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CHANGES IN LIPID AND PROTEIN PEROXIDATION IN BLOOD SERUM AND RESPIRATORY LUNGS HOMOGENATE IN EXPERIMENTAL IODINE DEFICIENCY, INSULIN RESISTANCE AND THEIR COMBINATION

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ЗМІНИ ПЕРЕКИСНОГО ОКИСНЕННЯ ЛІПІДІВ І БІЛКІВ У СИРОВАТЦІ КРОВІ ТА ГОМОГЕНАТІ РЕСПІРАТОРНОГО ВІДДІЛУ ЛЕГЕНЬ ПРИ ЕКСПЕРИМЕНТАЛЬНИХ ЙОДОДЕФІЦИТІ, ІНСУЛІНОРЕЗИСТЕНТНОСТІ ТА ЇХ ПОЄДНАННІ

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Abstract. The relevance of the study is the prevalence of diet-induced metabolic disorders, in particular, iodine deficiency and insulin resistance. The aim of the study was to find out the features of lipids and proteins peroxidation in blood serum and lung tissue in rats with iodine deficiency, insulin resistance and their combination. Thyroid profile indicators, carbohydrate metabolism markers, proteins and lipids peroxidation were determined in animals kept on a standard diet (1st, control group), iodine-deficient diet (2nd group), under high-fructose feeding conditions (3rd group) and combination of iodine deprivation and the high-fructose diet (4th group). Indicators lipids and proteins free radical oxidation were determined in serum and homogenate of the pulmonary respiratory part. Iodine insufficient diet caused hypothyroid dysfunction, as evidenced by a decrease level of thyroid hormones and increase TSH in the blood serum of rats (2nd and 4th experimental groups). Increase in serum insulin and HOMA-IR index reflects the insulin resistance (3rd and 4th groups). Long-term iodine deprivation and a high-carbohydrate diet (three months) caused a violation of thyroid homeostasis and the development of insulin resistance, which are potentiated in their combination. Under such conditions oxidative stress develops, reflecting elevation of lipid and protein peroxidation products in blood serum (by 16,40-83,10 %, $p<0,05$) and homogenate of the respiratory pulmonary section (by 39,61 % - 2,55 times, $p<0,05$) according to the control. Increase the intensity of peroxidation in a combination of iodine-deficient and high-carbohydrate diets suggests a synergism of comorbid pathology and an increase in bronchopulmonary risks.

Keywords: respiratory section of the lungs; changes in metabolism; insulin resistance; iodine deficiency.

Резюме. Актуальність дослідження зумовлена поширеністю дієтоіндукованих метаболічних порушень, зокрема, йододефіциту та інсулінорезистентності. Мета дослідження полягала у з'ясуванні особливостей пероксидації ліпідів та білків сироватки крові і тканини легень у щурів із йододефіцитом, інсулінорезистентністю та за умови їх поєднання. Показники тиреоїдного профілю, маркерів вуглеводного обміну та пероксидації білків і ліпідів визначали у тварин, яких утримували на стандартному харчовому раціоні (1-ша, контрольна група), на йододефіцитній дієті (2-га група), за умов високофруктозного вигодовування (3-тя група) та на тлі поєднання йодної депривації і навантаження дієти фруктозою (4-та група). Показники вільнорадикального окиснення ліпідів і білків визначали у сироватці крові й гомогенаті респіраторного відділу легень. Установлено, що дієта з недостатнім вмістом йоду спричинила порушення функції щитоподібної залози, про що свідчать зниження рівня тиреоїдних гормонів на тлі зростання ТТГ у сироватці крові щурів (2-га та 4-та дослідні групи тварин). Такі дані дозволяють стверджувати про розвиток гіпотиреоїдної дисфункції. Суттєве зростання вмісту інсуліну у сироватці крові та індексу HOMA-IR відображає розвиток інсулінорезистентності, особливо за умов навантаження дієти фруктозою (3-та і 4-та групи). Тривала йодна депривація (впродовж двох місяців) та високовуглеводна дієта (два місяці) зумовлюють порушення тиреоїдного гомеостазу і розвиток інсулінорезистентності, що потенціюються на тлі їх поєднання. За таких умов розвивається оксидативний стрес, що відображає суттєве зростання продуктів ліпідної і білкової пероксидації у сироватці крові (на 16,40-83,10 %, $p<0,05$) й гомогенаті респіратного відділу легень (на 39,61 % - у 2,55 раза, $p<0,05$) щодо контролю. Зростання інтенсивності пероксидації при поєднанні йододефіцитної та високовуглеводної дієт дозволяє припустити синергізм коморбідної патології та зростання бронхолегеневих ризиків.

Ключові слова: респіраторний відділ легень; зміни метаболізму; інсулінорезистентність; йододефіцит.

Introduction. The most common diseases of non-infectious origin include iodine deficiency followed by the development of hypothyroid dysfunction and insulin resistance. These endocrinopathies lead to changes in metabolism, and their comorbidity can be considered a trigger for the development of metabolic diseases, somatic chronic diseases, which also results in a high level of physical, social and psychological discomfort. Iodine deficiency states have been diagnosed in more than 2 billion people around the world, reaching up to 70% of the population in some of them [1]. Most often, a decrease in iodine intake is observed in residents of mountainous regions, as well as with certain physiological characteristics of the body, when the amount of iodine absorbed does not meet the needs of the body (pregnancy, lactation, adolescence, children of preschool and school age), reduced consumption of essential bioelements (selenium, zinc, iron) [2, 4, 9]. Thyroid hormones play a key role in protein and lipid metabolism, increase heat production, activate antioxidant enzymes, and cell uptake of nutrient substrates [2, 4]. Insulin plays an equally important role in carbohydrate, protein and fat metabolism. Known as an anabolic hormone, it stimulates cell growth and proliferation by enhancing the uptake of amino acids, ensures the synthesis of proteins of muscle and other cells, affects the synthesis of RNA and DNA, and activates growth factors of the epidermis, platelets, fibroblasts. In the liver, insulin enhances the synthesis of fatty acids, triglycerides, which are further transported to adipose tissue. In recent years, the incidence of metabolic syndrome, one of the pathogenetic links of which is insulin resistance, has become critical [3].

Attention is drawn to changes in the respiratory system in conditions of combined disorders of thyroid and carbohydrate homeostasis. It is known for certain that thyroid hormones are involved in the development of the pulmonary epithelium, gas exchange processes, enhance the synthesis of surfactant, ensure the course of respiratory processes at the cellular level, and provide the activity of respiratory muscles [4,10]. Therefore, it is justified to be interested in the state of the respiratory part of the respiratory system in case of insulin resistance, especially in combination with a decrease in the activity of the thyroid gland.

The aim of this study was to establish the features of peroxidation of lipids and proteins of blood serum and lung tissue in rats with iodine deficiency, insulin resistance and their combination.

Material and methods

The study was carried out on 80 male rats weighing 150-180 g, dividing them into four experimental groups. Animals of the 1st (control, n=20) The groups were kept on a regular vivarium diet with sufficient lighting conditions and frequent ventilation of the room. Animals of the 2nd group (n=20) kept on an iodine deficiency diet [11]. Group 3-rd rats (n=20) a 10% fructose solution was obtained instead of water [12]. Animals of the 4th group were on an iodine-deficient diet, and instead of water they received 10% fructose solution. To simulate iodine deprivation and insulin resistance, animals were kept on an iodine deficiency diet and fructose load for 3 months. The animals were kept in accordance with the requirements of the legislation of Ukraine on the protection of animals and the rules of the European Convention on the Treatment of Vertebrate Animals Used in Experiments and for Other Scientific Purposes. The exit from the experiment was carried out under ketamine anesthesia (100 mg/kg body weight) [13].

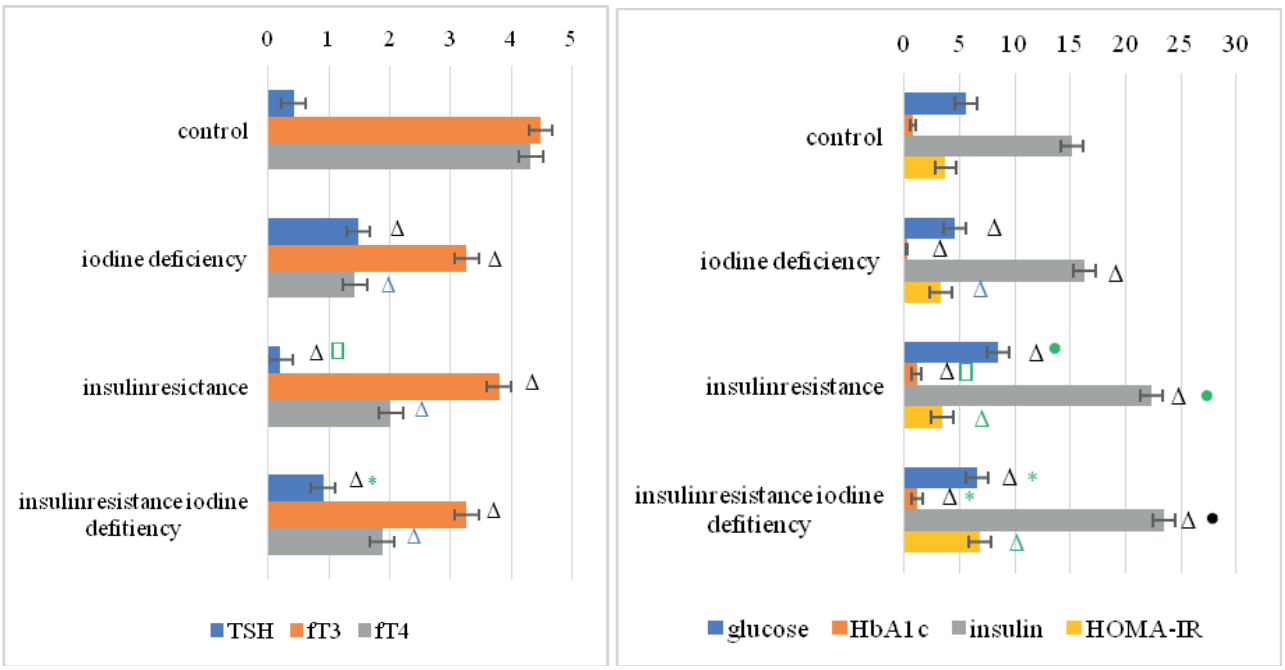
Thyroid status was assessed by the levels of thyroid-stimulating hormone (TSH), free triiodothyronine (fT3) and tetraiodothyronine (fT4). Carbohydrate metabolism was characterized by the content of glycosylated hemoglobin (HbA1c) in whole blood, serum glucose and insulin concentrations, and the insulin resistance index HOMA-IR was calculated. The thyroid profile was determined by enzyme-linked immunosorbent method using a standard test system Accu-Bind Elisa Microwells (USA), Serum insulin was determined using a standard test system Rat INS (Insulin) ELISA Kit (Elabscience, USA). The activity of lipid and protein peroxidation processes was characterized by the content of diene conjugates (DC) in serum and lung homogenate), active products that react to thiobarbituric acid (TBA-AP) and products of oxidative modification of proteins (OMP), which were determined by spectrophotometry of the studied tissues at a wavelength of 370 nm (for the determination of keto derivatives of a neutral nature) and 430 nm (for the determination of aldehyde derivatives of a basic nature) [14].

The results of the study were processed using the computer program Excel package Microsoft Office 365 ProPlus.

Results of the study

As a result of the study, the level of TSH in the serum of animals of the 2nd group increased by 3.52 times ($p_{1-2}<0,02$), 4th group – doubled ($p_{1-4}<0,05$), whereas in rats of the 3rd group – decreased by 19,05 % ($p_{1-3}<0,02$) relative to the original values. The serum level of fT3 in the experimental animals decreased by 27,30 % ($p_{1-2}<0,05$) in 2-nd, by 15,25 % – in 3-rd, by 27,30 % ($p_{1-4}<0,05$) – in the 4th groups of animals in terms of control. The content of fT4 decreased in the 2nd, 3rd and 4th groups of animals, respectively, by 67,13 % ($p_{1-2}<0,01$), 53,25 % ($p_{1-3}<0,01$) and 56,48 % ($p_{1-4}<0,01$) for reference values (pic. 1a). Such thyroid profile data characterize the development of thyroid dysfunction in animals of all experimental groups.

The changes in carbohydrate metabolism markers were as follows: Serum glucose concentration increased by 52,17 % ($p_{1-3}<0,05$) in animals of the 3rd group, by 17,93 % – 4-th ($p_{1-4}<0,05$) and decreased by 18,66 % ($p_{1-2}<0,05$) in rats of the 2nd group of animals according to the control. The HbA1c content had the same dynamic in the blood: increased in animals of the 3rd and 4th groups (respectively by 43,42 %, $p_{1-3}<0,05$ i 48,68 %, $p_{1-4}<0,05$) and declined (by 72,37 %, $p_{1-2}<0,05$) in rats of the 2nd group with respect to the initial values. The insulin content increased in the serum of animals of the 3rd and 4th experimental groups, respectively, by 54,15 % ($p_{1-3}<0,05$) and by 47,96 % ($p_{1-4}<0,05$) on data in intact animals. Attention is



a **b**

Pic.1 Thyroid status (a) and carbohydrate metabolism markers (b) in intact animals, under conditions of iodine deficiency, insulin resistance and their combination (M+m)

Notes: Δ p<0,05, Δp<0,01, Δp<0,001 – on data in intact animals, □p<0,05, □p<0,01, □p<0,001 – on data in animals with iodine deficiency, • p<0,05, • p<0,01, • p<0,001 – on data in animals on a high-carbohydrate diet, * p<0,05, * p<0,01, * p<0,001 – on the indicators in animals that were on an iodine-deficient diet with fructose load.

drawn to the dynamics of the HOMA-IR index: increased by 2,55 times (p_{1-3}<0,001) in animals of the 3rd and by 1.82 times (p_{1-4}<0,001) – 4th, but redused by 13.17 % (p_{1-2}<0,01) in rats of the 2nd experimental group (pic. 1b).

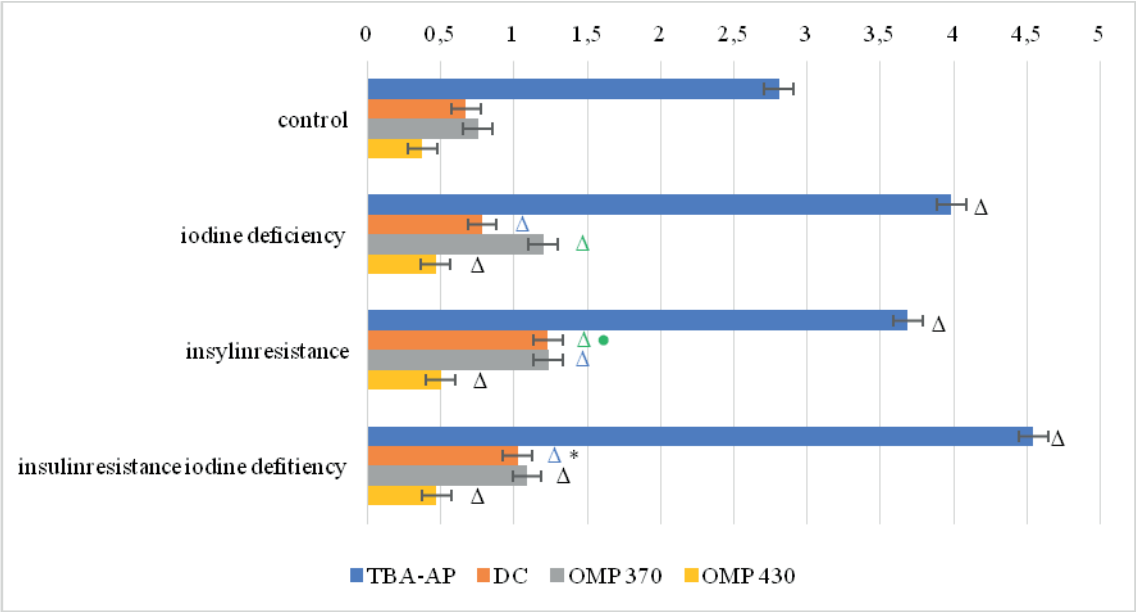
As a result of the study, an increase in lipid oxidation products in the blood serum of experimental animals was established. Thus, the content of the DC increased by 16,40 % (p_{1-2}<0,01) in the blood serum of animals of the 2nd group, by 83,10 % (p_{1-3}<0,001) – 3rd group and by the 52,20 % (p_{1-4}<0,01) – 4th group of experimental animals in comparison with the indicators of intact animals. The content of TBA-AP in the serum of animals of the 2nd group increased by 41,98 % (p_{1-2}<0,05), 3rd group – by 31,45 % (p_{1-3}<0,02) and the 4th group - by 62,01 % (p_{1-4}<0,05) compared to control. In the blood serum of animals of all experimental groups, unidirectional changes in the content of OMP products were found. In particular, in the serum of rats of the 2nd group, the content of neutral keto derivatives increased by 58,88 % (p_{1-2}<0,01), 3rd group- by 63,39 % (p_{1-3}<0,05) and the 4th group – by 43,76 % (p_{1-4}<0,05) in accordance with the control. The level of aldehyde derivatives of the main nature also increased in all groups: in rats of the 2nd group – by 25,00 % (p_{1-2}<0,05), 3rd group – by 34,41 % (p_{1-3}<0,05) and the 4th group – by 25,81 % (p_{1-4}<0,05) regarding to control (pic.2).

In the homogenates of the respiratory section of the lungs, the level of DC increased by 47,29 % (p_{1-2}<0,01) in rats of the 2nd group, by 51,12 % (p_{1-3}<0,01) – in animals of the 3rd group, by 63,65 % (p_{1-4}<0,001) – in rats of the 4th group for control. The content of TBK-AP exceeded the control data in rats of the 2nd group by 43,96 % (p_{1-2}<0,05), 3rd group – by 39,61 % (p_{1-3}<0,05), 4th group – by 52,85 % (p_{1-4}<0,05). In the homogenate of the respiratory part of the lungs, the changes in OMP were as follows: an increase in the content of neutral keto derivatives in the homogenate of tissues of the 2nd group was determined by 76,50 % (p_{1-2}<0,02), 3rd group – y 2,5 times (p_{1-3}<0,05), 4th group – three times (p_{1-4}<0,05) compared to control values. The content of aldehyde derivatives of the main nature in the homogenate of lung tissue was: an increase by 95,70 % (p_{1-2}<0,05) in the 2nd group, by 43,45 % (p_{1-3}<0,05) – in the 3rd group and by 64,55 % (p_{1-4}<0,05) – in the 4th group compared to the control group (pic. 3).

Discussion of the results

It was found that a diet with insufficient iodine content caused thyroid dysfunction, as evidenced by a decrease in the level of thyroid hormones against the background of an increase in TSH in the blood serum of rats (2nd and 4th experimental groups of animals). Such data suggest the development of hypothyroid dysfunction. Attention is drawn to changes in the thyroid panel under conditions of diet load with fructose. A decrease in the content of TSH in animals of the 3rd experimental group may indicate the peculiarities of the effect of hyperglycemia on metabolic processes in the hypothalamic-pituitary system, which reflects the tendency to decrease TSH in the blood serum of rats under the condition of insulin resistance relative to the values in intact animals.

Almost all links of carbohydrate metabolism are affected by thyroid hormones. It is known that hypersecre-

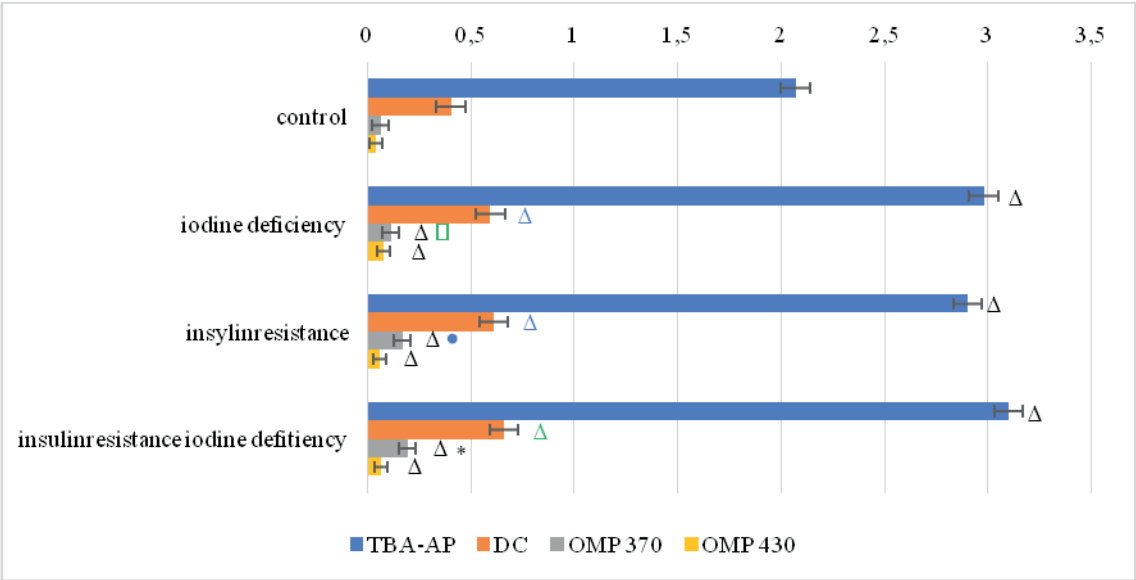


Pic. 2. Changes in the content of lipid and protein peroxidation products in the serum of intact animals, under conditions of iodine deficiency, insulin resistance and their combination (M+m)

Note: see pic. 1.

tion of thyroid hormones leads to increased absorption and synthesis of carbohydrates, activates their breakdown and use by organs and tissues, in particular, muscles. Conversely, with a decrease in the secretion of thyroid hormones, the absorption of carbohydrates in the gastrointestinal tract slows down and the consumption of glucose by muscle and adipose tissues decreases [4, 6, 7]. Therefore, hyperglycemia occurs and metabolic links in the development of insulin resistance are likely to undergo changes. A decrease in skeletal muscle's ability to utilize glucose is thought to be one of the main signs of insulin resistance. Today, the relationship between the content of free fatty acids and the development of insulin resistance has been confirmed. If serum fatty acid levels increase over a long period of time, fatty acid-induced insulin resistance develops. At the same time, persistent hyperglycemia is the result of the body's retention of carbohydrates as the main energy substrate for the nervous system [4, 7].

It should be emphasized that insulin resistance in combination with insufficient thyroid function has centrogenic effects on its hypothalamic-pituitary regulation, reducing the secretion of TSH. Under such conditions, multiple organ failure develops faster and requires more time to restore the functions of organs and systems in



Pic. 3. Changes in the content of lipid and protein peroxidation products in the homogenate of the respiratory section of the lungs of intact animals, under conditions of iodine deficiency, insulin resistance and their combination (M+m)

Note: see. pic. 1.

case of correction [9]. At that time, in animals that were on high-fructose feeding, persistent hyperglycemia, an increase in the content of glycosylated hemoglobin and insulin were observed. Such changes reflect the development of insulin resistance [3, 7, 8].

A decrease in the concentration of glucose in the blood serum of animals of the 2nd experimental group may be due to a slowdown in the absorption of carbohydrates in the gastrointestinal tract and a decrease in the processes of gluconeogenesis and glycogenolysis in the liver in hypothyroid dysfunction. Hyperinsulinemia in animals with iodine deficiency is indirect. It is known that thyroid insufficiency significantly affects fat metabolism, resulting in the accumulation of fatty acids. The body reacts to such changes by secreting insulin, which inhibits lipolysis by activating phosphodiesterase, which breaks down cAMP, which plays an essential role in fatty acid phosphorylation and lipid mobilization. As a result of such reactions, lipid metabolism changes to lipogenesis [4, 7].

Under conditions of excessive thyroid function, free radical processes are sharply intensified, which reflects the growth of LPO and OMP products [4, 5, 6]. The main points of application of thyroid hormones are the intracellular receptors of the mitochondria and the nucleus. Thyroid hormones have an effect on liponeogenesis, lipolysis and ensure the use of lipids as an energy source used by the mitochondria. Thyroid hormones activate lipase, which enhances lipolysis and reduces the amount of total fat, including deposited fat. There is a direct relationship between the thyroid profile and the fatty acid content. Therefore, inhibition of the secretory activity of the thyroid gland leads to a slowdown in lipolysis, a decrease in the synthesis of fatty acids and the development of hypercholesterolemia. Such data confirm the improvement of lipid metabolism after hormonal correction of hypothyroidism [4].

There are contradictory data on the peculiarities of the course of oxygen-dependent processes in conditions of hypothyroidism. It is known about a decrease in the level of reactive oxygen sorts due to the inhibition of metabolic processes in the body in conditions of hypothyroidism. In hypothyroid animals, the activity of antioxidant enzymes, in particular those belonging to the first line, decreases (superoxide desmutases – SOD, catalases and glutathione peroxidase – GP) [4, 5]. At the same time, oxygen tissues starvation and activation of LPO are reactions that characterize the course of most pathological processes, especially those that have a chronic course and are accompanied by the development of destructive changes in tissues and organs [4, 9]. We are inclined to believe that the intensity of LPO significantly depends on the degree and duration of thyroid dysfunction, the influence of a number of endogenous and exogenous concomitant factors, including disorders of fat and carbohydrate metabolism, an increase in the level of stress hormones, nutritional factors, lifestyle, etc [4, 6, 9].

An increase in the level of LPO and POP products in the serum and lung homogenate of experimental animals indicates the involvement of the respiratory part of the respiratory system in pathological processes. Apparently, these changes reflect damage to lung tissue caused by oxidative stress. It can be assumed that there are persistent pathological effects of these endocrinopathies on oxidative processes in the structures of the respiratory tissues of the lungs, the role of various lipid fractions in the structure of the lungs and those involved in their function. These are phospholipids and lecithin, which are the main components of surfactant, and are components of the cell membranes of alveolocytes and cells of the vascular wall. Therefore, the destruction of respiratory membrane tissues can significantly affect the processes of gas exchange [9].

Conclusions

Long-term iodine deprivation and a high-carbohydrate diet cause a violation of thyroid homeostasis and the development of insulin resistance, which are potentiated against the background of their combination. Under such conditions, oxidative stress develops, reflecting a significant increase in protein and lipid peroxidation products in serum and homogenate of the respiratory pulmonary region. An increase in the intensity of peroxidation with a combination of iodine deficiency and high-carbohydrate diets suggests a synergism of comorbid pathology and an increase in bronchopulmonary risks.

Conflict of Interest. The authors declare that they have no conflicts of interest.

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