Original article

The thiol-disulfide homeostasis and its role in the pathogenesis of the experimental alimentary obesity

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Abstract:

Objective: According to WHO, about 30 % of people in the world are overweight that allows to characterize this disease as a new non-infection "epidemic" of the XXI century. More than 500 million people in the world are overweight and 250 million are obese. There is a clear tendency to increasing of alimentary obesity among people with different age, sex and nationality. The aim of the study is to investigate the thiol-disulfide homeostasis in liver tissue, adipose tissue and erythrocytes in the pathogenesis of experimental alimentary obesity. ***Materials and methods*:** 60 males, non liner, white rats around 3 months of age with alimentary obesity were examined during the study. Experimental obesity was modeled by administering of sodium glutamate to the feed mixture in a ratio of 0.6: 100.0 and adding high-calorie diet. The glutathione redox-system activity in erythrocytes, liver and adipose tissue were analyzed by the level of reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR) and glutathione peroxidase (GP) activity. ***Results and Discussion:*** The data indicate a decrease in GSH level within 14 days of the experiment in all investigated tissues. The same trend was observed in animals on 28th day of the experiment: GSH index decreased in blood, adipose tissue and liver ($P < 0.05$). The index of GSSG have increased on 28th day of the experiment in all investigated tissues vs control group ($P < 0.05$). The ratio of the reduced and oxidized forms of glutathione contents was much lower vs control group in all the studied tissues within 28 days of the experiment. During additional investigation of the activity of thiol-disulfide system enzymes it was found that reducing the concentration of GSH in rats with alimentary obesity was due to the lack of thiol-disulfide system enzymes activity: GP and GR, which take part in the regeneration of GSH from GSSG. ***Conclusion:*** experimental alimentary obesity is characterized by a reduced redox state in blood, adipose and liver tissues, which is determinative in increasing the free radical reactions and accumulation of highly toxic lipoperoxides in the tissue substrates.

Keywords: alimentary obesity; glutathione redox-system; experiment

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Introduction. Obesity, as a global epidemic, in our days is observed both in high-income countries and in low-income[1,2]. In 2008, over 1.4 billion of adults were overweight, about 500 million of them had obesity, confirming that the worldwide prevalence of obesity has doubled from 1980 to 2008. In the US, more than 50 % of the population are overweight, herewith obesity is found in 35 % of women and 31 % of men. Thus, the increase of obese patients in America began in 1978 and

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continues to our days, gaining epidemic proportions [3,4]. In Europe, the incidence of obesity is 20 % in Switzerland, Bulgaria, Italy, France and Spain; 30 % in Germany, Finland, the UK and 40 % - in Romania. The growing number of people which are overweight is noticed in Japan, China and Korea, where the problem of obesity has not been recently so urgent.

In UK, it was estimated that about 61.3 % of adults are overweight or obese. The current cost of treating diseases related to obesity is approximately 5 billion pounds per year, and this figure will double by 2050. In Canada, the incidence of obesity almost has doubled, from 14 % in 1978/1979 to 26 % in 2009-2011, herewith 2 % of men and 5 % of women have a body mass index over 40. In general, more than two-thirds of Canadian men (67 %) and more than half of Canadian women (54%) are overweight or obese (Calgary: Canadian Task Force on Preventive Health Care; 2006).

In Ukraine, 52 % of people over 45 years are obese, 33 % – overweight and only 13 % have normal weight. In Ukraine, according to WHO estimates, 50.5 % of men are overweight, 16 % of them are obese and 56 % of women are overweight, 26 % of them are obese. In general, 45 % of people of working age in Ukraine are obese.

The latest investigation on epidemiology of alimentary obesity in Bangladesh indicate increasing of the incidence of this disease among school-age children in perspective will lead to the increasing in number of obese adults[5].

In Bangladesh, nearly 40 % of children < 5 years are suffering from malnutrition[6]. However, in recent years, multiple factors such as rapid urbanization, continually decreasing number of playgrounds, increasing purchasing power, and easy access to new technological devices have lead to less physical activity and more sedentary activity, and thereby have attributed to an emerging overweight and obesity problem among young children in urban settings, especially among affluent families in Dhaka[7].

Investigation, which was performed in the Diarrheal Disease Surveillance System (DDSS) at the Dhaka Hospital from 1993-2011, showed that over the last two decades the prevalence of overweight and obesity in Dhaka city has increased at least five folds and it was much higher among those with better socioeconomic status[8].

The analysis of epidemiology of alimentary obesity shows that obesity is an medical problem affecting people of all ages and incomes, everywhere and no one country has achieved successes in reducing of alimentary obesity. Therefore, the study of pathogenetic mechanisms of alimentary obesity and involvement of vital organs in the pathological process is an extremely important task today, because it will give reasons for developing algorithms of nutritional obesity prevention.

Molecular mechanisms of short-term and long-term adaptation to the pathological process is implemented by the participation of physiologically active substances, such as thiol-disulfide system. Glutathione is found in almost all tissues of the body and is involved in many biochemical and physiological processes: reduction and isomerization of disulfide bonds, the influence on the activity of enzymes and other proteins, maintenance of membrane and coenzyme functions, metabolism of eicosanoids, reservation of cysteine, influence on biosynthesis of nucleic acids and protein, regulation of the oxidation-reduction reactions, as a donor of SH-groups is very important in the mechanisms of detoxification. Therefore, the study of thiol-disulfide system is important for in-depth study of the mechanisms of alimentary obesity.

We conducted this study to investigate the thiol-disulfide homeostasis in liver tissue, adipose tissue and erythrocytes in the pathogenesis of experimental alimentary obesity.

Materials and methods.

Animals and experimental model**.** Experimental studies were conducted on 60 male, non liner, white rats around 3 months of age, which were housed at 25±3oC and humidity of 55±2%, under a constant 12 h light and dark cycle. Water was available ad libitum. Experimental obesity was modeled by administering of sodium glutamate to the feed mixture in a ratio of 0.6: 100.0 and adding high-calorie diet that consists of a standard meal (47 %), sweet concentrated milk (44 %), corn oil (8 %) and vegetable starch (1%) (diet # C 11024, Research Dietes, New Brunswick, NJ).

When we were selecting the optimal model of alimentary obesity, we focused that sodium glutamate affects ventrolateral nuclei of the hypothalamus, where the hunger center is located and thus stimulates appetite. Control of alimentary obesity model was performed by weighing animals, measurement of nasally-anal length and calculation of body mass index (BMI) (dividing body weight in kilograms by the length in meters squared).

The animals were divided into the following

Table I: The Indices of Thiol-Disulfide System in Rats' Tissues with Alimentary Obesity

Parameters		14th day (EG1), n=12	28th day (EG2), n=12
Blood			
GSH, μmol/gHb	CG	3,51±0,15	3,66±0,16
	EG	3,13±0,06*	2,34±0,09*#
GSSG, μmol/gHb	CG	0,16±0,1	0,17±0,01
	EG	0,15±0,01	0,23±0,01*#
GSH/ GSSG ratio	CG	19,3±1,51	21,37±0,50
	EG	20,78±1,00	10,08±0,61*#
Adipose tissue			
GSH, μmol/gHb	CG	6,79±0,11	6,76±0,11
	EG	5,58±0,13*	3,94±0,10*#
GSSG, μmol/gHb	CG	0,40±0,02	0,38±0,02
	EG	0,44±0,02	0,46±0,01*
GSH/ GSSG ratio	CG	17,65±0,97	18,37±0,99
	EG	12,95±0,64*	8,69±0,45*#
Liver tissue			
GSH, μmol/gHb	CG	7,75±0,10	6,96±0,11
	EG	4,88±0,09*	4,64±0,06*#
GSSG, μmol/gHb	CG	0,44±0,01	0,43±0,01
	EG	0,47±0,02	0,55±0,02*#
GSH/ GSSG ratio	CG	17,53±0,36	16,36±0,34
	EG	10,07±0,30*	8,59±0,25*#

*P<0,05 vs control group; # P<0,05 vs EG1group.

groups: 1st- the rats that were previously on an experimental diet for 14 days (EG1), 2nd – 28 days (EG2). The control groups (CG) consisted of 12 animals maintained on a standard diet for 14 days (CG1) and 28 days (CG2), respectively.

Animal euthanasia was carried out at 14 and 28 days of the experiment by decapitation, under the anesthesia in accordance with the requirements of the Animal Care Committee.

Material: Rat blood (erythrocytes and plasma), liver and adipose tissue samples were taken for the study in the morning on an empty stomach after decapitation.

Determination of the glutathione redox-system: The GRS activity in erythrocytes, liver and adipose tissue were analyzed by the level of reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (GR) and glutathione peroxidase (GP) activity. Briefly, GSH is oxidated by H_2O_2 to GSSG, then GR is recovered back to GSH using $NADPH^+H^+$. GSH and GSSG were measured according to the Ellman method. GR activity was measured according to the method described by Ramos-Martines I.L., et al., GP activity by Mills G.C. Protein concentration in tissue homogenate supernatant was estimated by the method of Lowry.

Statistic methods: All of the data was processed using the software package Statistica 6.1 for Windows. The mean (M) and standard error of the mean (SEM) were deduced. For data with normal distribution, inter-group comparisons were performed using Student's t-test. P value less than 0.05 was considered significant. Ethical approval was taken prior the study.

Results and Discussion: The data indicate a decrease in GSH level within 14 days of the experiment in all investigated tissues. The same trend was observed in animals of second experimental group: GSH index decreased to 36.1 % in blood and respectively 52.8 % and 33.3 % in adipose tissue and liver (P<0,05).

The index of GSSG have changed on 28th day of the experiment – increased in blood by 35.3 % in adipose tissue - by 21.1 % and liver tissue – 27.9 % vs control group (P<0,05).

The ratio of the reduced and oxidized forms of glutathione contents was much lower vs control group in adipose tissue and liver of animals in group EG1, and in all the studied tissues - in group

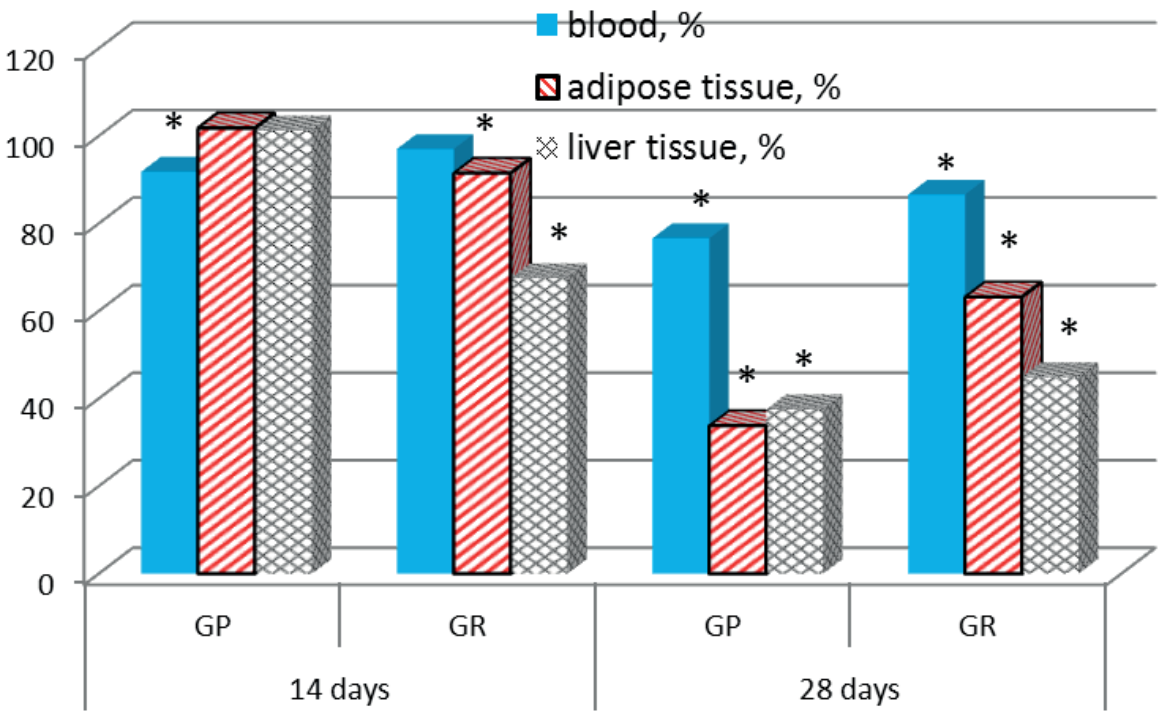

Fig. I : Dynamics of enzymes' activity of glutathione redox-system in case of alimentary obesity in rats. *P<0,05 vs control group.

EG2.

We know that GSH is used in the redox-reactions as a supplier of SH-groups that protect the cell from reactive oxygen species. Thus, it was found that in animals with alimentary obesity there is an imbalance in the thiol-disulfide system, which is associated with a significant increase in the consumption of GSH during neutralization of free radicals, which are formed due to activation of lipid peroxidation. Herewith was found almost identical in GSSG shift towards growth. The ratio of the reduced and oxidized forms of glutathione indicates about decreasing of total capacity of thiol-disulfide system in tissues of rats with alimentary obesity.

*P<0,05 vs control group; # P<0,05 vs EG1group.

During additional investigation of the activity of thiol-disulfide system enzymes it was found that reducing the concentration of GSH in rats with alimentary obesity was due to the lack of thiol-disulfide system enzymes activity: GP and GR, which take part in the regeneration of GSH from GSSG. Thus, in animals of group EG2 GP concentration in blood was (32,14±0,56) vs control group (41,94±0,53) μmol GSH/mgHb/min and, in accordance, in adipose tissue – (0,13±0,01) vs (0,37±0,03) nmol GSH/mg of protein/ min, in liver tissue – (0,19±0,02) vs (0,50±0,02) nmol GSH/ mg of protein/min. Index of GR in blood was (61,25±0,69) vs control group (70,72±0,62) μmol GSH/mgHb/min and, in accordance, in adipose tissue – (1,39±0,05) vs (2,20±0,07) nmol GSH/ mg of protein/ min, in liver tissue – (1,74±0,11) vs (3,87±0,13) nmol GSH/mg of protein/min (fig. 1).

The data show a significant decrease in activity of enzymes of thiol-disulfide system in animals with alimentary obesity, which is therefore completely unable to resist the damaging effect of excessive lipid peroxidation products. In a study of Zhang Y. et al. there are data that glutathione enzyme deficiency in the cells triggers damage to the proteins, lipid and DNA macromolecules[9]. Novo E. i Parola M. showed that the glutathione depletion in the hepatocytes determines the process of steatosis in the liver, necrosis and apoptosis of hepatocytes[10].

It was established that the sensitivity of biomarkers of oxidative damage are higher in individuals with obesity and correlate directly with BMI and the percentage of body fat[11]. In turn, Chrysohoou C. and co-authors show that antioxidant defense markers are lower according to the amount of body fat and central obesity[12]. The data indicate that adipose tissue is the source of free radicals, and thiol-disulfide system is exhausted and unable to protect against oxidative stress in case of alimentary obesity in rats.

Conclusion: Thus, taking into consideration our results and other reports, we can assume that experimental alimentary obesity is characterized by a reduced redox state in blood, adipose and liver tissues, which is determinative in increasing the free radical reactions and accumulation of highly toxic lipoperoxides in the tissue substrates. At 28th day of alimentary obesity, the rats experienced failure of the compensatory processes in the glutathione redox imbalance due to the exchange of thioldisulfide (the decrease of GSH, GSH/ GSSG ratio, glutathione enzymes activity in the cell and tissue substrates).

Conflict of interest: None

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