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Hibernation for space travel: Impact on radioprotection

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ABSTRACT

Hibernation is a state of reduced metabolic activity used by some animals to survive in harsh environmental conditions. The idea of exploiting hibernation for space exploration has been proposed many years ago, but in recent years it is becoming more realistic, thanks to the introduction of specific methods to induce hibernation-like conditions (synthetic torpor) in non-hibernating animals. In addition to the expected advantages in long-term exploratory-class missions in terms of resource consumptions, aging, and psychology, hibernation may provide protection from cosmic radiation damage to the crew. Data from over half century ago in animal models suggest indeed that radiation effects are reduced during hibernation. We will review the mechanisms of increased radioprotection in hibernation, and discuss possible impact on human space exploration.

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1. Introduction

Hibernation is a state in which the metabolic activity of the body is reduced. The drop can be as high as 98%. It is a solution used by some mammals to survive periods of scarcity of resources. *Obligate hibernators* are those mammals that seasonally decrease their metabolism and body temperature regardless of the surrounding environment. *Facultative hibernators* enter hibernation only when experiencing a negative energy balance. Hibernation is not necessarily linked to a decrease in body temperature, but always comprises a reduced metabolism (Heldmaier et al., 2004).

Considering the drastic reductions in all the metabolic function, it is not surprising that during hibernation cell proliferation is halted and mitochondrial respiration decreases. Hibernation seems to provide animals with a higher resilience to stress. For instance, a lethal hemorrhagic shock in a non-hibernator was shown to be non-lethal in hibernators (Bogren et al., 2014). Cells of hibernators are able to resist stress better than regular mammalian cells (Talaei et al., 2011). Serotonin and dopamine are some of the mediators involved in providing such protection, potentially acting on the H_2S pathway (Dugbartey et al., 2015).

Ionizing radiation is a powerful stressor, and therefore it can be asked whether hibernation also protects from the damage induced by exposure to high-energy electromagnetic and particle radiation. Pioneering experiments with X- or γ-rays were performed in the mid-XX century (see Table 1 and references therein). Most of the experiments gave strong indications of induced radioresistance during hibernation, yet the interest for humans was very limited, because of the impossibility to induce hibernation in non-hibernating animals.

Hibernation is an excellent tool for space travel: it ensures very low metabolic consumption, and therefore minimizes the need of storing large amounts of food and liquids for life support systems. It also relieves most of the psychological problems associated to isolation in very long missions. Reduced metabolism also mitigate aging. Hibernation is therefore an ideal solution for very long missions, exceeding 3–4 years, as it should be for Mars and beyond and was indeed proposed for exploratory space missions already many years ago (Hock, 1960; Cockett and Beehler, 1962). However, the initial enthusiasm, also reflected in so many popular science fiction works, was hampered by the simple problem that humans are non-hibernating animals, and inducing torpor with drugs does not generally reproduce the main features of "real" hibernation. Only recently, hibernation-like condition (synthetic torpor) was induced in non-hibernating animals (Cerri et al., 2013; Tupone et al, 2013). This new opportunity has immediately renovated the interest in hibernation for interplanetary human travel (Gemignani et al., 2015). NASA is studying the design of a specific habitat to host support crewmembers in hiberna-

Table 1

Hibernation/hypothermia and radioresistance. The table shows the main references for the experiments conducted to test the hybernation induced radioresistance.

Year	Authors	Species	Title	Key outcome
2015	Cheng et al.	Human cells	Modulation of radiation-induced cytogenetic damage in human peripheral blood lymphocytes by hypothermia	"This indicates that the temperature effect observed in peripheral blood lymphocytes after irradiation is not related to a temporary perturbation of the cell cycle. Also, it is not due to selective elimination of damaged cells by apoptosis"
2014	Lisowska et al.	Human cells	Effect of hypothermia on radiation-induced micronuclei and delay of cell cycle progression in TK6 cells	"…in conclusion the protective effect of hypothermia observed at the level of cytogenetic damage was not due to a modulation of cell cycle progression"
2006	Ignat'ev et al.	Rat	The effect of hypothermia on the rat radioresistance	"The cooling of Wistar rats up to 15–19 °C under a condition hypoxia-hypercapnia increased the radioresistance with a dose reduction factor (DRF) of 1.4″
1973	Musacchia	Hamster, Gerbil	Hibernation, stress, intestinal functions, and catecholamine turnover rate in hamsters and gerbils	"Radioresistance increased in hibernating as well as in hypothermic hamsters. Marked changes in hamster catecholamine turnover rates were observed during acclimatization to high temperature stress"
1972	Barr and Musacchia	Ground squirrel	Postirradiation and radiation response of ground squirrels: telemetry surveillance	"The results showed the highest levels of survival among squirrels irradiated while torpid and that additional increase in survival may have occurred due to postirradiation hibernation. No significant effect was noted relating to the three postirradiation environments"
1971	Musacchia et al.	Hamster	Radioresistance in hamster during hypothermic depressed metabolism induced with helium and low temperatures	"Increased radioresistance was evident in the metabolically depressed hypothermic hamsters… hypothermic hamsters showed increased mean survival times. Dose-reduction factors were approximately 1.22 and 1.31. The increased radioresistance was examined in light of limited hypoxia and general metabolic depression"
1969	Barr and Musacchia	Thirteen-lined ground squirrel	The effect of body temperature and postirradiation cold exposure on the radiation response of the hibernator Citellus tridecemlineatus	"Results indicated that squirrels irradiated while hibernating had higher percentages of survival than squirrels irradiated while active…complicated relationship existed between the effects of a postirradiation exposure to environments of 5 °C, 13 °C, or 23 °C for 90 days and the effects of irradiating squirrels while at body temperatures of 5 °C, 13 °C, 23 °C, or 37 °C"
1961	Mraz and Praslicka	Mouse	Influence of Hypothermia on the survival of mice after large doses of X-radiation	"…prolonged hypothermia at 22–25 °C, lasting 6–48 h, after irradiation also slows down destructive changes in the blood, spleen and bone marrow. After the ending of hypothermia and reaching the normal body temperature these changes, however, develop even more intensely than in controls subjected to irradiation only and the mean survival-time is shorter"
1951	Smith and Grenan	Marmot	Effect of hibernation upon survival time following whole-body irradiation in the Marmot (Marmota monax).	"Increased metabolic rate resulting from thyroid administration has been found to coincide with an increased mortality in mice following irradiation. On the other hand, the administration of antithyroid substances, which reduce the metabolic rate, does not alter radiation lethality nor increase the survival time in irradiated mice"

tion for the mission to Mars (Bradford et al., 2014) (Fig. 1). ESA has sponsored a dedicated study and included hibernation in the life sciences roadmap (Bereiter-Hahn et al., 2015). However, for very long exploratory-class missions, the main showstopper is generally considered the exposure to galactic cosmic radiation (Durante and Cucinotta, 2008). The recent Mars Science Laboratory (MSL) measurements demonstrate that the average dose-rate in deep space is about 1.8 mSv/day (Zeitlin et al., 2013). Therefore any missions of several years will exceed cumulative doses of 1 Sv. These recent measurements support previous estimates of the dose in deep space and confirm that countermeasures are indeed needed for a safe exploration program. Unfortunately, shielding is poorly effective against cosmic radiation, especially during the cruise where severe mass constraints have to be imposed (Durante, 2014). Biological countermeasures, such as radioprotective drugs (Kennedy, 2014), are as yet far from providing practical solutions, and they are indeed hardly used even in patients treated with radiotherapy for solid tumors.

Can hibernation reduce the radiation damage? If true, this effect will greatly expand the benefits of hibernation for space travel, and make it an essential tool for the safety of the crews. In this review, we will provide an overview of the past experiments on hibernation and radiation. Starting from the interpretation of previous results, we will evaluate potential advantages offered by the application of new approaches to induce a hibernation-like state for medical and space applications.

2. Biology of hibernation

2.1. Mammals

The term *torpor* identify a state of active suppression of metabolism, usually leading to a reversible and undefended state of hypothermia. Multiple episodes of torpor are at the base of *hibernation, aestivation*, in which hibernation takes place during the summer season, and *brumation*, that happens in ectothermic organisms such as reptiles or hibernacula (Fig. 2). All these peculiar animal states are characterized by the reduction of the metabolic rate, decrease of oxygen consumption, decrease of heart and respiratory rate, reduction of the body temperature in dependence of the ambient temperature and, at molecular level, gene expression and protein synthesis abatement (Table 2). The large decrease in body temperature, caused by the reduction in metabolic rate, does not activate homeostatic compensatory measures (Heldmaier et al., 2004). Moreover, hibernators, besides the reduc-
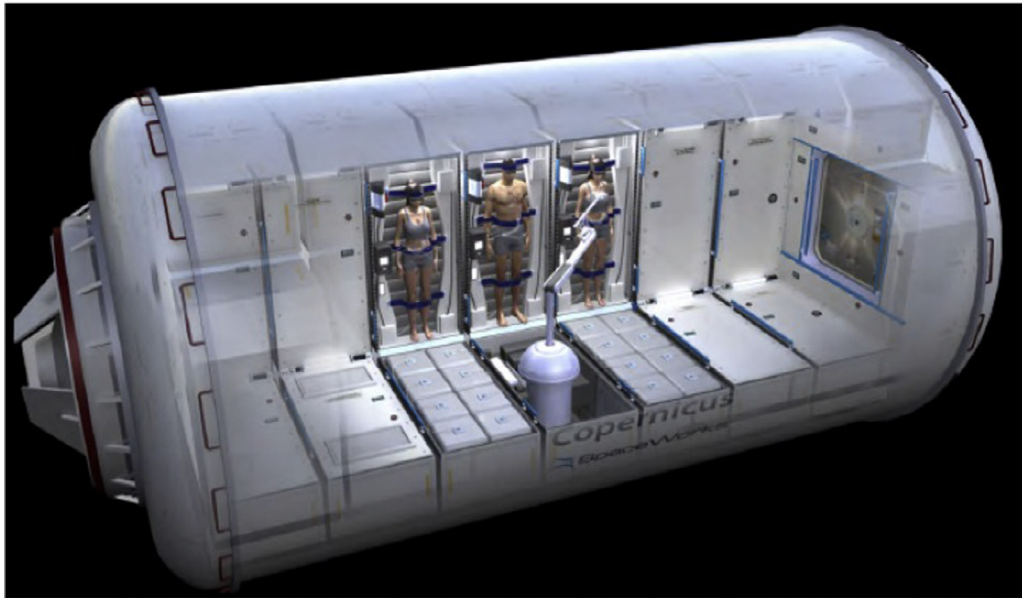
Fig. 1. Mars transfer habitat design by SpaceWorks Enterprises, Inc. (SEI). The habitat can support a crew of 6 in hibernation/stasis for a 180-day outbound mission transit, plus a 500-day aborted surface mission contingency, and 180-day Earth return transit. Robotic manipulator arms are used to manage the crew as needed, and neuromuscular electric stimulation (NMES) is used to prevent muscle atrophy. Total Parenteral Nutrition (TPN) is administered via tunneled central venous catheter in chest. The estimated weight and volume of the torpor stasis habitat is less than 1/2 of the NASA DRA 5 reference habitat for the same Mars mission. Courtesy of SpaceWork, study available online at https://www.nasa.gov/content/torpor-inducing- transfer-habitat-for-human-stasis-to-mars.

Fig. 2. Different states of metabolic suppression experienced by different animals: squirrel, snake, frog, bear.

tion in metabolic needs, show other relevant features that may be exploited in medical sciences, once a safe procedure to induce such state would be available for humans.

For centuries it has been known that several mammals can undergo hibernation, e.g. squirrels, hamsters, and bears. Only in 2004, a German research team discovered the first primate able to undergo hibernation: the fat tailed dwarf lemur *Cheirogaleus medius* (Dausmann et al., 2004). The ability to enter a hypometabolic state such as torpor, hibernation or aestivation is an ancestral trait that was present in early mammals, and that was later lost in some species. The widespread distribution of hibernators in the mammals family suggest that the gene set necessary to survive the process is probably common among mammals, while the regulatory mechanisms may have been lost in non-hibernators.

Table 2
Physiological changes induced by hibernation. In bold, the changes that can be exploited by crews of interplanetary missions.

General cell physiology	*General systems physiology*
1) **Enhanced radioprotection** 2) Preconditioning 3) Halt of proliferation	1) **Reduced metabolism** 2) Reduced breathing rate 3) Undefended hypothermia
Cardiovascular system	*Central Nervous System*
1) Reduced heart rate 2) Protection versus hypothermia-induced arrhythmia 3) Reduction of circulating platelets	1) Synaptic re-absorption 2) Tau Protein Hyperphosphorylation 3) Disappearance of EEG fast rhythms 4) **No consciousness**
Endocrine system	*GI tract*
1) Hypothyroidism 2) Hypogonadism 3) Insulin resistance	1) Stop of crypt cells proliferation 2) Leakage of the GI barrier 3) Inflammatory cells infiltrate the mucosa 4) Drastic changes in the bacteriome
Urinary system	
1) **Reduced urine production**	*Locomotor system*
Immune system	1) **Preservation of muscle strength** 2) **Preservation of bone mineralization** 3) Characteristic posture
1) Lymphocytes migration to the lymph node	

2.2. Molecular features of torpor and hibernation

Torpor induces many changes in the molecular biology of the cell in many animals (Storey and Storey, 2010), including primates (Faherty et al., 2016). Gene expression, for instance, is strongly affected during hibernation in many organs such as the brown adipose tissue (Ballinger et al., 2016; Biggar et al., 2014), skeletal muscle (Anderson et al., 2016), the heart (Vermillion et al., 2015), and the bone marrow (Cooper et al., 2016). Whereas gene expression studies provide relevant information, proteomics (Grabek et al., 2015) and epigenetics (Storey, 2015) are opening new possibility to unravel the complex molecular cascade that regulates cellular hypometabolic states. So far, gene expression related to hypometabolic states has been studied only during natural torpor. Transcriptomic and proteomic changes during synthetic torpor (see below Cerri, 2017) have not been reported yet.

Considering that the main feature of torpor is an active reduction in metabolic rate, and therefore in oxygen consumption (Heldmaier et al., 2004), the activity of mitochondria has been a relevant focus in hibernation research (Staples and Brown, 2008). The reduction in oxygen consumption could be in fact explained by some form of inhibition of the mitochondrial function (Mathers et al., 2016). The overall idea that mitochondria are the target of the mechanism of metabolic suppression is intriguing, but not free of objections. For instance, if mitochondrial activity was to be reduced first, cellular damage would probably arise for the unbalance between energy demand and energy supply. To solve such problem, it is possible to refer to the idea of *channel arrest* (Hochachka et al., 1986), according to which a reduction in cellular energy consumption, for instance by reducing membrane pumps activity, has to precede the reduction in energy production. If this idea was applied to torpor, the reduction in mitochondrial activity would not be the first target for metabolic suppression, but the consequence of a drastic reduction in energy demand.

2.3. Arousal

Hibernation is interrupted by periodic *arousals*. In these periods the increase of temperature and the acceleration of the metabolism allow the animal to restore almost normal vital and active functions.

The arousal could permit the activation of the immune system and subsequently initiate an immune response (Prendergast et al., 2002) or the periodic arousal may be needed by the animal to solve a slept-debt, as it occurs in the arctic ground squirrel (Daan et al., 1991). It has been further speculated that the arousal is needed to restore the energy deposit. The arousal is strictly dependent on the body temperature and in *Cheirogaleus medius* it occurs when the temperature exceeds 30 °C. However, there is a high energetic cost for the arousal. In this dwarf mammal the increased active thermogenesis necessary for the arousal is avoided using the temperature of the tropical situation in which they live. In the case of the *Cheirogaleus medius* they simply continue to stay in hibernation even over 30 ° and, suspending endogenous thermogenesis and becoming then ectotherm like a reptile, can go over 30 ° without any energy consumption (Dausmann et al., 2004).

2.4. Human hibernation

After many reported cases of humans that surviving a prolonged low body temperature, it was suggested that humans could also be facultative hibernators. There are examples of people surviving accident in situations where their whole body temperature fell to 13.7 °C without any serious damage.

Table 3
Different strategies used to induce an artificial state of torpor. "Synthetic torpor" indicates a state that mimics for the most part the natural features of the torpor occurring in hibernating species, which would be the ideal condition to translate for humans applications. It is noteworthy that the mouse is a facultative heterotherm, capable to enter torpor under specific circumstances, and therefore not the best model to be used.

Year	Species	Ibernator	Approach	Effects	Reference
2005	Mouse	Yes	H_2S inhalation	Induction of a state resembling torpor	Blackstone et al.
2008	Rat	No	H_2S inhalation	No synthetic torpor	Lou et al.
2008	Piglet	No	H_2S inhalation	No synthetic torpor	Li et al.
2011	Pig	No	H_2S iv	No synthetic torpor	Drabek et al.
2015	Sheep	No	H_2S inhalation	No synthetic torpor	Haouzi et al.
2006	Mouse	Yes	5′-amp systemic	Induction hypothermia but with different physiological characteristic compared to the state of torpor (Swoap et al., 2007)	Zhang et al.
2009	Rats	No	5′-amp systemic	No synthetic torpor	Zhang et al.
2013	Rats	No	Inhibition of RPa neurons	Synthetic torpor	Cerri et al.
2013	Rats	No	Central adenosine A1 receptor agonist	Synthetic torpor	Tupone et al.

Mitsutaka Uchikoshi is considered the first human being to undergo a hibernation-like state following an accident. According to his physicians, the hibernation-like state slowed many of his organs, yet his brain was protected.

Anna Elisabeth Johansson Bagenholm is a Swedish radiologist who survived a prolonged extreme hypothermia. Her body temperature was around 14.4 °C, the arterial blood sample showed normal serum potassium and oxygenation, the carbon dioxide (CO_2) concentration was higher than the standard level while the acidosis was severely high. A foamy pink fluid streamed from the endotracheal tube. She even reached the lowest body temperature ever recorded in a living human, i.e. 13.7 °C (Gilbert et al., 2000). However, besides small nerve injury and a slight tingling in her hands, Anna Elisabeth Johansson five months later recovered completely from this tragic experience and came back to her normal life and work.

Erika Nordby, a 13 months old Canadian baby left the house unattended wearing only a diaper. The ambient temperature outside was around −24 °C. When Erika was found, she was considered to be clinically dead. She had no heartbeat, no respiration and her body temperature was around 16 °C. Her conditions became normal after that she was placed under a warming blanket. No serious damage remained and her physician suggested that the low temperature could have brought her to a hibernation-like state.
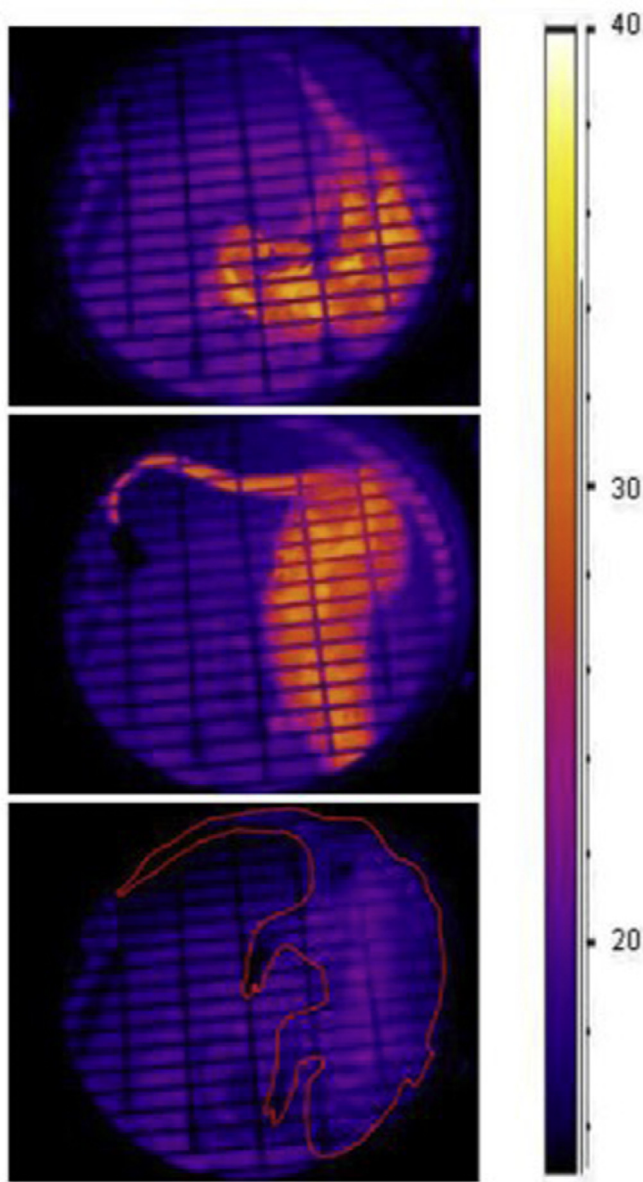
2.5. Synthetic torpor

Hibernation, torpor, aestivation, hibernation-like state, torpor-like state, pseudo-torpor, hypometabolism, hypothermia, deep hypothermia, suspended animation, stasis and probably others are some of the names that are used in the field. They all refer more or less to a state of hypometabolism that is artificially induced in a species that does not show any torpor or hibernation. With *synthetic torpor* we indicate not just an hypometabolic state, but a state that mimics for the most part the natural features of the torpor occurring in hibernating species, which would be the ideal condition to translate for humans applications (Cerri, 2017). Several methods to induce hibernation-like states are reported in Table 3. Mimicking hibernation may bring great benefits also to the clinical practice, in terms of neuroprotection after cerebral hypoxia, during major surgery, or to extend the window for organ transplantation. We also hypothesize that it can be a benefit in radiotherapy, for reducing radiotoxicity in normal tissues (see Section 3.3).

Since hibernation is characterized by a reduction of the metabolism, a possible method mimicking it would be to lower the metabolic rate by reducing the cellular energy production. This approach was tried in mice, and a suspended animation state was induced by using 5′-AMP (Zhang et al., 2006), or H_2S (Blackstone et al., 2005). All these substances reduce or block the energy produced in every cell by inhibiting the mitochondrial function, but all these approaches proved ineffective when applied to non-hibernating mammals (Haouzi et al., 2008). It is noteworthy that the mouse is a facultative hibernator able to enter torpor under specific circumstances, and that this could be the reason of the failure in translating those approaches to non-hibernators. In other words, the pharmacological treatments given to mice may have triggered the mechanism of torpor, but may not be effective in doing so in species that do not have the same underlying mechanisms.

It could be argued that hibernation is a trait of a specific group of species, and therefore may not be replicated in non-hibernators. For instance, flying is a trait of a specific group of mammals (bats), because they have evolved the biological structure (wings) to support that trait. But that is not the case of hibernation. Hibernators are, in fact not a homogeneous group of species, but as discussed above, they are present in every family of mammals, including primates. This suggests that the ability to suppress metabolic rate was probably a trait present in the proto-mammal (approximately 150 million years ago), an animal that, because of his homeothermy, was able to be nocturnal to reduce the risk of predation by dinosaurs. Such ability was critical to improve the survival chance of the newly-appeared mammal, since it provided the great energy saving skill, necessary to support the higher energy expenditure required by homeothermy. The trait was then lost in many mammals after the extinction of the dinosaurs, but remained present in all the species that relied on energy saving to survive (for instance species living in extreme climate). From this evolutionary reasoning, it can be argued that the set of genes and the biochemical regulation necessary to survive during the hypometabolic bout are shared among mammals, while only the regulatory mechanism was lost in non-hibernators.

To this day, only two procedures were shown to induce such conditions in non-hibernating rodents: (i) the inhibition of the sympathetic premotor neurons within the Raphe Pallidus (RPa) (Cerri et al, 2013); (ii) the activation of the central Adenosine A1 receptors (Tupone et al., 2013). The approach used differs substantially from what was done in mice: instead of acting on the whole organism metabolism, it inhibits the activity of specific neural pathways within the central nervous system. The physiology observed during such hibernation-like state strongly resembles spontaneous hibernation, in terms of cardiovascular, respiratory and neuro-physiology, as well as behavior. Fig. 3 shows the decrease in temperature in a rat following inactivation of the Raphe pallidus.

Fig. 3. Examples of infrared images taken during the procedure of induction of a synthetic torpor state by inhibition of RPa neurons. Top line: before the inhibition; middle line: shortly after the beginning of the procedure; bottom line: at the temperature nadir. In the right panel of the bottom line the rat shape is highlighted in red, since the animals shape is not very visible being in thermal balance with the environment. Experimental details in Cerri et al., 2013. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Radiation effects during hibernation

3.1. Experiments in hibernating animals

It is generally accepted that hibernation increases resistance to stress. In particular, many authors provided evidence indicating a relevant increase in radioresistance. This effect was intensively studied a few decades ago (1950–60) through animal experiments, mainly comparing the survival rate of squirrels after providing variable doses of gamma radiation (Table 1).

Smith and Grenan (1951) reported that lethal damage on exposed hibernating marmots seems to be just delayed after the arousal, where the same survival fractions are observed for marmots irradiated during or outside hibernation, if the survival time

is considered from the time of the arousal. The result is later confirmed by Doull and Dubois (1953) for ground squirrels. Since irradiation affects mitosis, the radioprotective effect caused by the stall of the cell cycle while in hibernation may be reduced after arousal. A mild increase of radiosensitivity was observed by Kuskin and coworkers (Kuskin et al., 1959), where a small decrease of body temperature was artificially induced on a population of mice by means of neuroplegic drugs. Hypothermia induced by neuroplegic drugs followed by physical cooling of the animal instead did not show a radioprotective effect. The authors suggested that the protective effect is not related to the depression of the metabolism alone but instead to a general depression of bodily processes.

Naturally occurring radioprotection in hibernating ground squirrels (*citellus tridecemlineatus*) has been reported by Musacchia and Barr (1968) over a broad range of doses (10–200 Gy). Jaroslow and colleagues (Jaroslow et al., 1969) reports the study of survival rates for hibernating squirrels exposed to a lethal dose from a ^{60}Co source. The highest survival rate is observed for squirrels aroused from hibernation one day after irradiation. Although the lack of food and water for longer hibernation periods may account in part for the difference in survival rates, an increased survival is observed also with respect to squirrels immediately aroused after irradiation.

An increased radiosensitivity of ileum crypt cells of ground squirrels was observed Jaroslow et al. (1976) comparing the cell survival fractions for irradiation during hibernation, arousal, and euthermic state. In squirrels irradiated 1 h after the initiation of the arousal, with core temperature in the range of euthermic controls, the survival was comparable with the one observed for irradiation during hibernation. The radioprotective effect disappears 3–7 h after irradiation. This study suggests hypoxia as a possible explanation for the radioprotective effect. The changes in the mitotic index of ileum cells during hibernation and arousal may also play a role.

More recent experiments investigated mechanisms of acquired resistance using in vitro systems. Baird et al. (2011) measured a suppressed DNA repair response in BJ-hTERT human cells irradiated in hypothermic conditions (irradiation at 13 °C, 20 °C and 30 °C). Hypothermia was observed to interfere with the cell cycle progression and a low fraction of cells in the S-phase. While repair of double strand break is temperature-dependent and suppressed at low temperature, the larger survival fractions for irradiation in hypothermia suggests that slowly repair of double strand breaks may not have a large negative impact on the survival of the cell.

Povinelli et al. (2015) reported that even a mild cold stress has a protective effect on hematopoietic stem and progenitor cells after both nonlethal and lethal total body irradiation on mice, prolonging the survival time. Injecting propanolol (non selective β-blocker) partially reduces the protective effect induced by cold stress, while it has no effect on mice kept at thermoneutral temperature. The proposed mechanism operates through the β-adrenergic signaling pathway, causing the inhibition of the apoptosis of hematopoietic stem and progenitor cells in bone marrow.

Taken together, all the experiments support the hypothesis that hibernation increases radioresistance. It is difficult to pool the data to give quantitative estimates of the observed effects. Fig. 4 reports a pool of the data on survival to high γ- or X-ray doses. In the panel (a) we pooled the data relative to ground squirrel, a hibernating animal, while the panel (b) shows the survival of rats, a non-hibernator, irradiated in hypothermia. The pooling of different experimental conditions produce a very significant spread in the data, but the trend clearly shows the increased survival of squirrels irradiated during hibernation. LD50 values were calculated with a probit fit:

$$M(\%) = \frac{1}{2}\left\{1 - \mathrm{erf}\left[\gamma\sqrt{\pi}\left(1 - \frac{D}{LD50}\right)\right]\right\} \qquad (1)$$
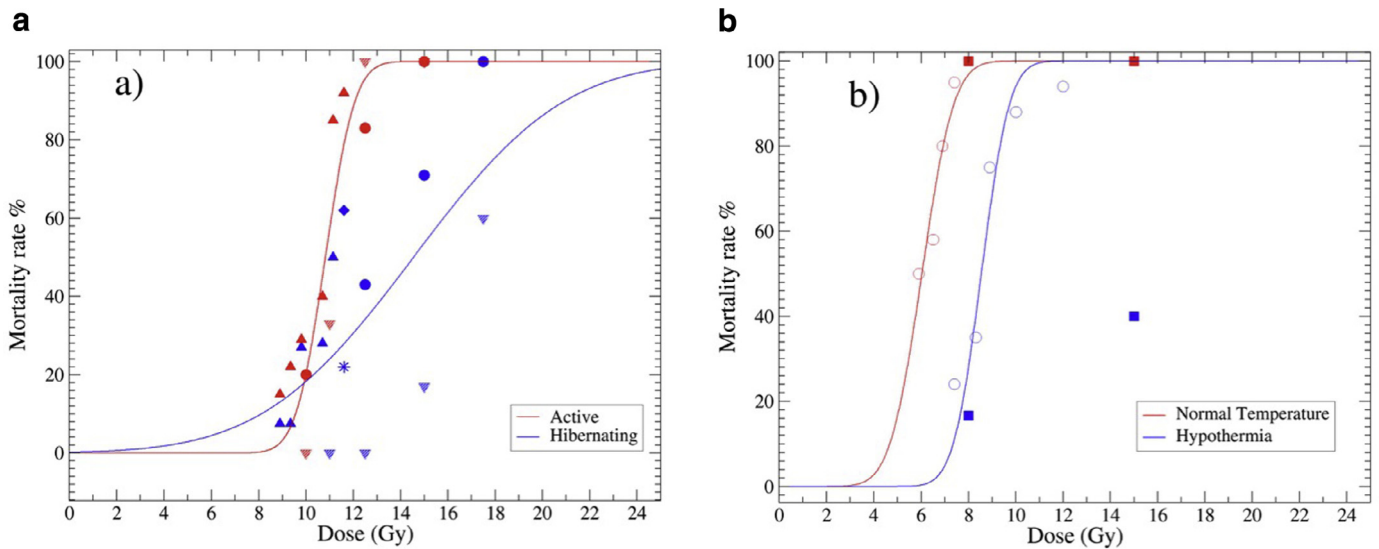
Fig. 4. Meta-analysis of observed lethality in animals exposed to graded γ- or X-ray doses. Lines are fits with a probit function to lethality of: (a) squirrels in hibernation (blue) or active (red); (b) rats in hypothermia (blue) or normal temperature (red). Experimental data are mortality data from different published papers. (a), squirrels: circles are mortality at 30 days from Musacchia et al., 1968: full circles, Summer hibernation; dashed circles, Winter hibernation; other symbols are mortality rates at 90 days from Jaroslow et al., 1969 ($\triangle$, normal hibernation; $\blacklozenge$ 2 weeks at 5 °C; ∗ 20 h at 5 °C). (b), rats: circles are mortality rates at 30 days from Ignat'ev et al. (2006), squares from Benvenuto and Lewis (1960). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Fitting parameters of the data in Fig. 4 using the probit formula (Eq. (1)). The protective factor is the ratio of the LD50 values. Uncertainties are standard errors of the fitting parameters calculated using the software package Wolfram Mathematica®.

Animal	Normal conditions		Hibernation/hypothermia		Protective factor
	γ	LD50 (Gy)	γ	LD50 (Gy)	
Squirrels	4.4 ± 1.2	10.82 ± 0.17	1.1 ± 0.4	14.6 ± 1.1	1.35 ± 0.07
Rats	2.3 ± 0.5	6.03 ± 0.13	3.6 ± 2.7	8.6 ± 0.4	1.42 ± 0.10

where M is the mortality rate, γ the slope parameter, *erf* the error function, D the dose, and LD50 the dose corresponding to 50% mortality. The fitting parameters are reported in Table 4. Even considering the large differences in experimental conditions, it is remarkable that the protective factor (ratio of LD50 values in hibernation/hypothermia and normal conditions) is around 1.4 for both squirrels (hibernators) and rats (non-hibernators). This value is high and makes it interesting for radioprotection. It is close to the protection factor of the few agents (amifostine and palifermin) in clinical use as radioprotectors during radiotherapy (Johnke et al. 2014). In a recent study in mice exposed to high X-ray doses and protected with amifostine (WR 2721) or its polymer complex, the protection factors calculated from LD50 values were 1.29 and 1.35, respectively (Koseva et al., 2014).

3.2. Possible mechanisms of radioresistance

Discrepancies are present in the interpretation of the different experiments. Some may be related to differences in the reactions of different species but also the differences in the realization of the experiments (like time in hibernation before and after irradiation, the ambient temperature) may have a non-negligible impact on the outcome. At the cellular level, it is well known that cells irradiated in resting state, and left undisturbed for 24 h after irradiation, are more resistant than cells re-plated immediately after exposure. This effect is known as delayed plating, and is generally attributed to the potentially lethal damage repair (PLDR) in resting phase (Marchese et al., 1987). PLDR is also observed in vivo, following exposure of xenografts in mouse (Little et al., 1973) but it

is unclear whether a reduced metabolism grants enhanced protection from radiation in a whole organism.

The oxygen level during hibernation may contribute to the enhanced radioresistance. Hypoxia is a well know protective mechanism, and is arguably the main reason of local control failure in radiotherapy. In fact, tumors are generally hypoxic compared to the normal tissues, and the ratio of the doses producing the same cell killing in hypoxic and aerobic atmosphere (oxygen enhancement ratio, OER) is close to 3 after X-rays (Wilson and Hay, 2011). The reduction in oxygen metabolism during hibernation may well provide an additional protection towards radiation damage. With respect to this latter point, it is important to notice that differences in the dynamics of awakening may affect the overall health status of the animal (Cerri et al., 2013), suggesting that the previously reported silent damage induced by radiation during hibernation may somehow be linked to the arousal process, the phase when oxygen metabolism (and therefore the production of reactive oxygen species (ROS)) is highest. The role of awakening in the resulting damage from irradiation is clearly relevant, but still unclear. For instance, it was also suggested that the protective effect might occur during the arousal from hibernation, following the increase of catecholamines that may occur in the arousal process and could cause severe vasoconstriction (Prewitt and Musacchia, 1975). Similar results were observed by Barr and Musacchia (1972), where different groups of squirrels were kept after irradiation in environments with three different temperatures (5 °C, 13 °C and 23 °C), without observing any significant difference in terms of radioresistance between the three groups. The temperature itself has a sensible effect on the radiosensitivity of many biological systems. The radiosensitivity is generally reduced under hypothermia, which is one of the main peculiarities shown by animals during hibernation. Living in a cold environment has an impact on the radioresistance of animals (see Sazykina and Kryshev, 2011 for a review). Imbalances in the temperature-dependent cell damage and repair processes for animals living in cold environment make the overall picture unclear and the topic is still an active research field.

3.3. Radiotherapy

Radiotherapy is an essential component of the cancer cure. Over 50% of the cancer patients are treated with high-doses of radia-

tion, either alone or in combination with surgery or chemotherapy. Radiotherapy has recently experienced many advances in methodology and techniques and the dose to the tumor is nowadays delivered with a much higher precision. Highly conformal dose distributions can be achieved with X-rays using intensity modulated deliver (IMRT), or accelerating charged particles (generally protons or C-ions) at very high energies (Thariat et al., 2013). Notwithstanding these excellent technological improvements, radiotherapy is still limited by the normal tissue toxicity. In fact, organ tolerance doses are thresholds to avoid unacceptable morbidity in the patient. This limits the maximum dose that can be used to control locally the tumor, and in some cases prevents treatments. For instance, multiple metastases in close proximity cannot be treated without exceeding the tolerance dose of the normal organ, even if their treatment with hypofractionated radiotherapy can substantially improve the survival of patients with stage IV cancers (Lo et al., 2011).

The torpor-induced reduced metabolic rate suggests that the tumors will stop growing during hibernation. This was indeed found in old experiments where human tumors where transplanted in hamsters (Lyman and Fawcett, 1954; Patterson et al., 1957). However, tumors return to normal proliferation as soon as the body temperature is restored. It is, however, unknown whether the radioresistance is differentially modulated by hibernation in normal and tumor tissues. If hypoxia plays a role, it can be expected that normal tissues (aerobic) will be more protected than tumors (normally already hypoxic). Hence, a hibernated patient could be treated, e.g. for multiple metastases, at doses that would not be acceptable in normal conditions, because the organ dose would be exceeded. At arousal, the patient will be cured. This is obviously a speculative hypothesis, and yet so attractive that we believe it deserves experimental verification.

4. Conclusions

Hibernation might be a promising strategy for putative future human interplanetary missions. Hibernated astronauts would reduce the spaceship's total energy consumption more than 50%, they would increase their lifespan, saving then the travelling time. Furthermore a hibernation-like state would help them with psychological issues resulting from the flight in a narrow spacecraft, spending much of their time in an "unconscious-like" state, and would reduce enormously the waste production. Moreover, hibernation may solve another crucial objection for manned space exploration, i.e. radiation exposure. The old observations pointing to increased radioresistance in torpor open new, fascinating scenarios. Exposure to cosmic rays is generally acknowledged as the main health threat in long-term exploratory-class missions. The problem is particularly critical for the cruise period, when thick shielding of the spacecraft is impossible. Drugs or dietary supplementary are, as yet, equally disappointing. Hibernation may represent an ideal solution. A radioprotective effect of hibernation is observed for several species and on different irradiation conditions. Our meta-analysis (Fig. 4) suggests a protective factor around 1.4, close to that provided by drugs used in radiotherapy (such as amifostine) to prevent normal tissue toxicity. The overall mechanisms at the basis of the increased radioresistance in hibernation or hypothermia are poorly understood and need further investigation. Old experiments are limited to natural hibernators exposed to high doses, with acute effects as main endpoints, generally survival. For space exploration, new experiments are needed, and they should explore late effects at low doses. The issue is extremely relevant not only for space travel, but also for cancer radiotherapy.

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