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BASIC RESEARCH

Effect of Prolonged High-Fat Diet on Thiol-Disulfide Homeostasis in Rats

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Abstract

The aim of this study was to determine the effect of a prolonged high-fat (HF) on thiol-disulfide homeostasis via the activity of the glutathione redox-system (GRS) in rat blood and liver.

Methods: The experiment was conducted on male Wistar rats. They were divided into groups and fed on the HF diet for 30, 90 and 180 days, respectively. The HF diet consisted of beef fat and cholesterol (19 % and 2 % of the total diet, respectively). The state of the GRS was assessed in the erythrocytes and liver tissue by the glutathione, glutathione reductase (GR) and glutathione peroxidase (GP) activity. The levels of the initial and final products of lipid peroxidation – lipid hydroperoxides (LOOHs), diene conjugates (DC) and malondialdehydes (MDA) in the blood and liver were investigated.

Results: Within 30 days, the HF diet inhibits the glutathione enzyme activity in the blood (GR: $P<0.01$; GP: $P<0.001$) and liver (GR, $P<0.01$). Within 90 days the HF diet kick-starts the beginning of the GRS compensatory response and restores the thiol-disulfide homeostasis. At 180 days, the HF diet shows failure of the compensatory processes in the glutathione system caused by the redox-imbalance in the thiol-disulfide exchange, which reveals lowered levels of glutathione, GR and GP activity ($P<0.001$ for all) in the blood and liver.

Conclusion: Our results suggest that the thiol-disulfide status of the cells depends upon the nature of the nutrition, a long-term breach of which triggers a compensatory response and a failure of the compensatory processes in the GRS.

Keywords**:** *high-fat diet; thiol-disulfide homeostasis; redox-glutathione system.*

Introduction

An important step in the metabolism accounts for the redox processes necessary to maintain the constancy of cellular and intracellular homeostasis under physiological conditions and under the effects of various stresses [1,2]. In a normally functioning body, there is a fine regulation of the intensity of the redox processes (free-radical processes) through the action of specific antioxidant enzyme systems. In recent years, the study of the thiol-disulfide regulation has attracted much attention [3]. Also, the regulation of important biological processes in the cells is associated with changes in the redox-state of the thiol groups in the proteins [4-7].

The role of glutathione as a redox-buffer and an antioxidant is well known and is recognized to have great importance in maintaining the thiol-disulfide status in proteins and protecting the cells from oxidative stress in aging and cancer, neurodegenerative and inflammatory diseases [8, 9]. The antioxidant effects of glutathione are catalyzed by GP and GR [10-12]. GR catalyzes the NADPH-dependent recovery of oxidized glutathione, which helps to maintain a high concentration of the glutathione without increasing its synthesis. The antioxidant action of GP is based on its ability to catalyze the reduction reaction of the hydrogen peroxide molecules to produce water and fatty acid hydroperoxides to the corresponding alcohol with two molecules of reduced glutathione (GSH) as a donor of protons and electrons. Glutathione enzymes form thioredoxin- and glutaredoxin-dependent systems [6]. These systems play a vital role in maintaining intracellular redox-homeostasis and protecting the cells from the destructive effects of the oxidative stress [2]. Glutathione is present in high concentrations inside each cell, as well as outside the cells [13]. Of particular importance is the glutathione system in the iron ion-containing red blood cells and in the hepatocytes - cells with high phosphorylation levels [14,15].

A significant impact on the change in the redox processes in the cell has a nutritional imbalance [12]. The HF has been proven to initiate lipid peroxidation and reduce the antioxidant status

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of the organism [16,17]. It is shown that two days of starvation reduces the glutathione concentration in the liver by one-third or one-half the original amount, while feeding restores the levels within a few hours [12]. This indicates a significant relationship between glutathione levels and nutrition. However, there is still insufficient information on the impact of the HF diet on thiol-disulfide homeostasis. The urgency of studying this issue is related to the establishment of the mechanism of the influence of the HF diet on obesity and atherosclerosis.

The objective of this study was to determine the effect of the prolonged HF diet on the thiol-disulfide homeostasis via the GRS activity in the blood and liver of rats.

Methods

Animals and Diets: Experimental studies were conducted on 60 male Wistar rats around 3 months of age. The animals were divided into the following groups: HF_{30} – the rats that were previously on an experimental diet for 30 days; HF_{90} – 90 days; HF_{180} – 180 days. The control groups (CG) consisted of 30 animals maintained on a standard diet for 30 days (CG 1), 90 days (CG 2), and 180 days (CG 3), respectively. The experimental HF diet consisted of beef fat (19% of the total diet) and cholesterol (2% of the total diet). The composition of beef tallow was found to be up to 66% of saturated fatty acids (FA) (12:0, 14:0, 16:0, and 18:0), 35% monoenic fatty acids, and 2% essential fatty acids (Table 1).

Table 1.
Composition of the HF diet (g/kg of animal weight)

Ingredients	HF diet	Standard diet
Tallow	**42.5**	5
Cholesterol	**4.3**	-
Sunflower oil	5	5
Grain mixture	50	50
Bread	20	20
Grits	13	13
Beef	20	20
Skim cheese	8	8
Carrot	33	33
Greens	33	33

Animal euthanasia was carried out at 30, 90 and 180 days of the experiment, respectively, by decapitation, under ether anesthesia in accordance with the requirements of the European Convention for the Protection of experimental animals, 86/609 EEC [18].

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

Determination of the GRS activity: The GRS activity in erythrocytes and liver tissue were analyzed by the level GSH, GR and GP activity. GSH was measured according to the Ellman method [19]. GR activity was measured according to the method described by Ramos-Martines IL, et al. [20], GP activity by Mills GC [21].

Determination of lipoperoxides: The intensity of lipid peroxidation in the blood and liver were investigated by spectrophotometry for the levels of the initial and end-products of lipid peroxidation (PLP) - (LOOHs), DC, and MDA [22].

Statistic methods: All of the data was processed using the software package Statistica 6.1 for Windows (series 1203C). The ANOVA method was applied. 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.

Results and Discussion

The assessment of the thiol-disulfide homeostasis and the intensity of the lipid peroxidation in rats on the HF diet revealed the following pattern (Table 2). The animals of the HF_{30} group showed inhibition of the blood enzyme activity of GRS: GR (*P<0.01*), GP (*P<0.001*), and reduced glutathione levels (*P<0.001*).

Table 2.
Effect of the HF diet on the activity of the glutathione redox-system in the blood and liver of the rats

Parameters	Days of the HF diet		
	30 days (HF_{30}) n=10	90 days (HF_{90}) n=10	180 days (HF_{180}) n=10
Blood			
GSH, *μmol/gHb*			
Standard diet	5.3±0.2	5.8±0.8	4.3±0.2
HF diet	***3.5±0.3	**4.1±0.1$^{\#}$	***2.0±0.2$^{\#\#}$
GR, *μmol NADPH/mgHb/min*			
Standard diet	75.1±1.5	74.1±1.1	69.1±0.9
HF diet	**68.0±1.5	73.3±1.6$^{\#}$	***50.1±2.4$^{\#\#}$
GP, *μmol GSH/mgHb/min*			
Standard diet	44.5±0.8	40.9±3.4	44.1±0.8
HF diet	***32.5±1.3	40.1±2.2$^{\#}$	***21.4±1.2$^{\#\#\#}$
Liver			
GSH, *μg/mg protein*			
Standard diet	8.03±0.22	8.25±0.19	7.12±0.17
HF diet	**4.96±0.11	***7.30±0.17$^{\#\#}$	***4.10±0.17
GR, *nmol NADPH/mg of protein/min*			
Standard diet	3.89±0.13	3.24±0.11	3.16±0.17
HF diet	***1.12±0.04	***1.55±0.11	***1.01±0.04
GP, *nmol GSH/mg of protein/min*			
Standard diet	0.53±0.07	0.55±0.04	0.49±0.05
HF diet	0.75±0.09	***0.12±0.01	***0.11±0.01$^{\#\#\#}$

*P<0.05, **P<0.01; ***P<0.001 *vs* control group;
#P<0.05, ##P<0.01, ###P<0.001 *vs* HF_{30} group.

The GRS response in the liver tissue after 30 days of the HF diet was expressed as a decrease in the GR activity (*P<0.01*), and GSH levels (*P<0.01*). GP activity was not changed (Table 3). The reduced redox-potential of the thiol-disulfide system after a 30-day HF diet was accompanied by an increase in the PLP in the blood (GPL: *P<0.001* and MDA: P<0.05) and liver (DC: *P< 0.001* and MDA: *P<0.01*) relative to the control group (Table 3).

On the 30th day of the HF diet, activation of lipid peroxidation, decreased antioxidant defense mechanisms and deterministic suppression activity of the GRS in blood and liver, indicating the development of the oxidative stress were identified. Reducing the level of glutathione indicates its attrition rate in antioxidant processe , compensating for the activation of free radical oxidation. GSH is used in the redox-reactions as a supplier of SH-groups that protect the cell from reactive oxygen species [2, 6, 23, 24]. It is known that glutathione can adjust the antioxidant response elemtent (ARE) [1, 4]. The maintenance of the glutathione peroxidase activity in the liver at the level of physiological norm, apparently, is necessary for the regulation of the redox-sensitive transcription factors and signaling systems.

After 90 days of the HF diet, increased glutathione levels ($P<0.05$) and the activity of GR and GP in the blood were observed (Table 2). In the liver, the glutathione content increased ($P<0.01$), while the GP and GR activity was reduced. In this case, normalization of GPL in the blood and liver MDA and DC was noted (Table 3).

Table 3.
Effect of the HF diet on content of PLPs in the blood and liver of the rats

Parameters	Days of the HF diet		
	30 days (HF_{30}), n=10	90 days (HF_{90}), n=10	180 days (HF_{180}), n=10
Blood			
LOOH, *U*			
Standard diet	0.66±0.07	0.73±0.08	1.06±0.07
HF diet	***3.1±0.1	0.81±0.12	*1.87±0.24$^{\#\#\#}$
MDA, *nmol /gHb*			
Standard diet	4.6±0.3	4.5±0.2	5.3±0.3
HF diet	*5.31±0.31	**8.12±0.2$^{\#\#\#}$	***10.1±0.2$^{\#\#\#}$
Liver			
LOOH, *U of optical density/g of tissue*			
Standard diet	0.40±0.05	0.45±0.09	0.95±0.05
HF diet	-	0.31±0.03	*1.28±0.02
DC, *nmol/mg of lipids*			
Standard diet	1.07±0.17	1.12±0.09	1.21±0.17
HF diet	***9.34±0.23	1.14±0.13$^{\#\#\#}$	***0.63±0.07$^{\#\#\#}$
MDA, *nmol/mg of protein*			
Standard diet	2.84±0.65	2.54±0.65	3.14±0.65
HF diet	***4.66±0.15	2.67±0.09$^{\#\#\#}$	***10.8±0.9$^{\#\#\#}$

*P<0.05, **P<0.01; ***P<0.001 *vs* control group;

#P<0.05, ##P<0.01, ###P<0.001 *vs* HF_{30} group.

It is known that the cell becomes sensitive to thiol-disulfide imbalance during the oxidative stress [2]. Any violation of the thiol-disulfide homeostasis affects the regulatory genes (nuclear factor kappa β − NF-κβ, ARE) expressing the glutathione enzymes and glutathione synthesis [7]. Thus, compensation is made for low antioxidant protection. Glutathione plays a significant role in the activation of redox-sensitive genes [9]. GSH induces the antioxidant and glutathione detoxification capacity of the enzymes involved in a redox-dependent expression of the genes regulating the intracellular signaling and other processes. The SH-groups of reduced glutathione act as scavengers of the hydroxyl radical and singlet oxygen, thereby reducing the destructive and cytotoxic effects of the reactive oxygen species [9,11]. The findings suggest that the compensatory response triggered in the antioxidant system on the 90th day of HF, targeted the disposal of the highly toxic LPP, reducing the intensity of OS, restoring the thiol-disulfide homeostasis and the deterministic amplification of the redox-signaling the glutathione system. It can be concluded that the state of the thiol-disulfide homeostasis is an important mechanism in maintaining an optimal balance between the pro- and anti-oxidant processes that regulate the intensity of the oxidative stress.

The GRS status on the 180th day of the experiment was characterized by a significant decrease in the activity of GP and GR, and the GSH level in the blood and liver (Table 2). Inhibition of the glutathione system redox-potential facilitated the accumulation of the initial and final PLP in the blood and liver of the rats (Table 3). Glutathione enzyme deficiency in the cells triggers damage to the proteins, lipid and DNA macromolecules [1,2]. The glutathione depletion in the hepatocytes determines the process of steatosis in the liver, inflammation, necrosis and apoptosis of hepatocytes, the activation of fibrogenesis with the development of fibrosis and cirrhosis [25]. Thus, at 180 days, the HF diet revealed a failure of compensatory processes in the glutathione redox system.

Our results suggest that the thiol-disulfide status of the cells depends upon the nature of the nutrition, a long-term interruption of which determines the formation of a compensatory response and the failure of the compensatory processes in the GRS. A short-term HF diet (30 days) provokes the development of the oxidative stress, accompanied by the activation of lipid peroxidation, reducing the GSH buffering capacity of antioxidant protection. Long-term maintenance of the effector states with a prolongation of the HF diet up to 90 days is characterized by the appearance of the compensatory-adaptive response of the GRS. There is an increase in the glutathione synthesis and the activation of the glutathione-dependent enzymes in the blood and liver compared with the rats fed with HF diet for 30 days. Induction of the reactions mentioned above reveal an antioxidant protection to arrest the development of the oxidative stress and to minimize the accumulation of the highly toxic lipoperoxides in the blood and liver. At 180 days of alimentary loads, the rats experienced failure of the compensatory processes in the glutathione redox imbalance due to the exchange of thiol-disulfide (the decrease of GSH, glutathione enzymes activity in the cell and tissue substrates). Inhibition of the GRS activity is deterministic in increasing the free radical reactions and accumulation of highly toxic lipoperoxides in the blood and liver. These data add weightage to the concept of the fundamental role of the antioxidant system played by the thiol-disulfide response under adverse environmental conditions.

Authors' contribution

Yulia Denisnko has made substantial contributions to the conception and design, and been involved in drafting

the manuscript and the final approval before publication. Yulia Dinisenko has done the data acquisition, analyzed, and interpreted it. Tatyana Novgorodtseva has been involved in critically revising the paper for important intellectual content. Tatyana Novgorodtseva has also been involved in drafting the manuscript, and has given final approval for publication. All authors have read and approved the final manuscript.

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