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Element Status of Organism under Influence of Food Stress in Wistar Rats

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ABSTRACT

Article presents the results of research of composition of elements in tissues of lab rats given the influence of food factors. Research was conducted on male Wistar rats from 2 months age (n = 51). Within record period animals were divided in 3 groups depending on the consumed diet. Animals of the control group (C) were fed with full-value semisynthetic diet within 60 days Basic Diet (BD). Animals of the test group I consumed semisynthetic diet I (SD I), consisting of mixture of major feed (50%), Instant Food (IF) (50%) and water, animals of the group II- Semisynthetic Diet II (SD II) consisting of a mixture of major feed, instant food (each 50%) and carbonated soft drink. Elements of researched biological substrates and food products were analyzed with atomic emission and mass spectroscopy with inductive-connected argon plasma using devices Optima 2000 DV and ELAN 9000 (PerkinElmer, USA). It was established that replacement of 50% major feed by instant food caused increase in levels of sodium, calcium, phosphorus, potassium in tissues of body. Consumption of instant food mixed with carbonated drinks caused the decrease of such essential microelements as zinc, manganese and selenium. Changes in element ratio were observed. Results may be used for assessment of influence of nutrition stress on exchange of mineral substances in organism of lab animals.

Key words: Food stress, macroelements, microelements, laboratory animals, rats

INTRODUCTION

Profound qualitative alterations in structure of diet of population took place in Russia recently. The basis of a healthy diet is a diet balanced with all nutrients (Tuteliyan *et al.*, 2004), but because of processing, use of edible raw materials poor according to their chemical composition and impact of other factors human body does not receive necessary quantity of indispensable components (Gres, 2008; Notova and Skalnaya, 2004). The situation is aggravated by low cultural level of population in matters of nutrition and lack of healthy behaviors. So, a questionnaire survey of Orenburg students that was carried out recently showed that percent of fast food and semi-finished food in daily diet of 53.4% respondents is 10%, 31.7% respondents it is from 10-30%, 10.5% respondents 50% (Moriyama *et al.*, 1988).

In the last decade more than 40% of food products are imported in Russia, it puts the state on the brink of food addiction. It is known that the use of imported products in the diet is a stress factor and causes long adaptation of organism to new composition of food (Katserikova, 2004).

Stress situations on the one hand promote mobilization of organism reserves and on the other hand they increase consumption of nutrients; that is why it is necessary to study the metabolism for subsequent prevention of diseases (Mustafina *et al.*, 2013; Notova, 2005).

The research objective is to study the influence of food stress on element composition of tissues of lab animals.

MATERIALS AND METHODS

Experimental research was carried out in experimental biological clinic (vivarium) of the Trace Element Institute of the Orenburg State University. Animal experiments were carried out in accordance with 1985 International Guiding Principles for Biomedical Research Involving Animals, with permission of the Committee on the Ethics of the Orenburg State University (Protocol No. 12 as of 22.01.2007).

Animals and rations: Research was carried on male Wistar rats starting from the age of 2 months ($n = 45$). During the record period the animals were divided into 3 groups. The first experimental group (I) consumed a Semisynthetic Diet I (SD I) consisting of a mixture of major feed (50%), Instant Food (IF) (50%) and water; the second group (II) a Semisynthetic Diet II (SD II) consisting of a mixture of major feed, IF (50%) and carbonated soft drinks. The control group (C) received balanced semisynthetic diet (BD) containing 58% corn starch, 25% casein, 5% unrefined sunflower oil, 5% of lard, 4% salt mixture, 1% vitamin mixture and 2% of microcrystalline cellulose. The IF mixture consisted of noodles, hot dog and porridge in equal proportion. Nutritional value of instant noodles (per 100 g): Protein-4.8 g, carbohydrates-30 g, fat-2 g, calories-270 kcal; porridge: protein-10.0 g, fat-5.0 g, carbohydrates 70.0 g, calories-350 kcal, hot dog: protein-10.6 g, fat-15.0 g, carbohydrates-27.5 g and calories-247 kcal.

Biological substrates: Samples of dry matter from body tissues of animals were used as biological substrates for the study of element status. Skeletal muscles, bones and internal organs were taken and subsequently minced, homogenized and dried up to a constant weight to form the average sample of organism. Three samples were taken from each animal; all in all, 135 measurements were recorded (n_i).

Analysis: Duration of the experiment was 60 days. The animals were weighed weekly before feeding and watering. Element analysis of the researched bio-substrates and feed of animals (major feed, IF) was carried out in the lab of ANO "Centre for Biotic Medicine", Moscow, Russia (accreditation certificate ROSS RU.0001. 513118 as of May 29, 2003; Registration Certificate of ISO 9001:2000, Number 4017-5.04.06) with a atomic emission and mass spectrometry with inductively-coupled argon plasma using Optima 2000 DV and ELAN 9000 equipment (PerkinElmer, USA).

Statistical analysis: Statistical analysis using Student t-test was performed with IBM program "SPSS Statistics Version 20", calculation of mean value (M), mean square deviation (σ) and standard deviation of error (m). Level of significance was considered probably true at $p < 0.05$. Student t-test and the Wilcoxon test were used for statistical analysis (Platonov, 2000).

RESULTS AND DISCUSSION

Analysis of mineral composition of major and semisynthetic diets used in the experiment attests that the content of chemical elements in them is significantly different (Table 1). So, semisynthetic

Table 1: Content of chemical elements in diets of the experimental animals

Elements	GD (mg kg ⁻¹)	SD I (mg kg ⁻¹)	Elements	GD (mg kg ⁻¹)	SD I (mg kg ⁻¹)
Ca	1390.00	1486.00	Co	0.31	0.2
K	551.00	2257.50	Zn	81.50	51.7
Mg	2179.00	1468.50	Mn	136.20	67.8
Na	688.10	25263.20	V	0.38	0.7
P	7501.00	6040.00	Ni	1.50	1.2
Cr	0.35	2.30	Se	0.51	0.5
Cu	8.20	6.30	Cd	0.09	0.2
Fe	130.00	88.20	Pb	0.07	0.1
I	0.31	0.96	Sr	28.50	18.5
As	0.21	0.39	Al	42.40	25.9

GD: General diet, SD I: Semisynthetic diet I

Table 2: Average values of macroelement content in tissues of the experimental animals

Statistical parameters (n = 15, n _i = 45)	Microelements (mg kg ⁻¹)				
	Ca	P	K	Mg	Na
Control (GD)					
M	22700	12500	4600	625	1800
m	1700	900	430	39	110
Group I (SD I)					
M	48300	28250	12650	1700	5800
m	2900	930	325	26	126
p _c	0.02	0.04	0.03	0.05	0.04
Group II (SD II)					
M	51800	28500	12000	1700	6700
m	3700	1500	340	52	204
p _{c, I}	0.05; 0.08	0.04; 0.68	0.03; 0.22	0.04; 0.89	0.02; 0.08

Hereinafter 0.02-0.05-statistically significant variations p_c: in comparison with control group, p_i: in comparison with the I experimental group, M: calculation of mean value, m: Standard deviation error, GD: General diet, SD I: Semisynthetic diet I, SD II: Semisynthetic diet II

diet differed by significantly higher (36.7 times) content of Na and K (4.1 times), lower level of Mg (1.5 times) against a background of practically constant level of Ca and P. Among essential and conventionally essential elements in semisynthetic diet lower content of Cu (1.3 times), Fe (1.5 times), Co (1.8 times), Zn (1.6 times), Mn (2 times) and higher levels of Cr (6.6 times), I (3 times) and V (1.9 times) was recorded. Higher content of such toxic elements as Cd (2 times) and Pb (1.3 times) was recorded in semisynthetic diet.

The results of the research showed that addition of instant food with water or carbonated drink in major feed influenced on growth and development of the experimental animals. It was observed that the experimental groups tended to reduce body weight as early as on the second week of the experiment. Reduction dynamics of group I was more evident, however, by the end of recording period weight of animals in group II was the lowest. Weight of animals fed with IF and water was significantly lower (p≤0.05) by 35.4% and weight of animals fed with IF and carbonated drink was lower by 36.3% (p≤0.05) compared with the control group.

Assessment of elemental composition of body tissues showed significant changes in element status of animals in the experimental groups (Table 2).

Addition of IF to major feed led to significant increase of all macroelements in tissues of lab animals of the I and II groups: calcium 2 and 2.3 times, phosphorus 2.3 times, potassium 2.8 and 2.6 times and magnesium 2.7 times. The increase of sodium content was the highest; its level exceeded the control values 3.2 and 3.7 times in I and II experimental groups respectively. First of all, it can be explained by high content of sodium in synthetic diets. Replacement of drinking water with carbonated drink in diet had not significant influence on content of macroelements.

Continuous consumption of IF including also its mixture with carbonated drinks led to significant decrease of such essential microelements as zinc by 12.1-20.6%, manganese by

Table 3: Average values of essential elements content in tissues of the experimental animals

Statistical parameters (n = 15, n _i = 45)	Essential elements (mg kg ⁻¹)						
	Zn	Mn	Cu	Fe	Co	Se	I
Control (GD)							
M	149	1.3	1.1	92	0.1	1.1	0.2
m	7.1	0.1	0.1	3.2	0.01	0.11	0.02
Group I (SD I)							
M	118	0.5	3	131	0.1	0.9	0.2
m	1.5	0.02	0.03	5.2	0.01	0.06	0.07
p _{k,1}	0.05	0.04	0.05	0.03	0.04	0.02	0.10
Group II (SD II)							
M	131	0.6	3.0	91	0.1	0.9	0.3
m	6.5	0.02	0.2	4.5	0.01	0.05	0.04
p _{k,1}	0.02; 0.02	0.04; 0.05	0.04; 0.13	0.89; 0.10	0.03; 0.45	0.04; 0.11	0.14; 0.08

Hereinafter 0.02-0.05-statistically significant variations p_k: in comparison with control group, p_i: in comparison with the I experimental group, M: calculation of mean value, m: Standard deviation error, GD: General diet, SD I: Semisynthetic diet I, SD II: Semisynthetic diet II

Table 4: Proportions of separate elements in tissues of experimental animals

Proportions	Control group	Group I	Group II
Ca/P	1.80	1.70	1.80
Ca/Mg	36.30	28.20	30.20
K/Na	2.50	2.20	1.80
K/ Mg	7.30	7.40	7.00
Cu/Fe	0.01	0.02	0.02
Cu/Zn	0.01	0.03	0.02

48.4-63.8% and selenium by 6.3-24.1% in body tissues of the experimental groups in comparison with the control (Table 3). At the same time significant increase of copper content (2.7 times) and cobalt (2.2 times) in the experimental groups was observed. Iodine level remained practically the same in all groups.

It was observed that levels of lead and cadmium were increasing during the comparison of contents of toxic elements. Level of lead was higher 1.2 and 1.3 times ($p \leq 0.05$) in the first and the second experimental groups respectively and cadmium-1.8 ($p \leq 0.05$) and 2.8 times.

Besides the assessment of mean values, changing proportions of separate elements are of some interest, among them proportions of Na/K, Ca/P, Ca/Mg, Cu/Zn, Cu/Fe are considered the most significant (Gres, 2008) (Table 4). So, the proportion Ca/Mg may influence on formation of bone tissue and K/Na reacts on changing secretion level of aldosterone and may be a surrogate of mineralocorticoid function of atrabiliary capsules (Yu, 2006).

Despite the significant change in calcium and phosphorus contents in the experimental groups, proportions of these elements practically did not change. In the experimental group proportion Ca/Mg was characterized by the decrease by 22.3 and 16.8% and K/Na by 12.0-28.0%. Proportion Cu/Zn increased 3.1-3.4 times in the experimental groups. Given the fact that copper is a functional antagonist of zinc, changing balance of these elements may lead to the disorder of Cu and Zn in dependent enzymatic systems. Proportion Cu/Fe increased 1.3-2.0 times in the experimental groups.

In experimental conditions element composition of wool changed with changing pool of the most substances in body. Concentrations of Ca, P, K, Mg, Na and Fe increased (1.1-8.7 times) in wool with the increase of pool and Zn, Mn (1.3-1.4 times) with the decrease of common pool.

The results of researches showed that addition of instant food with water or carbonated drink to major feed influenced on growth, development and element status of the experimental animals. Nutritional stress had a depressive effect on animals; it was manifested in significant weight loss.

The similar tendency was observed by Teegarden and Bale (2008), who registered the decrease of weight under stress. The influence of food stress on change of reproductive functions was established (Loginov, 2014; Loginov and Ivanov, 2014). The comparative analysis of peculiarities of chemical elements accumulation in tissues of the experimental animals revealed that addition of IF to the diet of animals led to change in content of practically all studied macro and microelements in tissues. Continuous consumption of IF including its mixture with carbonated drinks promoted significant increase in content of calcium, magnesium, phosphorus, potassium, sodium, copper, iron and decrease of such essential elements as zinc, manganese, selenium in tissues. Peculiarities of elemental composition of laboratory animals as affected by physical exercise and diet with different minerals were studied earlier (Alidzhanova *et al.*, 2009). Decrease in concentration of all macro and most of essential elements in rats' body were registered. Similar changes in elemental status of tissues of laboratory animals were found after the intake of ethanol and cadmium with food (Kiyaeva *et al.*, 2012). Change of body element status was quite expected and connected with change of chemical elements intake. Particularly, reduction of Zn, Mn in diet and the increased intake of K, Na, Cd and Pb led to practically simultaneous change in content of these elements in tissues of animals. Reduction of zinc and manganese content in bio-substrates may be also explained by the increased level of calcium and phosphorus (Tuteliyan *et al.*, 2004). Besides, microelements are important (Cu, Zn and Mn) elements of antioxidant system and the deficiency of some of them (entering organism with food) influences on efficiency of its work (Evans and Halliwell, 2001).

Against the background of decreasing intake of Mg, P, Co, Cu and Ni from synthetic diets the increasing content of these elements in tissues of animals was observed. It may be connected with more effective absorption of these elements in digestive tract, caused by intake deficiency. So, according to the opinion of many authors cobalt absorption in intestine may vary from 20-95%. The obtained data also may be the result of the excessive intake of Na and K in comparison with the control group, osmotic pressure and hormonal changes may be the consequences of it (Yu, 2006).

Change of pool of macro and essential elements influenced on proportion of most significant pairs of elements Ca/Mg, K/Na and Cu/Zn, it attested to the significant mineral metabolic imbalance.

Addition of carbonated drinks in diet had not significant influence on mineral metabolism. Significant differences between experimental groups were obtained only for zinc. Level of zinc was significantly higher by 10% in group of animals watered with carbonated drinks; it is probably connected with its binding by phosphoric acid and accumulation in tissues of body. In their turn, it was established by the researches that deficiency of Zn may lead to the increase in lipid peroxidation because of the oxidative stress caused by zinc deficiency (Gursel and Tekeli, 2009).

CONCLUSION

It was established that replacement of 50% major feed by instant food caused increase in levels of sodium, calcium, phosphorus, potassium in tissues of body. Consumption of instant food mixed with carbonated drinks caused the decrease of such essential microelements as zinc, manganese and selenium. Changes in element ratio were observed. Results may be used for assessment of influence of nutrition stress on exchange of mineral substances in organism of lab animals. Therefore, the obtained results demonstrate the change of element status of organism under food stress that is connected not only with excessive and deficient intake of separate elements, but also with change of their absorption and fixing in digestive tract.

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