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Antioxidant defenses, longevity and ecophysiology of South American bats ☆

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Abstract

Microchiropteran bats sustain very high oxygen consumption rates when active, but they also exhibit drastic daily drops in oxygen consumption when torpid. In addition, bats are also characterized by an extraordinary longevity considering their body mass and high specific metabolic rate when compared to other mammals of related size. Therefore, they consist of a very interesting group regarding the free radical theory of aging. The present study was carried out to measure the antioxidant defenses of several tissues of five South American bat species, attempting to correlate the antioxidant status, ecophysiology and longevity. Superoxide dismutase (SOD) and catalase (CAT) activities in blood, liver and kidney were higher compared to other tissues. The contents of α-tocopherol and β-carotene found in liver, heart, kidneys, and pectoral muscles were one to two orders of magnitude higher than those usually found in rat and mouse liver. Also, these contents in liver were generally inversely related to lipoperoxidation measured as TBARS contents. Blood GSH contents and the activities of SOD and CAT were higher in torpid *Sturnira lillium* compared to active ones, thus suggesting that the elevation of such antioxidants might be daily modulated to minimize the oxidative stress related to the transition from torpid to active state in bats. The lower ROS generation reported in the literature for other bat species, their high constitutive antioxidant defenses, and the daily energy sparing associated with torpor appear to be closely related to their ecophysiological adaptations and to their extended longevity.
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1. Introduction

Gershman and collaborators (1954) showed for the first time that reactive oxygen species (ROS) are harmful for aerobic organisms, whilst the free radical theory of aging, first advanced by Harman in 1956, proposes that ROS formation contributes by a cumulative and a deleterious effect to determine their lifespan (Harman, 1956, 1982). Production of ROS as by-products of oxidative metabolism is deleterious for important biological molecules and therefore must necessarily be counteracted by a panoply of antioxidant defenses (Chance et al., 1979; Halliwell and Gutteridge, 1999). These defenses seem to be higher in metabolically active species of thermoconforming vertebrates such as fish (Wilhelm Filho et al., 1993; Wilhelm Filho et al., 2000). On the contrary, in endotherms such as birds and mammals (Sohal and Orr, 1992; Sohal et al., 1993; Barja et al., 1994a,b), including bats (Brunet-Rossini, 2004), the rate of mitochondrial ROS production seems to determine basal endogenous levels of antioxidants and their longevity (Sohal and Orr, 1992; Sohal et al., 1993; Barja et al., 1994a,b; Brunet-Rossini, 2004).

Regarding mammals the information available on antioxidants deals mainly with laboratory animals (e.g. Barja et al., 1994a,b), and studies on wild mammals are relatively limited (Witas et al., 1984; Buzádzic et al., 1990; Sohal and Orr, 1992;

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Sohal et al., 1993; Selman et al., 2000), and only recently appeared a related information on bats (Brunet-Rossini, 2004).

Bats sustain very high oxygen consumption when active, similar to that exhibited by small birds when flying, a feature that contrasts with the drastic drops in oxygen consumption when they become torpid (Lyman, 1970). Additionally, as generally found in birds, they are also characterized by an extraordinary large longevity for their high metabolic rate when compared with other mammals of related size and oxygen consumption (Brosset, 1966; Altman and Dittmer, 1974).

The present study focused on the antioxidant defenses such as superoxide dismutase and catalase activities, and also α-tocopherol and β-carotene contents from several tissues and blood GSH contents of five microchiropteran bat species, attempting to correlate their antioxidant protection with their ecophysiological characteristics and their longevity.

2. Materials and methods

2.1. Materials

With the exception of the single specimen of *Tadarida brasiliensis* that was caught in Buenos Aires, the other four bat species were captured in bird nets during early night (between 20:00 and 22:00 h, during September, early spring in Southern Hemisphere) in a forest in the vicinity of Florianopólis, south Brazil. Two specimens of *Anoura caudifer*, three of *Myotis nigricans*, four of *Artibeus lituratus*, eight of *Sturnira lillium* were captured. Blood was immediately drawn by heart puncture. The animals were then killed by cervical dislocation in accordance with approved legal procedures appropriate for mammals. The three specimens of *Sturnira lillium* used for the torpor experiments were carefully monitored and maintained in accordance with ethical recommendations of the Brazilian Veterinary-Medicine Council and the Brazilian College of Animal Experimentation.

2.2. Sample preparation

Blood was drawn immediately following capture in heparinized syringes through heart puncture and analyzed for glutathione content and hematological parameters. All the hematological parameters (hematocrit, hemoglobin content and mean cell hemoglobin concentration) were measured according to conventional methodology. Hemoglobin concentration was measured in the form of ciano-methemoglobin, according to the following formula, and considering that in all vertebrate Hbs, excepting in lampreys, the MW is 64 kDa: $\mathrm{Hb}=A_{540}\times$ dilutions/ε, where $\varepsilon=40\ \mathrm{mM}^{-1}\ \mathrm{cm}^{-1}$. After being killed by cervical dislocation, liver and other organs were rapidly removed and perfused with ice-cold saline solution for 5 min before being homogenized (1:9 w/v) in a buffer containing 0.1% Triton X-100, 0.12 M NaCl, 30 mM Na-phosphate, pH 7.4, and also containing freshly prepared protease inhibitors (PMSF 0.3 mM and trypsin inhibitor 0.05 mM). Homogenization was carried out at 4 °C, employing 20 strokes of a Potter–Elvehjen homogenizer, followed by centrifugation at 10,000×*g* for 10 min. Supernatants were stored in liquid nitrogen until analysis by enzymatic assays, which were performed four days after sample collection.

2.3. Blood extracts for GSH measurements

Reduced glutathione was measured according to Beutler et al. (1963), using the Ellman's reagent (DTNB: 5,5′-dithio-2-nitrobenzoic acid). Blood acid extracts were obtained by the addition of one third of the blood volume of a solution containing a 1.67% metaphosphoric acid, 5% NaCl, and 0.2% DPTA, shaken manually for a few seconds and then centrifuged. Supernatants (1:4 v/v) from the acid extracts were added with 0.25 mM DTNB in 0.1 M phosphate buffer (pH 8.0), and the GSH–DTNB adduct determined at 412 nm after approximately 1 min of the equilibrium reaction.

To evaluate the reduced glutathione (GSH) contents and the activities of SOD and CAT in blood, as well these activities in other tissues during and immediately after arousal from torpor, three specimens of *Sturnira lillium* were maintained at the laboratory during one week. These animals stood quiet during the acclimation period and were fed with fruits meanwhile. Blood was sampled at early morning, when the bats were apparently torpid (quiet, starting to shiver when handled).

2.4. Antioxidant enzymes assays for blood

After plasma removal, red cells were washed thrice in saline solution. Hemolysates were obtained after addition of 20 mM Tris–HCl buffer (pH 8.0) (3:1 v/v), and centrifuged at 10,000×*g* for 10 min. Hemolysate supernatants were taken for enzymatic analysis. For measurements of catalase activity the hemolysates were further diluted 500 times (Aebi, 1984). Superoxide dismutase evaluations (see below) were carried out in hemolysates treated with a mixture of chloroform/ethanol (3:5 v/v).

2.5. Antioxidant enzyme assays for other tissues

Aliquots of the corresponding extracts were stored at liquid nitrogen and examined separately for each enzymatic assay, in order to minimize further lost in activity due to the freezing/thawing procedures. Superoxide dismutase (EC 1.15.1.1) activity was measured at 550 nm, according to Flohé and Ötting (1984), through the inhibition of the reduction rate of ferricytochrome *c*. Aliquots of supernatant were added to a cuvette containing a Na-phosphate buffer 50 mM pH 7.8, 0.1 mM in EDTA, 20 µM cytochrome *c*, 5 µM in xanthine, and 0.1 mU/mL xanthine oxidase, to reduce cytochrome *c* at a rate of about 0.025 nm/min. One SOD arbitrary unit inhibits ferricytochrome *c* to one half and is equivalent to 11 pmol (355 ng) of pure Cu–Zn superoxide dismutase (Flohé and Ötting, 1984; Wilhelm Filho et al., 1993). SOD activity is expressed as U SOD g^{-1} for the different tissues or U SOD g^{-1} Hb for blood (hemolysates). Catalase (EC 1.11.1.6) activity was evaluated by measuring the decrease in hydrogen peroxide concentration at 240 nm (Aebi, 1984). Decays in A_{240} were registered at intervals of 5, 10 or 15 s during the first minute, in

Table 1
Biological, hematological data, and reduced glutathione (GSH) contents in the blood of five South American bat species

Species	N	Sex	Mass (g)	Feeding habit	Hb (mM)	Ht (%)	MCHC (g%)	GSH (μmol/mL)	GSH/Hb (mmol/mmol)
A. caudifer	2	F(P)	10.8±3 .2	NEC	3.37±0.12	59.0±3.2	36.8±7.8	1.49±0.15	0.94±0.15
S. lillium	8	7M, 1F	21.3±1.1	FRU	1.86±0.60	51.5±4.4	36.0±9.6	1.90±0.32	1.06±0.20
A. lituratus	4	M	64.2±12.2	FRU	2.78±1.50	49.4±1.6	55.8±8.6	0.71±0.12	1.52±0.54
M. nigricans	3	F(P)	4.2±0.5	INS	1.91±0.43	55.5±3.1	12.3±4.4	0.97±0.20	0.94±0.16
T. brasiliensis	1	M	13.0	INS	n.e.	n.e.	n.e.	n.e.	n.e.

Mean values±standard errors. N=number of specimens analyzed; F(P) denotes pregnant females; n.e. means not examined; MCHC=mean cell hemoglobin content; GSH (whole blood values in μmol mL^{-1}). INS=insectivorous; NEC=nectarivorous; FRU=frugivorous.

a cuvette containing 50 mM Na-phosphate buffer pH 7.0, and freshly prepared 10 mM hydrogen peroxide. Hydrogen peroxide stock solution was previously titrated to ascertain the concentration. Catalase activity is expressed as mmol min^{-1} g^{-1} for the different tissues or mmol min^{-1} g^{-1} Hb for blood (hemolysates).

2.6. α-tocopherol and β-carotene assays

α-tocopherol and β-carotene were analyzed through reverse phase of high performance liquid chromatography (HPLC) with in line electrochemical and UV detection (Nierenberg and Nann, 1992). An aliquot of 200 μL of tissue homogenate was added to 500 μL of methanol and 4 mL of hexane. The mixture was vortexed for 1 min and then the tubes were centrifuged for 5 min at 1000×*g*. A 3-mL aliquot from the hexane layer was dried under N_2 flux, and the residue dissolved in 200 μL methanol:ethanol (1:1, v/v) and filtered through a 0.22-μm pore nylon membrane. To determine total ubiquinol 10 content, the extracts were reduced as follows: extract aliquots (100 μL) were mixed with 0.5 mL of methanol, 0.5 mL of MilliQ water, and approximately 20 mg of $NaBH_4$. The mixture was vortexed and incubated at room temperature in the dark for 30 min, and then extracted into 4 mL of hexane following the steps described above. Measurements of antioxidants in the methanol:ethanol extract were made by reversed phase HPLC, using a Supelcosil LC-8 column (33×4.6 mm; 3 μm packing material), and 20 mM $LiClO_4$ in methanol:H_2O (97.7:2.5, v/v) as mobile phase. An in line electrochemical detector with a glassy-carbon working electrode (Bioanalytical System LC4C) at an applied oxidation potential of +0.6 V, and UV detector at 290 nm (Waters model 460, Milford, MA, USA) were used. Standard solutions of antioxidants were prepared according to Nierenberg and Nann (1992).

2.7. Determination of thiobarbituric acid-reactive substances (TBARS)

Determination of TBARS contents was used for assessing endogenous lipid peroxidation in tissue according to Ohkawa (1979) and Bird and Draper (1984). Homogenates were treated with 0.2 mM butylated hydroxytoluene (BHT) and precipitated with TCA (30%, 1:1 v/v) immediately after obtained to stop additional free radical peroxidation reactions. Supernatants were centrifuged (10,000×*g* for 10 min), added with 0.67% thiobarbituric acid, maintained in boiling water for 45 min, cooled at 5 °C for 30 min, and then measured spectrophotometrically at 535 nm. Absorbances were expressed as equivalent to nmol/g tissue, using E_{535}=153 mM^{-1} cm^{-1}.

2.8. Statistics

Student's *t* test was used to compare differences (SOD and CAT activities in different tissues and GSH levels in blood; Table 6) between active and torpid specimens of *Sturnira lillium*, and $p<0.05$ were considered significant.

Table 2
TBARS contents (nmol g^{-1} wet tissue) in different organs and tissues from five South American bat species

Species	Liver	Kidney	Gut	Heart	Brain	Lung
A. caudifer (2)	37.0±4.1	17.3±2.1	490.7±23.1	281.8±71.0	94±15.6	0.0
S. lillium (5)	79.5±7.6	0.5±0.1	0.0	240.7±32.9	85±11.9	0.0
A. lituratus (4)	38.3±3.3	55.6±4.5	0.0	47.6±1.9	29±2.0	0.0
M. nigricans (3)	4.0±0.4	21.2±1.8	0.0	315.3±21.6	248±28.3	0.0
T. brasiliensis (1)	59.5	7.3	0.0	229.9	41.2	13.0
Rat (6)	30.8±2.3	n.e.	n.e.	n.e.	n.e.	n.e.
Mouse (6)	220.2±3.9	n.e.	n.e.	n.e.	n.e.	n.e.

Number of specimens examined in parenthesis; n.e.=not examined. Values from rat and mouse liver were included for comparison.

Table 3
α-tocopherol contents (nmol g^{-1} wet tissue) in different organs and tissues from five South American bat species

Species	Liver	Kidney	Gut	Heart	Brain	Lung
A. caudifer (2)	6.9±1.8	61.9±21.0	0.1±0.1	746.9±37.8	<	19.4±2.2
S. lillium (5)	1.7±0.3	162.5±30.6	<	10.3±3.0	<	<
A. lituratus (4)	5.3±2.1	0.2±0.1	<	24.2±6.7	0.1±0.1	<
M. nigricans (3)	307.8±23.6	12.6±2.3	0.2±0.1	69.6±6.5	<	19.0±2.8
T. brasiliensis (1)	15.5	3.0	16.9	0.6	<	1.5
Rat (6)	7.4±3.8	n.e.	n.e.	n.e.	n.e.	n.e.
Mouse (6)	10.6±4.2	n.e.	n.e.	n.e.	n.e.	n.e.

Number of specimens examined in parentheses; n.e.=not examined; < means concentration values lower than the detection limit (<0.1 nmol g^{-1} tissue).

Table 4
Superoxide dismutase activity (U SOD g^{-1} wet tissue or U SOD g^{-1} Hb in blood) of several organs and tissues from five South American bat species

Species	Blood	Liver	Kidney	Gut	Heart	Spleen	Brain	Lung	Pecm	Subm
A. caudifer (2)	5382.8±154.5	1269.1±87.7	173.3±32.6	585.5±52.8	486.4±111.4	801.9±77.3	485.1±81.5	405.5±39.8	585.5±86.3	756.4±44.8
S. lillium (5)	3177.3±165.9	945.5±72.6	882.8±56.1	396.9±44.8	333.7±41.3	351.9±31.9	234.6±33.6	108.2±12.9	450.0±54.7	336.7±23.9
A. lituratus (4)	3996.4±174.6	2178.2±74.3	1125.5±57.2	394.4±54.1	340.1±38.9	405.5±33.8	254.8±32.9	216.4±20.1	612.8±77.1	432.8±41.7
M. nigricans (3)	5508.2±243.6	2133.7±90.0	1628.0±39.3	1648.3±82.6	585.5±56.2	351.9±55.2	549.1±66.8	504.6±39.3	765.5±63.6	1456.2±65.7
T. brasiliensis (1)	n.e.	5449.1	5283.7	1051.7	459.1	639.1	1872.8	198.2	374.2	312.8
Rat (6)	n.e.	711.9±45.3								
Mouse (6)	n.e.	423.7±31.8								

Number of specimens examined in parentheses; n.e.=not examined; Pecm means pectoral muscle; Subm means sub pectoral muscle.

2.9. Chemicals

All the chemicals used in the present study were purchased from Sigma-Aldrich Co., St. Louis, MO, USA, and were of analytical grade.

3. Results

The average weight of the four specimens of *Artibeus* was approximately 15 times the average weight of the three specimens of the small *Myotis nigricans*. The blood contents of reduced glutathione and the hematological values did not show significant differences among the four species studied (Table 1).

With the exception of the gut from *Anoura*, the heart showed TBARS contents that were higher than the contents found in other tissues (Table 2). Liver peroxidation in all bat species examined here was essentially in the same level of those found in rat and lower compared to those found in mouse liver (Table 2).

Heart, liver and kidneys exhibited the highest α-tocopherol levels in all five species examined (Table 3). The α-tocopherol concentrations found in the liver from *Myotis nigricans* and in the kidneys from *Sturnira lillium* kidneys were more than one order of magnitude higher than those found in rat and mouse (Table 3).

β-carotene showed relatively high concentrations only in the heart from *Anoura* (65.7±28.2 nmol g^{-1} wet tissue), and also in the heart, liver, and pectoral muscles from one specimen of *Artibeus* (1.7, 4.4 and 57.2 nmol g^{-1} wet tissue, respectively) while other tissues seemed to be devoid of β-carotene, or the contents were under the detection limit (data not shown).

Blood SOD activities were high in each bat species, which correspond roughly to concentrations around 0.1 mM, and were slightly higher than those of other mammals including rat and mouse (Table 4). Blood revealed the higher catalase activities in all species examined (Table 5).

GSH contents in blood as well as the activities of SOD and CAT in blood were higher in resting/torpid bats compared to active bats (Table 6). However, liver SOD activity was decreased in torpid bats compared to active bats (Table 6).

4. Discussion

Excepting for the values found in the gut from *Anoura*, the heart showed the highest TBARS contents compared to the other tissues, a feature that could be associated with the high mitochondrial mass and the high oxygen consumption of bat myocardium where heart frequencies near thousand beats/min are common when flying (Thomas and Suthers, 1972; Jurgens and Prothero, 1991). Mitochondrial oxygen consumption seems to be well correlated with ROS generation (Boveris, 1977) and must be accompanied by a parallel antioxidant protection in vertebrates (Selman et al., 2000; Wilhelm Filho et al., 2000; Wilhelm Filho, 2007). A dynamic equilibrium between oxygen consumption and ROS generation was already demonstrated in long-term cold adaptation of the short-tailed field vole *Microtus agrestis* (Selman et al., 2000).

Heart, liver and kidneys exhibited the highest α-tocopherol levels in all five species examined, and these vertebrate tissues are characterized by very high oxygen consumption (Schmidt-Nielsen, 1977). The α-tocopherol concentrations found in the liver from *Myotis nigricans* and in the kidneys from *Sturnira lillium* (Table 3) were more than one order of magnitude higher than those found in rat and mouse (ca. 10 nmol g^{-1} wet tissue, this study; González-Flecha et al., 1991).

β-carotene showed relatively high concentrations in few tissues, particularly in the heart from *Anoura* (65.7±28.2 nmol

Table 5
Catalase activity (mmol min^{-1} g^{-1} wet tissue or mmol min^{-1} g^{-1} Hb in blood) of several organs and tissues from five South American bat species

Species	Blood	Liver	Kidney	Gut	Heart	Spleen	Brain	Lung	Pecm	Subm
A. caudifer (2)	93.48±26.3	6.07±3.08	11.70±3.66	8.98±3.18	2.77±0.73	6.93±1.29	2.06±0.09	6.90±3.34	3.08±0.11	2.77±0.13
S. lillium (5)	142.03±42.9	8.41±0.27	14.8±0.32	4.20±0.18	2.68±0.07	5.04±0.14	0.91±0.09	14.53±2.94	3.22±0.16	3.73±0.08
A. lituratus (4)	81.45±23.6	6.38±0.19	6.88±0.11	1.34±0.09	1.63±0.06	1.48±0.06	1.45±0.04	2.28±0.07	1.85±0.05	2.32±0.16
M. nigricans (3)	88.19±30.2	12.28±0.17	10.0±0.21	3.18±0.09	2.39±0.11	2.76±0.08	0.80±0.05	2.89±0.09	1.81±0.16	3.30±0.39
T. brasiliensis (1)	n.e.	39.24	7.86	6.74	3.77	2.25	2.32	9.46	5.87	4.34
Rat (6)	n.e.	31.96±8.7								
Mouse (6)	n.e.	15.93±9.3								

Number of specimens examined in parentheses; n.e.=not examined; Pecm=pectoral muscle; Subm=sub pectoral muscle.

Table 6
Superoxide dismutase (U SOD g^{-1} wet tissue or U SOD g^{-1} Hb in blood) and catalase (mmol min^{-1} g^{-1} wet tissue or mmol min^{-1} g^{-1} Hb in blood) activities in different tissues and GSH contents in the blood of active and torpid *Sturnira lillium*

Sturnira lillium	Blood	Liver	Heart	Brain	Lung
Active (n=5)					
SOD	80.12±13.44	25.28±3.42	4.30±1.72	3.28±1.26	2.85±0.35
CAT	1.31±0.38	2.53±0.80	0.19±0.04	0.18±0.09	0.31±0.12
GSH	1.01±0.07	n.e.	n.e.	n.e.	n.e.
Torpid (n=3)					
SOD	168.54±8.44**	13.8±0.52*	3.48±0.86	3.82±0.11	2.71±0.27
CAT	3.09±0.20*	4.28±0.22	0.18±0.02	0.15±0.05	0.30±0.18
GSH	1.28±0.05*	n.e.	n.e.	n.e.	n.e.

Number of specimens examined in parentheses; n.e.=not examined. Statistical differences (*p<0.05) and (**p<0.01) were obtained using Student's t test.

g^{-1} wet tissue), and also in the heart, liver, and pectoral muscles from one specimen of *Artibeus* (1.7, 4.4 and 57.2 nmol g^{-1} tissue, respectively), while in other tissues it was not detected. At low oxygen tensions that prevail in very active tissues such as pectoral muscles, β-carotene is a better chain terminator than α-tocopherol (Burton and Ingold, 1984; Rosseau et al., 1992). The vegetarian diet of these species probably provides large amounts of carotenoids, vitamins E and C, and also polyphenols that can offer a nutritional antioxidant protection for these tissues (Halliwell and Gutteridge, 1999).

In the present study high levels of α-tocopherol were generally found in tissues where the TBARS concentrations were low. This inverse relation between lipoperoxidation and the contents of β-carotene and α-tocopherol probably applies to other mammals (González-Flecha et al., 1991). On the other hand, the lack or restriction of fat deposits (Studier and Wilson, 1991) certainly contributes to minimize lipoperoxidation processes in bats, and this might explain why lipoperoxidation was not detected in some tissues such as lung and gut, except in *Anoura caudifer*. In addition, it was reported that the proportion between the dietary content of polyunsaturated fatty acids (PUFA) and the overall antioxidant capacity might be mandatory for the start and duration of torpor in heterothermic mammals (Munro and Thomas, 2004). Furthermore, insectivorous bats have a limited access to high PUFA levels in the diet and therefore, they appear to select their insect prey to maximize PUFA intake (Schalk and Brigham, 1995).

Interestingly, the two insectivorous species *Myotis nigricans* and *Tadarida brasiliensis* (Brosset, 1966) showed, in general, relatively high antioxidant capacity in their tissues compared to the other species. The relatively high SOD and CAT activities found in their gut might be related to their diet. While both species are insectivorous the others feed on plants, *Anoura caudifer* is nectarivorous and *Sturnira lillium* and *Artibeus lituratus* are both frugivorous bats (Brosset, 1966). According to the high enzymatic activities found in these two species, the highest liver and gut α-tocopherol concentrations were also found in *Myotis* and *Tadarida*, respectively.

Blood SOD activities were the highest revealed for each bat species, which correspond to concentrations around 0.1 mM, and were slightly higher than those of other mammals, including rat and mouse and the squirrel *Citellus citellus* (ca. 10 to 50 nmol g^{-1} Hb; this study; Buzádzic et al., 1990). On the other hand, liver SOD and CAT activities were near the range corresponding to mouse and rat (Chance et al., 1979; González-Flecha et al., 1991). Brain, lung and heart displayed relatively low SOD and CAT activities when compared to the other tissues. A similar profile was found for the α-tocopherol concentrations in the brain.

Despite the few individuals analyzed for this purpose, blood GSH concentrations showed higher values in *Sturnira lillium* kept in laboratory, which were killed early morning when

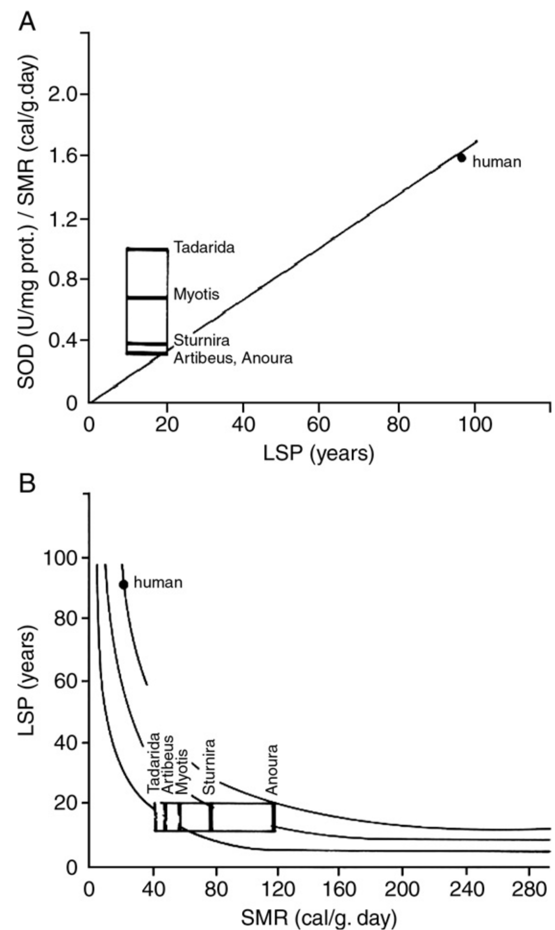

Fig. 1. A: Liver superoxide dismutase activity and approximate life span potential (LSP, in years) for the five South American bat species. Straight regression corresponds to 13 mammal species according to Cutler (1984). Bat values (box) refer to the range of standard metabolic rates (SMR; cal g^{-1} day^{-1}) corrected for torpor according to Jurgens and Prothero (1987). B: Approximate life span potential (LSP; range of 10 to 20 years) for bats and standard metabolic rates (SMR; cal g^{-1} day^{-1}) for the five South American bat species compared to the regression line of mammals (lower curve), primates (middle curve) and humans, lemur, and capuchin (upper curve), according to Cutler (1984). Bat values (box) refer to SMR corrected for torpor according to Jurgens and Prothero (1987).

torpid, when compared to active specimens captured and killed *in loco* when searching for food. In addition, roughly half SOD and CAT activities found in the erythrocytes and lower SOD activity found in the liver of torpid bats compared to active bats suggest that some antioxidants might be temporarily modulated. Daily modulations of antioxidants were already described in different tissues of vertebrates species (Wilhelm Filho et al., 1994, 2000). Therefore, the elevations found in GSH contents and in the activities of SOD and CAT in blood are probably recovered during torpor and might be under a circadian control. This antioxidant compensation probably minimizes oxidative stress originating from the daily transition from torpid to active state in bats (Geiser and Budinette, 1990), making the blood compartment part of a first antioxidant front (Halliwell and Gutteridge, 1999) in bats to counteract the high oxidative performance during flight.

The antioxidant adaptation found in the present study is in accord with other authors who proposed that hibernating bats tend to live longer than bat species that do not enter such arrested state (Wilkinson and South, 2002). However, SOD activities found in brain and heart of *Myotis lucifugus* were not different from two other mammalian species with distinct metabolic rates and longevities (Brunet-Rossini, 2004). Despite the fact that no statistical differences were found in SOD activities of *M. lucifugus*, the heart values were approximately two fold compared to the other two species examined, whilst brain is an organ that show generally low and similar SOD activities in vertebrates (Wilhelm Filho et al., 2000). In addition, in that study only SOD activity was evaluated as an antioxidant together with hydrogen peroxide production in the mitochondria of three different tissues, thus limiting appropriate conclusions.

Most bat species have an exceedingly large longevity (Brosset, 1966) for their high metabolic rate and small body size when compared to other mammals of the same weight (Altman and Dittmer, 1974; Perry, 1990; Studier and Wilson, 1991; Wilkinson and South, 2002). They generally possess a potential longevity of up to 10–30 years and some species even more (Hall et al., 1957; Griffin and Hitchcock, 1965; Brosset, 1966; Thomas and Suthers, 1972; Tuttle and Stevenson, 1982; Jurgens and Prothero, 1987; Wilkinson and South, 2002; Brunet-Rossini and Austad, 2004). Furthermore, bats share allometric characters such as respiratory surface and thickness of lungs better with passariform birds than non-flying mammals of same weight, both exhibiting exceedingly high oxygen consumption in flight compared to other vertebrates (Perry, 1990). Heterothermic bats may also be compared to birds (Brunet-Rossini, 2004; this study) in terms of high longevity associated with the balance between oxidants and antioxidants associated with their aerobic metabolism.

When the liver SOD activities found in the five bat species here studied are divided by their corresponding standard metabolic rates (SMR, corrected for torpor according to Jurgens and Prothero, 1987), and are plotted against their life span energy potential (LSP; Cutler, 1984), the values are similar or higher than the regression line for primates and humans (Fig. 1A). Also, considering this longevity range and the standard metabolic rate of the bat species here examined (ca. 1.0 to 2.0 mL O_2 g^{-1} h^{-1}; McNab, 1989), their life span energy potential (LSP; Cutler, 1984) will fall within or above the regression lines corresponding to mammals, primates and humans (Fig. 1B).

In summary, the relatively high antioxidant protection displayed by their tissues, considering both constitutive antioxidant enzymatic activities and nutritional antioxidants, together with the capability of some heterothermic bats becoming torpid, and therefore daily reducing their metabolic rate (Lyman, 1970; Jurgens and Prothero, 1987; McNab, 1969, 1989) seem to be determinant to their longevity. In addition to the above adaptations, the relatively lower rate of mitochondrial ROS production found in endotherms such as bats (Brunet-Rossini, 2004), other mammals (Ku et al., 1993; Sohal et al., 1993), and birds (Barja et al., 1994b), and also some important aspects of their life history and the genetic contribution (Wilkinson and South, 2002; Brunet-Rossini and Austad, 2004), are also important and probably act in concert to characterize their extended longevity.

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