Oxidative DNA damage and antioxidant defenses in the European common lizard (*Lacerta vivipara*) in supercooled and frozen states

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Abstract

The European common lizard (*Lacerta vivipara*) tolerates long periods at sub-zero temperatures, either in the supercooled or the frozen state. Both physiological conditions limit oxygen availability to tissues, compelling lizards to cope with potential oxidative stress during the transition from ischemic/anoxic conditions to reperfusion with aerated blood during recovery. To determine whether antioxidant defenses are implicated in the survival of lizards when facing sub-zero temperatures, we monitored the activities of antioxidant enzymes and oxidative stress either during supercooling or during freezing exposures (20 h at –2.5 °C) and 24 h after thawing in two organs of lizards—muscle and liver. Supercooling induced a significant increase in the total SOD and GPx activity in muscle (by 67 and 157%, respectively), but freezing had almost no effect on enzyme activity, either in muscle or in liver. By contrast, thawed lizards exhibited higher GPx activity in both organs (a 133% increase in muscle and 59% increase in liver) and a significant decrease in liver catalase activity (a 47% difference between control and thawed lizards). These data show that supercooling (but not freezing) triggers activation of the antioxidant system and this may be in anticipation of the overgeneration of oxyradicals when the temperature increases (while thawing or at the end of supercooling). Oxidative stress was assessed from the content of 8-oxodGuo and the different DNA adducts resulting from lipid peroxidation, but it was unaltered whatever the physiological state of the lizards, thus demonstrating the efficiency of the antioxidant system that has been developed by this species. Overall, antioxidant defenses appear to be part of the adaptive machinery for reptilian tolerance to sub-zero temperatures.

Keywords: Freeze tolerance; Supercooling; Reptile; Superoxide dismutase; Catalase; Glutathione peroxidase; Lipid peroxidation induced DNA adducts; 8-oxodGuo

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Several species of cold-blooded vertebrate that hibernate on land are commonly exposed to sub-zero temperatures. To ensure their survival during winter, these animals have developed cold hardiness strategies that are commonly divided into two main groups: freeze avoidance via an extensive supercooling capacity and freeze tolerance. The freeze avoidance strategy is characterized by various metabolic adaptations involving the release or masking of potent ice nucleators, the accumulation of low molecular weight carbohydrates and antifreeze proteins that depress the freezing point [39]. By contrast, the freeze tolerance strategy implies that animals can endure the conversion of a fraction of body water into ice. This second strategy is characterized by mechanisms such as the production of ice nucleators which allow the initiation of freezing at high sub-zero temperatures and the production of cryoprotectant substances, which allow controlled propagation of ice within the body [32]. Among the several species of Holarctic vertebrates, winter survival is mainly based on supercooling and the principal mechanisms developed involve the removal of active nucleating agents from body fluids and modification of the integument to provide a highly efficient barrier to the penetration of ice from frozen soil into the body compartments [26]. On the other hand, freeze tolerant vertebrates principally accumulate high concentrations of carbohydrates, such as glucose and/or glycerol [32]. In the supercooled state, the heart rate is known to be extremely low [3] which reduces tissue perfusion and causes accumulation of lactate within the body [16]. Freezing also induces ischemia and anoxia because when the extracellular body fluids freeze the circulation stops and the cells rely on internal fermentation of fuel reserves for survival. Increase in temperature and thawing then reintroduce oxygen into the tissues and organs, thereby restoring aerobic metabolism. However, reperfusion also potentially creates the conditions for oxidative stress.

Indeed, oxygen metabolism is known to generate reactive oxygen species (ROS), as by-products [6,12] and these can initiate free radical chain reactions that may damage cellular components such as DNA, proteins, and lipids. Both superoxide radicals (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) can lead to the formation of the hydroxyl radical (OH) which is the most reactive form of ROS. While the superoxide radicals are produced by the “leaky” mitochondrial respiratory chain, or from the activity of oxidases such as xanthine oxidase, aldehyde oxidase and NADPH oxidase, hydrogen peroxide (H$_2$O$_2$) is mainly formed by dismutation of O$_2^-$ [5,14]. To neutralize the oxidative effect of ROS, all aerobic organisms have developed biochemical defenses, including low molecular weight free radical scavengers and enzymatic antioxidant systems [38]. When the generation of ROS exceeds the capacity of the antioxidant defense systems, oxidative stress occurs in the cell. Many organisms are likely to experience wide variations in oxygen availability to their tissues when facing natural conditions like birth, exercise, hibernation, and overwintering [4,7,18,23,29]. To endure these situations, animals have developed different degrees of tolerance to ischemia and anoxia, including reliable antioxidant systems.

Questions concerning antioxidant defenses at sub-zero temperatures have received some attention lately, but only in two species of insect [23], a frog [22], and a reptile [20]. In spite of these three studies, no data are available to make direct comparisons between the biochemical adaptations associated with freeze tolerance and supercooling by examining these processes in the same species. Recent studies have shown that the European common lizard (Lacerta vivipara) tolerates both the supercooled state and the frozen state in its natural environment. Laboratory experiments have shown that this lizard can remain for at least 3 weeks in the supercooled state and for 3 days in the frozen state [9]. The maximum ice content (about 50% of the body water converted into ice) is attained after 5 h freezing and then remains constant [37]. Since these two physiological states potentially induce prolonged hypoxia, the present work aims to monitor the activities of the main antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)] during either supercooling or freezing induced by exposure to $-2.5\,^\circ C$ for 20 h, and 24 h after thawing at $5\,^\circ C$. Two organs were studied—muscle and liver. To assess the disturbance of the balance of prooxidant/antioxidant, we measured the oxidative damage to DNA in cells: that is extensively used as an indicator of the occurrence of oxidative stress [8]. We focused our interest on two types of DNA lesion: (i) 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo), which is a marker for direct DNA oxidation [17] and (ii) lipid peroxidation DNA adducts that come from the release of aldehydic products during the breakdown of lipid peroxides. The latter metabolites include malondialdehyde (MDA) and simple aldehydes such as α,β-unsaturated aldehydes like acrolein.
and 4-hydroxy-2-nonenal (4-HNE). Most of these aldehydes have been found to react with DNA bases, giving rise to adducts. MDA adds to 2'-deoxyguanosine to yield a pyrimidopurinone exocyclic adduct (M1dGuo). 4-HNE also converts guanine into an exocyclic propano adduct. In addition, the epoxidation product of 4-HNE has been shown to give rise to ethenobases from adenine and guanine [11].

Materials and methods

Animals

_Lacerta vivipara_ inhabits mainly damp habitats such as meadows, peatbogs, and heathlands, and hibernates in a shallow terrestrial hibernaculum. _L. vivipara_ specimens (2.94 ± 1.25 g) were collected from a highland population in the Cévennes mountains in France (latitude, 44.30°N; longitude, 3.45°E; altitude, 1450 m) in late September. They were held in boxes with sand and wet moss and cold acclimated at 4 °C for 6–7 weeks in the dark without feeding before use. The present investigation was carried out according to the ethical principles laid down by the French (Ministère de l’Agriculture) and European Convention for the protection of Vertebrate Animals used for Experimental and Scientific purposes (Council of Europe No. 123, Strasbourg, 1985).

Preparation of supercooled, frozen, and thawed animals

Freezing exposures were performed as previously reported [36,37]. Briefly, lizards were placed on a pad of absorbent wet paper, itself placed inside a 50 ml Falcon tube. Animals were then progressively cooled at a constant rate of 0.2 °C min⁻¹ down to −2.5 °C. They were then maintained at this temperature for 20 h.

After supercooling, freezing, and thawing the lizards were killed by decapitation and organs were quickly dissected out and frozen by immersion in liquid nitrogen. Organ samples were then stored at −80 °C until use.

Antioxidant enzyme activities

Frozen tissue samples were rapidly weighed and homogenized using a Potter–Elvehjem homogenizer at 4 °C, in a buffer containing KH₂PO₄ (100 mM), DTT (1 mM), and EDTA (2 mM), pH 7.4. After centrifugation (3000g, for 5 min), the supernatant was used for the enzymatic assays.

Superoxide dismutase

Superoxide dismutase (SOD) activity was assayed spectrophotometrically (at 550 nm) by monitoring the rate of acetylated cytochrome c reduction by superoxide radicals generated by the xanthine–xanthine oxidase system at 25 °C in reaction buffer (xanthine 0.5 mM, cytochrome c 0.2 mM, KH₂PO₄ 50 mM, and EDTA 0.1 mM, pH 7.8) [13]. One activity unit of SOD is defined as the amount of enzyme which inhibits the rate of acetylated cytochrome c reduction by 50%. To distinguish mangano-SOD (MnSOD), which is exclusively located in mitochondrial matrix, from cuprozinc-SOD (CuZnSOD), which is primarily located in the cytosol, SOD activity was determined after incubation with NaCN (1 mM). At this concentration, cyanide inhibits the CuZn isoform of the enzyme, but does not affect the MnSOD isoform [13]. SOD activity was expressed as unit per milligram of total protein.

Glutathione peroxidase

The total activity of glutathione peroxidase (GPx) activity was assayed with cumene hydroperoxide (1 mg ml⁻¹) as substrate by measuring spectrophotometrically the reduction of NADPH (ε = 6.22 × 10³ L mol⁻¹ cm⁻¹) at 340 nm (Tappel, 1978). Measurement was carried out at 37 °C in reaction buffer (GSH 0.25 mM, NADPH 0.12 mM, GR 1 U ml⁻¹, and NaCN 10 mM).

GPx activity was expressed as milliunit per milligram of total protein.
Catalase

The activity of catalase (CAT) was determined by the method of Aebi [1]. Each supernatant (200 µl) was incubated 30 min at 0°C with ethanol (95%, 2 µl). After adding Triton (1%, 2 µl) the sample was centrifuged at 5000 g for 5 min. The supernatant was used for measurement of CAT activity by using the first-order rate constant of the decomposition of H₂O₂ by tissue CAT at 20°C in buffer (pH 7.4) containing KH₂PO₄ (40 mM) and HNa₂PO₄ (60 mM). One unit of catalase activity was calculated by using

\[ k = \frac{2.3}{d_t} (\log A_1/A_2), \]

where \( k \) is CAT activity, \( d_t \) is change in time, \( A_1 \) is initial absorbance, and \( A_2 \) is final absorbance. CAT activity was expressed in millikelvin per milligram of total protein.

Nuclear DNA oxidative damage content

As previously described [27] determination of 8-oxodGuo needs some precautions to limit artefactual oxidation during DNA extraction.

Isolation of nuclei

Samples were homogenized with a Potter glass homogenizer in buffer (320 mM sucrose, 10 mM Tris, 5 mM MgCl₂, 0.1 mM desferroxamine mesylate, and 1% Triton 100, pH 7.5). After homogenization, the sample was centrifuged at 1500 g for 5 min. The supernatant was discarded and the pellet was washed before centrifugation at 1500 g.

DNA isolation

Nuclear pellets were resuspended in 600 µl extraction buffer (10 mM Tris, 5 mM EDTA, and 0.15 mM desferroxamine mesylate, pH 8) with addition of SDS (10%). RNase A (3 µl, 100 mg/ml) and RNase T1 (8 U) were then added and the samples were incubated for 15 min at 50°C. The samples were then incubated with Qiagen proteinase (20 mg/ml) for 60 min at 37°C. Isolation of nucleic acids was achieved by adding a NaCl solution (7.6 M NaCl, 40 mM Tris, and 20 mM EDTA, pH 8), and isopropanol (100%). The samples were centrifuged for 15 min at 5000 g at room temperature. The nucleic acids pellets were rinsed. Following the last centrifugation (5000 g, 10 min), the DNA pellet was solubilized in deionized water containing 0.1 mM desferroxamine mesylate.

Digestion

Phosphodiesterase II (Ph II, 1 µl, 0.004 U; Sigma, St. Louis, MO) and 5 U of nuclease P1 (5 µl in 300 mM of 10 µM ZnSO₄, pH 5, Sigma, St. Louis, MO) were added to the DNA solution (100 µl) together with 5 µl MN/SPD buffer (200 mM succinic acid, 100 mM CaCl₂, pH 6). The resulting solution was incubated for 2 h at 37°C. Then, 12 µl PA buffer (500 mM Tris, 1 mM EDTA, pH 8) was added together with 0.003 U phosphodiesterase I (Ph I, 0.25 µl, Sigma, St. Louis, MO) and 4 U alkaline phosphatase (0.2 µl, Roche Molecular Biochemicals). The sample was incubated at 37°C for 2 h. HCl (8 µl, 0.1 N) and chloroform (30 µl) were added. The sample was vortexed. The resulting solution was centrifuged (5000 g) and subsequently transferred into HPLC injection vials.

Samples were first analyzed by HPLC with electrochemical detection (HPLC-EC) to quantify 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) as described previously [28].

DNA samples were then analyzed for their content of lipid peroxidation adducts by HPLC–MS/MS as described previously [28]. For this purpose, the vials used for the HPLC-EC analysis were freeze-dried. DNA samples were then analyzed for their content of lipid peroxidation adducts by HPLC–MS/MS. For this purpose, the vials used for the HPLC-EC analysis were freeze-dried. The resulting residue was solubilized in 30 µl water containing 200 fmol of the [15N₅]-labeled derivatives of 1,N₂-ethdA, 1,N₂-edGuo, and 6-(1-hydroxyhexanyl)-8-hydroxy-1,N²-propano-2'-deoxyguanosine HNE-dGuo propano adduct. The latter compounds were used as internal standard for isotopic dilution calibration. M1dGuo was quantified by external calibration. The samples were then analyzed by reverse phase HPLC and an API 3000 mass spectrometer (Perkin-Elmer/SCIEX, Thornhill, Canada) This was used in the multiple reaction monitoring mode. 1,N⁶-Etheno-2′-deoxyadenosine (ethdA), 1,N⁶-etheno-2′-deoxyguanosine (ethdGuo), pyrimidopurinone malondialdehyde adduct to 2′-deoxyguanosine (M1dGuo), and 1,N²-propano adduct between dGuo and 4-hydroxy-2-nonenal (HNE-dGuo) were quantified by high performance liquid chromatography associated with tandem mass spectrometry (HPLC–MS/MS) in the positive mode. In each sample, the amount of DNA injected was inferred from the area of the peak corresponding to 2′-deoxyguanosine monitored in a UV spectrophotometer.

Protein measurement and statistics

Protein concentration was measured by the Lowry method [24].
Data are presented as means ± SE. Statistical analysis was performed with Statview computer statistical package. Data were analyzed by the non-parametric Kruskall–Wallis test and the Mann–Whitney test to compare two means. We used non-parametric tests because of violation of the parametric assumption of homogeneity of variance. A 5% ($p < 0.05$) level of significance was used in all tests.

**Results**

**Antioxidant enzyme activities**

Figs. 1–3 show the activities of enzymes involved in the antioxidant defenses of *L. vivipara*, in two tissues and in the following groups: control (4°C); frozen (20 h at $-2.5^\circ$C); supercooled (20 h at $-2.5^\circ$C);

![Graph 1](image1)

Fig. 1. (A) Total superoxide dismutase (SOD), (B) MnSOD, and (C) CuZnSOD activity in tissues of *L. vivipara*. Open bars, control lizards (4°C, $n = 5$); grey bars, supercooled (20 h at $-2.5^\circ$C, $n = 4$) lizards; crosshatched bars, frozen (20 h at $-2.5^\circ$C, $n = 5$) lizards, black bars, thawed (24°C at 5°C, $n = 5$) lizards. Data are means ± SE. Values were considered statically significant at $p < 0.05$. (*) Significantly different from corresponding control value.

![Graph 2](image2)

Fig. 2. Glutathione peroxidase (GPx) activity in tissues of *L. vivipara*. Details as in Fig. 1. (※) Significantly different from corresponding control value; (†) significantly different from corresponding frozen value.
thawed (24 h at 4 °C). Overall, the activities of antioxidant enzymes were always greater in liver than in muscle (between four- and sevenfold according to the enzyme) and they differed markedly between the different physiological states of *L. vivipara*.

The total SOD activity increased significantly during supercooling in muscle tissue (by up to 67% of control values—Fig. 1A). Such an increase was detected for both cytosolic and mitochondrial SOD (68 and 66%, respectively—Figs. 1B and C). On the other hand, total SOD activity in liver was unaffected by freezing and supercooling (Fig. 1A) although a significant increase in cytosolic SOD after freezing was observed (about 56% in comparison with controls—Fig. 1C). Mitochondrial SOD activity was equivalent irrespective the physiological state (Fig. 1B).

Muscle GPx activity was significantly higher with supercooling and 24 h after thawing (about 157 and 133%, respectively, Fig. 2). GPx activity in liver followed the same patterns but with a lesser effect of supercooling.

Hepatic catalase activity was not altered during freezing and supercooling but showed a significant decrease 24 h after thawing and that applied to all other groups (for instance, a 47% decrease in thawed lizards with respect to control. By contrast, the experimental treatment had no effect on catalase activity in muscles (Fig. 3).

**Oxidative stress status by nuclear DNA oxidative damages measurement**

Levels of modified bases within DNA were undetectable in muscle but this was explainable by the low DNA contents of these samples. However, with the liver, 8-oxodGuo and DNA adducts from lipid peroxidation were detectable but showed little or no oxidative stress induced by supercooling, freezing or thawing in *L. vivipara*. The five modified DNA bases assessed in this study did not show significant variations between the different physiological states (see Table 1).

**Discussion**

The European common lizard (*L. vivipara*) is perhaps the most widely distributed of extant lizards. It is one of the four palearctic reptiles inhabiting the subarctic region [30] and thrives in various habitats owing to its physiological plasticity [15]. Different studies have provided conclusive evidence of a well-developed capacity for supercooling and freezing tolerance in this species [9,37]. Its unusual cold hardiness, involving both supercooling and freezing, may promote its winter survival, which is exceptionally high (88–100% at all ages) even in severe winter [2]. Both physiological states induce prolonged ischemia due to the extreme reduction of the heart

**Table 1**

Liver DNA oxidative damage in *L. vivipara* after supercooling, freezing, and thawing

<table>
<thead>
<tr>
<th>Lacerta vivipara</th>
<th>Control</th>
<th>Supercooled</th>
<th>Frozen</th>
<th>Thawed</th>
<th><em>P</em> values</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OxodG</td>
<td>2.43 ± 0.21</td>
<td>2.33 ± 0.37</td>
<td>2.20 ± 0.45</td>
<td>1.88 ± 0.16</td>
<td>0.34</td>
</tr>
<tr>
<td>EthdA</td>
<td>4.43 ± 0.59</td>
<td>2.31 ± 0.61</td>
<td>3.72 ± 0.30</td>
<td>3.53 ± 0.64</td>
<td>0.18</td>
</tr>
<tr>
<td>EthdGuo</td>
<td>5.57 ± 0.21</td>
<td>3.70 ± 0.93</td>
<td>5.18 ± 0.49</td>
<td>5.19 ± 0.99</td>
<td>0.42</td>
</tr>
<tr>
<td>M1dGuo</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.81</td>
</tr>
<tr>
<td>HNE-dGuo</td>
<td>0.03 ± 0.02</td>
<td>0.07 ± 0.04</td>
<td>0.06 ± 0.03</td>
<td>0.03 ± 0.02</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Data are lesions per 10⁶ unmodified bases, means ± SE with *n* = 5 for frozen and thawed groups and *n* = 4 for control and supercooled groups.
pulse rate during supercooling [3] and trapping of cells by ice during freezing [31].

Our results suggest that the activation of antioxidant systems under sub-zero temperatures is a part of the survival strategy of the European common lizard when facing supercooling or freezing (see Figs. 1–3). The data show that SOD and GPx might play a role in this, with a threefold increase in GPx activity, that may be explained by its capacities to detoxify both H₂O₂ and the hydroperoxides resulting from lipid peroxidation [12]. Even if the antioxidant enzyme activity measurements were made far from their normal operating temperature, our results corroborate previous studies demonstrating that antioxidant defenses play key roles in tissue protection during freezing and thawing [20,22,23].

As far as weknow, the relations between antioxidant defenses and sub-zero temperatures have so far been studied in only one reptile: the garter snake (Thamnophis sirtalis). It has emerged that freezing at −2.5°C for 5h resulted in a significant rise in the activity of muscle and lung catalase (by 183 and 63%) and in muscle glutathione peroxidase (by 52%) [20]. This was interpreted as an adaptation to overcome the potentially injurious post-ischemic situation following thawing but the effect of recovery from freezing was not analyzed. The pattern is thus different for L. vivipara, which showed no significant difference between control and frozen individuals, whatever the enzyme studied (see Figs. 1–3). Such a discrepancy between the two reptiles may be explained by the different cold hardness capacities. Indeed, while spontaneous ice nucleation occurs within 1h of supercooling at high sub-zero temperature (−3.3°C) in garter snakes [10], supercooled L. vivipara can survive for at least 3 weeks at −3.5°C [9]. That suggests a “global” strategy in L. vivipara, whereby sub-zero temperatures induce an increase of the activity of at least two antioxidant enzymes (SOD and GPx) to control possible overgeneration of oxyradicals after supercooling or thawing. In contrast, the garter snake developed specific responses to freezing, the frozen state being the major risk for this species at sub-zero temperatures. To verify such an hypothesis, the activity of antioxidant enzymes should be assessed in animals that have endured significant supercooling, followed by freezing.

From the present data, we can also hypothesize that the liver and muscle cells of L. vivipara respond to the stress of sub-zero temperatures by anticipating an overgeneration of oxyradicals during the warming period after thawing or the end of supercooling. Similar responses have already been reported in other animals. For instance, Buzadie et al. [4] showed that the hibernating ground squirrel (Citellus citellus) increases its antioxidant defenses during arousal. In other respects, the land snail (Otalactea) shows increased antioxidant defenses in its organs while they are in a hypometabolic (estivating) state as a protective mechanism against oxidative stress during arousal [19]. Since similar patterns can be found in other groups such as fish [25,35], an anticipatory response to the reperfusion of O₂, that can follow estivation, anoxia, supercooling or freezing, may be considered a common trait among animals.

The oxidative stress encountered during sub-zero temperatures was assessed by the contents of 8-oxodGuo and different DNA adducts from lipid peroxidation that are useful biomarkers of oxidative DNA damages [11,17]. Interestingly, to our knowledge, it is the first study that measured 8-oxodGuo and DNA adduct content in ectotherms using HPLC–MS/MS and then ensuring very specific and high sensitive measurement (since less than 1 lesion/10⁶ DNA can be detected). Apparently, supercooling, freezing, and thawing do not induce variations of DNA damage, suggesting that a complete freeze–thaw cycle can be accomplished with very little oxidative stress. This lack of damage could be due to the effectiveness of enhanced antioxidant capacities but it is equally valid to suggest that free radical damage does not happen under the experimental conditions used in this experiment. Further investigations focused on longer periods of time and/or lower temperatures of supercooling and freezing are thus needed.

However, it is noteworthy that the values we have obtained in vertebrate ectotherms are superior to those found in endotherms such as the rat (Servais, unpublished data), which suggests higher DNA oxidation and lipid peroxidation. These observations are in complete agreement with other data comparing oxidative stress in the liver of different vertebrates [34]. Indeed, these authors have shown that the TBARS content (lipid peroxidation index) was twice as high in lizard liver than in rat liver. Such higher oxidative damage in ectotherms could result from an unfavorable prooxidant/antioxidant balance induced by a lower cell antioxidant capacity in ectotherms than in endotherms [34]. Previous work also demonstrated different functioning of the respiratory chain in ectotherms, with a lower proton leakage through the inner mitochondrial membrane [33], suggesting
that mitochondrial ROS production in these animals may be relatively high. Thus, it seems to be fundamental for the understanding of this high “basal” oxidative damage, to compare ROS production in ectotherms versus endotherms by focusing studies on the two major respiratory chain complexes implicated in free radical leak (complexes I and III).

Conclusions and perspectives

This study provides: (i) an unique comparison of the antioxidant defenses employed during freezing and supercooling in the same species and (ii) the first quantification of cellular oxidative damage, including DNA and lipid damage, by measuring respectively 8-oxo-dGuo and DNA adducts in a vertebrate ectotherm. It also shows that the overall maintenance/enhancement of antioxidant defenses during supercooling/freezing may account for the absence of an increase in oxidative damage in L. vivipara tissues. The increase in the activity of antioxidant enzymes observed in organs of supercooled and thawed lizards suggests that antioxidant defenses play a significant part in the adaptive machinery for ectothermal survival under sub-zero temperatures. Importantly, as observed in other cold-hardy animals, L. vivipara cells anticipate the reperfusion-linked radical overproduction, that will occur during thawing and at the end of supercooling. Such anticipation seems to be, at least partly, supported by higher activity of SOD and GPx. These findings might provide key information to ameliorate cryopreservation injury in organ transplantation.

However, questions remain unanswered and deserve further study. Indeed, the enzymatic antioxidant capacities cannot fully explain the higher oxidative damage reported in ectotherms. The quantification of non-enzymatic antioxidants such as urate, ascorbate, and other enzymes like peroxiredoxins [21] may bring understanding elements.

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