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Overeating in Early Postnatal Ontogenesis Forms Metabolic Memory and Reduces Lifespan

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Abstract

We investigated the role of metabolic memory in a choice of strategies for adaptation to stress influences. We used a model of rat overeating in early ontogenesis from birth to 21 days of life and investigated the maintenance of formed metabolic pattern in later ontogenesis.

The following characteristics were measured: somatometric indicators, resistance of animals to a temperature stress of 32°C, thyroxin and triiodothyronine content in blood serum, NO-synthase activity, the content of lipid hydroperoxides and antioxidant enzymes in different organs in animals of 3, 20 and 31 months of age under standard conditions of maintenance in control animals and after overeating in the early ontogenesis.

It is shown that the metabolic pattern formed on the background of overeating from birth to 21 days remains until the later stages of ontogeny. The forming metabolic pattern is characterized by elevated content of thyroxin, increase of NO-synthase activity in various tissues and particularly in the brain, a small increase of lipid hydroperoxides and reduced antioxidant activity of some enzymes. Animals that overeated in the early ontogenesis, are characterized by lower resistance to high temperatures and shorter life expectancy compared to the animals contained in the standard terms.

Keywords: Overeating; Metabolic memory; Lifespan; NO-synthase; Antioxidant activity

Introduction

The modern paradigm of gerontology is that with age, the ability of an organism to "adequately" (successfully) adapt to the constantly changing habitat conditions reduces. The loss by the organism of the ability to adapt may be accompanied by the formation of a variety of pathologies and increase the probability of death. One may assume the well-known fact that the increase of morbidity in the old age reflects the decline in the organism's ability to adapt to the biotic and abiotic environmental factors.

All existing concepts and hypotheses of gerontology essentially aim to explain the age-dependent reduction of adaptability or age-related degradation and the loss of safety of the systems of the organism.

Along with this, in a number of experimental studies it has been shown that with increasing age what is lost is not so much the organism's ability to adapt, but rather that other strategies are observed [1,2]. The ability to choose different adaptation strategies is the result of multifunctionality and hierarchy of biological systems [3]. We believe that the choice of adaptation strategies will determine the features of the development of chronic pathologies, the rate of aging and, as a result, the life span.

Based on this concept, it is necessary to determine how each adaptation strategy is selected, and which factors, mechanisms and principles determine the selection of new strategies of survival.

We assume that the leading, but not the only factor influencing the selection of strategies for adaptation of the organism to endogenous and exogenous factors is the currently existing epigenetic and metabolic memory, or the principle of the initial state.

Previously, it was suggested that the aging is a non-specific integrative process, which is implemented as a result of the accumulation of epigenetic-metabolic memory, which is formed as a result of a continuous process of adaptation. Formed epigenetic and metabolic memory determines the further choice of adaptation strategies. The metabolic memory inevitably leads to narrowing of further opportunities of choice of strategies and this leads to the formation of chronic conditions and an increase of the probability of death [4,5].

Formation of metabolic memory contributes, on the one hand, to the further distribution by definite types of the processes of adaptation, and on the other hand narrows the selection of new adaptation strategies, resulting in signs of aging.

As is known, as epigenetic memory we mean the ability of the system to maintain gene expression patterns formed in a series of cell generations [6]. The epigenetic pattern (Epigenome) forms an adequate metabolic pattern (Epigenotype). Formed interrelated metabolic cycles can be self-sustaining for a long time, i.e. can be stored and can influence the further choice of adaptation strategies [7].

To test the hypothesis about the role of the metabolic memory in the choice of adaptation strategies, the effects of overeating in the early ontogenesis (EO) were investigated, from birth to 21 days of age, in relation to the conservation of the metabolic pattern formed at this time and throughout the organism's lifetime. As metabolic parameters

the content of thyroxin and triiodothyronine, the activity of NO-synthesis, the content of lipid hydroxides and the activity of some antioxidant enzymes were assessed in animals at the ages of 3, 20 and 31 months fed with the standard diet and animals maintained in the same conditions, but after the period of overeating in EO. Along with this, the body weight, body temperature, weight of the liver, the ability to survive in conditions of hyperthermia (32°C -30 days) and lifespan were measured in animals of both groups.

Materials and Methods

Materials and methods, experimental facilities

The research was conducted on males of Wistar rats maintained at standard vivarium conditions and carried out as per the guidelines of the European Convention for the Protection of the Vertebrata using for the experimental and scientific aims [8].

Overeating in early postnatal ontogenesis (EO) was performed decrease in the number of newborn rats to two individuals per female. In one control group had 6-8 female rats.

After reaching 1 month of age animals in the control and experimental groups were reverted to standard conditions and feeding. Animals formed two groups: there were 72 animals in control group and 116 animals in the experimental. Animals were kept under identical vivarium conditions.

In the experiment of assessment of animals endurance to elevated temperature the animals of 20 months of age were maintained at 32°C, a pressure of 743-750 mm Hg, the light period-15 h, the dark-9 hours for 30 days. In the control group there were 22 animals in the test-16 animals.

For comparison, animals were on calorie restricted diet with one month of age, as described [9].

The body temperature of the rats was measured with a thermometer TW 2-193 2 Microtherma T Hand Held Thermometer (Braintree Scientific, Inc., USA). The body temperature was measured between 8 and 9 PM. Tissue sampling was carried out at the same time from 9-10 PM allowing excluding the influence of circadian rhythms.

Rats were euthanized via decapitated under ether anesthesia.

Upon reaching 3, 20 and 31 months of age the animals were used to determine certain biochemical characteristics.

Fractionation of the liver cells

The cooled liver samples were processed by the press and homogenized in 100 mM of Tris-HCl buffer, pH 7.4, at 4°C. Mitochondria, microsomes and cytosol fraction were obtained by differential centrifugation [10].

To obtain the serum the blood was collected in dried tube and was kept for 30 min at 4°C. After this time, the blood was centrifuged for 15 min at 1000 g, and serum was collected.

Serum was separated from the formed elements by centrifugation for 15 min at 1000 g.

The liver is perfused with cold 0.9% solution of NaCl, homogenized in 100 mM Tris-HCl, pH 7.4 for 1 min at 800 rev/min. The ratio of solution to weight of fabric 1:3.

Microsomes and mitochondria were isolated by differential centrifugation of the homogenate [10], in a medium such composition: 0.3 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4.

The obtained fraction was washed 2 times with mitochondria isolation medium without EDTA. The obtained fraction of mitochondria was diluted with isolation medium without EDTA, in order to achieve the final concentration of 60-80 mg of protein per ml.

Postmitochondrial fraction was isolated from the microsome fraction during centrifugation 100,000 g for 60 min at 4°C.

Analytical methods

Thyroxin and triiodothyronine concentration

The thyroxin and triiodothyronine concentration in serum were determined by radioimmunoassay using standard reagent kits "Total T4 RIA" and "Total T3 RIA" production IMMUNOTECH (Czech Republic). The thyroxin and triiodothyronine concentration was expressed in nmol/l.

Determination of the lipid hydroperoxide

The mitochondria swelling were recorded by changing the optical density in a thermostated (37°C) cuvette with constant agitation by spectrophotometer Specord UV VIS (Germany) at 610 nm. The incubation medium composition was the following: 10 mM Tris -HCl, pH 7.4, 0.25 M sucrose, 5 mM KH_2PO_4, 5 mM rotenone, 2 mM succinate and 25 mM $CaSO_4$. The lipid hydroperoxide (HPL) content in liver microsomes and mitochondria was determined by means of the Ohkawa et al. method [11]. The HPL content in serum was determined as previously described in [12]. The absorption spectrum of the colored product was recorded on double-beam spectrophotometer Specord UV VIS, measuring the difference in extinction at 535 and 520 nm [13]. The HPL content was expressed in equivalent amounts of using a molar extinction coefficient of $1.56 \times 10^5 \times M^{-1}$ cm^{-1}. The activity was expressed in nmol MDA/mg protein.

Glutathione peroxidase activity

Glutathione peroxidase activity (GP, CP 1.11.1.9) was determined in cytosolic fractions, and liver mitochondria serum spectrophotometrically at 340 nm with the help of the method of [14] in 50 mM K$^+$, Na$^+$ phosphate buffer (pH 7.4) containing 1 mM EDTA, 0.15 mM NADPH, 1 unit of yeast glutathione reductase, 0.2% Triton X-100 and 3 mM Na azide to inhibit KAT. 1.2 mM cumene hydroperoxide and 0.4 mM hydrogen peroxide were added. Incubation temperature was 37°C. The activity was expressed in nmol NADPH/min per mg of protein or ml of serum considering a molar extinction coefficient $6.22 \times 10^3 \times M^{-1}$ cm^{-1}.

Activity of glutathione reductase

The activity of glutathione reductase (GR EC 1.6.4.2) in homogenates and in liver mitochondria was measured spectrophotometrically by decrease of NADPH [15] in a medium containing 50 mM K$^+$ phosphate buffer, pH 7.4, 1 mM EDTA, 0.16 mM NADPH, 1 mM GSSG, 0.2% Triton X-100. The incubation temperature was 37°C. The activity was expressed in nmol NADPH/min • mg protein with consideration of the coefficient of molar extinction $6.22 \times 10^3 \times M^{-1}$ cm^{-1}.

Glucose-6-Phosphate Dehydrogenase Activity

Glucose-6-phosphate dehydrogenase activity (G6PDH, EC 1.1.1.49) was determined spectrophotometrically in liver cytosol by the rate of $NADP^+$ reduction [16] at a $37^{\circ}C$. The activity was expressed in nmol NADPH/min/mg protein with coefficient of molar extinction $6.22 \times 10^3 \times M^{-1}$ cm^{-1}.

Izocitrate-dehydrogenase activity

Izocitrate-dehydrogenase activity (IDH, EC 1.1.1.42) was determined in liver cytosol and mitochondria spectrophotometrically by the rate of $NADP^+$ reduction [17] in 34 mM Tris- HCI buffer, pH 7.4 containing 0.34 mM EDTA, 1.5 mM MnCI$_2$, 0.1 mM NADP$^+$, 1.5 mM isocitrate, 0.2% Triton X-100. The incubation temperature was $37^{\circ}C$. The activity was expressed in nmol NADPH/min/mg protein.

NADP$^+$-malatdehydrogenase activity

NADP$^+$-malatdehydrogenase activity (MDH, EC 1.1.1.40) was measured in liver cytosol and mitochondria spectrophotometrically by the rate of $NADP^+$ reduction [18] in 68 mM Tris-HCI buffer, pH 7.4, containing 0.85 mM MnCl$_2$, 2 mM Malate, 0.4 mM NADP$^+$, 0.2% Triton X-100. The incubation temperature was $37^{\circ}C$. The activity was expressed in nmol NADPH/ min•mg protein.

Determination of NO-synthase activity

Determination of NO-synthase activity (EC 1.14.13.39). NO-synthase (NOS) activity was determined in cytosol, mitochondria and post-mitochondrial fraction by spectrophotometer at 340 nm to reduce the level of NADPH in an environment that contained 0.1 M Tris-HCl buffer, pH 7.4, 1 mM CaCl$_2$, 0.08 mM NADPH and 0.011 mM L-arginine as described [19]. Registration activity performed at $37^{\circ}C$ versus controls, further to the above medium containing 0.05 mM NO-synthase inhibitor Nw-nitro-L-arginine. The activity was expressed in nmol NADPH/h•mg protein.

Assessment of the structural properties of microsomal membranes by Pyrene-Based Fluorescent Probe

The structural condition of microsomal membranes was assessed by pyrene (Pyrene, Sigma Co) fluorescent probe. Pyrene fluorescence spectra were recorded using spectrofluorometerVaryan Cary Eclipse (USA) at a wavelength of 337 nm excitation, in the medium, which contained 100 mM Tris-HCl buffer, pH 7.4, 0.25 mg of microsomal protein per 1 ml and of 2.4 μM probe. The probe can be used as a viscosity sensor for interior regions of membranes. Since excimer formation results in a spectral shift of fluorescence the probe may be useful for ratio imaging of molecular mobility. The excimerization of pyren (excimer-monomer ratio) was calculated by the ratio of excimer fluorescence (480 nm) and monomer fluorescence (390 nm) [20].

Rat survival

Rat survival was assessed using Kaplan-Meier method and comparison of survival curves was performed by Gehan method with Yates correction. The results were considered as significantly different at P<0.05.

Results

Dynamics of development of morpho-somatic indices, resistance to hyperthermia and life span of rats, overeating in early ontogenesis

Excessive food intake from birth to 21 days of life was accompanied by increasing of rat body weight in 1 month of age by 32% compared to the control diet (Table 1).

Further maintenance of the animals overeating in EO under standard vivarium conditions did not eliminate the superiority of their body weight compared with control during the ontogeny of up to 31 months of age (Table 1). With the increase of animals' age the variability of their body weight increases greatly (Table 1).

Variant	Age, months								
	1	2	3	4	5	11	14	20	31
Control	53.1 ± 1.8	123 ± 4.2	199 ± 3.7	246.1 ± 6.2	274.1 ± 6.2	403.2 ± 6.7	420 ± 5.6	467.8 ± 8.4	484.2 ± 44.9
Overeating	70.4* ± 1.5	159.9* ± 3.3	231.5* ± 3.9	278.1* ±4.4	319.7* ± 4.6	434.5* ± 7.1	451.0* ± 6.5	503.0* ± 7.9	509.2 ± 26.1
Overeating, excess above control, %	32	30	25	13	16	7	7	7	5
* - a significant difference between groups (P<0.05).									

Table 1: Rats body weight in control group and in group of animals with overeating in EO.

Therefore, the overeating in early postnatal ontogenesis is accompanied by nutritional programming of the metabolism, which was maintained for ontogenesis.

At the age of three months, the animals overeating in EO were superior to the control animals in body weight by 25%, and the mass of the liver was increased also. Thus, the mass ratio of the liver has been significantly higher than in the control animals (Table 2).

However, by the age of 31 months differences in body weight compared with control decreased and the liver mass ratio didn't differ from the control specimens (Table 2).

In order to test the ability of animals with food programming to overeating in the EO successfully adapt to extreme environmental conditions, we used 30 day-long maintenance of the animals at 32°C, 10°C above the normal temperature.

Index	3 Month		31 Month	
	Control	**Overeating**	**Control**	**Overeating**
Body weight (g)	199.0 ± 3.7	231.3 ± 3.9*	484.2 ± 44.9	509.2 ± 26.1
Liver weight (g)	7.5 ± 0.62	11.2 ± 0.7*	13.3 ± 1.4	15.8 ± 1.4
[1](Liver weight/Body weight) × 10^2	4.1 ± 0.21	5.1 ± 0.38*	2.7 ± 0.16	2.9 ± 0.14

Table 2: Influence of overeating at EO on some somatometric indexes in 3 and 31 month animals 1 coefficient (Liver weight/Body weight) × 10^2 was calculated for every animal individually and then the mean was calculated, therefore these values are different from the result of a simple division of mean liver weight to mean body weight.

It was found that when the rats of 20 months of age of the control group were kept at 32°C, the number of surviving animals was approximately 90% (Figures 1A and curve 1). At the same time, the survival in the same conditions of rats overeating in early ontogenesis was much less than for the control group and the number of surviving animals was slightly more 50% (Figures 1A and curve 2).

Assessment of coefficient of survival for the control and experimental animals with overeating in EO in case of their keeping under standard vivarium conditions showed a little but significant difference in their survival (Figure 1B).

The percentage of animals that survived to the 30th day at 32°C in the control group was 86%, while in the group with overeating there were only 56% (Figure 1C).

The results of the effect of feeding regimes on hyperthermia resistance were proved by determining the number of animals that survived at hyperthermia: in such circumstances 98% of the animals maintained at the CR survived after 30 days (Figure 1C). Consequently, the heat resistance is a good test system for the evaluation of adaptive abilities of the organism. The animals that overeated in EO, were poorly adapted to hyperthermia, and were inferior in the ability to survive to the animals in the control group by 30%, and to the animals kept at CR by 42%.

These significant differences in adaptive capacity to hyperthermia suggest that the metabolic changes that provide adaptation to hyperthermia are saved up to 20 months of age. In this regard, it was of interest to investigate the content of thyroid hormones in these animals since thyroxin is involved in thermogenesis and there is a strong relationship between thermogenesis and life span [21].

Thyroid hormone content in rats with overeating and caloric restriction

Contents of thyroxin in the blood serum of the control animals in onto-genesis of 3-31 months of age decreased (Figure 2A). Thus, in 31-month-old animals it was 32% less than in 3 month-old animals (Figure 2A). At the same time, its content in animals with overeating in the EO did not change with age and at 31 months of age the thyroxin content was 46% more than in the control animals of the same age (Figure 2A).

The content of thyroxin in blood serum of 3 months-old rats with overeating in EO was 18% more compared to control animals of the same age (Figure 2).
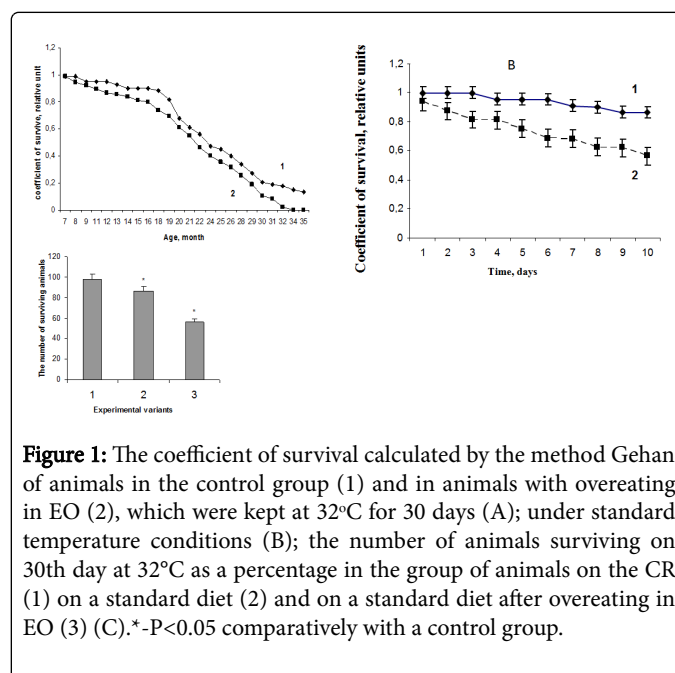

Figure 1: The coefficient of survival calculated by the method Gehan of animals in the control group (1) and in animals with overeating in EO (2), which were kept at 32°C for 30 days (A); under standard temperature conditions (B); the number of animals surviving on 30th day at 32°C as a percentage in the group of animals on the CR (1) on a standard diet (2) and on a standard diet after overeating in EO (3) (C).*-P<0.05 comparatively with a control group.

In 31 month aged animals triiodothyronine content in control almost linearly decreased by 2.2 times compared to 3 month animals (Figure 2B). The triiodothyronine content in the blood serum of rats overeating in EO did not differ of control animals (Figure 2B).

Therefore, overeating in EO is accompanied by increasing in the content of thyroxin in these animals. Elevated levels of this hormone persisted throughout the ontogenesis, and in the later stages the differences between the control and experimental animals even increased.

In the next stage of the work the "consistency" in the changes of survival control and experimental groups of animals and the content of thyroxin in their blood serum were determined. It was found that between these parameters there is a well-defined inverse relationship.

Thus, the survival rate of the rats after the 30th day of the maintenance at 32°C content was the highest in the CR group and they had the lowest content of thyroxin in blood serum (Figure 3). Reduction of survival rate after hyperthermia of animals of the control group and in the group overeating at EO was accompanied by linear increasing of thyroxin content in blood serum. At the same time the body temperature correlates positively with the thyroxin content and correlates in reverse to the life span (Figure 3).
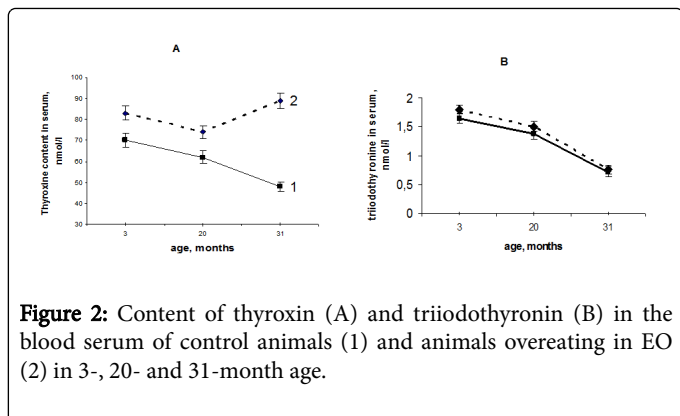
Figure 2: Content of thyroxin (A) and triiodothyronin (B) in the blood serum of control animals (1) and animals overeating in EO (2) in 3-, 20- and 31-month age.

Therefore, high concentrations of thyroxin coincided with decreasing in the survival rate of animals and an increase of body temperature and, conversely, the reduction of thyroxin content coincides with the increase in the survival rate and decrease of body temperature of animals. These results confirm the data obtained previously on different experimental models [21].

Some indicators of prooxidant system activity in ontogenesis of rats with overeating in EO

The hydroperoxide content in blood serum of 3 month old animals with overeating in EO exceeded, though slightly, but statistically significantly, that of the control level (Figure 4A). This superiority of experimental (overeating in EO) animals remained the same for 20 and 31 months-old animals (Figure 4A).

It should be noted that any significant developmental changes in the content of lipid hydroperoxides in the blood serum of rats at 3 and 31 months have not been identified, and its small decline in 20 months-old animals are well reproduced in the group of animals with overeating in EO (Figure 4A).

Therefore, age-related particularities in lipid hydroperoxide content in blood serum were completely retained in animals overeating in EO. Along with this, the content of lipid hydroperoxides in the mitochondrial fraction of liver of 3 months-old animals were below the reference level. The content of lipid hydroperoxide in liver microsomes of 3 months-old animals was significantly higher (29%) at the control level, and this difference persisted, though a less pronounced fashion, in 31 months-old animals (Figure 4B).

Contents of lipid hydroperoxides in the microsomes of the control old animals (31 months) was higher by 49%, compared with 3 months-old animals. In the group of animals with overeating in EO, this difference remained almost the same and amounted to 36% (Figure 4B).

Therefore, overeating of animals in EO was accompanied by an increase in the content of the products of free radical reactions in blood serum and liver microsomes and these characteristics remained elevated throughout ontogeny, but did not change in the mitochondria.
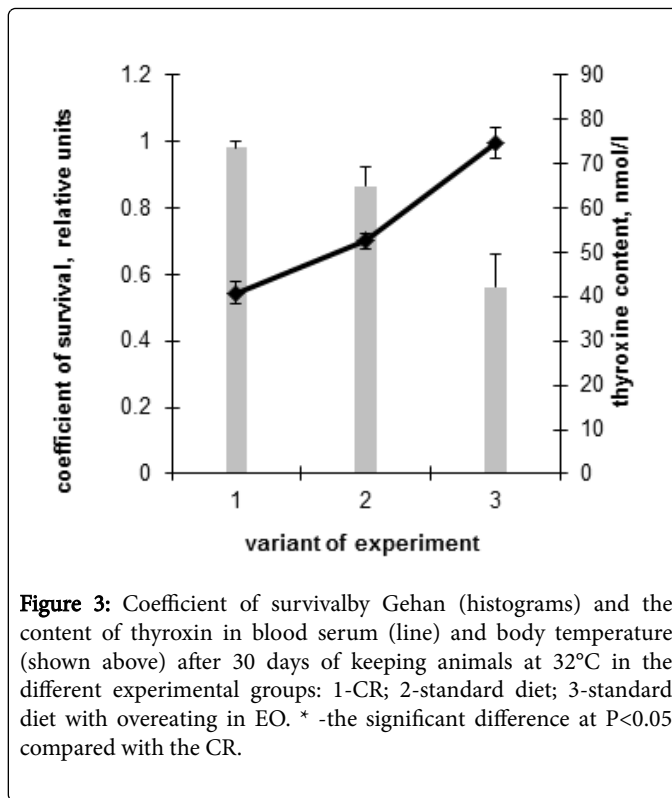

Figure 3: Coefficient of survivalby Gehan (histograms) and the content of thyroxin in blood serum (line) and body temperature (shown above) after 30 days of keeping animals at 32°C in the different experimental groups: 1-CR; 2-standard diet; 3-standard diet with overeating in EO. * -the significant difference at P<0.05 compared with the CR.

Determination of the activity of some antioxidant enzymes in rat ontogenesis with overeating in EO

The activity of glutathione reductase of liver cytosol of 3 months-old animals showed that overeating in the EO did not differ from the control level (Table 3). The malate dehydrogenase, G6PDH and IDH activity did not differ significantly (Table 3).
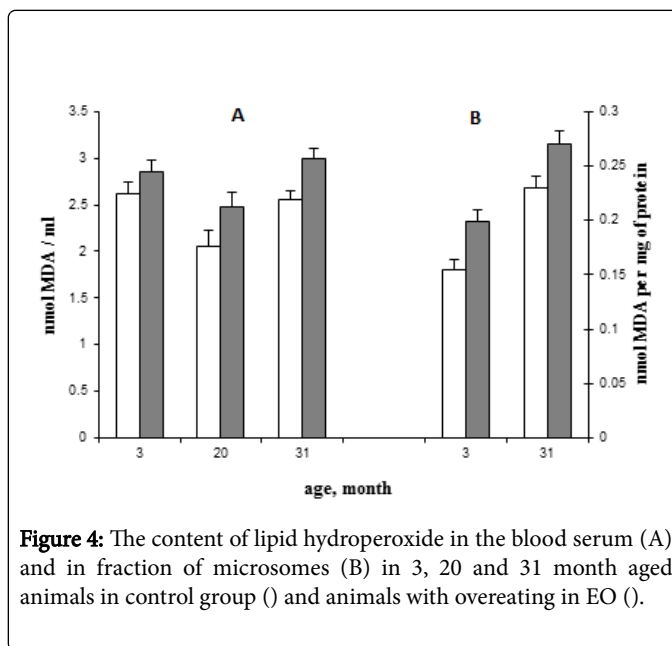

Figure 4: The content of lipid hydroperoxide in the blood serum (A) and in fraction of microsomes (B) in 3, 20 and 31 month aged animals in control group () and animals with overeating in EO ().

The activity of glutathione peroxidase (GP) in the cytosol of liver of 3 month-old rats overeating in EO was 27% lower than the control

value (Table 3). At the same time, the activity of NOS in cytosol of 3-month-old animals was 27% higher than the control levels (Table 3).

Animal Group	Enzyme activity (nmol NADPH/min per mg of protein)					
	GR	MDH	G6PDH	IDH	GP	NOS*
Control	108.6 ± 16.1	111.7 ± 7.2	65.7 ± 6.7	687.8 ± 61.2	547.9 ± 39.1*	30.3 ± 3.1*
Overeating in EO	100.4 ± 7.4	99.3 ± 9.3	72.4 ± 8.7	618.8 ± 43.3	397.9 ± 34.7	38.5 ± 1.4

Table 3: Activity of some antioxidant enzymesin the cytosol fraction of liver cells of 3 months animals in the control group and in group of animals with overeating in EO.

In the next series of experiments GP activity in blood serum of experimental animals was determined.

At the age of 3 months GP activity in the blood serum of overeating animals was 25% below of the control, in 20 months-old animals it didn't differ significantly, and at the age of 31 months it was significantly lower than the control (Figure 5A).

To a great extent activity of GP in animals with overeating was reduced compared to control in the liver microsome. So at the age of 3 months it was lower than the control by 22%, and at 31 months it was lower by 44% (Figure 5B).

Such reduction of the activity of GP in liver microsomes correlated with a significant increase of the degree of excimerization of pyren in animals with overeating (Figure 5C).

It is known that nitric oxide plays an important role in the functioning of organs and tissues of mammals. In 1989 NO-synthase (NOS), L-arginine NADPH: oxygen oxidoreductase - ES 1.14.13.39 was first described by [22], and from 1991-1994 three main forms of the enzyme were identified. The NOS catalyzes the formation of nitric oxide and citrulline from arginine, oxygen and NADPH.

Currently, it is shown that NOS is localized not only in immunocompetent cells but also in the nerve tissue, in the components of the cardiovascular system, epithelial and other tissues [23].

It was of interest to determine NOS activity in mitochondria and liver microsomes of rats, overeating in the EO and in some stages of ontogeny.
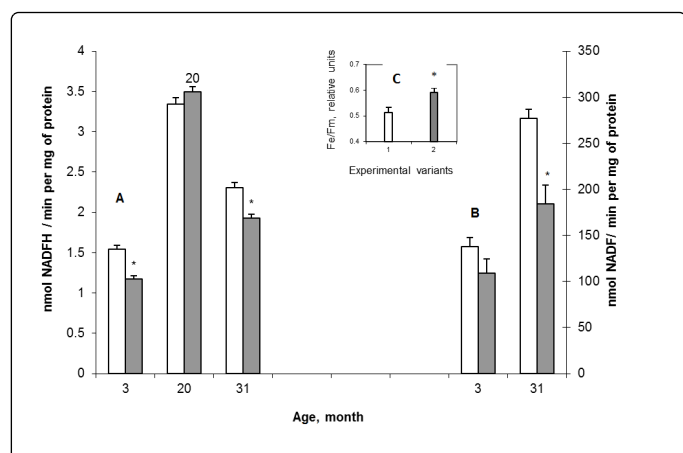

Figure 5: Glutathione peroxidase activity in the blood serum (A) and microsomes (B) of rat liver of the control group (□),in the group with overeating in EO (■), the degree of pyren excimerization (C) in control (1) and in animals with overeating in E(2).

Total activity of NOS in the mitochondria of 3 month-old rats overeating in the EO was higher by 37% than the corresponding control (Figure 6A).

In 31 month-old rats NOS activity in mitochondria was 54% higher than in similar aged rats maintained under standard conditions (Figure 6A).

The activity of the total NOS fraction in liver microsomes of 3 month-old animals with overeating was 51% higher than the corresponding control (Figure 6B), and in older animals these differences were not detected (Figure 6B).

In the next stage the NOS activity was determined in 20 months-old animals in the control and experimental groups in different tissues.
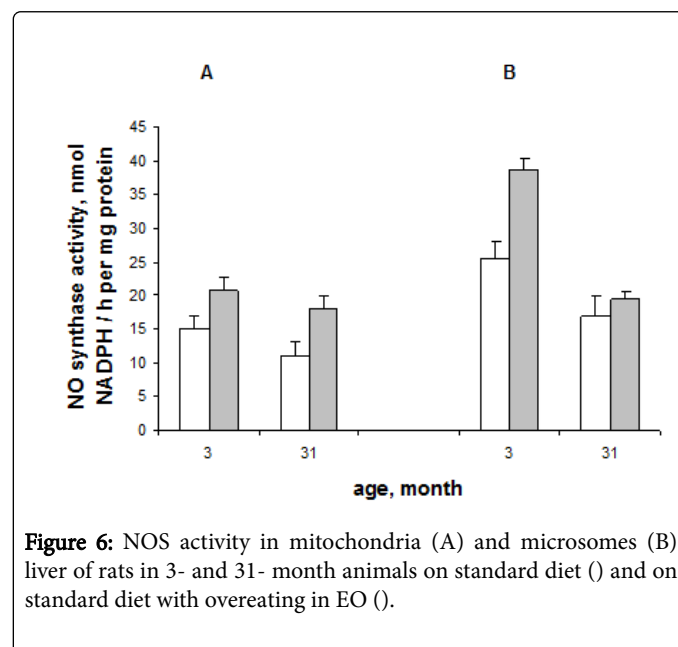

Figure 6: NOS activity in mitochondria (A) and microsomes (B) liver of rats in 3- and 31- month animals on standard diet () and on standard diet with overeating in EO ().

It has been found that the activity of NOS in the liver of 20 months-old animals with overeating in the EO was higher by 67% than the corresponding control (Figure 7). The NOS activity in the brain of 20 months-old overeating rats was 2 times higher than in the control animals; in the heart and kidney, it was also higher by 40 and 75% than the control level (Figure 7).

Consequently, the NOS activity in the liver, brain, heart, kidneys was increased significantly in animals overeating in EO.

The greatest increase of NOS activity induced by overeating was in the brain. Increased NOS activity in animals with overeating in EO persisted until the later stages of ontogeny.

The main conclusions

Overeating in EO was accompanied by increase in body weight which is concert at the age of 31 months. However, with the increase of age, the differences between the control and experimental animals decreased. Increasing of body weight of 3 months-old animals was accompanied by increasing of liver weight. However, at the age of 31 months this difference was absent.

Survivability of 20 months-old animals overeating in EO was 30% less compared to control after one-month-long maintenance at 32☐C and 42% less compared with the animal at CR.
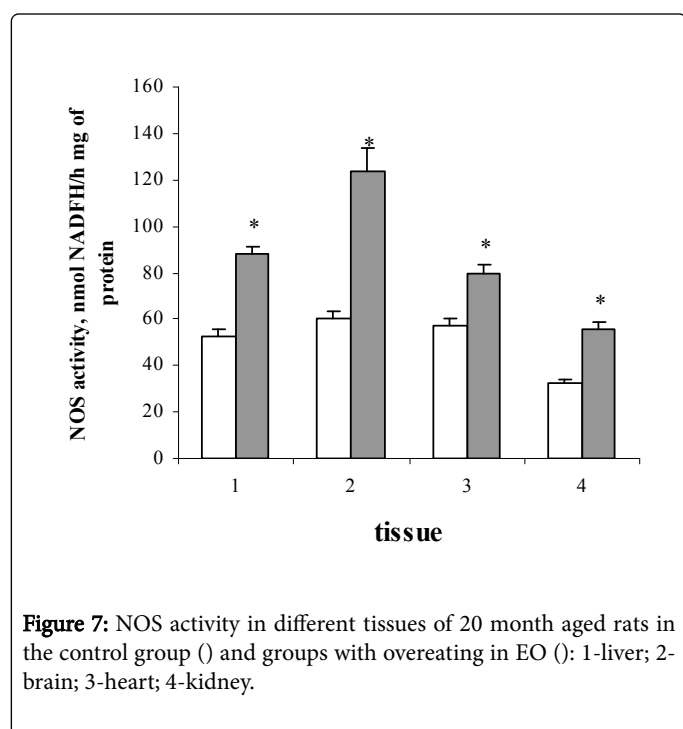

Figure 7: NOS activity in different tissues of 20 month aged rats in the control group () and groups with overeating in EO (): 1-liver; 2-brain; 3-heart; 4-kidney.

The contents of thyroxin in blood serum of the control animals significantly decreased during ontogeny and didn't change in the animals overeating in EO. As a result, the thyroxin content in animals overeating in the EO at the age of 31 months was 46% more compared with the control. The triiodothyronine content in ontogeny decreased to the same extent in the control animals and in animals overeating in EO.

There is a negative relationship between the survival rate of the animals, the content of thyroxin and body temperature. Increased body temperature was correlated with increased levels of thyroxin and with a decline of the survival rate.

In animals overeating in the EO, the lipid hydroperoxide content in serum and particularly in a fraction of liver microsomes increased and the liver was enlarged. These differences persisted up to 31 months of age.

Activity of some enzymes (glutathione reductase, MDH, G6PDH, ICH) in animals with overeating did not differ from those of the control animals. At the same time, the activity of GP was reduced in animals overeating in EO, especially in liver microsomes. Its decline was correlated with an increase of microviscosity of these membranes. Low activity of GP persisted throughout ontogeny.

The overeating in EO is associated with a significant increase in NOS activity in different tissues, particularly in the brain.

Discussion

These results supported the known fact that diet in early postnatal ontogenesis provides the metabolism programming [9]. The present work showed that metabolic patterns formed in EO can be conserved till the late stages of ontogenesis, i.e. can be stored and may determine the further particularities of response to the new influences and affect the lifespan.

One may confirm that overeating in EO forms the metabolic memory and is realized as the overeating syndrome (OS). The OS is characterized by:

- Increasing in body mass during all the ontogenesis, the difference from the control slowly decreases. The superiority in body mass and formed metabolic pattern is preserved independently on the further feeding regimes
- Decline in resistance to further extreme influences, in particular to the elevated temperature (32°C instead of 22°C)
- A small decrease of the lifespan of animals with overeating in EO and afterwards maintained under the standard conditions of vivarium compared to the control group was registered;
- A significant increase of thyroxin content in the blood serum and maintenance of its high level until the late ontogenesis stages;
- An increase of NOS activity in different organs, such as the liver, the kidneys and especially the brain; the stable changes of pro-antioxidant system of the liver: the increase of prooxidant activity and the decline of activity of some antioxidant enzymes.

The overeating in early ontogenesis induces the formation of the adipose tissue in them. It should be noted the adipose tissue under the standard conditions forms as a rule after puberty, i.e. in the later stages of ontogenesis [24].

The formation of the adipose tissue in the early ontogenesis may play the key role in metabolic memory and lifespan. It should be noted that under the calorie restriction the adipose tissue doesn't form even in old rats and it correlates with the lifespan [25].

The formed adipose tissue is the powerful endocrine organ and produces such humoral factors as leptin, adiponectin and adipocytokines [26]. Besides it the adipose tissue has the receptors for insulin, glucagon, growth hormone, thyreotrophin, and the metabolism of steroid hormones occurs there [27].

The formed adipose tissue forms the other than in control metabolic pattern and settles direct and reverse relations between adipocytes and other functional systems of organism, in particular the pancreatic gland, the liver, the nervous system and others (Figure 8). One may assume that the realization of the response to overeating on the system level will provide the formation of prolonged metabolic memory i.e. conservation of induced metabolic pattern for the relatively long period (Figure 8).

Unfortunately it is yet impossible to describe the entire metabolic configuration at OS, however the results of the present work allow to assess some characteristics of functional systems in the state of OS.

So, at OS the significant increase of thyroxin content in blood serum, especially in the late stages of ontogenesis, is observed (Figure 8). It is of paramount importance to determine whether there is an age-dependent decrease of thyroxin content in control, but in OS animals the thyroxin content in old animals was on the same level as in young ones.
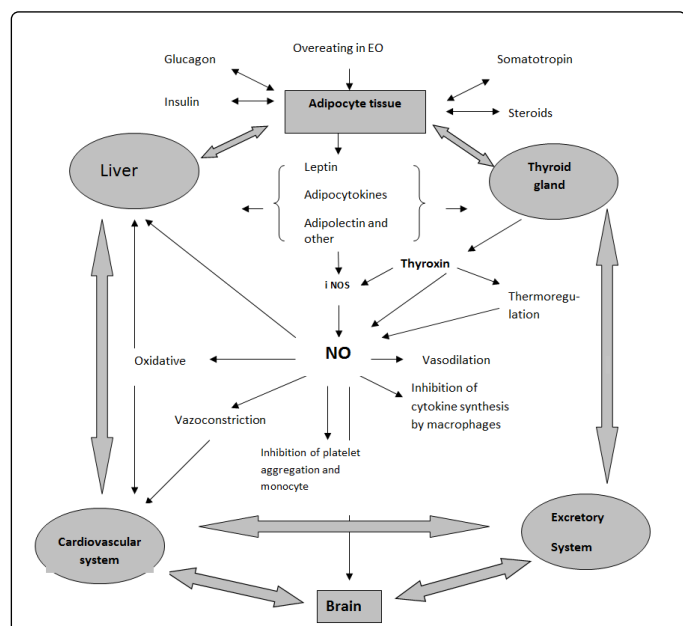

Figure 8: Diagram showing the formation of a specific metabolic pattern after overeating in EO. Overeating induces adipose tissue at a young age, which is a hormonal body. This is accompanied by changes in thyroid function, an increase in thyroxin, activation of iNOS. The nascent NO is a multifunctional regulatory molecule. The reactions at biochemical level are shown by arrows →and are accompanied by systemic transformation of many organs (gray background). Induction of adipose tissue is accompanied by a hierarchical restructuring of many systems of the body, which leads to the establishment of forward and backward linkages, i.e. to formation of new hypercycles or metabolic configurations (networks), which are characterized by ability of self-maintenance, even when changing the initial conditions, that may be associated with metabolic memory.

The data obtained in this work show that increase of thyroxin content at the late period of ontogeny is correlated with decrease of animals' lifespan. As it was demonstrated earlier, the experimentally induced hyperthyreosis is accompanied by the increase of body temperature and by decrease of lifespan [21]. The results of the present work indicate that overeating in the EO induce fat tissue development that in its turn influences the function of thyroid gland.

The increase in organisms of thyroxin content stimulates the whole metabolism and can suppress further "obesity" and provide metabolism 'retention' on the level that doesn't allow the overeating syndrome to become pronounced metabolic syndrome, that in its turn can easily become a pathology. At the same time it the increase of

thyroxin content leads to the increase of the NO-production. Earlier the increase of the NOS activity in the paraventricular and supraoptical nuclei of hypothalamus was shown at the hyperthyroidism [28].

It was found that on the model of overeating in EO the NOS activity in the brain increased twice compared to the control (Figure 8). The NOS activity increased on the system level in the whole organism (Figure 8).

The NO is known to be a universal and polyfunctional regulator of all the systems of the organism and regulates directly or indirectly all the organism systems on the system level (Figure 8).

Consequently the overeating in EO is accompanied by the changes in as few as three regulator systems of the organism: hormonal, nervous and immune. These regulator systems change the function of organism detoxication (liver, kidneys). Thus, the new metabolic configuration is forming. One may assume that it involves all the regulator systems and it is memorized and can be accompanied by the essential decrease of lifespan in stress situations, such as hyperthermia.

Consequently the overeating in EO can be accompanied by either the formation of metabolic syndrome and, consequently, by the development of pathologies (diabetes, heart failure, hyperthyroidism) and accelerated ageing, or by the increase of thyroxin content and its "retention" from becoming the metabolic syndrome at the cost of passing into the another metabolic state.

It is possible that the most important feature of such a state is its conservation, i.e. memorizing as is shown in our work.

One may assume that such metabolic memory, which is certainly connected with epigenetic memory, is conserved during all the ontogenesis, meaning that metabolic changes involve a big group of regulatory elements and the stable direct and indirect connections are settled between them (Figure 8).

One may assume that metabolic memory will be conserved for a long time when metabolic processes will form interrelated cycles or hypercycles. The more elements involved in the cycle, the more effectively the metabolic memory will be conserved (Figure 8).

Analyzing the data obtained in this work within the modern paradigm of aging based on the decrease of adaptability with ageing, it should be noted that the decrease of adaptability depends not so much on the age as on the ability to conserve metabolic programming, or to reprogram the metabolism quickly in the changing conditions of the environment. These features can be explained by the principle of the initial state, i.e. the initial state of metabolism always influences the forthcoming response of the organism. The metabolic memory can play a central role in the formation of responses to environmental changes.

Our previous data can explain the influence of CR diet on the formation of specific epigenotype associated with lifespan [9] and can also explain the influence of the pre-adaptation on the lifespan of cell cultures [7].

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