Arch. Tierz., Dummerstorf **45** (2002) 3, 307-314

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Influence of exogenous glutathione (GSH), as stressfactor, on the activity of lysosome enzymes in some organs of mice

Summary

The studies were carried on 30 random 8-week old Swiss male mice. The mice of I group received peritoneally 100 μg/g b.w. of reduced glutathione (GSH) in 250 μl 0.9% NaCl , II group 200 μg/ g of GSH in 250 μl 0.9% NaCl and mice of III control group received 250 μl of 0.9 % NaCl. In the lysosomal fraction of the liver, kidney and the skeletal muscle the activity of nine lysosomal enzymes were estimated. GSH injections caused in the liver a statistically confirmed increase of activity of estimated hydrolases, only the activity LL decreased significantly and NAGL did not change. After injection of both GSH doses increased significantly the activity LAP, Cat. D and L, AP, BGAL, BGLU and NAGL in the kidney. After injection 200 μg/g of GSH increased significantly activity AAP but an activity EL and LL decreased. In the skeletal muscle was observed a statistically confirmed increase of AP, LL, BGAL and NAGL activity, only the BGLU and Cat. D and L (after 100 μg/g dose) activity decreased.

Key Words: glutathione, kidney, liver, lysosomal enzymes, mice, muscle

Zusammenfassung

Titel der Arbeit: **Einfluss von exogenem Glutathion als Stressfaktor auf die Aktivität der lysosomalen Enzyme in der Leber, Niere und im Muskel bei Mäusen**

Nach 100 μg/g und 250 μg/g (Körpergewicht) Glutathioninjektion (GSH) wurde in der Leber eine Zunahme der lysosomalen Hydrolasenaktivität (ausgenommen lysosomale Lipase LL, welche sich verminderte) beobachtet. In der Niere erhöhte sich die Aktivität von LAP, Kat. D und L, AP, BGAL, BGLU und NAGL Enzymen. Im Muskel erhöhte sich die AP, LL, BGAL und NAGL Aktivität während sich BGLU, KAT D und L (nach 100 μg/g Dosis) verminderten.

Schlüsselwörter: Glutathion, Niere, Leber, lysosomale Enzyme, Maus, Muskel

Introduction

Glutathione (L- γ-glutamyl-L-cysteinyl-glycine; GSH) has emerged to be one of the most fascinating molecules present in all animal cells often in quite high (mM) concentrations. It is known to have multifaceted physiological functions including: antioxidant defense and detoxification of electrophilic xenobiotics (SIES, 1999); modulation of redox regulated signal transduction and gene expression (ITO et al., 1998); storage and transport of cysteine (SCHOFFNER et al., 1995); regulation of cell proliferation (HUANG et al., 1998); regulation of immune response (NAKAGAWA and RUDENSKY, 1999). The glutathione status may be used as a biological marker of aging (NOKATA et al., 1996). The ratio of GSH/GSSG is used as an index of the oxidative stress and may be the exponent of the organism's homeostasis (SAGARA et al., 1998). The function of several physiological proteins, including cellular enzymes and signaling molecules, is regulated by thiol-disulfide exchange between protein thiols and GSH mainly (FREEMAN et al., 1997). It was observed that oxidative

processes determined in sarcoplasmic and myofibillar proteins could be respectively implicated in meat colour stability (myoglobin autooxidation) and tenderness changes during maturation of meat (RENERRE et al., 1996).

Lysosomes are membrane-bound organelles with an acidic internal milieu containing hydrolytic enzymes for degradation of proteins, lipids, nucleic acids and saccharides. The lysosomal compartment has multiple functions and it may be indicator of adaptative abilities (HICKS, 1995). It is known that lysosomes are especially sensitive to oxidative stress (LI et al., 1998). It was investigated that hydroxyl radicals destabilize the lysosomal membranes and thereby cause leakage of lysosomal enzymes to the cytosol with ensuing cellular degeneration or even death (HELLQUIST et al., 1997; ÖLLINGER, 2000).

The aim of this study was in connection with these data to investigate the influence of exogenous glutathione (GSH) on the activity of lysosomal hydrolases in hepatocytes, kidney and skeletal muscle of mice.

Material and Methods

The studies were carried out on 30 Swiss male mice of body weight 20-22 g, aged 8 weeks, chosen at random from the population maintained at the Institute of Genetics and Animal Breeding, Polish Academy of Sciences in Jastrzębiec. The animals were kept in standard cages of the farm at temperature 22 ^{0} C, under 12 hours of light and 12 hours of darkness. They were fed with a homogenous industrial fodder (16% of protein) made by Animal Food Company in Łomna near Warsaw. The fodder and the water were available ad libitum.

The animals were divided into three groups, 10 mice in each. GSH solved in 0.9% NaCl solution was administered by peritoneal injection – 100 μg/g of body weight in 250 μl of solution for group I and 200μg/g of body weight in 250 μl of solution for group II. Animals of group III, treated as control, received an injection of 250 μl of 0.9% NaCl (pure).

All the injections were performed over a period of 7 days, twice daily, at 8.30 a.m. and 4.30 p.m.

After decapitation operated, 16 hours after last injection, immediately the liver, kidney and skeletal muscle of left thigh were taken and subjected to perfusion in 0.9% NaCl solution at +5^{0}C. After grinding tissues were placed in a 0.1 M phosphate buffer, pH 7.0, in ratio 1g/6 ml. Next, the liver and the kidney were homogenized in the Potter homogenizer with a teflon piston and the skeletal muscle was homogenized with a glass piston at 200 rotations/min. with four up-down movements. These homogenates were centrifuged at Sorvall centrifuge for 10 minutes at 3500 rpm. and again for 20 minutes at 14000 rpm. The precipitate containing lysosomes was diluted in a 0.1 M phosphate buffer, pH 6.0 with Triton X-100 and finally frozen at -20^0 C. The clear supernatant was subjected to determination of the activities of lysosomal enzymes.

In the lysosomal fractions of the liver, the kidney and the skeletal muscle was determine the activity: alanylaminopeptidase (AAP – E.C. 3.4.11.2); leucylamino-peptidase (LAP - E.C. 3.4.11.1); cathepsin D and L (Cat. D and L – E C. 3.4.23.5); acid phosphatase (AP – E.C. 3.1.3.2); lysosomal esterase (EL – E.C. 3.1.1.2); lysosomal lipase (LL – E.C. 3.1.1.13); beta – galactosidase (BGAL – E.C. 3.2.1.23); beta – glucosidase (BGLU – E.C. 3.2.1.21); N-acetyl-beta-glucosaminidase (NAGL – E.C. 3.2.1.30).

The activity of AP, EL, LL, BGAL, BGLU and NAGL was determined spectrophotometrically as 4-nitrophenyl derivatives at 420 nm according to BARRETT's and HEATH`s micro-method (1972). The activity of LAP and AAP was determined spectrophotometrically as Fast Blue BB salt (4-benzoyloamino-2.5–diethoxybenzene-diazonium chloride) derivatives at 540 nm according to the method of MC DONALD and BARRETT (1986). The activity of cathepsin D and L was determined spectrophotometrically as 2% azocasein in 6 mol urea derivatives at 366 nm according to the method of LANGNER et al. (1973). Spectrophotometer Lambda Bio 20/1998 (Perkin Elmer) was used. The reduced glutathione (GSH) and substrates for enzyme and protein determinations were purchased from SIGMA.
In the lysosomal fractions the protein concentration was determined by the LOWRY's method modified by KIRSCHKE and WIEDERANDERS, (1984). The activity of lysosomal hydrolases was expressed in nmol/mg of protein/hour. Moreover we have determined GSH level 4 and 16 hours after injection according to Ellman's method (1959) in the liver of 10 control mice which were peritoneally injected with 0.9% solution of NaCl and 10 experimental animals injected with solution of 100μg/g b.w. of GSH.
The results obtained were analysed by analysis of variance.
This experiment has been realized according to the agreement of Institute Ethic Commission for Animal Research.

Results

The results are presented in Tables 1-5. The percentage changes of the activity were calculated accepting values obtained for control animals as 100%.

Table 1
The activity (LSM ± SEM) and percentage changes of lysosomal enzymes (nmol/ mg of protein/ hour) in the liver of mice after GSH injection; (n in each subgroup = 10)

	Control		Experimental groups			
Enzymes	0,9% NaCl (C)	%	100 μg/g GSH (G1)	%	200 μg/g GSH (G2)	%
AAP	113.42 ± 6.09	100	164.77 ± 7.53	145	212.50 ± 12.19	187
LAP	73.41 ± 2.85	100	361.25 ± 17.18	492	390.75 ± 17.03	532
Cathepsin D and L	16.89 ± 0.88	100	26.62 ± 0.91	158	21.82 ± 0.71	129
AP	2648.83 ± 71.92	100	3177.47 ± 94.58	120	3738.56 ± 82.28	141
EL	707.39 ± 26.61	100	1346.07 ± 46.66	190	1447.67 ± 44.37	205
LL	1972.82 ± 89.46	100	1685.17 ± 93.13	85	1293.79 ± 49.44	66
BGAL	162.97 ± 5.20	100	208.22 ± 10.02	128	215.99 ± 7.34	133
BGLU	118.89 ± 5.48	100	227.23 ± 10.30	191	219.03 ± 8.15	184
NAGL	1499.52 ± 65.08	100	1400.10 ± 67.46	93	1417.05 ± 77.86	95

Table 1 informs that after both GSH doses in the liver increased significantly an activity of all studied lysosomal enzymes with the exception NAGL [93% and 95%]. Only the activity LL decreased significantly [85% and 66%].
In kidney (Table 2) was observed a significant increase of activity LAP, Cat. D and L, AP, BGAL, BGLU and NAGL. The larger GSH dose caused a significant increase of AAP activity and the decrease of EL and LL activity.

Table 2
The activity (LSM ± SEM) and percentage changes of lysosomal enzymes (nmol/ mg of protein/ hour) in kidney of mice after GSH injection; (n in each subgroup = 10)

	Control		Experimental groups			
Enzymes	0,9% NaCl (C)	%	100 μg/g GSH (G1)	%	200 μg/g GSH (G2)	%
AAP	1426.33 ± 68.17	100	1323.62 ± 76.06	93	2983.70 ± 71.45	209
LAP	1356.05 ± 63.57	100	2165.97 ± 72.55	160	1808.68 ± 41.45	133
Cathepsin D and L	9.86 ± 0.36	100	12.94 ± 0.46	131	13.65 ± 0.44	138
AP	2150.30 ± 90.58	100	8333.86 ± 604.51	388	5759.62 ± 291.57	268
EL	836.88 ± 21.80	100	781.24 ± 31.96	93	667.95 ± 19.40	80
LL	1481.57 ± 65.69	100	1482.18 ± 77.01	100	934.86 ± 47.83	63
BGAL	153.73 ± 5.65	100	213.16 ± 6.19	139	264.66 ± 13.08	172
BGLU	87.99 ± 3.20	100	134.19 ± 4.02	153	126.10 ± 3.70	143
NAGL	1431.30 ± 45.84	100	1769.61 ± 71.49	124	2047.16 ± 67.66	143

Table 3
The activity (LSM ± SEM) and percentage changes of lysosomal enzymes (nmol/ mg of protein/ hour) in the muscle of mice after GSH injection; (n in each subgroup = 10)

	Control		Experimental groups			
Enzymes	0,9% NaCl (C)	%	100 μg/g GSH (G1)	%	200 μg/g GSH (G2)	%
AAP	85.86 ± 4.31	100	75.63 ± 3.86	88	86.07 ± 4.76	100
LAP	57.76 ± 2.57	100	62.01 ± 2.95	107	61.06 ± 2.82	106
Cathepsin D and L	3.00 ± 0.09	100	2.33 ± 0.13	78	3.56 ± 0.20	119
AP	426.57 ± 12.98	100	537.09 ± 23.33	126	629.53 ± 25.97	148
EL	107.42 ± 4.17	100	109.79 ± 3.42	102	121.96 ± 5.02	114
LL	3.33 ± 0.20	100	18.02 ± 0.89	541	8.41 ± 0.43	253
BGAL	4.73 ± 0.21	100	6.22 ± 0.28	131	7.77 ± 0.44	164
BGLU	10.62 ± 0.57	100	9.42 ± 0.39	89	9.00 ± 0.21	85
NAGL	207.84 ± 10.64	100	252.56 ± 14.73	122	277.51 ± 12.06	134

Table 4
The GSH concentration (μM/mg of protein) in the liver of control and experimental mice 4 and 16 hours after of exogenous GSH injection (100 μg/g b.w.); (n in each group = 10)

Injection	Control		Experimental groups	
	4h	16h	4h	16h
0.9% NaCl (250 μl)	12.2[1] 11.6 - 12.9 (100%)	11.7[2] 10.9 - 12.1 (96%)		
GSH (250 μl)			21.1[1] 16.7 - 23.5 (173%)	15.2[2] 14.6 - 15.8 (130%)

1-1; 2-2; the statistically confirmed differences; P< 0.001

In the skeletal muscle (Table 3) after administration of first and second GSH dose was observed an increase activity of AP, LL, BGAL and NAGL. The smaller dose decreased of Cat. D and L [78%] activity but the larger GSH dose increased it [119%]

and EL activity [114%] somewhat unsignificantly. BGLU activity decreased significantly after two doses of GSH injection [89% and 85%].
Table 4 revealed, that GSH concentration in the liver of control mice after NaCl injections maintained on equal level after 4 and 16 hours [12.2 and 11.7 μM] but in experimental animals it increased to 173% after 4 hours and to 130% after 16 hours [21.1 and 15.2 μM].

Table 5
The analysis of variance for the examined lysosomal enzyme activities

Enzyme	AAP	LAP	CAT D,L	AP	EL	LL	BGAL	BGLU	NAGL
Liver									
C - G1									
MS	13184.6	414248	472.59	1397338	2039574	413718	10240.5	58657.8	49426.7
F value	16.33	209.42	67.03	20.07	126.06	6.49	16.93	86.91	1.00
F	0.0004***	0.0001***	0.0001***	0.0001***	0.0001***	0.0169*	0.0003***	0.0001***	0.3265
C - G2									
MS	49080.3	503520	121.43	5937612	2740102	2305395	14056.7	50144.1	34011.5
F value	60.77	254.55	17.22	85.28	169.36	36.17	23.24	74.26	0.69
F	0.0001***	0.0001***	0.0003***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.4144
G1 - G2									
MS	11388.4	4352.13	114.91	1574099	51614.8	765876	301.55	335.87	1436.34
F value	14.10	2.20	16.3	22.61	3.19	12.02	0.50	0.50	0.03
F	0.0008***	0.1496	0.0004***	0.0001***	0.0853	0.0018**	0.4862	0.4867	0.866
Kidney									
C - G1									
MS	52754.9	3279852	47.49	1911822	15477.9	1.88	17659	10670.3	572289
F value	1.02	89.27	26.86	125.05	2.48	0.001	21.95	80.01	14.56
F	0.3218	0.0001***	0.0001***	0.0001***	0.127	0.9947	0.0001***	0.0001***	0.0007***
C - G2									
MS	1212697	1024383	71.74	6513573	142675	1494465	61527.3	726101	1896418
F value	234.16	27.88	40.57	42.61	22.86	35.77	76.47	54.45	48.26
F	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***	0.0001***
G1 - G2									
MS	1377942	638270	2.49	3313378	64167.5	1497818	13261.8	327.1	385153
F value	266.07	17.37	1.41	21.67	10.28	35.85	16.48	2.45	9.80
F	0.0001***	0.0003***	0.2455	0.0001***	0.0034**	0.0001***	0.0004***	0.129	0.0042**
Muscle									
C - G1									
MS	523.78	90.40	2.26	61072.2	28.27	1078.5	11.09	7.20	10003
F value	2.80	1.17	10.61	13.21	0.16	320.55	10.51	4.14	6.31
F	0.106	0.2896	0.0030**	0.0012**	0.6957	0.0001***	0.0031**	0.0518	0.0183*
C - G2									
MS	0.21	54.35	1.56	205948	1058.22	129.23	46.06	13.09	24268.8
F value	0.001	0.70	7.34	44.54	5.85	38.41	43.68	7.52	15.30
F	0.9738	0.4096	0.0116*	0.0001***	0.02*	0.0001***	0.0001***	0.0107*	0.0006***
G1 - G2									
MS	544.76	4.56	7.58	42719.3	740.5	461.09	11.95	0.87	3110.3
F value	2.91	0.06	35.6	9.24	4.09	137.04	11.34	0.50	1.96
F	0.0995	0.8101	0.0001***	0.0052*	0.053	0.0001***	0.0023**	0.4846	0.1728

*P< 0.01; **P< 0.05; ***P< 0.001; C-G1- between control group and group I (100μg/g GSH), C-G2 between control group and group II (200 μg/g GSH), G1-G2- between group I and group II

We can see from Table 5 that F values for the majority of the examined enzymes were statistically high confirmed [P<0.001].

Discussion

The intracellular synthesis of glutathione and its extracellular and intracellular degradation take place by the reactions of the γ-glutamyl cycle and a system of specific enzymes (KELLY, 1999). The liver is very active in its synthesis de novo. It is limited by γ-glutamylcysteine synthetase (GCS) activity. However, the enzymatic capacity of the kidney to synthesis and degradation of the glutathione may be higher than that in the liver. Glutathione may be exported from the liver to the plasma and another tissues and it is controled by γ-glutamyltranspeptidase (WANG et al., 1998).
Supplementation of free GSH has to cause inhibition of GCS by high GSH level (MEISTER, 1991). However, several studies have reported that GSH administration can increase cysteine transport into the cells and it may change the enzymatic control of cell (LEEUWENBURGH and JI, 1998). Besides it has been suggested that the esters N-acetyl cysteine and iso-propyl-SG are easy transported into the living cells without specific transporter and can be the reductants as same as reduced glutathione (ENKVETCHAKUL and BOTTLE, 1995).
The results obtained indicate that the administration of exogenous GSH (both doses) caused the significant differences in the activity of the enzymes examined as compared to the control values. The studies conducted by KOŁĄTAJ et al. (1998), LOMBARDO (1996) indicate, that the lysosomal system constitutes the defence lines of the cells against stressors and it responds to disturbances in cell homeostasis.
Same studies indicate that sulphydryl groups have protective effect at lysosomal membrane and lysosomal sulphate transport is dependent upon these groups (BRUNK, et al., 1996; CHOU et al., 1998).
Differences in the activity of lysosomal hydrolases estimated in the control animals were the result of their tissue origin (FERLAND et al., 1990). In all estimated organs the biggest activity was observed for AP. This enzyme is typical for the lysosomal fraction (CHEN and CHEN, 1988).
It has been reported that GSH is transported out of renal proximal tubular across the brush-border membrane by a membrane potential-sensitive carrier (LASH et al., 1998). The highest activity of AAP and LAP was observed in the kidney, where they are placed first of all in the epithelium of renal tubule and are included in protein resorption from the tubuli fluid (MANTLE, 1991). NAGL is a hydrolase, which participates in the breakdown of glycoproteins. The activity of this enzyme proved higher in the liver and kidney than in the muscle. Some studies have reported that the GSH depletion can modulate processes of glycosylation (mainly proteins) (JAIN, 1998) and different doses of GSH can change activity of estimated lysosomal glycosydases.
Beside other numerous functions, the cell uses GSH for importing of γ- glutamyl-aminoacids and for the intracellular transport of cysteine, what among others, may cause an increase of the cathepsin activity. An increase of the proteolytic activity is linked with the activity of cathepsins and aminopeptidases too. Any type of stress burden results the significant fluctuations of cathepsin activity (SCHMIDT et al., 1993).
Activity of lysosomal esterases is an indicator of the cell lipolytic ability too. An increase of reduction processes caused by the administered GSH may decrease the rate of β- oxidation of fatty acids what, in turn, may result a decrease LL and EL activity in the kidney and LL in the liver (TIEN et al., 1982).

An exogenous reduced glutathione (GSH), independent from doses, introduced to an organism can demonstrate that the lysosomal enzymatic system plays an important role in the adaptative physiological response. These observed changes of the activity of examined lysosomal enzymes are probably on the basis of increasing of reducing environment in the cell. They are actually difficult to the explanation but they can have the pharmacological and physiological importance. It was also demonstrated a certain "organ specificity". The enzyme activity changes after GSH administration were somewhat differently in liver, kidneys and muscles, but we do not yet know the reasons for this differentiation.

References

BARRETT, A. J.; HEATH, M.F.:
Lysosomal enzymes. In: Lysosomes; A Laboratory Handbook. DINGLE, J. T. (Ed), North-Holland Publ. Co., Amsterdam, (1972), 19-135

BRUNK, U.T.; ZHANK, H.; ROBERG, K.; ÖLLINGER K.:
Lethal hydrogen peroxide toxicity involves lysosomal iron-catalyzed reactions with membrane damage. Redox Report **1**(1996), 267-277

CHEN, C.H.; CHEN, S.C.:
Evidence of acid phosphatase in the cytoplasma as distinct entity. Arch. Biochem. Biophs. **262** (1988), 427-438

CHOU, H.F.; PASSAGE, M.; JONAS, A.J.:
Lysosomal sulphate transport is dependent upon sulphydryl groups. Biochem. J. **330** (1998), 713-717

ELLMAN, G.L.:
Tissue sulfhydryl groups. Arch. Biochem. Biophys. **82** (1959), 70-77

ENKVETCHAKUL, B.; BOTTJE, W.G.:
Influence of Diethyl Maleate and Cysteine on Tissue Glutathione and Growth in Broiler Chickens. Poultry Sci. **74** (1995), 864-873

FERLAND, G.; PERA, A.; AUDET, M.; TUCHWEBER, B.:
Characterization of liver lysosomal enzyme activity in hepatocytes, Kupffer and endothelial cells during aging: effect of dietary restriction. Mech. Ageing Dev. **56** (1990), 143-154

FREEMAN, M.L.; HUNTLEY, S.A.; MEREDITH, M.J.; SENISTERRA, G.A.; LEPOCK, J.:
Destabilization and denaturation of cellular protein by glutathione depletion. Cell Stress and Chaperones **2** (1997), 191-198

HELLQUIST, H.B.; SVENSSON, I.; BRUNK U.T.:
Oxidant-induced apoptosis: a consequence of lethal lysosomal leak. Redox Rep. **3** (1997), 65-70

HICKS, J.J.:
Lysosomal system in hormonal mechanism. Ginecol. Obstet. Mex. **63** (1995), 68-73

HUANG, Z.Z.; LI, H.; CAI, J.; KUHLENKAMP, J.; KAPLOWITZ, N.; LU, S.C.:
Changes in glutathione homeostasis during liver regeneration in the rat. Hepathology **27** (1998), 147-153

ITO, H.; OKAMOTO, K.; KATO, K.:
Enhacement of expression of stress proteins by agents that lower the levels of glutathione in cells. Biochem. Biophys. Acta **1397** (1998), 223-230

JAIN, S.K.:
Glutathione and glucose-6-phosphate dehydrogenase deficiency can increase protein glycosylation. Free Rad. Biol. Med. **1** (1998), 197-201

KELLY, F.J.:
Glutathione: in Defence of the Lung. Food Chem. Toxicol. **37** (1999), 963- 966

KIRSCHKE, H.; WIEDERANDERS, B.:
Methoden zur Aktivitätsbestimmung von Proteinases. Martin-Luther-Universität, Halle-Wittenberg, Wissenschaftl. Beitr. Halle/Saale, (1984), 11-17

KOŁĄTAJ, A.; SOMMER, A.; WITEK, B.; NITRAY, J.; FLAK, P.:
The effect of exogenous glucose on the activity of lysosomal enzymes, the glucose and insulin concentration in the blood plasma of young bulls. Arch. Tierz., Dummerstorf **41** (1998), 371-377

LANGNER, J.; WAKIL, A.; ZIMMERMANN, M.; ANSORGE, S.; BOHLEY, H.; KIRSCHKE, H.; WIEDERANDERS, B.:
Aktivitätsbestimmung proteolytischer Enzyme mit Azokasein als Substrat. Acta Biol. Med. Germanica **31** (1973), 1-18

LASH, L.H.; VISARIUS, T.M.; SALL, J.M.; QIAN, W.; TOKARZ, J.J.:
Cellular and subcellular heterogeneity of glutathione metabolism and transport in rat kidney cells. Toxicology **130** (1998), 1-15

LEEUWENBURGH, CG.; JI, L.L.:
Glutathione and glutathione ethyl ester supplementation of mice alter glutathione homeostasis during exercise. J. Nutr. **128** (1998), 2420-2427

LI, W.; YUAN, X. M.; BRUNK, U.T.:
OxLDL-induced macrophage cytotoxicity is mediated by lysosomal rupture and modified by intralysosomal redox-active iron. Free Rad. Res. **29** (1998), 389-398

LOMBARDO, A.; BAIRATI, C.; GOI, G.; ROGGI, C.; MACCARIANI, L.; BOLLINI, D.; BURLINA, A.:
Plasma lysosomal glycohydrolases in a general population. Clin. Chim. Acta **247** (1996), 39-49

MANTLE, D.:
Characterization of dipeptidyl and tripeptydyl aminopeptidases in human kidney soluble fraction. Clin. Chim. Acta **196** (1991), 135-142

MC DONALD, J.K.; BARRETT A.J.:
Exopeptidases. In: Mammalian Proteases: A Glossary and Bibliography, 2, eds. London, UK, Academic Press (1986), 111-144

MEISTER, A.:
Glutathione deficiency produced by inhibition of its synthesis and its reversal: applications in research and therapy. Pharmacol. Ther.**51** (1991), 155-194

NAKAGAWA, T.Y.; RUDENSKY, A.Y.:
The role of lysosomal proteinases in MHC class II-mediated antigen processing and presentation. Immunological Rev. **172** (1999), 121-129

NOKATA, K.; KAWASE, M.; OGINO, S.; KINOSHITA, C.; MURATA, H.; SAKAUE, T.; OGATA, K.; OHMORI, S.:
Effect of age on levels of cysteine, glutathione and related enzyme activities in livers of mice and rats and attempt to replenish hepatic glutathione level of mouse with cysteine derivatives. Mech. Ageing Develop. **90** (1996), 195- 207

ÖLLINGER, K.:
Inhibition of cathepsin D prevents free-radical-induced apoptosis in rat cardiomyocytes. Arch. Biochem. Biophys., **373** (2000), 346-351

RENERRE, M.; DUMONT, F.; GATELLIER, PH.:
Antioxidant Enzyme Activities in Beef in Relation to Oxidation of Lipid and Myoglobin. Meat Sci. **43** (1996), 111-121

SAGARA, Y.; DARGUSCH, R.; CHAMBERS, D.; DAVIS, J.; SCHUBERT, D.; MAHER, P.:
Cellular mechanisms of resistance to chronic oxidative stress. Free Radical Biol. Med. **24** (1998), 1375-1390

SCHMIDT, M.; KOŁĄTAJ, A.; BULLA J.:
Die lysosomalen Enzyme der Leukozyten als Adaptationsindikator einiger Stresseinwirkungen beim Schwein. Beiträge, Kolloquium „Adaptation bei Nutztieren" (Dummerstorf), **1** (1993), 3-11

SCHOFFNER, J. M.; VOLJAVEC, A. S.; DIXON, J.; KAUFMAN, A.; WALLACE, D. C.; MITCH, E. E.:
Renal amino acid transport in adult with oxidative phosphorylation diseases. Kidney Int. **47** (1995), 1101-1107

SIES, H.:
Glutathione and its role in cellular functions. Free Rad. Biol. Med. **27** (1999), 916-921

TIEN, M.; BUCHER, J. R.; AUST, S. D.:
Thiol-dependent lipid peroxidation. Biochem. Biophys. Res. Commun. **107** (1982), 279- 285

WANG, S.; BOTTJE, W.G.; CAWTHON, D.; EVENSON, C.; BEERS, K.; MC NEW, R.:
Hepatic export of glutathione and uptake of constituent amino acids, glutamate and cysteine in broilers *in vivo*. Poultry Sci. **77** (1998), 1556-1564

Received: 2000-11-27

Accepted: 2002-04-15

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