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Antioxidant defenses in three vesper bats(Chiroptera: Vespertilionidae) during hibernation

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Abstract: Hibernation of bats is characterized by considerable changes in the oxygen supply during the torpor–arousal cycles. The purpose of this study was to examine the antioxidant defenses in tissues of the hibernating bats (*Eptesicus nilssonii*, *Myotis brandtii*, and *Plecotus auritus*), attempting to relate the tissue antioxidant protection with the ecophysiological characteristics of the studied species. We found that the superoxide dismutase (SOD) activity in the heart and the catalase activity in the skeletal muscle were higher in *E. nilssonii* than in *P. auritus* and in *M. brandtii*. In comparison with studied hibernating bats, *P. auritus* had the lowest activities of SOD and catalase in the heart. In addition, the level of glutathione was higher in the liver, kidneys, and muscle in *M. brandtii* than in the corresponding tissues of both *P. auritus* and *E. nilssonii*. We conclude that the differences in the mechanisms of adaptation to hibernation of bats are the result of various ecophysiological characteristics among species.

Key words: Hibernation, *Eptesicus nilssonii*, *Myotis brandtii*, *Plecotus auritus*, adaptation, catalase, superoxide dismutase, glutathione

1. Introduction

Mammalian hibernators represent a unique model for the study of adaptation to changes in oxygen consumption (Carey et al., 2003; van Breukelen and Martin, 2015). Physiological parameters such as metabolism and oxygen supply are reduced during torpor (Storey, 2010; Yin et al., 2016). However, torpor is replaced by periods of intensive metabolic activity (arousal), which are characterized by quick warming of the organism (Carey et al., 2003; Storey, 2010). The great burst of reactive oxygen species (ROS) in mitochondrial respiration during the torpor-arousal cycles of bats may lead to the oxidative damage to sensitive tissues (Carey et al., 2003; Sanderson et al., 2013; Yin et al., 2016). The glutathione (GSH) and antioxidant defense enzymes (superoxide dismutase (SOD) and catalase) reduce the effects of ROS (Galano and Alvarez-Idaboy, 2011; Conde-Pérezprina et al., 2012).

Along with numerous studies on torpid ground squirrels (Sciuridae) (Toien et al., 2001; Carey et al., 2003; Astaeva and Klichkhanov, 2009; Orr et al., 2009; Storey, 2010; James et al., 2013) there are very few data on the antioxidant status in bats (Wilhelm Filho et al., 2007; Lilley et al., 2014; Yin et al., 2016). Some authors (Wilhelm Filho et al., 2007; Yin et al., 2016) consider that bats are likely to cope with oxidative stress through effective antioxidant defenses during hibernation. However, the findings demonstrated from field studies stem from small data sets or a single bat species or organ (Wilhelm Filho et al., 2007; Conde-Pérezprina et al., 2012; Lilley et al., 2014; Yin et al., 2016).

Among the five vesper (Vespertilionidae) bat species, including *Eptesicus nilssonii, Myotis brandtii*, *Myotis daubentoni*, *Myotis mystacinus*, and *Plecotus auritus*, that hibernate in Karelia, *E. nilssonii* inhabits regions further north than the other bats (Belkin et al., 2015). The present research focuses on the activities of antioxidant enzymes and the GSH content in tissues (liver, kidney, heart, lungs, and skeletal muscle) of three species of bats (*E. nilssonii*, *M. brandtii*, and *P. auritus*) in the deep torpid state (February–March).

2. Materials and methods

2.1. Animals and tissue collection

The experimental protocols were approved by the special permission of the Local Ethic Committee of the Institute of Biology. The animals were captured in the Republic of Karelia (61–63°N, 30–36°E) in February–March of 2016 under the terms of permits issued by the Game Management Directorate of the Republic of Karelia (10№ 00011). The easily differentiated species were identified

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visually (*Plecotus auritus* and *Eptesicus nilssonii*), whereas *Myotis brandtii* was examined for the position of the wing membrane attachment to the hind limb—the main trait for in situ differentiation between species in the myotids group. Differentiation between *M. brandtii* and *M. mystacinus* was performed based on the analysis of craniological material and penis shape (for males) (Schober and Grimmberger, 1997). The three species of vesper bats were weighed, and then induced to a hibernating state in a cold room (4–7 °C). Samples of fresh tissue of torpid bats—*E. nilssonii* (2 males and 10 females), *M. brandtii* (1 male and 2 females) and *P. auritus* (1 males and 2 females)—were analyzed. Samples of the liver, kidneys, heart, lung, and pectoral muscle of bats were obtained after decapitation of animals, placed in Eppendorf tubes, and stored at –80 °C until analyzed.

2.2. Antioxidant enzyme activities

2.2.1. Superoxide dismutase (EC 1.15.1.1)

The total SOD activity was measured by the adrenochrome method based on the spontaneous autoxidation of epinephrine with the formation of product with an absorbance peak at 480 nm (Misra and Fridovich, 1972). This reaction depends on the presence of superoxide anions and is specifically inhibited by SOD. The amount of enzyme that caused 50% inhibition of epinephrine autoxidation is defined as 1 unit (U). SOD activity was expressed as U per mg protein after normalization with estimated total protein in milligrams in the respective tissues.

2.2.2. Catalase (EC 1.11.1.6)

The catalase activity was evaluated by measuring the decrease in H_2O_2 concentration at 240 nm (Bears and Sizes, 1952). One enzyme unit (IU) is defined as the amount of catalase capable of transforming 1.0 μmol of H_2O_2 for a minute. Catalase activity was expressed as IU per mg protein after normalization with estimated total protein in milligrams in the respective tissues.

2.3. Total protein assay

Results for antioxidant enzymes' activities were standardized to total soluble protein content in tissue homogenates. Total tissue protein content was determined by the method described by Lowry et al. (1951) using bovine serum albumin as standard.

2.4. Glutathione measurement

The level of GSH was measured using the Ellman method (Sedlak and Lindsay, 1968). The GSH levels are presented in μmol GSH per 1 g of wet tissue (see Sergina et al., 2015 for details).

2.5. Treatment of data

Statistical analysis was carried out using the nonparametric Mann–Whitney U-test. Differences between species were considered to be significant when the P value was less than 0.05.

3. Results

3.1. Antioxidant enzyme activities

No significant differences in the SOD activity were found among hibernating bats for the liver, kidneys, lungs, and skeletal muscle (Figure 1). In the heart the SOD activity of *E. nilssonii* was higher ($P < 0.05$) as compared to the same tissue of *P. auritus* and *M. brandtii*. Among the studied tissues in bats the skeletal muscle exhibited the highest SOD activity (Figure 1).

There were no significant differences in the catalase activity among bat species for the liver, kidneys, lungs, or skeletal muscle. However, the heart catalase activity was lower ($P < 0.05$) in *P. auritus* than in other mammals studied. In comparison with studied hibernating bats (*P. auritus* and *M. brandtii*), *E. nilssonii* had higher catalase activity in the skeletal muscle (Figure 2).

3.2. Glutathione

Results of the GSH assay are summarized in the Table. In *M. brandtii*, the GSH content was higher ($P < 0.05$) in the liver, kidneys, and skeletal muscle as compared to the same tissues of both *P. auritus* and *E. nilssonii*. Level of GSH was significantly lower ($P < 0.05$) in the liver and skeletal muscle of *E. nilssonii* than in the corresponding tissues of both *P. auritus* and *M. brandtii*.

4. Discussion

Significant differences were found in antioxidant defenses among the three species of vesper bats. Previously it was shown (Lyman, 1970) that bats maintain very high oxygen supply when active, a characteristic that contrasts with the quick changes in oxygen consumption when they become torpid. Generation of ROS depends on mitochondrial oxygen supply (Boveris, 1977) and is followed by simultaneous antioxidant defenses in mammals (Wilhelm Filho et al., 2007). Our results demonstrated that *E. nilssonii* differs from the other bat species, especially regarding higher ($P < 0.05$) the SOD activity in the heart and the catalase activity in the skeletal muscle but lower level of GSH in liver and skeletal muscle. In addition, the *P. auritus* had the lowest activity of SOD and catalase in the heart compared to the other bat species sampled. Wilhelm-Filho et al. (2007) have documented that the antioxidant capacities were higher in torpid little yellow-shouldered bats (*Sturnira lillium*) in comparison with active bats. It is suggested that this antioxidant response reduces oxidative stress during the daily transformation from torpid to the active state in *S. lillium* (Wilhelm-Filho et al., 2007).

To provide an adequate level of antioxidant capacity, bats, like other hibernating mammals, preferred for low-molecular antioxidants (Wilhelm-Filho et al., 2007). Our results showed that in *M. brandtii* the GSH content in the liver, kidneys, and skeletal muscle were higher ($P < 0.05$) than in *P. auritus* and *E. nilssonii*. It is assumed

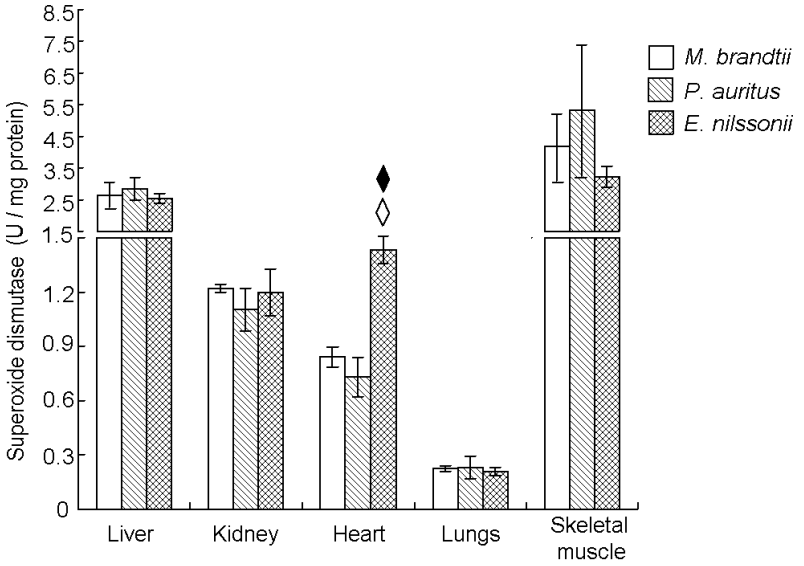

Figure 1. The activity of SOD in the bats' tissues. Results (in U/mg protein) are expressed as mean ± SEM. ♦ Significant difference from *M. brandtii*, ◊ from *P. auritus* in the same tissue. ♦, ◊ $P < 0.05$.

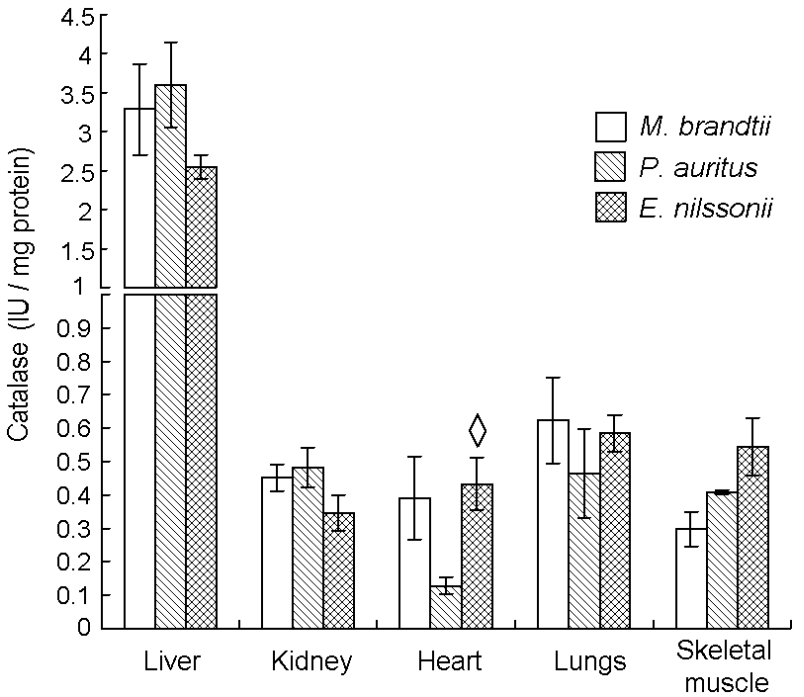

Figure 2. The activity of catalase in the bats' tissues. Results (in IU/mg protein) are expressed as mean ± SEM. ◊ Significant difference from *P. auritus* in the same tissue. ◊ $P < 0.05$.

that the GSH system is a key defense mechanism against oxidative damage by increasing the production of ROS (Cantú-Medellín et al., 2011). The ratio between reduced and oxidized glutathione species (GSSG/GSH) increased during reoxygenation (Yin et al., 2016). In accordance with previous results, *M. brandtii* had significantly higher glutathione S-transferase activity (GST) and ratio of GSH/GSSG in blood compared to *E. nilssonii*, *P. auritus*, *M. daubentoni*, and *M. mystacinus* (Lilley et al., 2014). GST is connected with biotransformation of exogenous

Table. The GSH content (μmol/g wet tissue) in the tissues of bats.

Species	Tissue				
	Liver	Kidney	Heart	Lungs	Skeletal muscle
M. brandtii (n = 2–3)	35.59 ± 5.30	63.66 ± 3.36	-	17.17 ± 2.82	28.87 ± 4.13
P. auritus (n = 1–3)	24.91 ± 0.37♦	40.11 ± 3.64	42.65	34.22	23.86 ± 5.23
E. nilssonii (n = 7–12)	16.56 ± 2.30♦◊	46.57 ± 2.93♦	31.32 ± 4.07	22.09 ± 1.93	19.81 ± 1.08♦◊

♦ Significant difference from *M. brandtii*, ◊ from *P. auritus* in the same tissue. ♦, ◊ $P < 0.05$. The GSH content was not measured in the heart of *M. brandtii*.

compounds, reducing the effects of oxidative stress and endogenous intoxication (Lilley et al., 2014). As noted earlier, Carey et al. (2003) demonstrated that during arousal the rate of metabolism increases to near normal levels. Perhaps during bats' arousal the intensification of circulation processes and excretion from the kidneys and other organs to the blood of different metabolites of low-molecular antioxidants make the blood compartment part of a first antioxidant front in bats (Wilhelm-Filho et al., 2007). Moreover, previous studies have demonstrated that the GSH content in blood of the Syrian hamster (*Mesocricetus auratus*) is increased by awakening (Ohta et al., 2006).

The differences in the antioxidant capacity among the studied species can be explained by ecophysiological characteristics of vesper bat species and indicate that *M. brandtii*, *E. nilssonii*, and *P. auritus* have different defense mechanisms against ROS formation during torpor–arousal cycles. *E. nilssonii* lives in colder areas with a shorter photoperiod and hibernation of this species occurs at lower temperatures (2.0 ± 0.1 °C) and relative humidity (78.0 ± 0.6%) as compared with other species of bats (*M. brandtii*, *P. auritus*, and *M. daubentoni*), affirming that this species is well adapted to the north's severe conditions (Siivonen and Wermundsen, 2008; Belkin et al., 2015). In contrast, *M. brandtii* hibernated in the warmer and more comfortable locations and used both energy-saving ways (crevices and clusters) for torpor (Siivonen and Wermundsen, 2008). Previously it was shown (Anufriev and Revin, 2006) that *E. nilssonii* has longer torpor duration than *P. auritus* and *M. daubentoni* in similar natural environments. Furthermore, *E. nilssonii* typically hibernated on the ceiling/wall (without using any energy-saving ways), whereas *M. brandtii* and *P. auritus* preferred crevices (Siivonen and Wermundsen, 2008).

Our results demonstrated that the skeletal muscle of bats has higher SOD activity compared with other tissues. It has previously been shown that there is a comparatively low degree of muscle dystrophy in hibernating mammals in comparison with the artificial models of muscle resting (Hudson and Franklin, 2002). A variety of mechanisms promote the resistance of bats to muscle atrophy, including enhanced antioxidant defenses (James et al., 2013). In accordance with previous results, the torpid *S. tridecemlineatus* had significantly higher antioxidant capacity of muscle compared to summertime specimens (James et al., 2013). Presumably, skeletal muscles during hibernation feature high rates of aerobic oxidation of lipids, which provide thermogenesis during arousal (Storey, 2010). Previously it was shown (Armstrong et al., 1977) that all fibers of *M. lucifugus* muscle possessed high myofibrillar ATPase activities and aerobic potentials, indicating fast contractile properties.

In summary, understanding the defense mechanisms against ROS formation and organ injury during torpor–arousal cycles may provide insights into possible methods of treatment for organ injury during cold storage (Jani et al., 2013). Significant differences were found among bat species for SOD and catalase activities and GSH content in the tissues studied. We found that the SOD activity in the heart and the catalase activity in the skeletal muscle were higher in *E. nilssonii* than in *P. auritus* and in *M. brandtii*. In addition, *P. auritus* had lower activity of SOD and catalase in the heart compared to the other vesper bat species sampled. The GSH level was significantly higher in the liver and skeletal muscle in *M. brandtii* than in *P. auritus* and *E. nilssonii*. We conclude that the differences in the mechanisms of adaptation to hibernation of bats are the result of various ecophysiological characteristics among species. Obviously, further more extensive research (including other species of bats and physiological parameters) is necessary to clarify their antioxidant protection with their ecophysiological characteristics.

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References

Anufriev AI, Revin YuV (2006). Bioenergetics of hibernating bats (Chiroptera, Vespertilionidae) in Yakutia. Plecotus et al. 9: 8-17.

Armstrong RB, Ianuzzo CD, Kunz TH (1977). Histochemical and biochemical properties of flight muscle fibers in the little brown bat, *Myotis lucifugus.* J Comp Physiol 119: 141-154.

Astaeva MD, Klichkhanov NK (2009). Oxidative modification of proteins and blood antioxidant activity of ground squirrels during induced awakening from hibernation. Biology Bulletin 36: 562-567.

Bears RF, Sizes IN (1952). A spectral method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 195: 133-140.

Belkin VV, Panchenko DV, Tirronen KF, Yakimova AE, Fedorov FV (2015). Ecological status of bats (Chiroptera) in winter roosts in eastern Fennoscandia. Russian Journal of Ecology 46: 463-469.

Boveris A (1977). Mitochondrial production of superoxide radical and hydrogen peroxide. Adv Exp Med Biol 78: 67-82.

Cantú-Medellín N, Byrd B, Hohn A, Vázquez-Medina JP, Zenteno-Savín T (2011). Differential antioxidant protection in tissues from marine mammals with distinct diving capacities. Shallow/short vs. deep/long divers. J Comp Biochem Physiol 158: 438-443.

Carey HV, Andrews MT, Martin SL (2003). Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. Physiol Rev 83: 1153-1181.

Conde-Perezprina JC, Luna-Lopez A, Gonzalez-Puertos VY, Zenteno-Savín T, León-Galván MÁ, Königsberg M (2012). DNA MMR systems, microsatellite instability and antioxidant activity variations in two species of wild bats: *Myotis velifer* and *Desmodus rotundus*, as possible factors associated with longevity. Age 34: 1473-1492.

Galano A, Alvarez-Idaboy JR (2011). Glutathione: mechanism and kinetics of its non-enzymatic defense action against free radicals. Rsc Advances 1: 1763-1771.

Hudson NJ, Franklin CE (2002). Maintaining muscle mass during extended disuse: aestivating frogs as a model species. J Exp Biol 205: 2297-2303.

James RS, Staples JF, Brown JC Tessier SN, Storey KB (2013). The effects of hibernation on the contractile and biochemical properties of skeletal muscles in the thirteen-lined ground squirrel, *Ictidomys tridecemlineatus.* J Exp Biol 216: 2587-2594.

Jani A, Martin SL, Jain S, Keys D, Edelstein CL (2013). Renal adaptation during hibernation. Am J Physiol Renal Physiol 305: F1521-F1532.

Lilley TM, Stauffer J, Kanerva M, Eeva T (2014). Interspecific variation in redox status regulation and immune defence in five bat species: the role of ectoparasites. Oecologia 175: 811-23.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275.

Lyman CP (1970). Thermoregulation and metabolism in bats. In: Wimsatt WA, editor. Biology of Bats. New York, NY, USA: Academic Press, pp. 301-330.

Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247: 3170-3175.

Ohta H, Okamoto I, Hanaya T, Arai S, Ohta T, Fukuda S. (2006). Enhanced antioxidant defense due to extracellular catalase activity in Syrian hamster during arousal from hibernation. Comp Biochem Physiol 143: 484-491.

Orr AL, Lohse LA, Drew KL, Hermes-Lima M (2009). Physiological oxidative stress after arousal from hibernation in Arctic ground squirrel. Comp Biochem Physiol A Mol Integr Physiol 153: 213-221.

Sanderson TH, Reynolds CA, Kumar R, Przyklenk K, Hüttemann M (2013). Molecular mechanisms of ischemia–reperfusion injury in brain: pivotal role of the mitochondrial membrane potential in reactive oxygen species generation. Mol Neurobiol 47: 9-23.

Schober W, Grimmberger E (1997). The Bats of Europe and North America. Neptune, NJ, USA: T.F.H. Publications.

Sedlak J, Lindsay RH (1968). Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25: 192-205.

Sergina S, Antonova E, Ilyukha V, Łapiński S, Lis M, Niedbała P, Unzhakov A, Belkin V (2015). Biochemical adaptations to dive-derived hypoxia/reoxygenation in semiaquatic rodents. Comp Biochem Physiol B 190: 37-45.

Siivonen Y, Wermundsen T (2008). Characteristics of winter roots of bat species in southern Finland. Mammalia 72: 50-56.

Storey KB (2010). Out cold: biochemical regulation of mammalian hibernation – a mini-review. Gerontology 56: 220-230.

Toien O, Drew KL, Chao ML, Rice ME (2001). Ascorbate dynamics and oxygen consumption during arousal from hibernation in Arctic ground squirrels. Am J Physiol Reg Integr Comp Physiol 281: 572-583.

Van Breukelen F, Martin SL (2015). The hibernation continuum: physiological and molecular aspects of metabolic plasticity in mammals. Physiology 30: 273-281.

Wilhelm Filho D, Althoff SL, Dafre AL, Boveris A (2007). Antioxidant defenses, longevity and ecophysiology of South American bats. Comp Biochem Physiol 146: 214-220.

Yin Q, Ge H, Liao CC, Liu D, Zhang S, Pan Y (2016). Antioxidant Defenses in the Brains of Bats during Hibernation. PLoS One 11: 1-17.