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MORPHOFUNCTIONAL STATE OF THE LIVER IN RATS WITH FATTY HEPATOSIS MODELING AND ALTERED THYROID STATUS

© Albina A. Kondratenko[1], Natalia D. Prokhorova[1], Aleksandr A. Minchenko[1], Anton I. Poloskov[1], Nikolay S. Dedanishvili[2], Sarng S. Pyurveev[2], Ruslan I. Glushakov[1]

[1] Military Medical Academy named after S.M. Kirov. 6 Akademician Lebedev str., Saint Petersburg 194044 Russian Federation
[2] Saint Petersburg State Pediatric Medical University. 2 Lithuania, Saint Petersburg 194100 Russian Federation

Contact information: Ruslan I. Glushakov — Doctor of Medical Sciences, Assistant Professor of the Department of Pharmacology with the course of Clinical Pharmacology and Pharmacoeconomics SPbSPMU; Head of the Department Medical and Biological research of the Research Center of Military Medical Academy named after S.M. Kirov. E-mail: glushakoffruslan@yandex.ru
ORCID: https://orcid.org/0000-0002-0161-5977 SPIN: 6860-8990

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Abstract. ***Introduction.*** The prevalence of thyroid pathology, along with the increasing incidence of hepatobiliary diseases, necessitates studying the influence of thyroid status on the "natural history" of liver disease development. ***Objective.*** To evaluate the morphofunctional state of the liver in rats with drug-induced hypo- and hyperthyroidism using a model of chronic fatty hepatosis. ***Materials and methods.*** Models of hyperthyroidism (I) and hypothyroidism (II) were reproduced. Animals in the experimental groups received 15 and 30% fructose solutions instead of drinking water. Decapitation was performed after 45 days. Liver fragments were fixed in 10% neutral formalin for 24 hours. Sections with a thickness of 3–4 μm were prepared and subjected to histological examination. ***Results.*** The vascularization index was highest in group I with a 15% fructose load. Liver sinusoids occupied the maximum area relative to the area of the liver tissue image. With a 30% fructose load against a background of hypo- and hyperthyroidism, the lumen of the sinusoids appeared narrowed. The relative content of connective tissue in the liver parenchyma of the experimental groups did not statistically significantly depend on the thyroid status and the level of fructose load. The inflammatory activity index averaged 5–6 points in all experimental groups. Condition I influenced the volume of infiltration by neutrophils, while dystrophic changes in hepatocytes were more dependent on the level of fructose load. Pronounced granular dystrophy of hepatocytes was revealed in all experimental groups, as well as a decrease in glycogen stores. In group II, already at a 15% fructose load, individual cells were in a state of ballooning degeneration. With a twofold increase in fructose consumption, discomplexation of hepatic plates, granular protein structures, and significant lipid accumulation in the cytoplasm of hepatocytes were observed in groups I and II. ***Conclusions.*** A high level of thyroid hormones significantly affects the indicators of inflammatory and proliferative activity of liver tissue. A low level of thyroid hormones affects the severity of dystrophic changes in hepatocytes. With an increase in fructose load, both with hypo- and hyperthyroidism, hepatocytes undergo intense dystrophic changes.

Keywords: non-alcoholic fatty liver disease, thyroid status, rats

МОРФОФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ ПЕЧЕНИ КРЫС ПРИ МОДЕЛИРОВАНИИ ЖИРОВОГО ГЕПАТОЗА И ИЗМЕНЕННОМ ТИРЕОИДНОМ СТАТУСЕ

© Альбина Александровна Кондратенко[1], Наталья Дмитриевна Прохорова[1], Александр Александрович Минченко[1], Антон Иванович Полосков[1], Николай Сергоевич Деданишвили[2], Сарнг Саналович Пюрвеев[2], Руслан Иванович Глушаков[1]

[1] Военно-медицинская академия им. С.М. Кирова. 194044, г. Санкт-Петербург, ул. Академика Лебедева, 6
[2] Санкт-Петербургский государственный педиатрический медицинский университет. 194100, г. Санкт-Петербург, ул. Литовская, 2

Контактная информация: Руслан Иванович Глушаков — д.м.н., доцент кафедры фармакологии с курсом клинической фармакологии ифармакоэкономики СПбГПМУ, начальник отдела медико-биологических исследований научно-исследовательского центра ВМедА им. С.М. Кирова. E-mail: glushakoffruslan@yandex.ru ORCID: https://orcid.org/0000-0002-0161-5977 SPIN: 6860-8990

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Резюме. ***Введение***. Распространенность тиреоидной патологии наряду с повышением инцидентности болезней гепатобилиарной системы определяет необходимость изучения влияния тиреоидного статуса на «естественную историю» развития заболеваний печени. ***Цель*** — оценить морфофункциональное состояние печени крыс при медикаментозно индуцированном гипо- и гипертиреозе на модели хронического жирового гепатоза. ***Материалы и методы.*** Воспроизведены модели гипертиреоза (I) и гипотиреоза (II). Животные экспериментальных групп (крысы) вместо питьевой воды получали 15 и 30% раствор фруктозы. Через 45 суток осуществляли декапитацию. Фрагменты печени фиксировали в 10% нейтральном формалине в течение 24 ч. Изготавливали срезы толщиной 3–4 мкм и проводили гистологическую оценку. ***Результаты.*** Индекс васкуляризации имел наибольшие значения при состоянии I с 15% фруктозной нагрузкой. Синусоиды печени занимали максимальную площадь относительно площади снимка ткани печени. При 30% фруктозной нагрузке на фоне гипо- и гипертиреоза просвет синусоидов выглядел суженным. Относительное содержание соединительной ткани в паренхиме печени опытных групп статистически значимо не зависело от тиреоидного статуса и уровня фруктозной нагрузки. Индекс воспалительной активности в среднем составил 5–6 баллов во всех опытных группах. Состояние I влияло на объем инфильтрации нейтрофильными лейкоцитами, в то время как дистрофические изменения в гепатоцитах сильнее зависели от уровня нагрузки фруктозой. Выявлены выраженная зернистая дистрофия гепатоцитов во всех опытных группах и снижение запасов гликогена. При II — уже при 15% фруктозной нагрузки отдельные клетки находились в состоянии гиалиново-капельной дистрофии. При увеличении потребления фруктозы в два раза наблюдали дискомплексацию печеночных пластинок, зернистые белковые структуры и значительное накопление липидов в цитоплазме гепатоцитов в I и II группах. ***Выводы.*** Высокий уровень тиреоидных гормонов значимо влияет на показатели воспалительной и пролиферативной активности ткани печени. Низкий уровень тиреоидных гормонов влияет на выраженность дистрофических изменений в гепатоцитах. При увеличении фруктозной нагрузки как при гипо-, так и при гипертиреозе гепатоциты подвергаются интенсивным дистрофическим изменениям.

Ключевые слова: неалкогольная жировая болезнь печени, тиреоидный статус, крысы

INTRODUCTION

Thyroid gland (thyroid) diseases are the most common among all endocrine diseases. Hypo- and hyperthyroid conditions are in the first and the third place in the structure of endocrinologic pathology [7, 8], while epidemiologic studies demonstrate that the proportion of subclinical thyroid pathology is several times higher than the official statistics [1, 9].

Thyroid hormones regulate the expression of more than 100 genes, with tissue-specific changes in the gene expression [3, 10]. In turn, the products of thyroid-dependent transcription interact at the level of the whole organism, and the resulting effects determine comorbidity in thyroid pathology [5, 11].

In addition, liver disease is currently a serious threat to public health due to the high prevalence of viral hepatitis, increased alcohol consumption, obesity epidemic and somatic pathology [4, 12, 13].

Thus, the high prevalence of thyroid pathology along with the increased incidence and prevalence of hepatobiliary system disease determines the need to study the influence

of thyroid status on the "natural history" of liver disease development.

AIM

To evaluate the morphofunctional state of rats liver in the drug-induced hypo- and hyperthyroidism on the model of fatty change of liver.

MATERIALS AND METHODS

Discription of the general design of the experimental study. Two series of experimental observations were performed with the inclusion of 90 Wistar rats (Federal State Unitary Enterprise "Rappolovo Laboratory Animal Nursery", Leningrad Region, Russia) aged 30±10 days of both sexes of 3–4 months old, weighing 250±35 g at the beginning of the experiment. The number of laboratory animals was determined by the calculated number of rats necessary to test the statistical hypothesis (Table 1). In each series of experiments, each group of laboratory animals was composed of same-sex individuals. Thus, the experiment was performed on an equal number of males and females. The duration of quarantine (an acclimatization period) for all animals was at least 14 days. During the quarantine, each animal was examined (their behavior and general condition) twice a day (in the morning and evening hours). Laboratory animals suspected of any disease and/or having behavioral changes were excluded from the study during quarantine. Rats within each gender were randomized into three equal groups after the end of quarantine. Randomization of laboratory animals was performed using the closed envelope method. The total duration of the experiment excluding quarantine was 45 days.

Animal housing conditions. The food and the maintenance of laboratory animals were in accordance with the norms of the order of the USSR Ministry of Health 1179 of October 10th, 1983 "Sanitary rules for the arrangement, equipment and maintenance of experimental-biological clinics". Each group of animals was kept with no more than 4–6 same-sex individuals per group and had access to water and food ad libidum.

Drug induction of hypo- and hyperthyroidism. Laboratory animals of the first (I, hyperthyroid, n=32) and the second (II, hypothyroid, n=32) groups were reproduced the model of drug-induced hyperthyroidism and hypothyroidism, respectively, by administration of the investigated substances. The following drugs were administrared: L-thyroxine (dry substance, RUP "Belmedpreparaty", Republic of Belarus) at a dose of 100±10 μg per 100 g of animal body weight once a day, propylthiouracil (dry substance, Merck Selbstmedikation GmbH, Germany) 2.0±0.15 mg per 100 g of animal body weight once a day, intragastrically through atraumatic polyurethane probe daily, starting from the first day of the experiment. After weighing the laboratory animals (at least once every 3 days), the required amount of the tested substance was dissolved in 1.0 ml of indifferent food gelatin gel (Henan Boom Gelatin Co., Ltd., PRC) and administered to individuals of the corresponding group. In order to create the same stress factor, laboratory animals of comparison group III (conditional normothyroidism) also received 1.0 ml of gel intragastrically through a probe.

A model of fatty change of the liver. Animals were divided into equal subgroups within groups I, II and III (hypo-, hyperthyroidism, conditional normothyroidism) in which instead of drinking water rats received 15 and 30% fructose solution throughout the research. In the comparison group animals against the background of induction of drug-induced hypo- and hyperthyroidism received drinking water. 8 laboratory animals were absolute control group.

Blood sampling and withdrawal from the experiment. After 45 days the animals were removed from the experiment

Table 1

Distribution of animals by groups. Number of series of experiments (total number of animals in the group)

Таблица 1

Распределение животных по группам. Количество серий экспериментов (суммарное количество животных в группе)

Экспериментальные группы / Experimental groups	Номер группы / Group number		
	I	II	III
Тиреоидный статус / Thyroid status	Гипертиреоз / Hyperthyroidism	Гипотиреоз / Hypothyroidism	Условный нормотиреоз / Conditional normothyroidism
15% фруктоза в поилке, n / 15% fructose in the drinker, n	12	12	10
30% фруктоза в поилке, n / 30% fructose in the drinker, n	12	12	10
Интактные, n / Intact, n	8	8	6

by ether vapor overdose. Mixed arteriovenous blood was collected by puncture from the heart, transferred into a clean plastic tube and left at room temperature for an hour. Blood was centrifuged for 15 min at 3000 rpm to obtain serum. Serum from each animal was examined individually. The content of transaminases (alanine aminotransferase and aspartate aminotransferase) of serum was analyzed by UV kinetic method without pyridoxal phosphate. Total protein was analyzed by biuret method, total bilirubin was analyzed by diazosulfanil method ("Vector-Best", Russia). The analysis was performed according to the instructions. The levels of the studied hormones were determined by solid-phase enzyme immunoassay using standard kits produced by "NVO Immunotech" for free T_4 (ImmunoFA-T4), free T_3 (ImmunoFA-T3) and TSH (ImmunoFA-TSH).

Morphological study description. Liver fragments were fixed in 10% neutral formalin for 24 h, after which they were embedded in paraffin according to the standard technique. Slices 3–4 microns thick were made and stained with hematoxylin and eosin, Van Gieson's picrofuchsin, Gaidengain's azocarmine, and Periodic Acid — Schiff (PAS) reaction. Histological preparations were analyzed using a light-optical microscope Carl Zeiss Axio Scope A1 at different magnifications. Morphometric evaluation was performed in ImageJ program, plugins segmentation. Five blocks of liver tissue were made from each animal, 5 sections were made from each block, 20 fields of view were examined in each tissue section. A total of 500 measurements of each studied parameter were made. Total relative areas of sinusoidal capillaries (C) and parenchyma (P) were determined. Hepatocyte necrosis was measured in points (0 to 10), the severity of dystrophic changes and inflammatory infiltration in points (0 to 4). Images with an area of 64 000 μm2 (S set) were divided into 80 squares (N node=63). The following parameters were determined: the number of mitoses (NM), the number of bi-nucleated cells (BNC), the number of hepatocytes with one nucleolus in the nucleus (NON), the number of whole nucleated cells (NC), and the number of grid crossing points (GCP) not falling on the slices of hepatocytes and their nuclei.

According to the results of these measurements, the following was calculated: Parenchymatous density index (PD = 1 – GCP/N node). Functional cell mass index (FCM = = (NC/Sset) × PD × 100 000), characterizing parenchymatous-stromal relations in a unit volume of tissue. Nuclear mass index (NM = (NC+BNC)/Sset) × PD × 100,000), which characterizes parenchymatous-stromal relations per unit volume of tissue. Binuclear cell mass index (BCMI = = ((BNK/NC)/Sset) × PD × 100 000), indicating the degree of implementation of the restorative reserves of a unit of liver tissue volume. Mass-mitotic index (MMI = ((NM/NC)/Set) × × PD × 100 000), showing the proliferative activity of the liver tissue volume unit. Functional karyocellular index (FKCI = NM/FCM), which characterizes the amount of nuclear material in a cell per unit volume of liver tissue. The average hepatocyte slice area index (AHSA = (Sset/NC) × PD), which is proportional to its functional activity. Mass index of cells with one nucleolus in the nucleus (MION = ((NON/NC)/SSet) × PP × 100 000), indicating the degree of realization of protein-synthetic function of a unit volume of hepatic tissue. Vascularization index — VI = C/P. Inflammatory activity index (IAI) as the sum of scores of necrosis, dystrophy and inflammatory infiltration severity measurements.

Statistical analysis of the study results. The quantitative characteristics studied in the study were presented as an average value (M±m) or median (Me) with 95% confidence interval boundaries (or 25 and 75% quartiles). The hypothesis of distribution type was tested using the Shapiro–Wilk test. Comparison of data in subgroups was carried out depending on the variant of the distribution of the trait in the groups. The results of morphometry were recorded in tables with subsequent statistical processing of the results in STATISTICA 7.0, modules Nonparametric, ANOVA and Discriminant analysis.

Ethical rules and regulations. The work was conducted in accordance with the ethical principles established by theEuropean Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (adopted in Strasbourg on 18.03.1986 and confirmed in Strasbourg on 15.06.2006) and approved by the Local Ethical Committee.

RESULTS

Thyroid stimulating hormone (TSH) levels in intact animals were: 1.43±0.06 (1.28–1.57) ulU/mL. In model animals with hyperthyroidism the level of TSH in blood was not determined, which is probably due to the peculiarity of sensitivity. In the hypothyroidism model it amounted to 6.24±0.84 (4.67–6.88) ulU/mL. The levels of T4 and T3 free fractions and transaminases in the serum of experimental and intact animals are presented in Table 2.

The vascularization index had the highest values in hyperthyroidism with 15% fructose load (0.24±0.0036). The liver sinusoids occupied the maximum area relative to the liver tissue imaging area (9.1±0.25%). When animals were fed 30% fructose on the background of hypo- and hyperthyroidism, the lumen of sinusoids looked narrowed (Fig. 1).

The relative content of connective tissue in the liver parenchyma of the experimental groups was statistically significantly independent of thyroid status and the level of fructose load ($p < 0.05$) (Fig. 2).

Table 2

Free thyroid hormone and transaminase fractions content M±m (95%CI), p <0.05

Таблица 2

Содержание фракций свободных тиреоидных гормонов и трансаминаз M±m (95%CI), p <0,05

Экспериментальные группы/ Experimental groups	fT4, pmol /L	fT3, pmol/L	ALT, U/L	AST, U/L
Гипертиреоз, 15% фруктоза / Hyperthyroidism, 15% fructose	24,20±1,66 (20,44–27,95)	3,76±0,08 (3,57–3,94)	35,90±0,80 (34,07–37,73)	234,10±29,34 (167,74–300,46)
Гипертиреоз, 30% фруктоза / Hyperthyroidism, 30% fructose.	52,45±3,26 (45,08–59,12)	4,07±0,16 (3,70–4,44)	49,10±1,05 (46,70–51,49)	374,30±3,49 (366,40–382,20)
Гипотиреоз, 15% фруктоза / Hypothyroidism, 15% fructose	8,28±0,16 (7,90–8,65)	5,40±0,13 (5,09–5,70)	32,70±2,05 (28,05–37,35)	150,10±2,32 (143,49–156,71)
Гипотиреоз, 30% фруктоза / Hypothyroidism, 30% fructose	5,26±0,11 (5,02–5,49)	<1,64	54,50±1,49 (51,12–57,87)	320,90±7,34 (304,29–337,51)
Условный нормотиреоз, 15% глюкоза / Conditional normothyroidism, 15% glucose	23,40±1,75 (19,82–23,67)	5,56±0,16 (4,57–5,98)	35,70±2,15 (28,05–37,35)	140,10±2,46 (143,49–156,71)
Условный нормотиреоз, 30% глюкоза / Conditional normothyroidism, 30% glucose	24,79±2,09 (18,29–24,38)	5,47±0,28 (3,98–5,84)	44,60±1,49 (41,16–52,63)	265,70±5,34 (227,43–287,11)
Интактный контроль / Intact control	20,74±0,77 (18,99–22,49)	5,78±0,08 (5,59–5,96)	27,60±1,15 (24,98–30,22)	120,50±1,91 (116,18–124,82)

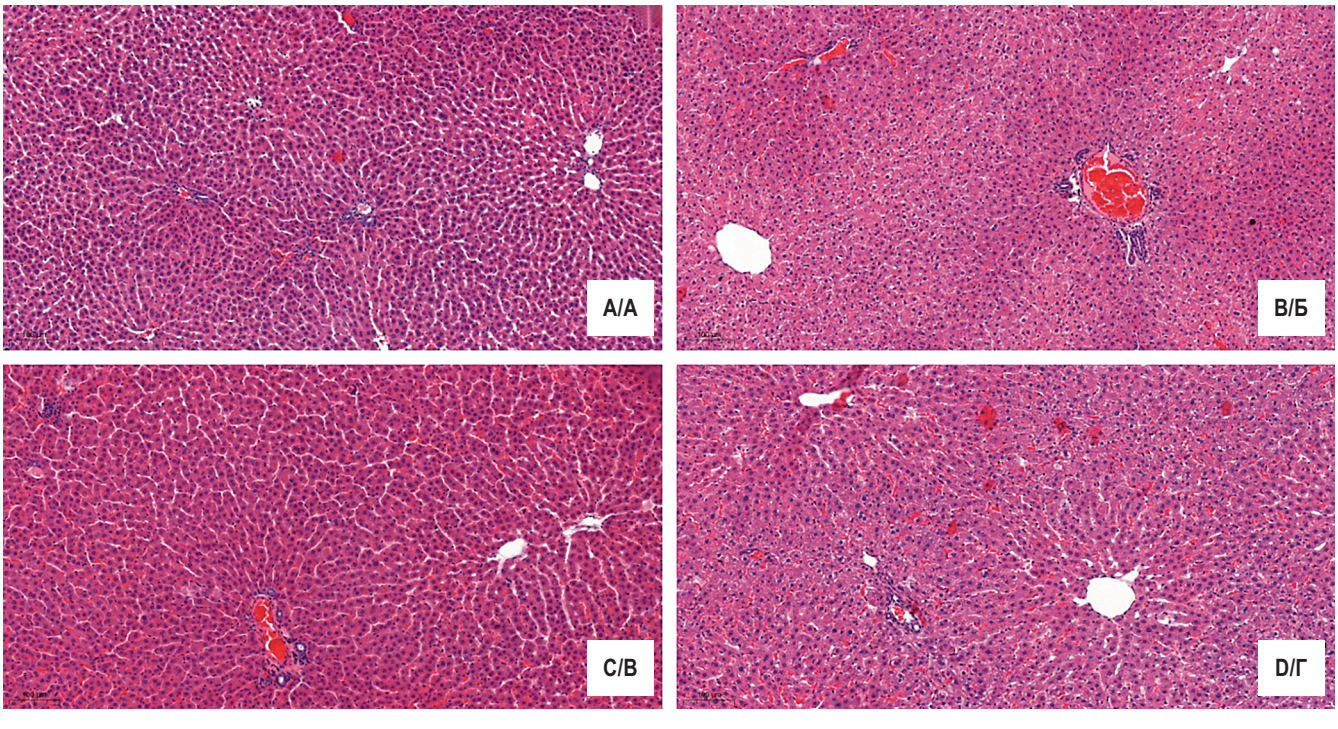

Fig. 1. **Liver tissue of rats of experimental groups: A — received 15% fructose in the drink on hypothyroidism background; B — received 30% fructose in the drink on hypothyroidism background; C — received 15% fructose in the drink on the background of hyperthyroidism; D — received 30% fructose in the drinker on the background of hyperthyroidism. Hematoxylin and eosin staining, ×100**

Рис. 1. **Ткань печени крыс экспериментальных групп: А — получали 15% фруктозу в поилке на фоне гипотиреоза; Б — получали 30% фруктозу в поилке на фоне гипотиреоза; В — получали 15% фруктозу в поилке на фоне гипертиреоза; Г — получали 30% фруктозу в поилке на фоне гипертиреоза. Окраска гематоксилин и эозин, ×100**

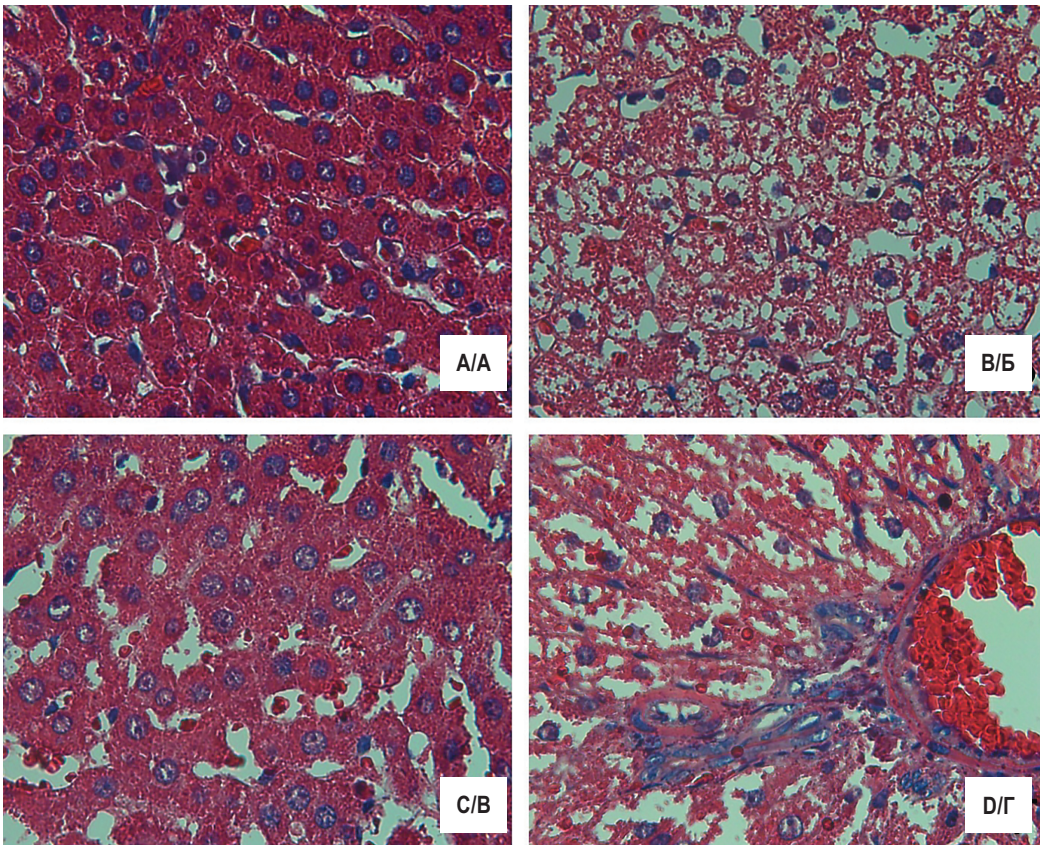

Fig. 2. **Liver tissue of rats of experimental groups: A — received 15% fructose in the drink on hypothyroidism background; B — received 30% fructose in the drink on hypothyroidism background; C — received 15% fructose in the drink on the background of hyperthyroidism; D — received 30% fructose in the drinker on the background of hyperthyroidism. Azocarmine staining by Gaidengain, ×400**

Рис. 2. **Ткань печени крыс экспериментальных групп: А — получали 15% фруктозу в поилке на фоне гипотиреоза; Б — получали 30% фруктозу в поилке на фоне гипотиреоза; В — получали 15% фруктозу в поилке на фоне гипертиреоза; Г — получали 30% фруктозу в поилке на фоне гипертиреоза. Окраска азокармином по Гайденгайну, ×400**

The index of inflammatory activity averaged 5–6 points in all experimental groups and statistically significantly differed from this index in intact animals. The state of hyperthyroidism most significantly affected the volume of infiltration by neutrophilic leukocytes, while dystrophic changes in hepatocytes were more strongly dependent on the level of fructose loading ($p < 0.005$). Azocarmine staining of slices according to Gaidengain (Fig. 2) and Periodic Acid — Schiff (PAS) reaction reaction (Fig. 3) revealed pronounced granular dystrophy of hepatocytes in all experimental groups and decreased glycogen stores. In the hypothyroid state, already at 15% fructose load individual cells were in a state of hyaline granular dystrophy. Liver plate dyscomplexation, granular protein structures and significant lipid accumulation in the cytoplasm of hepatocytes in hypo- and hyperthyroidism were observed with doubled fructose intake.

The indices of nuclear mass, BCMI, MMI and FKCI in the state of hyperthyroidism were statistically significantly higher than the values in hypothyroidism regardless of the level of fructose load.

AHSA when drinking 30% fructose instead of drinking water in hypothyroidism state was the highest (345.04±1.40). The total relative number of cells containing single nucleoli was maximum in hyperthyroidism irrespective of the level of fructose load (0.113±0.00046).

As a result of stepwise selection, all studied morphometric parameters were statistically significantly informative by Fisher's F-criterion. The BCMI, FKCI and MION signs changed most significantly. The changes in IAI and AHSA parameters were less pronounced (Table 3).

DISCUSSION

Our reproduced model of chronic liver damage associated with excessive fructose consumption leads to pathologic

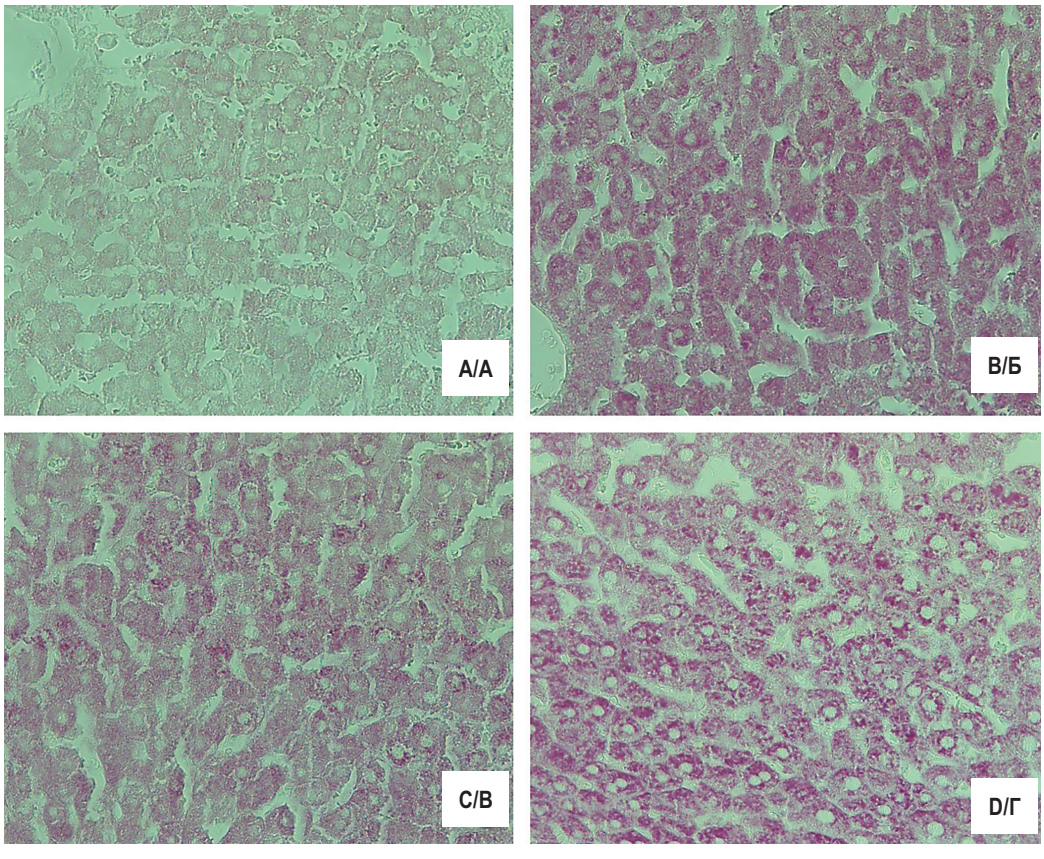

Fig. 3. **Liver tissue of rats of experimental groups: A — received 15% fructose in the drink on hypothyroidism background; B — received 30% fructose in the drink on the background of hypothyroidism; C — received 15% fructose in the drink on the background of hyperthyroidism; D — received 30% fructose in the drink on the background of hyperthyroidism. SHIC reaction, ×400**

Рис. 3. **Ткань печени крыс экспериментальных групп: А — получали 15% фруктозу в поилке на фоне гипотиреоза; Б — получали 30% фруктозу в поилке на фоне гипотиреоза; В — получали 15% фруктозу в поилке на фоне гипертиреоза; Г — получали 30% фруктозу в поилке на фоне гипертиреоза. ШИК-реакция, ×400**

Table 3

Morphometry of liver tissue of intact animals and rats of experimental groups, M±m (95% CI), p<0.005, M±m (95% CI), p <0,005

Таблица 3

Морфометрия ткани печени интактных животных и крыс опытных групп M±m (95% CI), p <0,005, M±m (95% CI), p <0,005

Морфометрические показатели / Morphometric indices	Гипертиреоз / Hyperthyroidism		Гипотиреоз / Hypothyroidism		Условный нормотиреоз / Conditional normothyroidism		Интактные / Intact
	15% фруктоза / 15% fructose	30% фруктоза / 30% fructose	15% фруктоза / 15% fructose	30% фруктоза / 30% fructose	15% фруктоза / 15% fructose	30% фруктоза / 30% fructose	
ИВА / IVA	5,96±0,045 (4,61-–6,65)	6,18±0,04 (5,60–6,63)	5,42±0,04 (4,61–6,64)	6,10±0,03 (5,57–6,83)	2,46±0,16 (2,21–3,09)	4,35±0,05 (3,96–4,75)	0,60±0,035 (0,30–0,65)
ИМДК / IMDC	0,22±0,005 (0,21–0,23)	0,23±0,011 (0,20–0,25)	0,16±0,008 (0,17–0,21)	0,15±0,011 (0,16–0,21)	0,15±0,008 (0,17–0,21)	0,17±0,009 (0,17–0,22)	0,03±0,002 (0,02–0,03)
ФККИ / FCCI	1,19±0,004 (1,18–1,20)	1,21±0,009 (1,18–1,23)	1,17±0,008 (1,15–1,19)	1,17±0,009 (1,15–1,19)	1,16±0,008 (1,15–1,19)	1,17±0,017 (1,15–1,18)	1,16±0,008 (1,15–1,17)
СПСГ / SPSG	308,22±5,58 (296,07–320,37)	296,10±8,20 (278,23–313,97)	287,90±5,38 (276,18–299,62)	288,72±4,87 (278,00–299,44)	280,90±4,48 (274,28–289,12)	282,60±6,12 (277,58–294,37)	279,11±4,20 (274,23–309,07)
ИМОЯ / IMOJA	0,11±0,003 (0,09–0,15)	0,09±0,005 (0,06–0,13)	0,06±0,005 (0,05–0,09)	0,08±0,003 (0,06–0,010)	0,06±0,005 (0,05–0,09)	0,06±0,005 (0,05–0,09)	0,05±0,003 (0,04–0,09)

transformation and dystrophy of hepatocytes. Insufficiency of adenosine triphosphate (ATP) synthesis and decreased redox potential leads to mitochondrial dysfunction and altered fat and carbohydrate metabolism [14]. Presumably, the activity of hydrolytic enzymes of lysosomes leads to coagulation of cytoplasm proteins with the appearance of cellular inclusions in the form of "grains". When fructose load is doubled, the excessive amount of carbohydrates is transformed into triacylglycerides, damaging hepatocytes [15], which is laboratory expressed in a significant increase in the level of serum transaminases.

Modern knowledge about the mechanisms of action of iodothyronines has been extended to the understanding of proangiogenic activation by iodothyronines due to non-genomic effects arising from interaction with $\alpha_V\beta3$ integrin, and the quantitative effect of angiogenesis activation at supraphysiological concentrations of iodothyronines is comparable to the effect of vascular endothelial growth factor and fibroblast growth factor [6, 16]. The immunomodulatory effects of T_4 have also been studied, which consist in an increase in the expression of tissue-specific proinflammatory genes, while a reduced cytotoxic activity of NK-cells and a decrease in chemotaxis and macrophage phagocytosis are noted, which at the systemic level leads to the restoration of the balance between pro- and anti-inflammatory factors [17]. In our study, the index of inflammatory activity had maximum values in the hyperthyroid state, which agrees with the data of other authors on the proinflammatory properties of iodothyronines.

Our study also demonstrates that the degree of realization of regenerative reserves and proliferative activity were higher in the state of hyperthyroidism, as evidenced by the indices of the amount of nuclear material per unit volume of liver tissue and the index of the proportion of hepatocytes with a single nucleus in their nucleus. A significant part of tumor cells in the liver of transgenic mice contain a single nucleus in the nucleus. According to various studies, thyroid hormones are potent mitogens of hepatocytes, activating the E2F family of transcription factors. It leads to overexpression of cell cycle inducers — cyclins and cyclin-dependent kinases (CDK) and promotes the transition of hepatocytes from G1 to S-phase. Thyroid hormones increase the levels of cyclins A, D1 and E and the activity of complexes of cyclin A with cyclin-dependent kinase 2 (CDK2) and cyclin D1-CDK4 and decrease the levels of CDK inhibitors p16 and p27. Expression of vascular endothelial growth factor (VEGF) and nuclear antigen Ki-67 in the liver against the background of thyroid hormone preparations increases [16]. However, this increase in proliferative activity is transient and in long-term hyperthyroidism leads to a decrease in the proliferative potential of functional tissue and the development of fibrosis [19]. We have previously demonstrated that long-term hyperthyroidism in reproductive organs leads to pronounced fibrotic changes, while hypothyroidism leads to fatty dystrophy [20].

Discussing the relationship between the functional activity of the liver and the thyroid gland, it should be noted that osteopontin secretion is induced by polarization of M1-fraction of macrophages, which due to paracrine mechanisms can inhibit the expression of TRb-receptor in hepatocytes, on the one hand, suppressing the action of thyroid hormones and, accordingly, aggravating lipid deposition in the liver, on the other hand, compensatory increasing the level of TSH in serum. Elevated TSH levels promote osteopontin secretion by the M1 fraction of macrophages. This study demonstrates a positive feedback between the thyroid gland and the liver, possibly playing an important role in maintaining and enhancing the pathologic process of nonalcoholic fatty liver disease [2, 18].

Given the different effector actions of thyroid hormones after interaction with different isoforms of TRa and TRb nuclear receptors, including with respect to carbohydrate and lipid metabolism in the liver, future treatment strategies are aimed at weakening the effects of TRa stimulation and enhancing the effects of TRb targeting. Currently, about 10 drugs have been patented whose affinity to TRb is 10–40 times higher than to TRa. At the same time, experimental data confirm the efficacy of selective TRb-thyromimetic action in the treatment of experimental non-alcoholic fatty liver disease [19]. The altered thyroid status modulates the transformation of functional liver tissue in two directions: toward the enhancement of dystrophic changes and fatty transformation in hypothyroidism and toward the activation of inflammation and fibrotic changes in hyperthyroidism.

CONCLUSION

1. High level of thyroid hormones most significantly affects the indices of inflammatory and proliferative activity of liver tissue.

2. Low level of thyroid hormones affects the severity of dystrophic changes in hepatocytes.

3. In the process of fructose load increase both in hypo- and hyperthyroidism hepatocytes undergo intensive dystrophic changes.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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Experiments with animals. The work was carried out in accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (adopted in Strasbourg on March 18, 1986 and confirmed in Strasbourg on June 15, 2006), and approved by the Local Ethics Committee.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Эксперименты с животными. Работа проведена в соответствии с этическими принципами, установленными Европейской конвенцией по защите позвоночных животных, используемых для экспериментальных и других научных целей (принятой в Страсбурге 18.03.1986 г. и подтвержденной в Страсбурге 15.06.2006 г.), и одобрена Локальным этическим комитетом.

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