TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2015) 39: 129-138
© TÜBİTAK
doi:10.3906/biy-1405-52

Activity of antioxidant enzymes in the liver of wild boars (*Sus scrofa*) from a selenium-deficient area depending on sex, age, and season of the year

Dorota JANKOWIAK[1], Renata PILARCZYK[2], Radosław DROZD[1,*], Bogumiła PILARCZYK[3], Agnieszka TOMZA-MARCINIAK[3], Gabriela WYSOCKA[4], Izabella RZĄD[5], Arleta DROZD[6], Jarosław KUBA[3]

[1]Department of Immunology, Microbiology, and Physiological Chemistry, West Pomeranian University of Technology, Szczecin, Poland
[2]Laboratory of Biostatistics, West Pomeranian University of Technology, Szczecin, Poland
[3]Department of Animal Reproduction, Biotechnology, and Environmental Hygiene, West Pomeranian University of Technology, Szczecin, Poland
[4]Department of Horticulture, Laboratory of Storage and Processing, Faculty of Environmental Studies and Agriculture, West Pomeranian University of Technology, Szczecin, Poland
[5]Department of Ecology and Environmental Protection, University of Szczecin, Szczecin, Poland
[6]Department of Biochemistry and Human Nutrition, Pomeranian Medical University, Szczecin, Poland

Received: 21.05.2014 • **Accepted:** 27.07.2014 • **Published Online:** 02.01.2015 • **Printed:** 30.01.2015

Abstract: The aim of this study was to compare the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and total and selenium-dependent glutathione peroxidase (total GSH-Px and Se-GSH-Px) in the liver of wild boars (*Sus scrofa*) selected from a selenium-deficient area with respect to season of the year, sex, and age. Only season had a significant effect on liver Se content. Liver selenium concentration in the wild boars was highest in spring (0.292 µg/g wet weight) and lowest in winter (0.185 µg/g wet weight) ($P < 0.05$). The highest mean activity of total GSH-Px, Se-GSH-Px, and CAT (404.0, 309.9, and 1170.0 U/mg protein, respectively) was found in the spring season, and the respective highest mean activity of SOD was found during autumn (31.5 U/mg protein). Females showed markedly higher mean activities of total GSH-Px (388.2 and 314.7 U/mg protein) and CAT (1151.0 and 765.8 U/mg protein) compared to males. In comparison to other groups of animals, those aged between 1 and 1.5 years were characterized by higher mean activity of total GSH-Px and Se-GSH-Px, and those over 2 years of age showed higher activity of CAT and SOD. The analysis showed that the strongest oxidative stress and the greatest risk of peroxidative damage to the liver occurred during the winter.

Key words: Liver, wild boar, selenium, antioxidant enzymes, seasons of the year

1. Introduction

The liver is particularly susceptible to the deleterious effects of reactive oxygen species (ROS) and their derivatives, because of its role in many aspects of bodily function. Normal liver function is one of the major requirements for the organism's adaptation to the changing conditions of the external environment and for the maintenance of homeostasis (Van den Berghe, 1991). The crucial role of the liver in energy homeostasis, biotransformation of numerous endogenous molecules, and xenobiotics is determined by intense aerobic metabolism. The result is a great abundance and variety of ROS-generating systems (Limón-Pacheco and Gonsebatt, 2009). The main source of ROS in liver tissues is the mitochondrial respiratory chain. The initial substrate for the production of all endogenous ROS, including free radicals and their derivatives, is the superoxide radical anion ($\bullet O_2^-$). Hence, superoxide dismutase (SOD, EC 1.5.1.1) is the primary enzyme that protects cells against the effects of free radicals. This enzyme catalyzes the dismutation of the superoxide radical anion to hydrogen peroxide (H_2O_2). While not being a free radical, this molecule is the main source of hydroxyl radical (•OH), which is highly reactive and toxic to cellular macromolecules (Limón-Pacheco and Gonsebatt, 2009). Hydrogen peroxide is reduced to water and molecular oxygen due to glutathione peroxidase (GSH-Px, EC 1.11.1.9) and/or catalase (CAT, EC 1.11.1.6) (Lykkesfeldt and Svendsen, 2007). GSH-Px has a greater affinity for H_2O_2 than does CAT, eliminating H_2O_2 at its already low concentrations in the cell in contrast to CAT, which acts when H_2O_2 concentrations are high (Simmons and Jamall, 1988). Glutathione peroxidases are glutathione-dependent enzymes. Both selenium-dependent (Se-GSH-Px, EC 1.11.1.9) and nonselenium-dependent (non-Se-

* Correspondence: rdrozd@zut.edu.pl

GSH-Px, EC 1.11.1.7) glutathione peroxidases are present in mammals (Davies, 2000). The former reduces both hydrogen peroxide and organic peroxides, while the latter only reduces organic peroxides, including lipid peroxides. It should be noted, however, that Se-GSH-Px is more effective in eliminating organic hydroperoxides compared to non-Se-GSH-Px due to the lower K_M value for these substrates (Kim and Mahan, 2003). In most animal cells, mitochondria lack CAT, which in effect makes Se-GSH-Px the only enzyme that eliminates hydrogen peroxide from these organelles (Antunes et al., 2002). The activity of this enzyme is determined by the presence in its catalytic center of reduced selenium found in selenocysteine. The liver is the main organ responsible for the homeostasis of selenium in the body. Selenium is stored in hepatocytes, which synthesize and distribute not only Se-GSH-Px but also other enzymatic selenoproteins (Gromer et al., 2005; Papp et al., 2007).

Issues concerning oxidative stress in wild animals, changes in the activity of the enzymes of the antioxidant triad related to succession of the seasons and thus reproductive seasonality, changes in physical environmental conditions, and access to food and its quality have received little attention. Therefore, this study was designed to compare the activity of antioxidant enzymes (SOD, CAT, total GSH-Px, and Se-GSH-Px) in the liver of wild boars from a selenium-deficient area depending on season of the year, sex, and age.

2. Materials and methods

2.1. Materials

The materials used in this study consisted of livers harvested from 34 wild boars (*Sus scrofa*) that were shot by hunters during the hunting season of 2008–2009 in compliance with hunting regulations in northwestern Poland. The material was stored at –80 °C until chemical analysis was performed. The wild boars examined were aged between 0.5 and 4 years. Differences between male and female boars were determined in animals older than 1 year (16 males and 13 females). Age and sex were determined after the animals were shot. The age of the animals was estimated using fur characteristics (Lochman, 1987).

2.2 Methods

2.2.1. Determination of selenium concentration

Selenium concentrations were determined using the spectrofluorimetric method (Pilarczyk et al., 2010). Samples (0.5–1.5 g) were digested in HNO_3 at 230 °C for 180 min and in $HClO_4$ at 310 °C for 20 min. They were then hydrolyzed with 9% HCl. Selenium was derivatized with 2,3-diaminonaphthalene (Sigma-Aldrich) under conditions of controlled pH (pH 1–2) with the formation of selenodiazole complex. This complex was extracted into cyclohexane. EDTA and hydroxylamine hydrochloride were used as masking agents. Se concentration was determined fluorometrically using a Shimadzu RF-5001 PC spectrofluorophotometer. The excitation wavelength was 376 nm and the fluorescence emission wavelength was 518 nm.

All chemicals used were of analytical reagent grade. The accuracy of the method was verified using certified reference material BCR-185R (bovine liver) (European Commission Joint Research Centre, Institute for Reference Materials and Measurements). A reference sample was analyzed in triplicate. The mean Se concentration was 94.8 ± 3.1% of the reference values. The detection limit was 0.003 µg/g wet weight (ww).

2.2.2. Antioxidant enzymes assay

2.2.2.1. Sample preparation

Samples of liver tissue were homogenized in a glass homogenizer on ice in 10 volumes of 50 mM Tris-Cl buffer (pH 8.1), 1 mM PMSF, and 2 mM EDTA. Homogenates were centrifuged for 15 min at 15,000 rpm at 4 °C. Supernatants were collected and stored until analysis at –80 °C. Total protein was assayed by Bradford method with bovine serum albumin as a standard (Bradford 1976).

2.2.2.2. Total and selenium-dependent GSH-Px assay

GSH-Px activity was assayed according to Pagila and Valentine (1967) using a RANSEL kit (Randox Laboratories Ltd). Cumene hydroperoxide was used to determine the activity of total GSH-Px as substrate. Se-GSH-Px was assayed using hydrogen peroxide as a substrate at a final concentration of 0.25 mM in the presence of 1 mM sodium azide for CAT inhibition. Activity was defined as amount of total GSH-Px or Se-GSH-Px that oxidized 1 nmol of NADPH per minute per milligram of extracted protein at 37 °C and pH 7.2.

2.2.2.3. SOD activity

SOD activity was determined by its ability to inhibit the autoxidation of pyrogallol using the method of Marklund and Marklund (1974) modified to microplate assay. To 270 µL of buffer (50 mM Tris-Cl, pH 8.2; 1 mM EDTA) was added 9 µL of 10 µM bovine erythrocyte CAT solution in 50 mM Tris-Cl (pH 8.2) and 9 µL of 24 mM pyrogallol solution (in 10 mM HCl). Subsequently, 6 µL of appropriately diluted sample was added to the reaction mixture. Increases in absorbance at 420 nm were monitored (Tecan, Infinite m200 PRO) after 2 min at 15-s intervals after a 30-s lag phase at 30 °C.

One unit of SOD decreased the autoxidation of pyrogallol to 50% at pH 8.5 at 30 °C.

2.2.2.4. CAT activity

CAT activity was determined according to Li and Schellhorn (2007). Ten microliters of sample diluted in assay buffer (50 mM phosphate buffer, pH 7.0) was added to 300 µL of 5 mM hydrogen peroxide solution in

assay buffer and brought to 30 °C on a flat-bottomed 96-well UV-transparent microtiter plate (Greiner Bio-One, GmbH). Immediately after adding the sample, decreases in absorbance at 240 nm were monitored for 2 min at intervals of 15 s (Tecan, Infinite m200 PRO). One unit of CAT split 1 μmol of hydrogen peroxide to oxygen and water per minute at pH 7.0 at 30 °C.

2.2.3. Statistical data analysis

Statistical calculations were performed using Statistica PL 7.1. All data are expressed as an arithmetic mean ± SEM and also as geometric mean and median. The concentrations of Se were log-transformed to attain or approach a normal distribution of the data. The effect of sex, season, and age on activity of total GSH-Px, Se-GSH-Px, CAT, SOD, and Se concentration in the liver of wild boars was analyzed using generalized linear models. Differences were considered as significant at the levels of $P < 0.05$, $P < 0.01$, and $P < 0.001$. Relationships between total GSH-Px, Se-GSH-Px, CAT, and SOD activity and the Se concentrations in the liver were evaluated by calculating the coefficients of correlation. Pearson's correlation coefficient ($r_{x,y}$) was calculated for each sex, season, and weight class separately and together for all data. Statistical significance of the coefficients of correlation was tested at the levels of $P < 0.05$ and $P < 0.01$.

3. Results

The highest mean concentration of selenium in the liver of wild boars was found in the spring season (0.292 μg/g ww) and it was significantly ($P < 0.05$) higher than in winter, during which mean Se concentration was at its lowest (0.185 μg/g ww) (Table 1). Mean selenium concentrations in the liver of females (0.247 μg/g ww), males (0.247 μg/g ww), and in wild boars representing different age groups (0.223–0.252 μg/g ww) were at similar levels.

No statistically significant differences were observed between the mean activities of antioxidant enzymes in the liver of wild boars in different seasons, in males and females, and in the age groups analyzed. The highest mean activity of total GSH-Px, Se-GSH-Px, and CAT (404.0, 309.9, and 1170.0 U/mg protein, respectively) was noted in the spring, and that of SOD in the autumn (31.5 U/mg protein). Females, compared to males, were characterized by a markedly higher mean activity of total GSH-Px (388.2 vs. 314.7 U/mg protein) and CAT (1151.0 vs. 765.8 U/mg protein). Compared to the other groups, animals between 1 and 1.5 years of age were characterized by higher mean activity of total GSH-Px and Se-GSH-Px, and wild boars older than 2 years showed higher activity of CAT and SOD.

Analysis of the correlations between activity of different enzymes and between enzyme activity and liver selenium concentration in wild boars (Table 2) revealed that, depending on the season, the activity of total GSH-Px correlated highly significantly and positively with Se-GSH-Px and CAT activity in summer (0.92 and 0.91; $P < 0.001$ and $P < 0.01$), autumn (0.86 and 0.85; $P < 0.01$), and winter (0.82 and 0.84, respectively; $P < 0.05$ and $P < 0.01$). There was also a significant ($P < 0.001$ and $P < 0.01$) and almost completely positive correlation between Se-GSH-Px and CAT activity in all seasons (coefficient of correlation: >0.93). SOD activity was highly negatively correlated with selenium concentration in the winter (–0.74; $P < 0.05$).

In both females and males, highly significant positive correlations were found between the activities of total GSH-Px and Se-GSH-Px (0.82 and 0.80; $P < 0.001$) and total GSH-Px and CAT (0.80 and 0.77; $P < 0.001$), and almost completely positive correlations were seen between Se-GSH-Px and CAT activity (0.97 and 0.99; $P < 0.001$).

The analysis of correlations depending on wild boar age revealed that the activity of total GSH-Px was highly significantly and positively correlated with Se-GSH-Px and CAT activity in the liver of wild boars aged from 1 to 1.5 years (0.83 and 0.82; $P < 0.01$), from 1.5 to 2 years (0.91 and 0.92; $P < 0.001$), and over 2 years (0.91 and 0.90, respectively; $P < 0.01$). A significant ($P < 0.001$) and almost completely positive correlation was also noted between Se-GSH-Px and CAT activity in all age groups (coefficient of correlation: >0.94). The analysis of correlations between the activities of total GSH-Px, Se-GSH-Px, CAT, and SOD and between the activity of individual enzymes and selenium concentration in the liver of all wild boars analyzed together demonstrated significant correlations between the activities of total GSH-Px and Se-GSH-Px (0.80; $P < 0.001$), total GSH-Px and CAT (0.77; $P < 0.01$), and Se-GSH-Px and CAT (0.98; $P < 0.001$).

4. Discussion

Selenium status in wild boars is strictly dependent on the amount and bioavailability of this element from the soil to plants. Wild boars are mainly herbivores. Around 90% of their diet consists of plant food (acorns, beech nuts, nuts, berries and other fruit, some grasses, and other plants) and around 10% of animal food (various invertebrates, earthworms, insects and their larvae, mollusks, small rodents, and carrion) (Pinna, 2007).

Due to the lack of reference values for liver selenium content in wild boars, the results of our research were interpreted using recommendations for pigs. According to Puls (1994), the biochemical criteria used to diagnose Se deficiency in pig liver are as follows: less than 0.11 μg/g ww = deficiency; 0.12–0.39 μg/g ww = marginal level; more than 0.40 μg/g ww = normal (optimal) level. When comparing the determined Se concentrations to the biochemical criteria of the body's status of this element, marginal levels of Se were found in the wild boars throughout the year. This trace element had the

Table 1. Mean values of activity for total glutathione peroxidase (GSH-Px), selenium dependent GSH-Px (Se-GSH-Px), catalase (CAT) and dismutase (SOD) and selenium concentration in the liver of wild boar.

Factor	N	Total GSH-Px (U/mg protein)			Se-GSH-Px (U/mg protein)			CAT (U/mg protein)			SOD (U/mg protein)			Se (µg/g ww)		
		Mean	GM	SEM	Mean	GM	SEM	Mean	GM	SEM	Mean	GM	SEM	Mean	GM	SEM
Season																
Spring	7	404.0	382.8	54.89	309.9	240.6	59.66	1170.0	627.8	298.75	26.1	13.4	9.02	0.292[a]	0.278	0.035
Summer	9	344.1	277.2	73.40	270.9	200.4	59.38	782.7	464.5	213.29	28.2	23.5	4.96	0.223	0.203	0.029
Autumn	10	349.4	247.0	99.03	288.4	219.3	68.56	990.6	610.7	235.68	31.5	20.8	6.54	0.263	0.255	0.019
Winter	8	325.5	252.4	86.72	227.6	177.9	59.94	978.8	727.8	229.64	24.9	21.0	6.51	0.185[a]	0.174	0.023
Sex																
Female	13	388.2	301.5	62.46	275.3	195.6	48.74	1151.0	830.1	157.48	29.2	21.1	4.36	0.234	0.213	0.022
Male	16	314.7	257.8	50.16	272.4	222.3	37.02	765.8	409.6	167.47	26.6	18.2	4.91	0.247	0.236	0.018
Age (years)																
>1	5	291.5	242.5	92.38	167.4	118.3	69.45	902.0	492.9	335.22	28.1	27.0	4.34	0.242	0.210	0.063
1–1.5	10	458.8	384.5	76.26	365.5	294.7	54.86	991.5	701.9	197.69	29.9	25.4	6.16	0.252	0.229	0.029
1.5–2	10	316.4	230.8	86.50	226.9	171.2	56.93	853.0	464.9	229.74	23.1	12.8	6.43	0.223	0.210	0.024
<2	9	312.5	264.7	66.35	283.5	238.9	56.92	1112.8	724.9	255.86	31.2	20.0	7.22	0.245	0.241	0.015
Total	34	353.6	280.1	40.53	273.9	207.8	30.68	969.7	595.4	117.83	28.0	19.7	3.22	0.240	0.223	0.014

The same superscripted letters denote statistically significant differences at $P < 0.05$; GM: geometric mean, SEM: Standard error of the mean.

Table 2. The correlation ratio for studied enzymatic antioxidants and Se concentration depending on animal age, weight, and yearly season.

Factor	N	Total GSH-Px - Se-GSH-Px	Total GSH-Px - CAT	Total GSH-Px - SOD	Total GSH-Px - Se	Se-GSH-Px - CAT	Se-GSH-Px - SOD	Se-GSH-Px - Se	CAT - SOD	CAT - Se	SOD - Se
Season											
Spring	7	Ns	ns	ns	ns	0.99***	ns	ns	ns	ns	ns
Summer	9	0.92***	0.91**	ns	ns	0.99***	ns	ns	ns	ns	ns
Autumn	10	0.86**	0.85**	ns	ns	0.99***	ns	ns	ns	ns	ns
Winter	8	0.82*	0.84**	ns	ns	0.93**	ns	ns	ns	ns	−0.74*
Sex											
Female	13	0.82***	0.80***	ns	ns	0.97***	ns	ns	ns	ns	ns
Male	16	0.80***	0.77***	ns	ns	0.99***	ns	ns	ns	ns	ns
Age (years)											
>1	5	Ns	ns	ns	ns	0.99***	ns	ns	ns	ns	ns
1–1.5	10	0.83**	0.82**	ns	ns	0.99***	ns	ns	ns	ns	ns
1.5–2	10	0.91***	0.92***	ns	ns	0.94***	ns	ns	ns	ns	ns
<2	9	0.91**	0.90**	ns	ns	0.99***	ns	ns	ns	ns	ns
Total	34	0.80***	0.77***	ns	ns	0.98***	ns	ns	ns	ns	ns

***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$, ns: nonsignificant coefficient of correlation.

lowest content during winter (0.187 µg/g ww) and a markedly higher content sequentially in summer (0.223 µg/g ww), autumn (0.263 µg/g ww), and spring (0.292 µg/g ww). The area inhabited by the animals has long been considered deficient in selenium (Pilarczyk et al., 2009), which was reflected in the Se content of the liver. In an earlier study, Pilarczyk et al. (2010) also demonstrated marginal liver selenium levels in the wild boars living in West Pomerania in different seasons. The authors found Se deficiency in 31% of animals in the autumn, and in 20.9%, 15.2%, and 12.0% of animals in the summer, winter, and spring, respectively. The differences in liver selenium concentrations in the wild boars observed in the study under discussion should be attributed to differences in their food intake during different seasons and to different contents and bioavailability of Se in the food. The content of this trace element in plants depends on species, type of soil, amount, and chemical form, as well as climatic and vegetation conditions (Germ and Stibilj, 2007). The absorption of selenium by plants increases with increasing ambient temperature. Selenium uptake from Se-deficient soils may be several times higher at >20 °C compared to <14 °C (Lum et al., 2009). Se uptake also decreases with increasing air humidity and rainfall. The actively growing plant tissues generally contain larger amounts of selenium, accumulating more of this trace element in sprouts and leaves than in underground parts (Germ and Stibilj, 2007). Valdez Barillas et al. (2012) reported that, in spring, selenium is translocated from the roots to the developing sprouts and leaves, where its content, mainly in organic form, peaks in the middle of this season. Leaf selenium concentration decreases gradually during the summer and autumn, with increases in the percentage content of the inorganic form in leaves and the entire plant. Selenomethionine is the main selenium compound in plants that do not accumulate this trace element (Kim and Mahan, 2003; Germ and Stibilj, 2007). The greater amounts of total selenium and predominantly organic forms of Se in younger compared to older leaves are more easily absorbed in the digestive tract compared to inorganic selenites. Inorganic Se compounds are absorbed in the small intestine by passive diffusion, while the organic forms are absorbed by active amino acid transport mechanisms (Wang et al., 2011). Both selenium forms are available for biosynthesis of selenoproteins, but only selenomethionine can be nonspecifically incorporated into proteins in place of methionine (Met) because Met-tRNA does not discriminate between Met and Se-Met. Selenomethionine that was not quickly metabolized after absorption is incorporated into tissues characterized by intensive protein biosynthesis, namely the liver, pancreas, kidneys, skeletal muscles, and gastrointestinal tract mucosa. Selenomethionine accumulation in the body of humans and animals is greater (74%) than that of inorganic selenium (32%–40%). Normally absorbed Se-Met passes through the liver and pancreas several times before being eliminated from the body; for this reason, tissue selenium content is higher after selenomethionine accumulation compared to selenite.

Considering the differences in the quantity and quality of plant food consumed by wild boars in different seasons of the year, the facts stated above sufficiently explain the highest liver selenium concentration observed in the investigated animals in spring, intermediate concentration in summer and autumn, and the lowest in winter. The content of both total selenium and that occurring in the form of selenomethionine in the parts of plants consumed by the wild boars during summer was definitely higher than that in autumn. In the autumn, however, these animals consume more food to store in the form of adipose tissue, the energy reserves needed to maintain homeostasis and survive the winter period, during which the amount of available food is very limited. Although plant selenium content is lower in the autumn (with older parts of plants available, decreased temperature, increased humidity and rainfall) compared to the summer, the level of this element in the liver of wild boars during autumn was slightly higher than in summer. This leads us to assume that due to the consumption of much greater amounts of food, the total supply of selenium in the wild boars was not less in the autumn than in the summer, and even exceeded it. Autumn is the reproductive period for these animals, and selenium deficiency results in clear differences in its tissue distribution. The brain, endocrine glands, and reproductive organs have a higher priority for Se retention compared not only to the heart, skeletal muscles, and erythrocytes, but also to the liver (Lyons et al., 2007).

The present study showed that the sex of the animals had no considerable effect on liver selenium concentration. The lack of a relationship between selenium concentration and sex was also found in wild boars by Pilarczyk et al. (2010), as well as in red deer from northwestern Poland (Pilarczyk et al., 2011) and in polar bears (Rush et al., 2008), although such a correlation was reported in Norwegian red deer (Vikøren et al., 2005) and in red foxes and mongooses (Millán et al., 2008).

Neither did we find a significant relationship between wild boar age and liver selenium concentration; the concentration of this element in different age groups was highly similar (0.223–0.252 µg/g ww). Such a correlation was reported by Pilarczyk et al. (2010) in an earlier study with wild boars from the same area. They found that mean liver Se concentration increased significantly with age, from 0.14 µg/g ww in wild boars less than 1 year of age to 0.24 µg/g ww in animals older than 2 years. The results of the relevant studies performed by other authors

with different species of mammals are inconclusive. For example, no relationship was found between liver selenium levels and age in female elks aged 1–23 years (Stussy et al., 2000), whereas the level of this trace element was found to increase with age in Norwegian red deer (Vikøren et al., 2005) and in Greenland polar bears (Dietz et al., 2000). Under physiological conditions, liver selenium levels are mainly determined by the dietary supply. Considering the numerous biological functions of selenium, it seems justified to assume that an increase in liver selenium content with age is only possible in animals in which Se supply during that time satisfies the body's requirement. This was proven by Matsumoto et al. (2009) in a study with rats aged between 1 and 50 weeks. The increase with age in liver selenium levels was only observed in animals receiving the required amount of dietary selenium. In deficient rats, Se concentration not only failed to increase, but was often below detection level.

Statistical analysis showed no significant differences in liver enzymatic activity for any of the analyzed enzymes of the wild boars grouped according to season. Nevertheless, several marked differences noted between some groups in the mean activity of GSH-Px, CAT, and SOD are worth discussing and interpreting. The highest activity of both total GSH-Px and Se-GSH-Px (404.0 and 309.9 U/mg protein) was found in the liver of wild boars in spring, and the lowest (325.5 and 227.6 U/mg protein) during winter. SOD activity was also at its lowest in the winter. Winter is the season when animals living in the wild find it most difficult to maintain systemic and cellular homeostasis, because the ambient temperature is low and limited access to food results in quantitative and qualitative malnutrition (including considerable Se deficiency, as well as deficiency of Mn, Zn, and Cu, which are cofactors of the active center of Se-GSH-Px and SOD) (Bonda-Ostaszewska et al., 2012). All these factors are strong stressors and can even act separately; each one may increase the generation of ROS and impair antioxidant defense mechanisms, leading to oxidative stress of varying intensity.

The maintenance of normothermia during chronic cold stress requires increased production of endogenous heat through shivering and nonshivering thermogenesis in amounts that balance its loss from the body. The main factor responsible for heat production in nonshivering thermogenesis is 3,5,3'-triiodothyronine (T_3), and one of the basic target organs for this thyroid hormone is the liver (Saičić et al., 2006; Varela et al., 2006; Venditti et al., 2010). T_3 increases the rate of aerobic metabolic processes, resulting in increased ROS generation, consumption of low-molecular-weight cellular antioxidants, and reduced expression and activity of antioxidant enzyme triad (Kaushik and Kaur, 2003; Saičić et al., 2006; Venditti et al., 2010). In all vertebrates, mitochondria are considered the main target for thyroid hormones as regards the regulation of cellular respiration and energy metabolism (Venditti et al., 2010). Chronic cold stress and the associated constant stimulation of the hypothalamic–pituitary–thyroid axis considerably increase oxygen consumption by hepatocytes. More than 90% of this oxygen is consumed by mitochondria (Viña et al., 2006). Long-lasting elevations of thyroid hormone secretion increase both the amount and the volume of individual mitochondria in liver cells by increasing the content of the components of the electron transport chain complex (Venditti et al., 2010). However, there are 2 aspects to the increased oxygen consumption by mitochondria. On the one hand, it increases the production of ATP and of the heat necessary for maintaining normothermia; on the other hand, it is responsible for increasing the generation of ROS. Around 2%–5% of O_2 consumed by mammalian mitochondria does not undergo the complete 4-electron reduction to water and, as a result of electron leakage from the respiratory chain, it forms $\cdot O_2^-$ (Venditti et al., 2010). In a dismutation reaction catalyzed by superoxide dismutase, this free oxygen radical is reduced to H_2O_2, which, in turn, is a substrate for Se-GSH-Px (there is no CAT in mitochondria), which transforms it into water and molecular oxygen (Lykkesfeldt and Svendsen, 2007; Limón-Pacheco and Gonsebatt, 2009). A major role in the whole system is played by selenium. Deficiency of this trace element limits biosynthesis and thus the activity of Se-GSH-Px (Kaur and Bansal, 2004; Wu et al., 2010; Oštádalová, 2012), causing a quantitative decrease in hydrogen peroxide reduction. This increases substrate availability for the Fenton and Haber–Weiss reactions that generate the most reactive and toxic hydroxyl radical (•OH) for cellular macromolecules (Kaushik and Kaur, 2003). The excessive amounts of H_2O_2 and •OH secondarily inhibit the activity of superoxide dismutase, which increases the accumulation of $\cdot O_2^-$ (Venditti et al., 2010). All the ROS mentioned above cause peroxidative damage to proteins, nucleic acids, and liver cell lipids. Organic peroxides are the products of this damage. Their need for neutralization additionally exhausts the cellular reserves of low-molecular-weight antioxidants (including reduced glutathione and vitamins A, E, and C) and reduces the activity of nonselenium-dependent GSH-Px. Because the wild boars under study showed the lowest selenium liver concentration during the winter, the lowest level of activity of total GSH-Px, Se-GSH-Px, and SOD during the same period was not surprising in the context of the sequence of events described above.

Selenium deficiency during chronic cold stress not only increases oxidative stress, but possibly also reduces the efficiency of nonshivering thermogenesis in maintaining normothermia. Selenium is a cofactor of thyroxine

5'-deiodinase (EC 3.8.1.4), the enzyme responsible for the conversion of the less active thyroxine (T_4) into T_3, which is the main biologically active thyroid hormone. The hepatic activity of this enzyme decreases considerably when selenium concentration is reduced by just 20% (Van Bakel et al., 2000; Khoshvaghti et al., 2012).

The factor that increases oxidative stress caused by chronic cold during the winter is a prolonged negative energy balance, a state in which the energy needed for the normal bodily functions of wild animals is obtained primarily from the oxidation of free fatty acids (FFAs) mobilized from adipose tissue accumulated during autumn. The mechanism of oxidative stress during prolonged malnutrition is similar to that occurring under chronic cold and essentially also involves increased ROS generation and decreased antioxidant capacity of low-molecular-weight cellular and enzymatic antioxidants (Vázquez-Medina et al., 2010). The negative energy balance causes a considerable increase in production of H_2O_2 in hepatocytes, the main source of which is β-oxidation of FFAs in peroxisomes and the mitochondrial matrix. Because cellular ATP content in undernourished animals decreases, the process of oxidative phosphorylation increases in mitochondria, as does their consumption of oxygen and ROS generation. Similar to cold stress, all of the above result in a decrease (especially in selenium deficiency) in the expression and activity of antioxidant enzymes. An important factor that enhances oxidative stress in the liver of malnourished animals is the considerable decrease in the content of reduced glutathione (GSH). This is the consequence of its limited biosynthesis, regeneration from the oxidized (GSSG) to the reduced form (GSH), and increased consumption in conditions of food deficiency and oxidative stress (Lu, 1999; Amutha and Subramanian, 2012). The level of hepatic GSH is heavily dependent on food supply. As reported by Lu (1999), even a 2-day considerable food restriction reduces the GSH content of rat liver to 33%–50% of its normal level. The low concentration of GSH decreases the activity of both selenium-dependent and nonselenium-dependent GSH-Px, leading to the accumulation in the hepatocytes of superoxide anion radical, hydrogen peroxide, and hydroxyl radical and organic peroxides, in particular lipid peroxides and their derivatives (Amutha and Subramanian, 2012).

The evidence outlined above leads us to suggest that considerable selenium deficiency can already be regarded as a primary source of oxidative stress in liver tissue (and other tissues). Together with chronic cold stress and malnutrition during winter, this may produce a specific "vicious circle" and, with time, a gradual accretion of oxidative stress and peroxidative damage to cellular macromolecules (Figure).

In the present study, the liver of female wild boars showed a markedly greater mean activity of total GSH-Px (388.2 vs. 314.7 U/mg protein) and CAT (1115.0 vs. 765.8 U/mg protein) compared to males, but the differences were not significant. The mean activity of SOD in females only slightly exceeded that of males, while the activity of Se-GSH-Px was very similar in both sexes. No significant differences were reported in the hepatic activity of GSH-Px in male and female pigs by Balagh et al. (2012), nor in red deer by Pilarczyk et al. (2011). Significantly higher CAT and Mn-SOD activity was observed by Carrillo et al. in the liver of female mice (1992), and considerably higher GSH-Px and CAT activity was reported by Wu et al. (2003).

A study of rats by Valle et al. (2007) showed that hepatocyte mitochondria generate 40%–50% more hydrogen peroxide in males compared to females; in females, these organelles show a greater expression of the genes coding for antioxidant enzymes. The mitochondrial activity of GSH-Px and Mn-SOD is 50%–60% higher in female than in male rats (Viña et al., 2006). This effect is attributed to the influence of estrogenic hormones, in particular 17β-estradiol. Because the promoter region of the SOD and GSH-Px genes does not contain estrogen-responsive elements, estradiol acts on the expression of the genes coding for these enzymes indirectly through the intracellular signaling cascade with the contribution

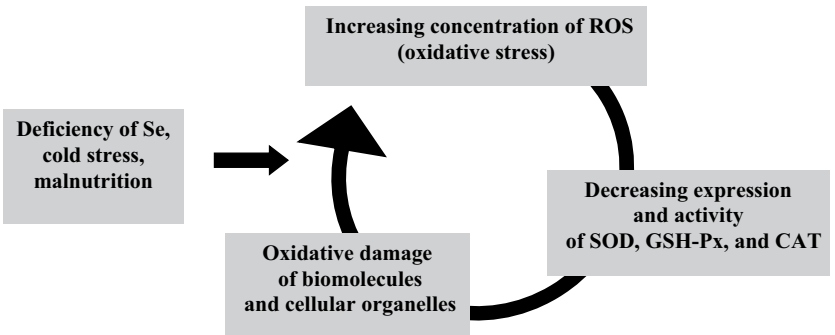

Figure. The potential mechanism of a "vicious circle" leading to rise in winter oxidative stress in hepatocytes.

of mitogen-activated protein kinase. This kinase activates nuclear factor kappa B, which increases the transcription of both SOD and GSH-Px genes because their promoters contain motifs that bind them (Viña et al., 2006). It can be concluded from the results of the present study that the higher activity of GSH-Px in female compared to male wild boars is the result of the greater activity of the nonselenium-dependent form of this enzyme. Se-GSH-Px activity was almost identical in both sexes, which is likely explained by their similar liver selenium levels. In Poland, the female wild boar hunting season runs from 15 August to 15 January, which is also the period of initial and full ovarian activity. The estrogen secreted by the ovaries probably increases the expression of the genes of both selenium-dependent and nonselenium-dependent GSH-Px, but the magnitude of Se-GSH-Px biosynthesis at the posttranscriptional level depends on selenium availability and is considerably reduced when Se is deficient (Wu et al., 2003; Lum et al., 2009).

In our study, the wild boars aged between 1 and 1.5 years were characterized by a clearly higher (although nonsignificantly so) mean activity of total GSH-Px and Se-GSH-Px compared to the other groups. The activity of CAT and SOD was the highest in animals aged 2 years or more. None of the studied enzyme activity showed an upward or downward trend with age. In a study of rats, Saičić et al. (2006) found that the liver activity of the enzymatic antioxidant defense system increases with age during the first postnatal period and is markedly higher in animals aged between 60 and 90 days compared to 30-day-old animals. The increased liver activity of GSH-Px in rats aged between 1 and 50 days was also reported by Matsumoto et al. (2009), but they clearly indicated that it is determined by providing a supply of selenium with food or water that meets the bodily needs. Carrillo et al. (1992) noted a sex-dependent direction of changes in the rat liver activity of antioxidant enzymes with age. For example, CAT and Mn-SOD activity in the liver of 30-month-old males was significantly lower than that of 7-month-olds, while the activity of Cu and Zn-SOD followed an opposite pattern. As a result, the activity of total SOD and GSH-Px in younger and older animals was comparable. Balogh et al. (2012) reported that, in growing pigs, the liver activity of GSH-Px is highly negatively correlated to the magnitude and rate of body weight gain.

Based on the analysis of correlations, significant positive relationships were only found between total GSH-Px and Se-GSH-Px, total GSH-Px and CAT, and Se-GSH-Px and CAT. In the liver of pigs, Se-GSH-Px activity accounted for approximately 40%–55% of total GSH-Px activity. Kim and Mahan (2003) reported that the activity of nonselenium-dependent peroxidase depends to a considerable degree on the level of Se supply, and its percentage in total activity of GSH-Px increases with increasing deficiency of this trace element. The increase in total GSH-Px activity largely reflects the increase in the selenium-dependent form of the enzyme (Kaur and Bansal, 2004; Oštádalová, 2012). These data provide sufficient evidence to explain the high and positive correlation between the activity of total GSH-Px and Se-GSH-Px, which was found when the wild boars were grouped according to season, sex, and age, and also when they were not grouped.

It is also relatively easy to explain the high and positive correlation between GSH-Px and CAT activity. It is well known that both Se-GSH-Px and CAT catalyze the reduction of hydrogen peroxide to water. However, these enzymes differ considerably in their Michaelis constants (K_M) and thus in the affinity for this substrate. Peroxidase is located in the cytosol and mitochondria of the cell and acts when H_2O_2 concentrations are low (of the order of micromoles), whereas CAT is found mainly in peroxisomes and acts when the concentration of this peroxide is high (of the order of millimoles) (Simmons and Jamall, 1988). Therefore, both enzymes are used when hydrogen peroxide concentration in the cell is high. This results in an even greater accumulation of H_2O_2 and in generation of hydroxyl radical in the Fenton and Haber–Weiss reactions, and, under its influence, of the organic peroxides. In turn, their neutralization exhausts both selenium-dependent and nonselenium-dependent GSH-Px (Davies, 2000; Kaushik and Kaur, 2003; Kim and Mahan, 2003).

The lack of a significant positive relationship between liver selenium concentration and Se-GSH-Px activity may seem debatable. It should be noted, however, that the wild boars exhibited considerable selenium deficiency during all seasons of the year. Liver Se-GSH-Px is only 1 of at least 25 selenoproteins present in the body of mammals. Each of them contains selenocysteine (Sec) residues in their polypeptide chains (Gromer et al., 2005). The position of Sec in the polypeptide chains is determined by the UGA codon in the mRNA of a given selenoprotein. When selenium deficiency is paralleled by a lack of $tRNA^{(Sec)}$ transport of Sec to the ribosomes, this codon is read as a nonsense codon, which means that translation is terminated and mRNA is degraded into a given selenoprotein (Carrillo et al., 1992; Valle et al., 2007). Most selenoproteins are biosynthesized in the liver. Decrease in Sec availability, resulting from a low dietary supply of selenium, means that it is first incorporated into selenoproteins, which play a key role in the body. Thus, selenoprotein P and thyroxine 5'-deiodinase have priority (Gromer et al., 2005; Papp et al., 2007). In this system, Se-GSH-Px occupies a more distant position, because its deficiency and functions can be at least partly replaced with increased expression and activity of CAT and a nonselenium-dependent form of GSH-Px. Compared to

Se-GSH-Px, selenoprotein P incorporates selenium more quickly and contains as many as 10 Sec residues in its polypeptide chain (Papp et al., 2007). For this reason, it is suggested that the hepatic levels of selenoprotein P are a better indicator of the body's selenium status compared to Se-GSH-Px activity (Gromer et al., 2005).

The results of our study demonstrate that selenium content in the forest soils of northwestern Poland is low and does not meet the wild boar's requirement for this trace element, even in the seasons when plant food is most abundant. The wild boars from this area had only marginal liver selenium levels throughout the year, with the highest values found in the spring and lowest in the winter. This contradicts the theory of Fiedler (1986) that wild animals that have lived in selenium-deficient areas for many generations could develop alternative adaptive mechanisms that would compensate for the low dietary content and bioavailability of this element.

The analysis of selenium concentration and the activity of total and selenium-dependent GSH-Px, CAT, and SOD showed that the strongest oxidative stress and the greatest risk of peroxidative damage to liver tissue (and other tissues) occurs in winter. It is possible that the considerable selenium deficiency, through increased accumulation of reactive oxygen species, and especially in mitochondria, is already the primary reason for gradual accretion of oxidative stress in these organelles and later in the cell, the entire organ, and the body.

References

Amutha C, Subramanian P (2012). Studies on the seasonal changes in antioxidant enzymes activity on differently, polluted areas along the Bay of Bengal employing *Perna viridis* as an animal model. Am J Anim Vet Sci 7: 96–103.

Antunes F, Han D, Cadenas E (2002). Relative contributions of heart mitochondria glutathione peroxidase and catalase to H_2O_2 detoxification in in vivo conditions. Free Radical Bio Med 33: 1260–1267.

Balogh K, Weber M, Heincinger M, Kollár G, Mézes M (2012). Correlations between daily weight gain, lipid peroxidation and glutathione status of liver and kidney in different pig genotypes. Arch Tierz 55: 263–271.

Bonda-Ostaszewska E, Włostowski T, Krasowska A, Kozłowski P (2012). Seasonal and photoperiodic effects on lipid droplet size and lipid peroxidation in the brown tissue of bank voles (*Myodes glareolus*). Acta Theriol 57: 289–294.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.

Carrillo MC, Kanai S, Sato Y (1992). Age-related changes in antioxidant enzyme activities are region and organ, as well as sex, selective in the rat. Mech Ageing Dev 65: 187–198.

Davies KJ (2000). Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 50: 279–289.

Dietz R, Riget F, Born EW (2000). Geographical differences of zinc, cadmium, mercury and selenium in polar bears (*Ursus maritimus*) from Greenland. Sci Total Environ 245: 25–47.

Germ M, Stibilj V (2007). Selenium and plants. Acta Agric Sloven 89: 65–71.

Gromer S, Eubel JK, Lee BL, Jacob J (2005). Human selenoproteins at a glance. Cell Mol Life Sci 62: 2414–2437.

Kaur P, Bansal MP (2004). Effect of selenium-induced oxidative stress on the oxidation reduction system and reproductive ability of male mice. Biol Trace Elem Res 97: 83–93.

Kaushik S, Kaur J (2003). Chronic cold exposure affects the antioxidant defense system in various rat tissues. Clin Chim Acta 333: 69–77.

Khoshvaghti A, Nazifi S, Mirabad MM, Akbarpour B (2012). The relationship between serum level of thyroid hormones and antioxidant enzymes in clinically healthy ostriches. Int J Poult Sci 11: 202–208.

Kim YY, Mahan DD (2003). Biological aspects of selenium in farm animals. Asian Austral J Anim 16: 435–444.

Li Y, Schellhorn HE (2007). Rapid kinetic microassay for catalase activity. J Biomol Tech 18: 185–187.

Limón-Pacheco J, Gonsebatt ME (2009). The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutat Res 31: 137–147.

Lochman J (1987). Określanie Wieku Zwierzyny. Warsaw, Poland: PWRiL (in Polish).

Lu SC (1999). Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J 13: 1169–1183.

Lum GE, Rowntree JE, Bondioli KR, Southern LL, Williams CC (2009). The influence of dietary selenium on common indicators of selenium status and liver glutathione peroxidase-1 messenger ribonucleic acid. J Anim Sci 87: 1739–1746.

Lykkesfeldt J, Svendsen O (2007). Oxidants and antioxidants in disease: oxidative stress in farm animals. Vet J 173: 502–511.

Lyons MP, Papazyan TT, Surai PF (2007). Selenium in food chain and animal nutrition: lessons from nature. Asian Austral J Anim 20: 1135–1155.

Marklund S, Marklund G (1974). Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47: 469–474.

Matsumoto K, Terada S, Ariyoshi M, Okajo A, Hisamatsu A, Ui I, Endo K (2009). The effect of long-running severe selenium deficiency on the amount of iron, and zinc in the organs of rats. Molecules 14: 4440–4453.

Millán J, Mateo R, Taggart MA, López-Bao JV, Viota M, Monsalve L, Camarero PR, Blázquez E, Jiménez B (2008). Levels of heavy metals and metalloids in critically endangered Iberian lynx and other wild carnivores from Southern Spain. Sci Total Environ 399: 193–201.

Oštádalová I (2012). Biological effect of selenium compounds with a particular attention to the ontogenetic development. Physiol Res 61: 19–34.

Paglia DE, Valentine WN (1967). Studies on the quantitative and qualitative characteristics of erythrocyte glutathione peroxidase. J Lab Clin Med 70: 158–169.

Papp LV, Lu J, Holmgren A, Khanna KK (2007). From selenium to selenoproteins, identity, and their role in human health. Antioxid Redox Sign 9: 775–806.

Pilarczyk B, Balicka-Ramisz A, Ramisz A, Adamowicz E, Pilarczyk R, Tomza-Marciniak A, Bąkowska M (2009). Selenium concentration in liver and kidney of free living animals (roe and red deer) from West Pomerania (Poland). Eur J Wildlife Res 55: 279–283.

Pilarczyk B, Drozd R, Pilarczyk R, Tomza-Marciniak A, Jankowiak D, Hendzel D, Kuba J, Kowalska J (2011). Glutathione peroxidase (GSHPx) activity in the liver of red deer in relations to hepatic selenium concentration, sex, body weight and season of the year. Biol Trace Elem Res 144: 560–569.

Pilarczyk B, Hendzel D, Pilarczyk R, Tomza-Marciniak A, Błaszczyk, B, Dąbrowska-Wieczorek M, Bąkowska M, Adamowicz E, Bujak T (2010). Liver and kidney concentrations of selenium in wild boars (*Sus scrofa*) from northwestern Poland. Eur J Wildl Res 55: 797–802.

Pinna W, Nieddu G, Moniello G, Cappai MG (2007). Vegetable and animal ford sorts fund in the gastric content of Sardinian Wild boar (*Sus scrofa meridionalis*). J Anim Physiol An N 91: 252–255.

Puls R (1994). Mineral Levels in Animal Health: Diagnosis Data. 2nd ed. Clearbrook, BC, Canada: Sherpa International.

Rush SA, Borgå K, Dietz R, Born EW, Sonne C, Evans T, Muir DC, Letcher RJ, Norstrom RJ, Fisk AT (2008). Geographic distribution of selected elements in the liver of polar bears from Greenland, Canada and the United States. Environ Pollut 153: 618–626.

Saičić ZS, Mijalković DN, Nikolić AL, Blagojević DP, Spasić MB (2006). Effect of thyroxine on antioxidant defense system in the liver of rats of different age. Physiol Res 55: 561–568.

Simmons TW, Jamall IS (1988). Significance of alterations in hepatic antioxidant enzymes. Biochem J 251: 913–917.

Stussy RJ, Findholt SL, Johnson BK, Noyes JH, Dick BL (2000). Selenium levels and productivity in three Oregon elk herds. Northwest Sci 74: 97–101.

Valdez Barillas JR, Quinn CF, Freeman JL, Lindblom SD, Fakra SC, Marcus MA, Gilligan TM, Alford ÉR, Wangeline AL, Pilon-Smits EA (2012). Selenium distribution and speciation in the hyperaccumulator *Astragalus bisulcatus* and associated ecological partners. Plant Physiol 159: 1834–1844.

Valle A, Guevara R, García-Palmer FJ, Roca P, Oliver J (2007). Sexual dimorphism in liver mitochondrial oxidative capacity is conserved under caloric restriction conditions. Am J Physiol-Cell Ph 293: 1302–1308.

Van den Berghe G (1991). The role of the liver in metabolic homeostasis: implications for inborn errors of metabolism. J Inherit Metab Dis 14: 407–420.

Varela P, Tapia G, Fernández V, Videla L (2006). The role of thyroid hormone calorigenesis in the redox regulation of gene expression. Biol Res 39: 611–617.

Vázquez-Medina JP, Crocker DE, Forman HJ, Ortiz RM (2010). Prolonged fasting does not increase oxidative damage or inflammation in postweaned northern elephant seal pups. J Exp Biol 213: 2524–2530.

Venditti P, Di Stefano L, Di Meo S (2010). Oxidative stress in cold-induced hyperthyroid state. J Exp Biol 213: 2899–2911.

Vikøren T, Bernhoft A, Waaler T, Handeland K (2005). Liver concentration of copper, cobalt, and selenium in wild Norwegian red deer (*Cervus elaphus*). J Wildlife Dis 41: 569–579.

Viña J, Sastre J, Pallardó FV, Gambini J, Borrás C (2006). Role of mitochondrial oxidative stress to explain the different longevity between genders: protective effect of estrogens. Free Radical Res 40: 1359–1365.

Wang YX, Zhan XA, Zhang ZW, Wu R, Yuan D (2011). Comparison of different forms of dietary selenium supplementation on growth performance, meat quality, selenium deposition, and antioxidant property in broilers. Biol Trace Elem Res 143: 261–273.

Wu A, Sun X, Wan F, Liu Y (2003). Modulations by dietary restriction on antioxidant enzymes and lipid peroxidation in developing mice. J Appl Physiol 94: 947–952.

Wu X, Huang K, Wei C, Chen F, Pan (2010). Regulation of cellular glutathione peroxidase by different forms and concentrations of selenium in primary cultured bovine hepatocytes. J Nutr Biochem 21: 153–161.