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Glomerular filtration and diuresis are related to the state of lipids peroxidation in female rats exposed to water-salt loads accompanied by chronic stress

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Summary

Background. It is known about the existence of close functional link between adequate working kidney nephrons and bone metabolism. Focused monitoring of renal function is able to provide substantial assistance in the organization of preventive measures against latently emerging osteoporosis. Activation of lipoperoxidation is known to inhibit glomerular filtration, whereas antioxidants prevent this. We set ourselves the goal of clarifying correlations between glomerular filtration, as well as tubular water reabsorption and daily diuresis, on the one hand, and lipoperoxidation parameters, on the other hand. **Materials and Methods**. Experiment was performed on 50 healthy female Wistar rats 240-290 g, both intact and exposed to water-salt loads. By the size of the diuresis and the level of creatinine in plasma and urine, glomerular filtration and tubular reabsorption were calculated. The plasma levels of diene conjugates and malondyaldehide as well as catalase of plasma and superoxide dismutase of erythrocytes were determined. The levels of the first three parameters determined in urine too. **Results**. In conditions of water-salt loads accompanied by chronic stress, glomerular filtration is downregulated by malondialdehyde and superoxide dismutase; diuresis is also downregulated by malondialdehyde, but upregulated by catalase. Lipoperoxidation parameters determine kidney function by 52,8%. **Conclusion**. The obtained data indicate the role of lipoperoxidation as a accompaniment of chronic stress in the regulation of kidney functions.

Keywords: glomerular filtration, diuresis, lipid peroxidation, relationships, water-salt loads, chronic stress, rats.

INTRODUCTION

It is known about the existence of close functional link between adequate working kidney nephrons and bone metabolism. Focused monitoring of renal function is able to provide

substantial assistance in the organization of preventive measures against latently emerging osteoporosis [8]. Activation of lipoperoxidation, particularly by nitrates, is known to inhibit glomerular filtration, whereas antioxidants, particularly alpha-tocopherol, prevent this [5,10]. It is known that stress also affects the state of lipoperoxidation [3,20]. Based on the above, we set ourselves **the goal** of clarifying correlations between glomerular filtration, as well as tubular water reabsorption and daily diuresis, on the one hand, and lipoperoxidation parameters, on the other hand, in rats exposed to water-salt loads accompanied by chronic aversive stress [15-19].

MATERIAL AND METHODS

Experiment was performed on 50 healthy female Wistar rats 240-290 g divided into 5 equals groups. Animals of the first group remained intact, using tap water from drinking ad libitum. Rats of the second group for 6 days loaded a with tap water through the tube at a dose of 1,5 mL/100 g of body mass. In the third group was given daily load of animals with water Sophiya of the Truskavets' field. The rats of the next groups received the native water from the Hertsa field and its artificial salt analogue. The chemical composition of the applied waters (according to Truskavetsian Hydrogeological Regime-operational Station data) is given in Table 1 [23].

Table 1. The chemical composition of the applied mineral waters

	Daily Water	Sofiya	Hertsa	Salt analogue
Na^+	0,5	156	196,7	196,7
Cl^-	3,4	142	205	205
HCO_3^-	2,9	7,5	5,6	5,6
Ca^{2+}	3,4	5,3	3,40	3,40
Mg^{2+}	0,5	4,3	3,44	3,44
K^+	0,4	0,3	0,4	0,4
SO_4^{2-}	1,2	13,1	0,1	0,1
H_2SiO_3	5	4,43	9,88	0
H_3BO_3	0,25	8,39	42,76	0
Br	8,3	6,7	21,17	0
J	0,025	1,29	6,62	0
F	0,95	0,52	0,57	0
C org	5,0	5,5	34	0
N org	0,02	0,8	0,14	0

The day after the completion of the drinking course animals were placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible.

By the size of the diuresis and the level of creatinine (determined by Jaffe's color reaction by Popper's method [7]) in plasma and urine, glomerular filtration and tubular reabsorption were calculated.

The plasma levels of the lipid peroxidation products as diene conjugates (by spectrophotometry of the heptane phase of the lipids extract [6]) and malondyaldehide (in the test with thiobarbituric acid [1]) as well as antioxidant enzymes catalase of plasma (by the rate of decomposition of hydrogen peroxide [13]) and superoxide dismutase of erythrocytes (according to the degree of inhibition of reduction of nitroblue tetrazolium in the presence of N-methylphenazonium metasulphate and NADH [4,14]) were determined. The levels of the diene conjugates, malondyaldehide and catalase were determined in urine too.

The analyzers "Tecan" (Oesterreich), "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets.

RESULTS AND DISCUSSION

Following the algorithm of the Truskavetsian Scientific School of Balneology [9,19], at the first stage, four groups were retrospectively formed by the method of cluster analysis [2]. The members of the groups differ minimally from each other in the levels of glomerular filtration and lipoperoxidation parameters, but maximally - from the members of other cluster groups (Table 2).

Table 2. Euclidean Distances between Clusters

Cluster Number	Distances below diagonal Squared distances above diagonal			
	No. 1	No. 2	No. 3	No. 4
No. 1	0	1088	493	7393
No. 2	33	0	1072	3237
No. 3	22	33	0	7948
No. 4	86	57	89	0

The variance analysis shows that glomerular filtration makes the maximum contribution to the distribution of the sample into clusters. Further, according to criterion F, malondialdehyde and diene conjugates of urine, malondialdehyde and catalase of plasma follow, while the role of the remaining parameters in clustering is insignificant (Table 3).

Table 3. Analysis of Variance

Variable	Between SS	df	Within SS	df	F	signif. p
GF	327151	3	54654	46	91,78	0,000000
SOD	461,6	3	3874	46	1,83	0,155
KAT	0,015	3	0,094	46	2,37	0,083
KATU	0,009	3	0,068	46	2,09	0,115
MDA	5634,4	3	28377	46	3,04	0,038
MDAU	32728	3	18768	46	26,74	0,000000
DC	0,875	3	9,184	46	1,46	0,237
DCU	2,301	3	8,994	46	3,92	0,014

Another approach to elucidating characteristic satellites of glomerular filtration is discriminant analysis [12]. The forward stepwise program included 6 variables in the discriminant model (tables 4 and 5), of which only the first two coincided in ranking with such a variance analysis, while diene conjugates of urine were found to be outside the model.

Table 4. Discriminant Function Analysis Summary
Step 6, N of Variables currently in the model: 6; Grouping: 4 groups
Wilks' Lambda: 0,0300; approx. $F_{(18,1)}$=15,9; $p<10^{-6}$

Variables currently in the model	Clusters (n)					Parameters of Wilks' Statistics				
	Intact rats (10)	IV (6)	II (8)	I (16)	III (20)	Wilks' Λ	Partial Λ	F-remove (3,41)	p-level	Tolerancy
Glomerular Filtration, µL/min•100 g BM	86,01 0	324 3,77 +7,72	167 1,94 +2,63	88,0 1,02 +0,07	72,1 0,84 -0,45	0,215	0,139	84,4	10^{-6}	0,680
Malondialdehyde Urine, µM/L	92 1 0	75 0,81 -0,49	71 0,78 -0,48	126 1,37 0,78	67 0,73 -0,58	0,072	0,416	19,2	10^{-6}	0,819
Superoxide Dismutase Erythrocytes, un/mL	58,0 1 0	53,1 0,92 -0,45	50,4 0,87 -0,70	54,9 0,92 -0,29	58,8 1,01 +0,07	0,038	0,793	3,57	0,022	0,755
Malondyaldehide Plasma, µM/L	63,2 1 0	54,9 0,87 -0,39	68,8 1,09 +0,25	84,2 1,33 +0,96	62,7 0,99 -0,03	0,033	0,914	1,29	0,290	0,294
Diene conjugates Plasma, E^{232}/mL	1,34 1 0	1,53 1,14 +0,47	1,41 1,05 +0,17	1,62 1,20 +0,69	1,31 0,98 -0,08	0,033	0,910	1,36	0,270	0,302
Katalase Activity Plasma, µM/h•L	103 1 0	128 1,24 +0,87	134 1,29 +1,08	101 0,97 -0,10	140 1,36 +1,32	0,032	0,924	1,12	0,353	0,871
Variables currently not in the model		IV (6)	II (8)	I (16)	III (20)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Katalase Activity Urine, µM/h•L	123 1 0	156 1,27 +1,23	158 1,28 +1,27	122 0,99 -0,04	150 1,22 +0,98	0,029	0,977	0,32	0,814	0,270
Diene conjugates Urine, E^{232}/mL	1,86 1 0	1,99 1,07 +0,21	1,52 0,82 -0,51	2,10 1,13 +0,37	1,66 0,89 -0,30	0,029	0,957	0,59	0,623	0,556
Canalicular Reabsorbtion, %	98,7 1 0	99,4 1,01 +0,90	99,0 1,00 +0,43	99,0 1,00 +0,38	98,4 1,00 -0,41					
Diuresis, mL/24h•100 g BM	1,44 1 0	2,55 1,77 +1,24	2,24 1,56 +0,90	1,22 0,85 -0,24	1,58 1,10 +0,16					
Creatinine Urine, mM/L	6,41 1 0	7,36 1,15 +0,51	6,73 1,05 +0,17	7,92 1,24 +0,82	6,31 0,98 -0,06					
Creatinine Plasma, µM/L	72,5 1 0	42 0,57 -1,28	61 0,84 -0,47	78 1,07 +0,21	96 1,33 +0,98					

Note. In each column, the first line is the average value, the second is the fraction of the norm, and the third is the Z-score. The intact group is not subject to discriminant analysis.

Table 5. Summary of Stepwise Analysis. Variables ranged by criterion Lambda

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Glomerular Filtration, µL/min•100 g BM	91,8	10^{-6}	0,143	91,8	10^{-6}
Malondialdehyde Urine, µM/L	28,2	10^{-6}	0,050	52,2	10^{-6}
Superoxide Dismutase Erythrocytes, un/mL	4,12	0,012	0,039	33,3	10^{-6}
Diene conjugates Plasma, E^{232}/mL	1,22	0,316	0,036	24,0	10^{-6}
Malondyaldehide Plasma, µM/L	1,44	0,245	0,032	19,1	10^{-6}
Katalase Activity Plasma, µM/h•L	1,12	0,353	0,030	15,9	10^{-6}

The dividing information contained in 6 variables is condensed in 3 canonical discriminant roots (Table 6). The first root contains 74,3% of discriminative opportunities (r*=0,940; Wilks' Λ=0,030; $\chi^2_{(18)}$=154; p<10^{-6}), the second root - 24,5% (r*=0,844; Wilks' Λ=0,256; $\chi^2_{(10)}$=60; p<10^{-6}), the third - 1,2% only (r*=0,329; Wilks' Λ=0,892; $\chi^2_{(4)}$=5; p=0,284).

The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients (Table 6) to the individual values of discriminant variables together with the constant enables the visualization of each rat in the information space of the roots (Fig. 1).

Table 6. Standardized and Raw Coefficients for Canonical Variables

Coefficients	Standardized			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Glomerular Filtration, µL/min•100 g BM	-1,194	0,104	0,038	-0,035	0,003	0,001
Malondialdehyde Urine, µM/L	-0,090	0,991	-0,234	-0,004	0,049	-0,012
Superoxide Dismutase Erythroc., un/mL	-0,302	-0,415	-0,808	-0,033	-0,045	-0,088
Diene conjugates Plasma, E^{232}/mL	0,447	0,121	-1,017	1,001	0,272	-2,277
Malondyaldehide Plasma, µM/L	-0,293	0,256	1,261	-0,012	0,010	0,051
Katalase Activity Plasma, µM/h•L	-0,186	-0,281	-0,0003	-0,004	-0,006	-6•10^{-6}
			Constants	6,333	-2,549	5,559
			Eigenvalues	7,53	2,48	0,12
		Cumulative Proportions		0,743	0,988	1

In the Table 7 together with discriminant variables are also variables that carry identifying/ separating information, but were outside the model due to its duplication/redundancy. For ease of comparison, the values of the variables are transformed into Z-scores [9,19].

Table 7. Factor Structure Matrix and Means of Roots and Variables

	Correlations Variables-Canonical Roots			IV (6)	II (8)	I (16)	III (20)
Root 1 (74,3%)	R1	R2	R3	-6,61	-1,27	+1,13	+1,59
Glomerular Filtration	-0,891	0,012	0,112	+7,72	+2,63	+0,07	-0,45
Canalicular Reabsorbtion				+0,90	+0,43	+0,38	-0,41
Diuresis				+1,24	+0,90	-0,24	+0,16
Creatinine Plasma				-1,28	-0,47	+0,21	+0,98
Root 2 (24,5%)				-0,11	-0,44	+2,09	-1,46
Malondialdehyde Urine	0,107	0,813	-0,346	-0,49	-0,48	+0,78	-0,58
Malondyaldehide Plasma	0,072	0,244	0,303	-0,39	+0,25	+0,96	-0,03
Diene conjugates Plasma	-0,029	0,189	0,013	+0,47	+0,17	+0,69	-0,08
Diene conjugates Urine				+0,21	-0,51	+0,37	-0,30
Creatinine Urine				+0,51	+0,17	+0,82	-0,06
Katalase Activity Plasma	-0,011	-0,248	0,100	+0,87	+1,08	-0,10	+1,32
Katalase Activity Urine				+1,23	+1,27	-0,04	+0,98
Root 3 (1,2%)				-0,34	+0,74	-0,06	-0,15
Superoxide Dismutase Erythr	0,075	-0,092	-0,676	-0,45	-0,70	-0,29	+0,07

The localization of the members of the **fourth** cluster in the left zone of the axis of the major (contains ¾ information) root (Fig. 1) reflects their drastically high level of glomerular filtration, which is accompanied by a less pronounced, but maximal for the sample, levels of tubular reabsorption of water and diuresis and maximally reduced creatinineemia.

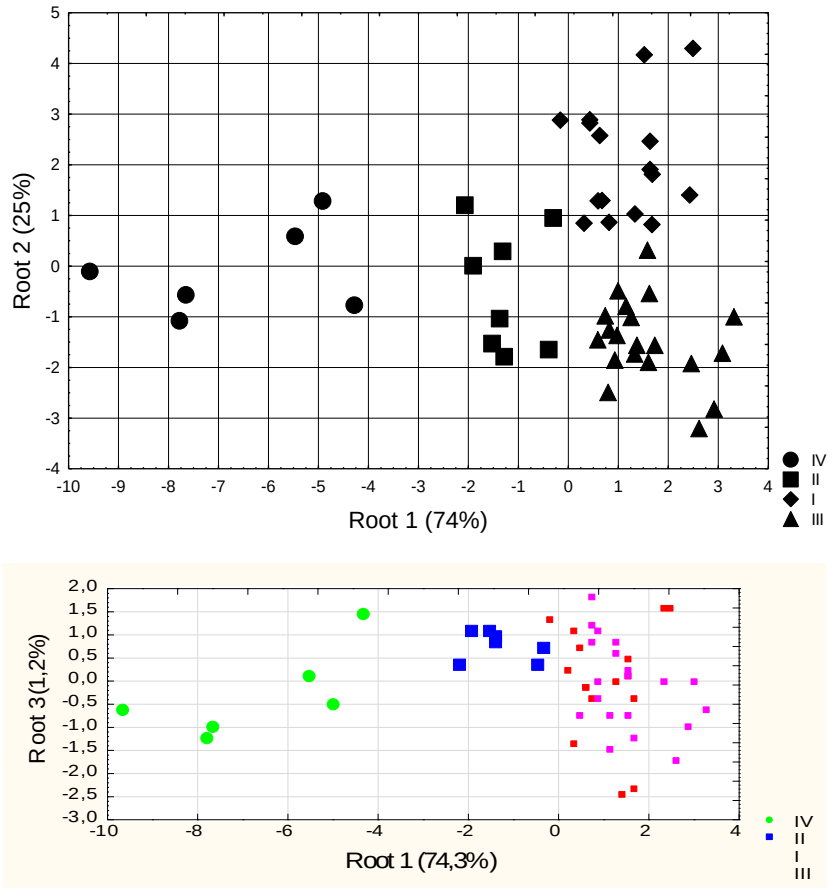


Fig. 1. Individual values of the first and second (above) and the first and third (below) roots of the endocrine and metabolic parameters in intact rats

The shift along the root axis to the right of the localization of the rats of the **second** cluster reflects a less pronounced increase in glomerular filtration and diuresis and a decrease in the level of creatinine. Instead, the levels of these parameters in the rats of the **first** cluster located even to the right are within the normal range, and in the rats of the extreme **third** cluster, the levels of glomerular filtration and tubular reabsorption are moderately reduced in combination with an increase in the level of creatinineemia.

The reason for high glomerular filtration becomes obvious when finding out the composition of water-salt loads. It turns out that 2 animals of the fourth cluster each received Sofiya water, Hertsa and its salt analogue, i.e., to a first approximation, an isotonic NaCl solution. In the second cluster, the distribution looked like 2+2+3, and only one animal received tap water. This is in excellent agreement with the known fact about the ability of both semi-isotonic (to a greater extent) and isotonic (to a lesser extent) NaCl solution to increase glomerular filtration [11].

Instead, the share of salt loads in the first cluster is only 62,5% due to the presence of 2 rats loaded with tap water and another 4 intact, so the average levels of the parameters do not differ from the norm. And in the third cluster, 35% of the composition is represented by rats loaded with tap water and 30% -intact.

It is known that the loading procedure is aversive for the animal. Therefore, chronic stress develops over the course of 6 days [19], one of the manifestations of which is a prevention of increase in glomerular filtration and diuresis, caused by salt loads [22].

The projections of the members of the first and third clusters on the axis of the major root are still mixed, and their demarcation occurs along the axis of the second root (Fig. 1 above). The top position of members of the first cluster reflects their maximally elevated levels of lipoperoxidation products in both plasma and urine, as well as creatinineuria, combined with normal levels of plasma and urine catalase activity. Additional delimitation of the second cluster occurs along the axis of the third root, despite its poor discriminating ability, due to the maximum inhibition of SOD (Fig. 1 below). In the information field of the three discriminant roots, all four clusters are clearly delineated, as documented by the Mahalanobis distances between them (Table 8).

Table 8. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=6,4) and p-levels (under diagonal)

Clusters	IV (6)	II (8)	I (16)	III (20)
IV (6)		29,8 ,045	64,9	69,2
II (8)	15,2 10^{-6}		12,9	10,0
I (16)	42,0 10^{-6}	10,2 10^{-6}		12,9
III (20)	47,4 10^{-6}	8,5 10^{-5}	16,9 10^{-6}	

The application of the classifying functions (Table 8) enables the retrospective identification of rats with a single error only (Table 9).

Table 8. Coefficients and Constants for Classification Functions

Variables currently in the model	IV (6)	II (8)	I (16)	III (20)
Glomerular Filtration, µL/min•100 g BM	0,464	0,279	0,203	0,176
Malondialdehyde Urine, µM/L	0,250	0,198	0,321	0,145
Superoxide Dismutase Erythrocytes, un/mL	1,071	0,815	0,692	0,846
Diene conjugates Plasma, E^{232}/mL	-1,015	1,788	6,699	6,400
Malondyaldehide Plasma, µM/L	0,036	0,024	-0,019	-0,065
Katalase Activity Plasma, µM/h•L	0,097	0,077	0,052	0,072
Constant	-121,6	-59,85	-56,49	-44,33

Table 9. Classification Matrix

Group	Rows: Observed classifications Columns: Predicted classifications Percent Correct	IV p=,12000	II p=,16000	I p=,32000	III p=,40000
IV	100,0	6	0	0	0
II	75,0	0	6	1	1
I	100,0	0	0	16	0
III	100,0	0	0	0	20
Total	96,0	6	6	17	21

At the final stage, on the basis of the correlation matrix (Table 10), the relationships between kidney function parameters and lipoperoxidation parameters were analyzed.

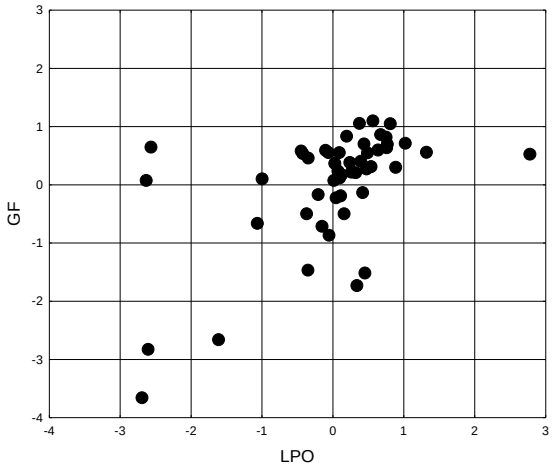
Table 10. Correlations Matrix

Variable	Correlations											
	Diu	CrP	CrU	GF	Can Reab	SOD	Kat P	Kat U	MDA P	MDA U	DC P	DC U
Diu	1,00	0,12	-0,32	0,47	-0,26	-0,17	0,43	0,57	-0,13	-0,31	0,03	0,06
CrP	0,12	1,00	-0,04	-0,60	-0,78	0,38	0,37	0,29	0,08	0,08	0,14	0,01
CrU	-0,32	-0,04	1,00	0,13	0,62	-0,10	0,39	0,24	0,12	0,45	0,05	0,08
GF	0,47	-0,60	0,13	1,00	0,54	-0,33	0,04	0,13	-0,20	-0,23	0,14	0,01
CReabsor	-0,26	-0,78	0,62	0,54	1,00	-0,35	0,49	0,33	-0,03	0,21	0,06	0,00
SOD	-0,17	0,38	-0,10	-0,33	-0,35	1,00	0,14	0,03	0,12	0,15	0,07	0,23
KatP	0,43	0,37	-0,39	-0,04	-0,49	0,14	1,00	0,85	0,14	-0,29	0,07	0,05
KatU	0,57	0,29	-0,24	0,13	-0,33	0,03	0,85	1,00	0,03	-0,26	0,03	0,00
MDAP	-0,13	0,08	0,12	-0,20	-0,03	0,12	0,14	0,03	1,00	0,33	0,77	0,29
MDAU	-0,31	0,08	0,45	-0,23	0,21	0,15	0,29	0,26	0,33	1,00	0,15	0,54
DCP	-0,03	-0,14	0,05	0,14	0,06	-0,07	0,07	0,03	0,77	0,15	1,00	0,42
DCU	-0,06	-0,01	0,08	0,01	-0,00	0,23	0,05	0,00	0,29	0,54	0,42	1,00

Table 11. Regression Summary for Dependent Variable: Glomerular Filtration

N=50	R=,562; R^2=,316; Adjusted R^2=,255; F(4,45)=5,19; p<,0016; Std.Error of estimate:76					
	Beta	St. Err. of Beta	B	St. Err. of B	t(45)	p-value
Intercpt			165,76	85,49	1,94	0,059
SOD	-0,215	0,128	-2,02	1,20	-1,68	0,101
MDA	-0,676	0,201	-2,27	0,67	-3,37	0,002
DC	0,644	0,200	125,39	38,97	3,22	0,002
KATU	0,140	0,123	311,18	274,27	1,13	0,263

It was found that glomerular filtration is downregulated by superoxide dismutase (r=-0,33) and malondialdehyde (r=-0,20). Together with catalase and diene conjugates included in the regression model, the degree of determination reaches 31,6% (Table 11 and Fig. 2).



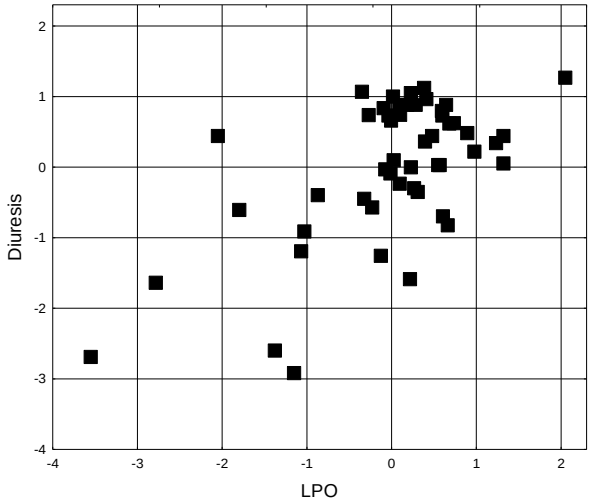
R=0,562; R^2=0,316; $\chi^2_{(4)}$=17,4; p=0,0016; Λ Prime=0,684

Fig. 2. Scatterplot of canonical correlation between lipids peroxidation parameters (X-line) and glomerular filtration (Y-line)

The diuresis is also downregulated by malondialdehyde (r=-0,31) and SOD (r=-0,17), but upregulated by catalase (r=0,57). The degree of determination is 37,8% (Table 12 and Fig. 3).

Table 12. Regression Summary for Dependent Variable: Diuresis

N=50	R=,614; R^2=,378; Adjusted R^2=,337; F(3,46)=9,30; p<,00006; Std.Error of estimate:,665					
	Beta	St. Err. of Beta	B	St. Err. of B	t(46)	p-value
Intercpt			1,2571	0,7084	1,77	0,0826
SOD	-0,165	0,118	-0,0143	0,0102	-1,39	0,1697
KATU	0,533	0,121	10,9658	2,4833	4,42	0,0001
MDAU	-0,149	0,122	-0,0038	0,0031	-1,22	0,2274



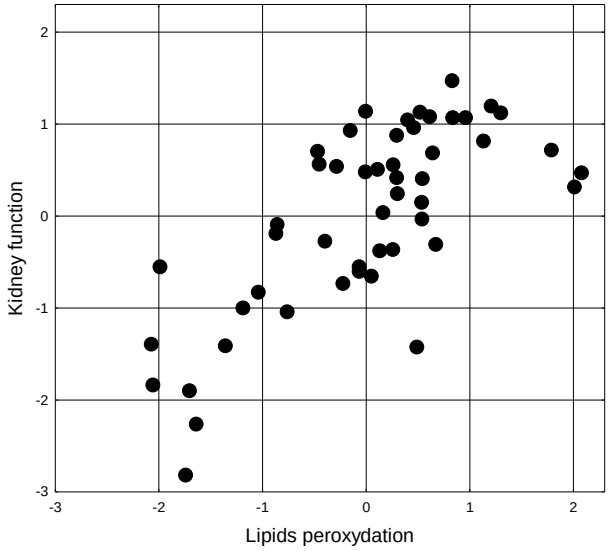
R=0,614; R^2=0,378; $\chi^2_{(3)}$=22,0; p<10^{-4}; Λ Prime=0,622

Fig. 3. Scatterplot of canonical correlation between lipids peroxidation parameters (X-line) and diuresis (Y-line)

Taken together, lipoperoxidation parameters determine kidney function parameters by 52,8% (Table 12 and Fig. 4).

Table 12. Factor structure of the lipids peroxidation and kidney function canonical Roots

Lipids peroxidation	**R**
Malondialdehyde Urine	**0,718**
Malondyaldehide Plasma	**0,268**
Katalase Plasma	**-0,580**
Katalase Urine	**-0,463**
Diene conjugates Plasma	**-0,111**
Superoxide Dismutase	**-0,119**
Kidney function	**R**
Glomerular Filtration	**-0,350**
Diuresis	**-0,495**
Creatinine Urine	**0,743**
Canalicular Reabsorbtion	**0,517**
Creatinine Plasma	**-0,064**



R=0,727; R^2=0,528; $\chi^2_{(35)}$=80; p<10^{-4}; Λ Prime=0,153

Fig. 4. Scatterplot of canonical correlation between lipids peroxidation (X-line) and kidney function (Y-line) parameters

CONCLUSION

The obtained data indicate the role of lipoperoxidation as a accompaniment of chronic stress in the regulation of kidney functions.

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

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