ISSN 0022-0930, Journal of Evolutionary Biochemistry and Physiology, 2022, Vol. 58, No. 2, pp. 465–475. © Pleiades Publishing, Ltd., 2022.
Russian Text © The Author(s), 2022, published in Rossiiskii Fiziologicheskii Zhurnal imeni I.M. Sechenova, 2022, Vol. 108, No. 3, pp. 325–338.

EXPERIMENTAL PAPERS

Reactivity of the Thyroid System to Short-Term Stress in Wistar Rats with Visceral Obesity and Restricted Social Activity

T. A. Mityukova[a],*, E. N. Chudilovskaya[a],, and A. A. Basalai[a]**

[a] *Institute of Physiology, National Academy of Sciences of Belarus, Minsk, Belarus*
**e-mail: mityukovat@gmail.com*
***e-mail: e.chudilovskaya@gmail.com*

Received December 16, 2021

Revised January 21, 2022

Accepted January 22, 2022

Abstract—The obesity problem requires a study of its pathophysiological consequences affecting hormonal regulation and organism's reactivity to extreme exposures. The study was aimed first to examine the effect of a high-calorie diet and social isolation, as well as their combination for 4 months, on the development of obesity, its metabolic and behavioral sequelae, features of the thyroid status, while at the second stage, to assess the reaction of hormonal indices of the thyroid status to short-term stress in rats. The experiments were carried out on male Wistar rats and at the first stage focused on the effects of a high-calorie diet and social isolation, as well as their combinations for 4 months. At the end of the experiment, behavioral reactions, metabolic syndrome indices, thyroid status, and cortisol levels were evaluated. At the second stage, the animals were exposed to short-term acute stress, and the shifts in the hormonal indices were recorded one hour later versus the initial background. A high-calorie diet led to the development of metabolic syndrome, signs of depression, increased thyroid-stimulating hormone (TSH), thyroxine and triiodothyronine serum levels, as well as iodothyronine deiodinase type 1 (D1) activity, in the rat liver. At the same time, there was a decrease in thyroperoxidase activity and an increase in thyroid levels of triglycerides and malondialdehyde. The physiological response to stress in the control rat group included an increase in cortisol and TSH serum levels, however, against the background of a high-calorie diet, no cortisol release into the bloodstream was recorded. Social isolation did not alter normal reactivity of the adrenal cortex, but reduced TSH release in response to acute stress, since the initial level of this hormone was slightly elevated against the background of chronic social isolation stress. Thus, excessive nutrition and the deficit of social activities in male Wistar rats led to significant changes in the organism's reactivity to acute stress.

DOI: 10.1134/S0022093022020156

Keywords: rat, high-calorie diet, social isolation, stress, thyroid system, cortisol

INTRODUCTION

Sociological studies suggest that the number of overweight and obese people is increasing worldwide, while obesity increases the risk of metabolic syndrome, cardiovascular disease, thyroid dysfunction, cognitive and psychoemotional disorders, including depression [1–3]. The most common causes of obesity are an excessive diet and unhealthy lifestyle, which includes insuffi-

cient physical and social activities [1–3]. The state of thyroid status has its own features in obesity and attracts the attention of researchers, because thyroid hormones are involved in the regulation of basal metabolism, thermogenesis and lipid metabolism [2]. Most clinical and epidemiological studies converge in associating thyroid dysfunction with overweight and obesity. In euthyroid patients, there is a positive correlation between body weight and thyroid-stimulating hormone (TSH) indices, although data on peripheral hormone levels in obesity are ambiguous. It should be admitted that thyroid status plays an important role not only in the pathogenesis of obesity, but also in the system of regulatory processes that provide organism's adaptation to physical loads and stress [2, 4, 5]. Nevertheless, the issue of the possible modulating effect of obesity and restricted physical and social activities, as well as their combination, on the reactivity of regulatory systems, including the thyroid status, remains poorly understood and requires appropriate experimental studies.

Experimental rat models of obesity and metabolic syndrome are thoroughly described in the literature, their choice and conduct do not cause difficulties [6]. However, the concept of unhealthy lifestyle is more complex and ambiguous. At present, such factors as a decrease in social activity due to the development of distance learning and remote work practices, as well as the expansion of the virtual entertainment sphere and forced lifestyle changes, are attracting particular attention against the background of the COVID-19 pandemic. This model can be implemented in an experiment by keeping animals in individual cages. According to Mumtaz et al. [7], the social isolation factor can act as a chronic stressor leading to changes in social behavior, the functioning of the neurochemical and neuroendocrine systems, and reactivity to acute stress. Based on this, we adopted an individual maintenance as a variant of social activity restriction in rats (social isolation).

The study was aimed to explore first the effect of a high-calorie diet and social isolation on the development of obesity, its metabolic and behavioral consequences, as well as the thyroid status features, and then to assess the reaction of thyroid status hormonal indices on short-term stress in rats.

MATERIALS AND METHODS

All experiments with laboratory animals was carried out in compliance with the legislation, in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experiments or other scientific purposes (ETS N 123), as well as the principles and regulations approved by the Bioethics Committee of the Institute of Physiology of the National Academy of Sciences of Belarus (IP NASB). The experiments were performed on sexually mature male Wistar rats. Animals were introduced into the experiment at the age of 2 months and a body mass of 180–200 g. The following types of experimental exposures were examined: a high-calorie diet (HCD) and a social isolation (SI), as well as their combinations. The rats were divided into 4 groups: (1) Control ($n = 20$)—standard vivarium diet and optimal housing conditions (by 6–7 individuals per common cage); (2) Control-SI ($n = 20$)—standard diet and individual cage housing; (3) HCD ($n = 36$)—a diet was added with animal fat as a lard (45% of food caloric value) and carbohydrates (drinking water was replaced with 10% fructose solution at an ad libitum access to drinkers), rats were housed under standard conditions (by 6–7 individuals per common cage); (4) HCD-SI ($n = 36$)—a high-calorie diet and individual cage housing. HCD included a lard added in accordance with animal's body weight, while observing 45% of an additional contribution to a standard vivarium diet as the body weight increased, and according to a caloric value of the basal diet. Lard consumption per animal was recorded under both group and individual housing. Techniplast cages with a floor area of 1500 cm^2 ($48 \times 38 \times 21$ cm) were used for a group housing, while for individual housing we used Zoonlab Gmbh cages with a floor area of 360 cm^2 ($26 \times 20 \times 14$ cm) for used for an individual housing. Both variants complied with the ETS No. 123 requirements, with the floor area per rat being always no less than 200–250 cm^2.

The duration of the experiment was 4 months. In the last week, the Porsolt forced-swim test for

rodent depressive behavior was performed [8]. Each rat was placed for 6 min in a vessel filled with water up to the mark at a height of 30 cm; water temperature was 24–25°C. The duration of the first act of active swimming, the number and duration of freezes (no swimming movements, full immobility) were recorded. The refusal from active swimming characterized the state of "despair", being a sign of depression [8].

Rat body mass was estimated on Scout Pro scales (China), and systolic blood pressure (SBP) was measured on a PanLab system (Spain). At the final stage of the experiment, half of the animals in each of the four groups were exposed to acute short-term stress, forced swimming in cold water ($t = 13$°C) for 5 min. The animals were withdrawn from the experiment 1 h after stress, under thiopental anesthesia. All other animals were also decapitated using sodium thiopental. The visceral adipose tissue, liver, thyroid, and blood were sampled for serum separation and further analysis of biochemical and hormonal indices.

Blood serum biochemical indices, such as total cholesterol, triglycerides, glucose, and total protein, were determined by enzymatic methods using Diasens commercial kits (Belarus) on a BS-200 Automated Biochemistry Analyzer (China) with BS-330 software. Blood serum hormones were determined by enzyme immunoassay: triiodothyronine (T3), thyroxine (T4), and free T4 (fT4) with Chema kits (Russia), free T3 (fT3) and cortisol with Diagnostic Systems kits (Russia), thyroid-stimulating hormone (TSH) with FineTest kits (China).

Thyroperoxidase (TPO) activity in thyroid tissue was assayed as described elsewhere [9]. Thyroid tissue was homogenized using an IKA T10 basic Ultra-Turrax homogenizer (Germany) and 0.05 M sodium phosphate buffer (pH 7.4). The homogenate (diluted 1 : 80) was centrifuged at 4°C and 5000 rpm (10700 g) on an Allegra 64R Centrifuge Beckman Coulter (USA). Supernatants were separated, and a protein content was determined by the biuret method on a BS-200 Automated Biochemistry Analyzer (China) using Diasens kits (Belarus). The supernatants for TPO activity assay were kept on an ice bath (melting ice temperature) and used during 3–4 h. Spectrophotometric studies were carried out on a SOLAR CM 2203 spectrofluorimeter. The buffer and other components were placed into a thermostatted cuvette (1 cm). Incubation mixture composition: 0.05 M sodium phosphate buffer (pH 7.4)—2.7 mL, KI solution (0.6 M)—75 μL, supernatant—200 μL. The contents of the cuvette were held for 3 min at 25°C. The reaction was initiated by adding H_2O_2 (10 μL of 40 mM solution), the sample was stirred, and the optical density was recorded at a wavelength of 353 nm for 3 min. The enzyme activity was expressed in international units per mg of protein per minute of incubation (IU/mg/min).

The activity of iodothyronine deiodinase type 1 (D1) in the liver was assayed as described previously [10] with some modifications, that of malonic dialdehyde (MDA)—by the reaction with thiobarbituric acid [11]. The procedure of D1 assay [10] is based on the determining hepatic D1 activity by a conversion of T4 into T3 in the presence of a dithiothreitol (DTT) cofactor. The following reagents were used: 0.05 M Tris-HCl buffer (pH 7.8), 0.01 M sodium phosphate buffer (pH 7.6) containing 0.25% bovine serum albumin (BSA), 320 mM DTT in distilled water, T4 (I) standard in methanol—1000 μM added with 0.5 N NaOH (100 μL per 5 mL of solution); T4 (II) standard was obtained by diluting T4 (I) 20-fold on BSA-phosphate buffer added with 0.5 N NaOH to pH 8.0 (final concentration 50 μM). Liver homogenate was prepared on Tris-buffer (1 : 5) at a melting ice temperature, then centrifuged for 15 min on a BioSan LMC-3000 centrifuge at 3000 rpm (1700 g).

Incubation mixture composition: supernatant—700 μL, DTT—40 μL, T4 (II) standard—40 μL. Incubation at 37°C for 1 h was performed in duplicates: 2 experimental and 2 control samples (control samples without DTT and T4). The reaction was stopped by adding 700 μL 96% ethanol, while DTT and T4 were added to the control samples. After stirring, the samples were left in a refrigerator for 2 h or overnight for protein precipitation. The supernatant was separated by centrifugation and used for the determination of T3 by ELISA (storage was possible at –20°C until analysis). The resulting supernatant contained about 48% ethanol. It was found that, when determining T3 by ELISA in an alcohol-containing superna-

Table 1. The effect of high-calorie diet and social isolation on body mass, visceral fat mass, and systolic blood pressure (SBP) in male Wistar rats

Indices	1) Control	2) Control-SI	3) HCD	4) HCD-SI
Body mass, g	373.9 ± 7.7	370.6 ± 8.6	411.1 ± 9.0*#	416.2 ± 9.3*#
Visceral fat mass, g	5.5 ± 0.5	5.2 ± 0.5	21.8 ± 1.2*#	10.7 ± 0.8*#^
SBP, mm Hg	131.1 ± 2.2	134.0 ± 1.4	145.3 ± 2.1*#	153.6 ± 2.5*#^

The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group.

tant, it is necessary to mix it with a "zero serum" (pooled human serum treated with activated charcoal), not allowing the ethanol concentration in the sample to exceed 5%. The effect of ethanol concentration on T3 determination by ELISA was tested on pooled human serum. When adding ethanol to this serum with a final concentration of no more than 5%, the T3 level was determined as a baseline, obtained in the native serum. While performing this technique, the supernatant was added to the "zero serum" at a ratio of 10 : 100 L in such a way that the ethanol content in the sample was about 4.8%, which was maximum allowable.

Statistical analysis was carried out using Statistica 6.0. The normality of data distribution was checked using the Shapiro–Wilk test. If the distribution was normal, the ANOVA was applied using the Fisher exact test (data were presented as $M \pm SEM$). For non-normal distributions, the nonparametric methods, Kruskal–Wallace test, were used (data were presented as Me (25th–75th percentile)). Differences were considered significant at $p < 0.05$.

RESULTS

At the first stage, the experiment focused on the effect of a high-calorie diet and social isolation. The body mass indices in Control and Control-SI rat groups were almost identical at the end of the experiment (Table 1). The high-calorie diet led to a statistically significant increase in the body mass by an average of 10% versus Control group, while the combined effect in HCD-SI led to a 12% increase on average versus Control-SI group. These rat groups (HCD and HCD-SI) were practically indistinguishable from each other in average body mass values. The visceral fat mass did not reveal significant differences in the rats of Control and Control-SI groups. The rats of HCD group showed a 4-fold gain in the visceral fat mass versus Control group, while this elevation was less pronounced in HCD-SI group: approximately 2-fold versus Control-SI group. At the same time, the visceral fat mass in HCD group was 2-fold higher versus HCD-SI group. Obviously, in social isolation, the gain in rat visceral fat mass was less pronounced versus group housing, although fat intake in these rat groups was almost the same (6.7 and 6.8 g per day, respectively). The mean SBP values in both groups fed a standard diet (groups 1, 2) were practically identical, while in the rats fed an excessive diet (groups 3, 4), they significantly increased by 11 and 15%, respectively, reaching comparable values (Table 1).

As seen from Table 2, the values of biochemical blood indices were almost identical in Control and Control-SI groups. Feeding a high-calorie diet led to an increase in the total serum cholesterol level in the rats of HCD-SI group. The triglyceride level increased significantly only in HCD, but not HCD-SI, group. The glucose level increased significantly in both groups versus Control group.

The determination of triglyceride levels in thyroid tissue showed comparable values in the first two groups of rats fed a standard diet and revealed a significant increase in the index with a high-calorie diet regardless of the housing conditions versus Control group. However, the accumulation of triglycerides in thyroid tissue was most pronounced in HCD group, significantly exceeding (1.5-fold) this index in the rats exposed to a combined effect (HCD-SI group). The MDA level in thyroid tissue significantly increased (1.4-fold) with a high-calorie diet in group 3 versus Control, while in group 4, there was only an upward ten-

Table 2. Biochemical indices of blood serum and thyroid tissue in rats of experimental groups ($M \pm SEM$)

Indices	1) Control	2) Control-SI	3) HCD	4) HCD-SI
	Blood serum			
Total cholesterol, mmol/L	1.43 ± 0.09	1.50 ± 0.08	1.58 ± 0.07	1.65 ± 0.08*
Triglycerides, mmol/L	0.55 ± 0.05	0.57 ± 0.09	0.97 ± 0.09*#	0.65 ± 0.07^
Glucose, mmol/L	5.41 ± 0.30	5.86 ± 0.38	6.89 ± 0.27*#	6.39 ± 0.28*
	Thyroid tissue			
Triglycerides, mmol/g tissue	4.52 ± 0.57	3.30 ± 0.63	9.63 ± 0.88*#	6.23 ± 0.51#^
MDA, mmol/g tissue	59.71 ± 5.13	60.85 ± 8.44	86.49 ± 5.22*#	74.07 ± 5.15

The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group.

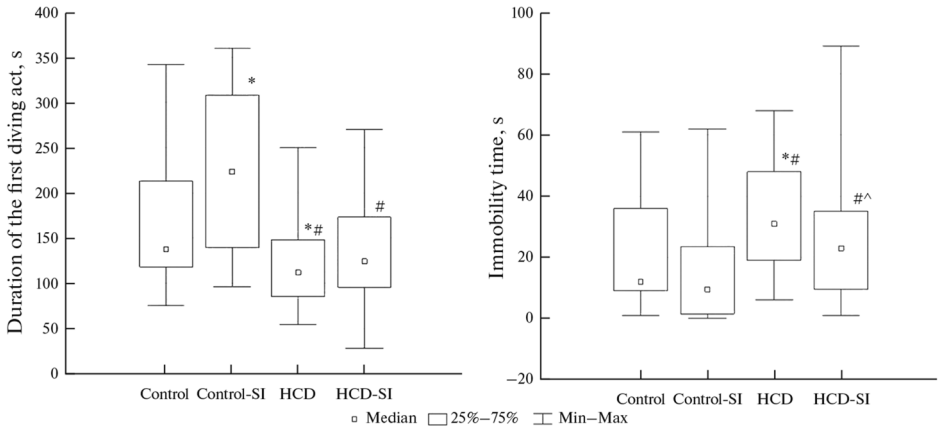

Fig. 1. The results of the Porsolt forced-swim test in experimental animals (Me (25; 75). The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group.

dency for the its level (Table 2).

Considering the increase in visceral fat mass, SBP elevation and metabolic shifts, we can state that the animals fed a high-calorie diet developed an experimental metabolic syndrome. The consumption of almost equal amounts of lard under conditions of social isolation and in group housing led to a more pronounced accumulation of visceral fat and greater metabolic abnormalities under conditions of group housing (Tables 1, 2).

The assessment of the rat psycho-emotional status was carried out using the Porsolt forced-swim test, which is designed to detect depressive behavior in rodents [8]. As seen from Fig. 1, the rats of Control-SI group differed by a reliable, almost 2-fold, increase in the duration of the first active swimming act versus Control group. The rats fed a high-calorie diet showed shifts in the opposite direction, namely a significant decrease in the duration of the first active swimming act and an increase in the freezing (immobility) time, indicative an increasing depression.

At the first stage of the experiment, along with hormonal indices, the characteristics of the activity of some enzymes of thyroid status were also studied (Table 3). TPO activity in thyroid tissue was almost indistinguishable in the first two groups. Against the background of a high-calorie diet, there was a significant decrease in TPO activity, almost half of the control level, regardless of the housing conditions (Table 3). In contrast, hepatic D1 activity significantly increased with excessive nutrition.

Serum hormonal indices of the thyroid status are presented in the Figures as light bars (rats without stress) and hatched bars (rats after stress).

Table 3. Indices of TPO activity in thyroid tissue and D1 activity in liver tissue in rats of experimental groups

Indices	1) Control	2) Control-SI	3) HCD	4) HCD-SI
	Thyroid			
TPO, IU/mg protein/min	0.69 ± 0.12	0.70 ± 0.13	0.37 ± 0.04*#	0.40 ± 0.08*#
	Liver			
D1, nmol/g tissue/h	90.00 ± 8.27	71.33 ± 7.97	152.56 ± 20.29*#	136.88 ± 14.33

The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group.

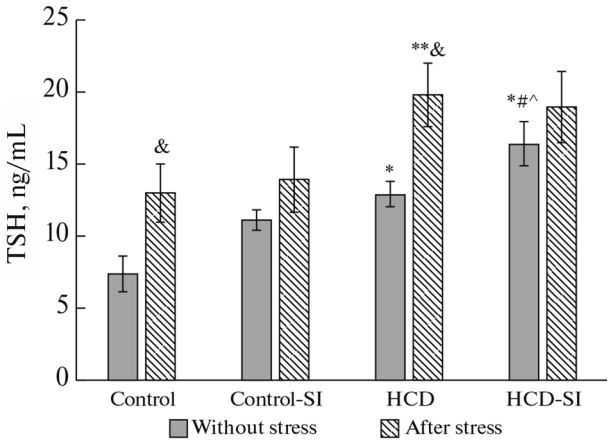

Fig. 2. The experimental exposure on the TSH level in the rats of experimental group. The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group; &—vs unstressed animals; **—vs Control group after stress; ##—vs Control-SI group after stress; ^^—vs HCD group after stress.

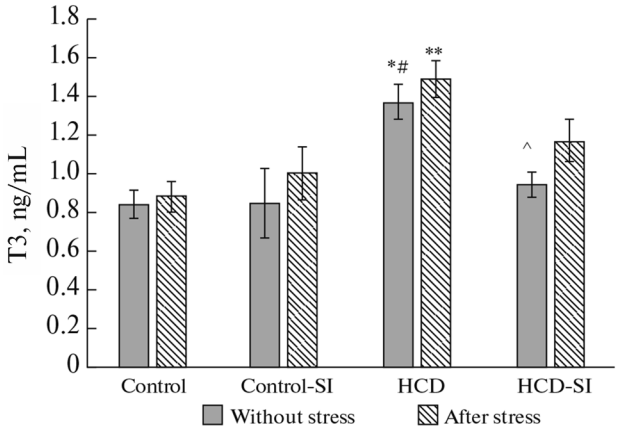

Fig. 3. The experimental exposure on the T3 level in the rats of experimental group. The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group; &—vs unstressed animals; **—vs Control group after stress; ##—vs Control-SI group after stress; ^^—vs HCD group after stress.

First of all, it is necessary to consider the effect of a high-calorie diet and social isolation on the studied indices.

As seen from Fig. 2, TSH values are not significantly different among the animals of the first two groups (Control and Control-SI) without stress, although in group 2, there was an upward tendency for the TSH level ($p > 0.05$). A high-calorie diet led to a significant (1.8-fold) increase in the TSH level versus Control, while HCD-SI group showed a 2-fold increase in the index versus Control group and a 1.5-fold increase versus Control-SI group. A significant (1.8-fold) increase in the TSH level was observed under the influence of short-term stress in Control group; a significant hormone increase (1.5-fold) was also detected in HCD group. However, in the rats kept in isolation (Control-SI and HCD-SI), the shifts in TSH levels did not reach statistical significance (Fig. 2).

Against the background of excessive feeding, there were a significant 1.6-fold increase in the total T3 level and a 1.4-fold increase in the total T4 level, which was paralleled by a 1.7-fold elevation of hepatic D1 activity versus Control group (Figs. 3, 4; Table 3). When a high-calorie diet was combined with social isolation, T3 and T4 levels remained within control values, despite a significant increase in TSH levels versus Control. Serum T3 and T4 levels in HCD significantly exceeded those in HCD combined with social isolation.

The poststress T3 level (Fig. 3) remained relatively stable in almost all experimental groups, whereas the serum T4 significantly increased in Control-SI group against the background of stress (Fig. 4).

As seen from Fig. 5 and 6, the feeding type and

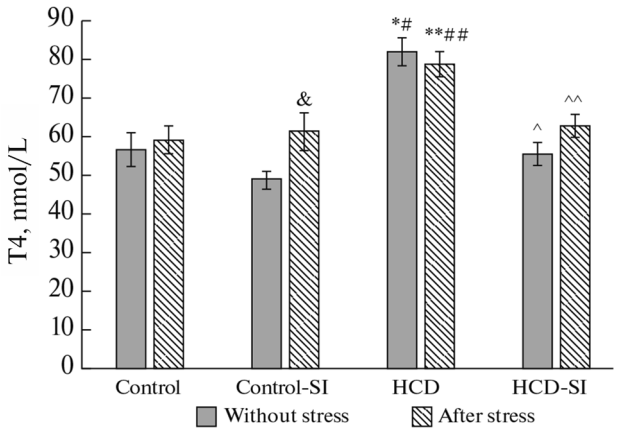

Fig. 4. The experimental exposure on the T4 level in the rats of experimental group. The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group; &—vs unstressed animals; **—vs Control group after stress; ##—vs Control-SI group after stress; ^^—vs HCD group after stress.

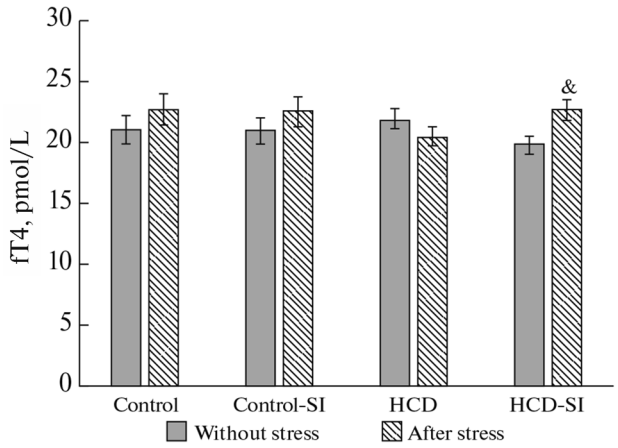

Fig. 6. The experimental exposure on the fT4 level in the rats of experimental group. The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group; &—vs unstressed animals; **—vs Control group after stress; ##—vs Control-SI group after stress; ^^—vs HCD group after stress.

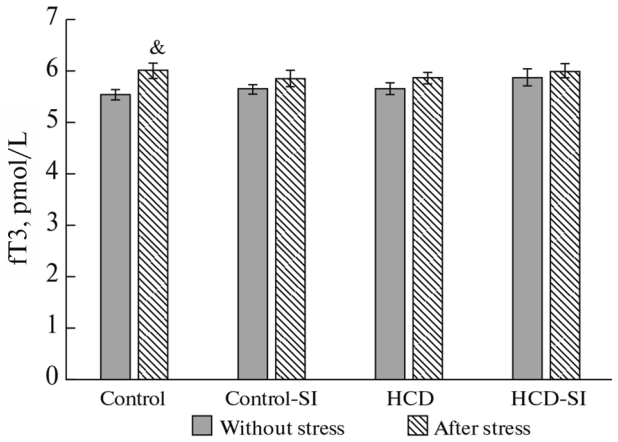

Fig. 5. The experimental exposure on the fT3 level in the rats of experimental group. The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group; &—vs unstressed animals; **—vs Control group after stress; ##—vs Control-SI group after stress; ^^—vs HCD group after stress.

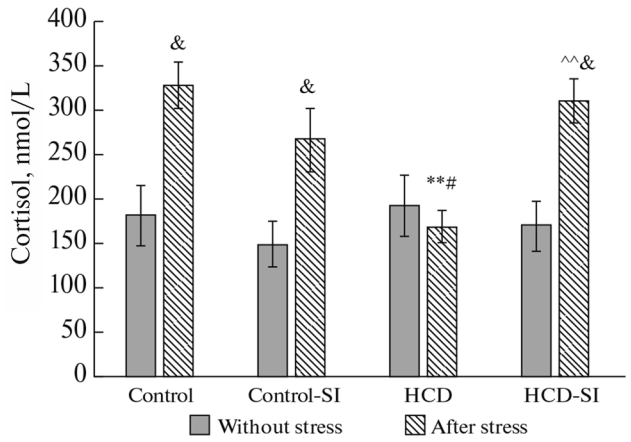

Fig. 7. The experimental exposure on the cortisol level in the rats of experimental group. The differences are significant at $p < 0.05$: *—vs Control group; #—vs Control-SI group; ^—vs HCD group; &—vs unstressed animals; **—vs Control group after stress; ##—vs Control-SI group after stress; ^^—vs HCD group after stress.

housing conditions had no effect on levels of free thyroid hormone fractions. A poststress elevation of fT3 was only observed in Control group (Fig. 5), and of fT4 only in HCD-SI group (Fig. 6).

A high-calorie diet and social isolation caused no significant shifts in serum cortisol levels (Fig. 7), but there was a downward tendency for its index in Control-SI group.

A short-term stress exposure caused a sharp (1.8-fold) increase in the serum cortisol level in Control group rats versus those not exposed to cold water swimming, hence unstressed (Fig. 7).

It is worthwhile to characterize stress-induced changes in the indices in Control group, i.e. an increase in levels of TSH (1.8-fold) and cortisol (1.8-fold), as well as a slight increase in the serum fT3 level, which appears to be an adequate physiological reaction to a short-term acute stress.

In Control-SI group, there was also a signifi-

cant increase in serum cortisol levels (1.8-fold), but there were no changes in such indices as TSH and fT3, which is typical for Control group. The mean serum TSH value in Control-SI group after swimming-induced stress matched that in Control group after swimming. However, at the same time, the magnitude of the response to acute stressor exposure was decreased.

In HCD group, there was only observed a 1.5-fold increase in serum TSH levels in the stressed versus unstressed animals. No cortisol release into the bloodstream was detected 1 h after stress.

In HCD-SI group, there was a poststress increase in cortisol levels similar to that in Control group (1.8-fold), but there was no reaction from TSH. The latter may be due to the initially elevated level of the index, which exceeded the control value by more than 2-fold.

DISCUSSION

It is known from the literature that the isolation of rodents in early childhood causes later on a pronounced anxiety and depression accompanied by a decrease in corticosterone blood levels (chronic isolation stress) and changes in neuroplasticity indices [12, 13]. When isolating adult animals, no such characteristic shifts may occur, but more often, a hyperactive phenotype ensues [12, 13]. In our experiments, when tested in the Porsolt test, animals in Control-SI group showed a significant increase in the duration of the first swimming act versus Control group. The animals of HCD group showed opposite shifts, namely a decrease in the time of the first swimming act and an increase in the freezing time. These characteristics seem to indicate the development of an active phenotype against the background of isolation stress and the signs of depression in a high-calorie diet under conditions of group housing.

There is ambiguous information in the literature concerning the effect of social isolation on the body mass of animals. Some authors point out that social isolation in overfed adult rodents promotes an increase in obesity and concomitant alterations of metabolic functions [14], while others report the opposite results [15, 16]. The social isolation model that we used in our experiments restricted an increase in visceral obesity and characteristic metabolic and hormonal shifts and also increased animal activity in the Porsolt test. The HCD group rats had a most pronounced visceral obesity and exhibited a tendency toward depression in the Porsolt test.

As for the peculiarities of the thyroid status in HCD, our results confirmed the clinical observations stating an increase in the TSH level in obesity [2]. This fact can be interpreted as evidence of hypothyroidism in obese patients, however, in our experiments, an increase in the TSH level was recorded against the background of elevated T3 and T4 levels. Current studies indicate that leptin produced by adipocytes has a regulatory effect on TSH secretion as it activates the hypothalamic-pituitary-adrenal (HPA) axis by affecting the neurons residing in the paraventricular nucleus and responsible for TSH production [17]. It has been suggested that an increased TSH level in obesity is not a consequence of hypothyroidism, but serves as a mechanism aimed at the activation of basal metabolism [17]. Our work also revealed other adaptive components of the response aimed at the activation of metabolism in excessive high-calorie diet and visceral obesity, such as an activation of hepatic D1 and an increase in T3 and T4 blood levels. T3 is known to increase the intensity of lipolysis in adipose tissue through cAMP-dependent mechanisms, and thus affects lipolysis synergistically with the adrenergic system [17]. However, under conditions of social isolation, serum T3 and T4 shifts characteristic of HCD were absent in spite of elevated TSH levels.

The fact of a decrease in TPO activity in thyroid tissue in HCD and HCD-SI groups deserves attention. It may be explained by an accumulation of triglycerides in the thyroid gland in obesity and their toxic effect on the thyroid function, which was also noted in other studies [18]. Alternatively, this may be associated with an increase in free-radical processes, as evidenced by an increase in the MDA level (Table 2). Inhibition of TPO activity with prolonged overfeeding and visceral obesity may indicate a downward tendency for the thyroid function, which in the long run may entail the development of hypothyroidism.

There are few publications in the literature addressing the problem of obesity in the light of its

influence on the reactivity of the organism's regulatory systems under stress, although it is well known that a number of neuroendocrine processes are definitely altered in obesity. In our experiments, a study of the reaction to short-term stress showed that in the rats with a severest visceral obesity (HCD group), there is no stress-induced increase in blood cortisol levels after 1-h exposure. It can be assumed that this is due to the neurobiological mechanisms of depression in obese animals. According to the literature, depressed patients are characterized by substantial changes in the HPA axis, with attenuated HPA and sympathetic responses to acute stress [19–21] being typical for depressed patients. There is evidence of a biological link between type 2 diabetes, which is characteristic of obesity, depression and HPA axis dysregulation, including stress response [22].

As for the social isolation model, Control-SI rat group showed an increased activity in the Porsolt test and an upward tendency for the TSH level, presumably due to chronic isolation stress. Against the background of prolonged social isolation (Control-SI and HCD-SI groups), TSH release in response to short-term stress, which was characteristic of Control group, did not show up.

Thus, the feeding type, as well as the deficit of social activity, are factors that modulate the state of regulatory systems and alter their reactivity in response to acute stressor exposure.

CONCLUSIONS

1. A high-calorie 4-month diet causes a significant gain in the body and visceral fat mass, as well as an elevation of systolic blood pressure, glucose and triglyceride serum levels, in male Wistar rats. Visceral obesity and triglyceride levels were increased most pronouncedly when the animals were housed collectively as opposed to their individual housing under social isolation conditions.

2. The results of the Porsolt test indicate an increase in depression in rats fed a high-calorie diet and a manifestation of an active phenotype in animals housed in conditions of social isolation.

3. A high-calorie diet leads to a significant increase in TSH levels, total serum fractions of thyroxine and triiodothyronine, and hepatic D1 activity, which seems to be an adaptation aimed at the activation of metabolism. Under conditions of combined exposure to a high-calorie diet and social isolation, no increase in thyroxine and triiodothyronine blood levels was observed.

4. Against the background of a high-calorie diet, there is a decrease in TPO activity and an increase in triglyceride and malondialdehyde levels in thyroid tissue, which in the long run may lead to a decrease in the thyroid function.

5. The reaction to short-term acute stress in animals of Control group includes a sharp elevation of cortisol and TSH, as well as a slight increase in the serum level of free triiodothyronine.

6. The rats fed a high-calorie diet also showed an increased TSH release into the bloodstream 1 h after stress, but the cortisol level remained virtually unchanged in the meantime.

7. When animals were housed individually (social isolation), regardless of the type of their diet, a typical stress response was observed in the form of a cortisol release into the bloodstream after 1-h exposure. However, in these animals, TSH response to acute stress was not manifested, since the level of this index was already somewhat elevated against the background of chronic stress of social isolation.

Thus, social isolation and a high-calorie diet evoke oppositely directed changes in behavioral reactions of male Wistar rats. These exposures, as well as their combinations, lead to specific shifts in the functional activity of the pituitary-thyroid-adrenal axis, which, in turn, corrects the physiological response to short-term stress exposure.

AUTHORS' CONTRIBUTION

Conceptualization (T.A.M.), experimental design and data collection (T.A.M., E.N.Ch., A.A.B.), data processing (E.N.Ch.), writing and editing the manuscript (T.A.M., E.N.Ch., A.A.B.).

FUNDING

The work was supported by the governmental budget funding to the Institute of Physiology of

the National Academy of Sciences of Belarus within the SSRP "Fundamental and applied sciences for medicine", research theme "To investigate neuroendocrine mechanisms of rat propensity to obesity" (reg. no. 20200320 of 16.03.2020).

CONFLICT OF INTEREST

The authors declare that they have no evident or potential conflict of interest related to the publication of this article.

REFERENCES

1. Engin A (2017) The definition and prevalence of obesity and metabolic syndrome. Adv Exp Med Biol 960: 1–17. https://doi.org/10.1007/978-3-319-48382-5_1
2. Biondi B (2010) Thyroid and Obesity: An Intriguing Relationship. J Clin Endocrinol Metab 95(8): 3614–3617. https://doi.org/10.1210/jc.2010-1245
3. Blasco BV, Garcна-Jiménez J, Bodoano I, Gutiérrez-Rojas L (2020) Obesity and Depression: Its Prevalence and Influence as a Prognostic Factor: A Systematic Review. Psychiatry Invest 17(8): 715–724. https://doi.org/10.30773/pi.2020.0099
4. Fortunato RS, Ignácio DL, Padron ÁS, Peçanha R, Marassi MP, Rosenthal D, Werneck-de-Castro JPS, Carvalho DP (2008) The effect of acute exercise session on thyroid hormone economy in rats. J Endocrinol 198(2): 347-353. https://doi.org/10.1677/JOE-08-0174
5. Helmreich DL, Tylee D (2011) Thyroid hormone regulation by stress and behavioral differences in adult male rats. Horm Behav 60(3): 284–291. https://doi.org/10.1016/j.yhbeh.2011.06.003
6. Wong1 SK, Chin K-Y, Suhaimi FH, Fairus A, Ima-Nirwana S (2016) Animal models of metabolic syndrome: a review. Nutrition and metabolism 13: 65–76. https://doi.org/10.1186/s12986-016-0123-9
7. Mumtaz F, Khan MI, Zubair M, Dehpour AR (2018) Neurobiology and consequences of social isolation stress in animal model-A comprehensive review. Biomed Pharmacother 105: 1205–1222. https://doi.org/10.1016/j.biopha.2018.05.086
8. Porsolt RD, Berlin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Intern Pharmacodyn 229: 327–336.
9. Mityukova TA, Chudilovskaya EN, Migalevitch AS (2020) Determination of thyroid peroxidase activity in thyroid tissue (experimental study). Lab Diagnost; Eastern Europe 9(3): 285–293 (In Russ).
10. Kaplan MM, Utiger RD (1978) Iodothyronine metabolism in rat liver homogenates. J Clin Invest 61:459–471. https://doi.org/10.1172/JCI108957
11. Müller GA, Mathias B (1986) Thiobarbituric acid positive substances as indicators of lipid peroxidation. Z Gesamte Inn Med 41(24): 673–676.
12. Ieraci A, Mallei A, Popoli M (2016) Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. Neural Plast 2016: 6212983. https://doi.org/10.1155/2016/6212983
13. Begni V, Sanson A, Pfeiffer N, Brandwein C, Inta D, Talbot SR, Riva MA, Gass P, Mallien AS (2020) Social isolation in rats: Effects on animal welfare and molecular markers for neuroplasticity. PLoS One 15(10): e0240439. https://doi.org/10.1371/journal.pone.0240439
14. Sun M, Choi EY, Magee DJ, Stets CW, During MJ, Lin ED (2014) Metabolic effects of social isolation in adult C57BL/6 mice. Int Sch Res Notices 2014: 690950. https://doi.org/10.1155/2014/690950
15. Balsevich G, Uribe A, Wagner KV, Hartman J, Santarelli S, Labermaier C, Schmidt MV (2014) Interplay between diet-induced obesity and chronic stress in mice: potential role of FKBP51. J Endocrinol 222(1): 15–26. https://doi.org/10.1530/JOE-14-0129
16. Patterson ZR, Abizaid A (2013) Stress induced obesity: lessons from rodent models of stress. Front Neurosci 7: 130. https://doi.org/10.3389/fnins.2013.00130
17. Santini F, Marrullo P, Rotondi M, Ceccarini G, Pagano L, Ippolito S, Chiovato L, Biondi B (2014) The crosstalk between thyroid gland and adipose tissue: signal integration in health and disease. Eur J Endocrinol 171(4): R137–R152. https://doi.org/10.1530/EJE-14-0067
18. Shao S, Zhao Y, Song Y, Xu C, Yang J, Xuan S, Yan H, Yu C, Zhao M, Xu J, Zhao J (2014) Dietary high-fat lard intake induces thyroid dysfunction and abnormal morphology in rats. Acta Pharmacol Sin 35(11): 1411–1420. https://doi.org/10.1038/aps.2014.82
19. Yoon KL, Joormann J (2012) Stress reactivity in social anxiety disorder with and without comorbid depression. J Abnorm Psychol 121(1):250–255. https://doi.org/10.1037/a0025079
20. Steudte-Schmiedgen S, Wichmann S, Stalder T,

Hilbert K, Muehlhan M, Lueken U, Beesdo-Baum K (2017) Hair cortisol concentrations and cortisol stress reactivity in generalized anxiety disorder, major depression and their comorbidity. J Psychiatr Res 84: 184–190. https://doi.org/10.1016/j.jpsychires.2016.09.024

21. Funke R, Eichler A, Distler J, Golub Y, Kratz O, Moll GH (2017) Stress system dysregulation in pediatric generalized anxiety disorder associated with comorbid depression Stress Health 33(5): 518–529. https://doi.org/10.1002/smi.273622
22. Joseph JJ, Golden SH (2017) Cortisol dysregulation: the bidirectional link between stress, depression, and type 2 diabetes mellitus. Ann NY Acad Sci 1391(1): 20–34. https://doi.org/10.1111/nyas.13217

Translated by A. Polyanovsky