsky, Institute of Chemistry of the Ufa Federal Research Centre of the Russian Academy of Sciences)

Cytochrome oxidase assay

8) dimethyl-p-phenyldiamine (Angene-international Ld)

Malate dehydrogenase

9) NAD^+ (Sigma-Aldrich)

Succinate dehydrogenase

10) Potassium ferricyanide (Sisco Research Laboratories)

Malonic dialdehide

11) "TBA -AGATE" (LLC Agate-Med, Russia)

Protein carbonyls

12) 2,4-dinitrophenylhydrazine (Koehler Chemie GmbH)

Statistical analysis

Statistical analysis of quantitative data was performed using the STATISTICA-12 software package by calculating mean and standard deviation (M±ó). The normality of the distribution of the obtained data was checked using the Shapiro-Wilk criterion. In case of abnormal data distribution, a nonparametric test was used to calculate the median (Me), upper and lower quartiles [Q_1-Q_3]. The reliability of the differences between the groups was assessed using the Mann-Whitney U test. The differences were considered significant at pd"0.05.

RESULTS

Serum and tissue concentration of thyroid hormones

LID

The first stage of establishing thyroid status is determination of the level of circulating thyroid hormones. The level of total thyroxine in the blood serum of rats kept on the LID for 3 months and not receiving replacement doses of potassium iodide (Group 2) amounted only 42% of the level of the control group (Table 1). Such a pronounced decrease in the concentration of the primary thyroid hormone indicates a violation of the synthesis of thyroid hormones in the thyroid gland. The concentration of the more active form of thyroid hormones (free T_3) in the blood serum decreased slightly - to only 92% of the control, which apparently occurs due to activation of peripheral deiodination of T_4. At the same time, a noticeable decrease in the tissue hepatic concentration of T_3 (up to 73 % of the control) makes it possible to confidently state the development of a hypothyroidism in LID-rats.

MMI-hypothyroidism

Three-week administration of the antithyroid drug (MMI) led to a rapid and significant

decrease in the level of circulating thyroid hormones (Table 2). The serum concentration of total and free thyroxine in MMI-rats (Group 2) amounted 67.4% and 66.7% of the control (euthyroid rats, p=0.0002 for both tT_4 and fT_4). The level of the sensitive indicator of thyroid dysfunction -total T_3 - also decreased and amounted to 75.6% of the level of control animals (p=0.0001). The administration of the iodine polysaccharide complex during the recovery period (Group 4) led to a faster normalization of the level of thyroid hormones compared with the group of animals who were for a month on the standard vivarium diet after 3 weeks MMI-administration (Group 3). This indicates that the iodine polysaccharide complex "RebA-iodine" possesses a specific physiological activity toward the thyroid system and can be effective in supplementing the iodine deficiency.

Oxidative metabolism

LID

The effect of thyroid hormones on mitochondrial respiration, mediated by the regulation of gene expression and activity of Krebs cycle enzymes and various components of the mitochondrial chain, is widely known[27-28].

The activity of the terminal link of the electron transport chain - cytochrome oxidase in the mitochondrial fraction of liver homogenate

Table 1. Concentration of thyroid hormones in the blood serum and liver tissue of LID-rats (Me, $[Q_1$-$Q_3,]$, n=14)

Thyroid hormones	Group 1 (euthyroid)	Group 2 (LID)	Group 3 (LID+KI)	Group 4 (LID+ Iodine-Chitosan)
t Ò$_4$, nM/L (blood ser.)	57.4 [52.0; 59.1]	24.3 [20.7;29.4] p=.0121	57.0 [56.8;58.3] p*=.0128	57.6 [55.7;60.6] p*=.0231
f Ò$_3$,pM/L (blood ser.)	5.0 [4.8;5.2]	4,6 [4.5;4.7] p=.0367	4,8 [4.8;5.1]	4.7 [4.5;5.2]
t Ò$_3$, ng/g of tissue(methanol liver tissue extract)	6.25 [6.1;6.5]	4,4 [4.1;4.5] p=.0051	6,2 [5.7;6.5] p*=.0082	6.1 [5.1;6.9] p*=.0123

Legend: p – p-Value vs group 1 (euthyroid); p*-p-Value vs group-2 (LID), only statistically significant p-values are presented

Table 2. Concentration of thyroid hormones in the blood serum of MMI- rats (M±ó, n=14)

Thyroid hormones	Group 1 (euthyroid)	Group 2 (MMI)	Group 3 (MMI+recovery period)	Group 4 (MMI+ RebA-iodine in recovery period)
t Ò$_4$, nM/L	76.9±4.53	51.8±6.02 p=.0002	63.8±5.68 p=.0142 p_2=.0039	78,5±6,08 p_2=.0002 p_3=.0030
f Ò$_4$,pM/L	16.2±1.71	10.8±2.14 p=.0002	12.6±2.11 p=.0038 p_2=.0613	17,8±0,34 p_2=.0001 p_3=.0067
t Ò$_3$nM/L	3.12±0.57	2.36±0.19 p=.0001	2.91±0.41 p_2=.0012	3,21±0,34 p_2=.0001 p_3=.00671

Legend: p – p-Value vs group 1 (euthyroid); p_2-p-Value vs group 2 (MMI), p_3-p-Value vs group 3 (MMI+recovery period), only statistically significant p-values are presented

Table 3. Cytochrome oxidase activity in the mitochondrial fraction of liver homogenate of LID-rats (nM/ min per mg of protein, Me, [Q_1 –Q_3], n=14)

Experimental group	Enzyme activity
Group 1 (euthyroid)	165.5[161.0; 230.0]
Group 2 (LID)	113.5 [104.0;161.0] p=.0202
Group 3 (LID +KI)	163.5[112.0;229.0] p*=.0456
Group 4 (LID + (Iodine-Chitosan)	161.6 [110;221.3] p*=.0487

Legend: p – p-Value vs group 1 (euthyroid); p*- p-Value vs group 2 (LID), only statistically significant p-values are presented

of LID-rats significantly decreased, amounting to 68.5% of the enzyme activity in euthyroid animals (p=0.0202), whereas in the group receiving additional potassium iodide, it practically did not differ from the control animals (Table 3). The mitochondrial respiratory chain is the main source of reactive oxygen species that initiate free radical oxidation. TBA reactive products (predominantly malondialdehyde) are secondary products of lipid peroxidation. Concentration of one of the indicators of the intensity of free radical oxidation – malondialdehyde in LID-rats was only 58% of the level of control euthyroid animals (p=0.0127) (Table 4). At the same time, the content of protein carbonylation products (aliphatic ketondinitrophenylhydrazones) in animals kept on a low-iodine diet was slightly higher than that in

Table 4. Lipid peroxidation products (malondialdehyde) and protein carbonyls in liver of LID-rats (Me, [Q_1-Q_3], n=14)

Oxidative modification products	Group 1 (euthyroid)	Group 2 (LID)	Group 3 (LID+KI)	Group 4 (LID+ Iodine-Chitosan)
Malondialdehyde, nM/g of tissue	6.7343 [6.2699;7.3131]	3.8922 [2.9257; 4.4074] p=.0127	7.7105 [7,2322;8.4555] p*=.0182	7.14 [5,2811;8.2398] p*=.0202
Protein carbonyls, nM/mg of protein	3.55[3.2;3.7]	4.10[3.9;4.6] p=.0411	3.00[2.7;3.2] p*=.0198	3.20[2.8;3.5] p*=.0253

Legend: p- p-Value vs group 1; p*- p-Value vs group 2 (LID), only statistically significant p-values are presented

Table 5. Succinate- and Malate dehydrogenase activity in liver of MMI-rats, (M±Ã, n=14)

Experimental groups	Succinate dehydrogenase, nM sec^{-1}/g of protein	Malate dehydrogenase, nM sec^{-1}/g of protein
Group 1 (euthyroid)	9,9 [8,5-11,8]	2363 [2160-2524]
Group 2 (MMI)	16,2 [14,4-17,0] p=.016	1675 [1438-1748] p=0.017
Group 3 (MMI+recovery period)	28,4 [25,3-29,7] p=.028 p_2=0,032	2211 [1868-2448] p_2=.017
Group 4 (MMI+ RebA-iodine in recovery period)	28,0 [26,0-31,8] p=.028 p_2=.016	3212 [3048; 3341] p=0.026 p_2=.024 p_3=.029

Legend: p – p-Value vs group 1 (euthyroid); p_2-p-Value vs group 2 (MMI), p_3-p-Value vs group 3 (MMI+recovery period), only statistically significant p-values are presented

Table 6. Lipid peroxidation products in blood plasma and liver tissue of MMI-rats, (M±σ, n=14)

MDA in recovery period)	Experimental groups			
	Group 1 (euthyroid)	Group 2 (MMI)	Group 3 (MMI+recovery period)	Group 4 (MMI+ RebA-iodine in recovery period)
Blood plasma, μM/L	1,81±0,08	2,20±0,14 p=.0016	1,91±0,11 p_2=0,0634	1,83±0,07 p_2=.0021
Liver tissue, nM/g of tissue	3,51±0,21	5,41±0,32 p=.0001	4,31±0,32 p=.0002 p_2=.0002	3,84±0,22 p=.0119 p_2=.0001 p_3=.0005

Legend: p – p-Value vs group 1 (euthyroid); p_2-p-Value vs group 2 (MMI), p_3-p-Value vs group 3 (MMI+recovery period), only statistically significant p-values are presented

control group and amounted to 116% of the level in the euthyroid animals (p=0.0411).

MMI-hypothyroidism

The used model of MMI- hypothyroidism (Group 2) was accompanied by a significant decrease in malate dehydrogenase activity – up to 70.9% (p=0.017) with simultaneous activation succinate dehydrogenase up to 163.63% (p=0.016) of the activity of control euthyroid animals (Table 5).

The content of TBA-reactive products in the blood and liver tissue of rats with MMI-hypothyroidism (Table 6) increased up to 121.5% and 154.1% of the level of euthyroid animals (p=0.0016 and p=0.0001, respectively). One-month intake of RebA iodine-polysaccharide complex after 3-week administration of MMI (Group 4) promoted faster normalization of thyroid hormone concentration and oxidative metabolism than in animals receiving standard vivarium diet during the recovery period (Group 3). Such organomineral complexes, in which iodine is incorporated into plant polysaccharide matrix and being a kind of analog of the main natural sources of iodine (algae), represent promising means for correction of iodine deficiency[29].

DISCUSSION

The significant decrease in serum thyroxine level is the first indicator of developing hypothyroid changes in LID-rats. At the same time, according to most authors, the development of systemic hypothyroidism can be stated only by reducing the level of more active T_3. The concentration of the free T_3 in the LID-rats blood also decreased, but slightly – up to 92% of the level in euthyroid animals. Maintenance of the more active T_3 level under these conditions is apparently provided by activation of peripheral T_4-deiodination. Compensatory shifts are aimed at maintaining the free form of a more active form of thyroid hormones - triiodothyronine, which is often observed in the early stages of adaptation to iodine deficiency[30-32]. The main extrathyroid pool of T_4 is contained in the blood plasma, while about 2/3 of the total T_3 is in the intracellular space. Local, tissue thyroid status may be relatively independent of the concentration of thyroid hormones in the blood[33-35]. Liver tissue demonstrates the greatest independence in this regard due to presence of a powerful deiodination system and a special role in maintaining the total pool of thyroid hormones in the body. Based on the above, along with the serum concentration of thyroid hormones, their tissue concentration is of no less interest. Despite the absence of pronounced changes in the serum concentration of T_3, the concentration of the most active of thyroid hormones in methanol extracts of liver tissue of LID-rats decreased more significantly and amounted to 73% of the control level.

Thus, the detected shifts allow us to state the development of hypothyroidism in LID-rats.

Shifts in the serum concentration of thyroid hormones in MMI-rats also allow us

to conclude the development of a hypothyroid condition, but hypothyroidism of a less pronounced severity, than that in LID-rats.

A natural consequence of the revealed decrease in concentration of serum and the tissue concentration of thyroid hormones is a decrease in the number of occupied receptors and the intensity of thyroid signaling in the target tissues.

Traditionally, thyroid-dependent metabolism in target tissues is assessed by the activity of energy metabolism enzymes (tricarboxylic acid cycle enzymes, respiratory cytochromes)[32,36]. Taking into account the above, a marked decrease in the activity of cytochrome oxidase, detected in the liver tissue of LID-animals, and amounting to only 68.5% of the activity in the control group, can be considered the result of a decrease in the intensity of thyroid signaling, especially since in animals treated in addition to in a low-iodine diet of potassium iodide, the activity of the enzyme almost did not differ from the activity in the group of control animals.

Cytochrome oxidase is the terminal link of the respiratory chain that transfers electrons from cytochrome c to oxygen, thereby directly determining the intensity of cellular aerobic metabolism. In this regard, the decrease in the concentration of an indicator of the intensity of free radical oxidation processes - malondialdehyde (MDA), can be considered as a logic consequence of the decrease in the activity of one of the most powerful enzymes of aerobic metabolism. Along with TBA-reactive products, the determination of products of oxidative modification of proteins is widely used as indicators of oxidative stress. In the experimental model used, in parallel with the decrease in the concentration of MDA, certain increase (up to 116% of the control) in the level of protein carbonyl was detected. Protein carbonylation products are comparatively more stable, in contrast to lipid peroxidation products, which have a significantly shorter half-life ô ½. Protein carbonyls are produced at earlier stages of oxidative stress[37]. An increase in the persistence of protein carbonyls may also result from a decrease of activity of the cellular protease systems, the rate of protein renewal, increased production of aberrant proteins in translation disorders, chaperone deficiency[37]. Taking into account the critical role of thyroid hormones in the control of protein synthesis, all of the above may occur as a result of a decrease in the intensity of thyroid stimulation.

The study of the activity of Krebs cycle oxidative enzymes in the liver of MMI-rats revealed divergent changes - a decrease of activity of malate and an increase of the activity of succinate dehydrogenases. The succinate oxidation pathway is important in adaptation to hypoxia and hypoergosis[38]. The data obtained allow us to conclude that the used model of MMI- hypothyroidism is not accompanied by a collapse of the universal compensatory reaction of mitochondria with a switch to the succinate oxidation pathway. Simultaneously with the activation of the succinate pathway, an increase in the concentration of secondary lipid peroxidation products - TBA reactive products - was also detected in liver tissue, as well in the blood serum of MMI-rats.

Numerous sources report the increase in the concentration of MDA, as well as other products of free radical oxidation (protein carbonyls) in patients with various forms of hypothyroidism (primary, subclinical), as well as in the tissues of animals with experimental hypothyroidism[39-42].

The development of oxidative stress in hyperthyroidism is easily explained. Activation of oxidative processes and oxygen consumption by tissues (and, consequently, the production of ROS) is one of the specific manifestations of the action of thyroid hormones on cellular metabolism[20,36]. The development of oxidative stress was also detected in various forms of hypothyroidism[39-42], which can be explained by a decrease in antioxidant defense, as well as by the formation of a pro-oxidant environment in hypothyroid tissues. A decrease in the intensity of lipid metabolism, changes in the lipid composition and availability of substrates for the lipid peroxidation process can increase susceptibility to oxidative stress and provide the increase in the intensity of free oxidation processes in hypothyroidism[43]. Undoubtedly, the severity of thyroid dysfunction, subtle feedback mechanisms and many other factors determine the sometimes-contradictory results obtained when studying the intensity of free radical processes in hypothyroidism. When comparing the results, we obtained, it is impossible not to take into account the longer duration and more pronounced degree

of suppression of thyroid function in LID-rats, compared with MMI-animals. At the same time, there is no doubt that the contradictory data obtained in the study of the oxidant status in thyroid system abnormalities is to some extent due to the various experimental models used. A comparative analysis of changes in free radical processes in the modeling of hypothyroidism by thyroidectomy and administration of anti-thyroid drugs revealed the following results. Hypothyroidism caused by thyroidectomy was accompanied by a decrease in the production of ROS in the myocardium and liver, while hypothyroidism caused by PTU and MMI was accompanied by the development of oxidative stress in the same tissues[44]. It is reported that pathochemical changes in liver tissue in MMI–induced hypothyroidism develop due to damage to liver by toxic products of the CYP_{450}-dependent biotransformation of the drug – 4,5-epoxdome, as well as products of its subsequent hydrolysis - glyoxal and N-methylthiourea[44-45]. Thus, in the chemically induced hypothyroidism, pathochemical shifts can be partly caused by the extra thyroid effects of injected xenobiotic.

Chemical methods of induction of hypothyroidism, the most widely used in experimental thyroidology, in a short time make it possible to achieve pronounced and stable changes in the thyroid system, but to what extent are these changes identical to those complex subtle mechanisms of multilevel control and adaptation that are triggered in the thyroid system with iodine deficiency and hypothyroid abnormalities of thyroid status of a different genesis? Most researchers report activation of free radical processes, in particular, lipoperoxidation in hypothyroidism caused by MMI[15-19], while the data obtained in the model using a low-iodine diet allow us to conclude that the intensity of free radical oxidation and lipoperoxidation process in iodine and thyroid hormones deficiency decreases, which, taking into account the specific role of thyroid system in the regulation of oxidative metabolism can be considered a natural consequence of iodine and thyroid hormones deficiency in target tissues.

CONCLUSION

An appropriate experimental design is crucial for obtaining reliable data that are more close to clinical conditions. Among the wide range of methods (surgical, chemical, immunological, etc.), each of which may have its advantages and disadvantages depending on the purpose and objectives of the study, low iodine diet is the most preferable for studying iodine deficient disorders as a model that most accurately reproduces changes in target tissues in hypothyroidism caused by iodine deficiency.

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Conflict of Interest

The authors do not have any conflict of interest.

Ethics Approval

All experiments have been examined and approved by the Ethics committee of the Bashkir State Medical University.

Informed Consent Statement

Consent is not applicable.

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