Mammalian Hibernation: Relevance to a Possible Human Hypometabolic State

Final Report

Authors: Carlo Zancanaro
Affiliation: University of Verona

Authors: Marco Biggiogera
Affiliation: University of Pavia

Authors: Manuela Malatesta
Affiliation: University of Urbino

ESA Researcher: Mark Ayre
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Contacts:
Carlo Zancanaro
Tel: +39 045 802 7155
Fax: +39 045 802 7163
e-mail: Carlo.zancanaro@univr.it

Advanced Concepts Team
Fax: +31(0)715658018
e-mail: act@esa.int

Available on the ACT website
http://www.esa.int/act

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1. Introduction: Natural Hypometabolism in Vertebrates: Why, Who, How

1.1 Why?

Endotherms have evolved the ability to produce endogeneous heat for maintenance of a high and constant body temperature and thus have achieved a high degree of independence from the restrictions imposed by the environment. However, this advantage comes at high energetic costs. In many, especially small animals, the cost for the thermoregulatory heat production can exceed the available energy and, to overcome this problem, they enter a state of torpor. The natural torpor in response to adverse environmental conditions (cold, food or water scarcity) is characterised by a drastic reduction in body temperature and other physiological functions which may last from a few hours to some weeks (reviews in Nelson, 1980; Storey and Storey, 1990; Hoffman, 1964; Lyman et al., 1982; Wang, 1987; French, 1988; Geiser and Ruf, 1995). Unlike ectotherms (e.g. frogs and snakes), torpid endotherms are able to leave the depressed metabolic state at any time, using endogeneously produced heat to restore normal body temperature.

1.2 Who

Members of at least six mammalian orders are capable of exhibiting torpor. Some examples are echidna of *Monotremata*, many dasyurids of *Marsupialia*, tenrecs, shrews of the subfamily *Crocidurinae,* hedgehogs of Insectivora, many bats of both sub-orders *Megachiroptera* and *Microchiroptera*, dwarf and mouse lemurs of *Primates* and the various sciurids, cricetids, heteromyids, murids and zapodids of *Rodentia*.

1.3 How

Depending on the timing, duration and depth, torpor in mammals may be characterised as seasonal and non-seasonal.

1.3.1 SEASONAL TORPOR is represented by aestivation and hibernation, and is characterised by subsequent torpor bouts of several days or weeks generally concentrated into one torpor season which can last many months. In fact, the seasonal torpor never spans the entire hibernating season, but it is interrupted by periodic arousals and brief normothermic periods (French, 1985, 1988). Some common groups which exhibit seasonal torpor include the hedgehogs (*Erinaceous*), marmots and woodchucks (*Marmota*), ground squirrels (*Spermophilus* or *Citellus*) and bats (*Eptesicus* and *Myotis*). In these animals two distinct states can be identified annually: a non-hibernating phase, during which body weight is relatively constant and exposure to cold results in both increased heat production and maintenance of euthermia, and a hibernating phase, during which a drastic, rapid weight gain occurs, and the exposure to cold results in hibernation with body temperature capable of decreasing to near 0°C.

1.3.2 NON-SEASONAL TORPOR is a torpor inducible at any time of the year by proper stimuli (i.e. cold and/or food shortage). A typical form of non-seasonal torpor is the daily torpor, with a duration of less than 24 hours and a body temperature decrease generally to 10°C-25°C (considerably higher that that found during hibernation). Moreover, the daily torpor appears to be integrated into the normal circadian rhythm of activity and rest, although the torpor is not restricted only to the normal rest phase of the animal (Körtner and Geiser, 2000). Several groups, such as marsupials, insectivores, chiropterans, primates and rodents, contain members which exhibit daily torpor. A further type of non-seasonal torpor is typical of the Syrian (golden) hamster (*Mesocricetus auratus*), which exhibits deep torpor (body temperature decreasing to about 5°C and lasting a few days) but requires a long preliminary period of cold exposure.

A controversy concerns whether the WINTER SLEEP in bears can be considered a true hibernating state. During winter dormancy, which lasts from 3 to 7 months, bears do not eat, drink, defecate or urinate and they use fat exclusively as their energy and water source. The bear hibernates at a near normal body temperature ranging from 31°C to 35°C, its metabolic rate decreases to 50-60% of the euthermic level and heart rate drops from 40 to 10 beats/minute. This metabolic depression is much

less than that found even in the daily torpor. In addition, unlike torpid animals, when disturbed the bear is easily aroused into a mobile, reactive state, able to defend itself, and female bears give birth and nurse cubs during denning. However, calculation based on theoretical considerations between body size, fat reserve and energy requirement under fasting condition have demonstrated that the shallow torpid state of the bear is optimal for this animal to survive during denning.

1.4 Natural hypometabolism: general aspects

Despite the different torpor types, the patterns of metabolism, heart rate, respiratory rate and body temperature of the torpid state are basically similar, differing only in quantitative aspects. Thus, in spite of the polyphyletic origin in evolution of torpor in mammals, the specific ecological features of their niches and the great diversity of torpor patterns, there is a physiological convergence in achieving a hypometabolic state for energy conservation. Typically, a torpor bout consists of entry into, maintenance of and arousal from torpor. During entry into torpor, a progressive inhibition of heart rate, an increase in vasoconstriction to maintain blood pressure, a decrease in respiratory rate with irregular periods of apnoea and a significant decrease of oxygen consumption occur. Body temperature falls following decrease of heat production, with periodic shivering to counterbalance a too rapid cooling. During torpor, which may last from hours to weeks, all physiological functions are kept at minimum. Heart rate may decrease to 1/30 or less and oxygen consumption to 1/100 or less of their respective euthermic levels. Prolonged apnoea (40-150 minutes) as well as Cheyne-Stoke breathing (apnoea followed by bursts of breathing) occurs in rodents. Body temperature is maintained near ambient temperature (sometimens near 0 °C), however there is a “critical” level below which a further ambient temperature decrease could result in an adaptation (metabolic rate increase or arousal) or death of the hibernating organism. Arousal from torpor is an “explosive” event requiring from 20-30 minutes in small rodents and bats to a few hours in marmots: the substrate are mobilized for energy production, the cardiovascular system is stimulated for tissue perfusion and the non-shivering thermogenesis in the brown adipose tissue starts.

Unfortunately, in spite of extensive studies on hibernation, the basic mechanisms of the natural hypometabolism are still unknown. The scientific literature is in fact heterogeneous (numerous mammal species have been studied under diverse experimental conditions) and fragmentary (in each species studied only few aspects have been investigated by different technical approaches).

2. Overview of natural hypometabolism mechanisms as applied to the human

2.1 A Premise

Before discussing potential utilisation of natural hypometabolism mechanisms in the human, the concept of homeostasis should be stressed. **Homeostasis** (the "wisdom of the body" according to the physiologist Walter Cannon) is the body's ability to maintain relatively stable internal conditions in the presence of continuous change in the external world. The literal translation of homeostasis is "unchanging"; however, the term does not actually mean a static, or unchanging state. Instead, it indicates a dynamic state of equilibrium, in which internal conditions can change, but always within relatively narrow limits. Maintaining homeostasis is much more complex than it appears at first glance. Virtually every organ system plays a role in maintaining the constancy of the internal environment. A variety of neural, thermal and chemical factors interact in a complex manner to maintain homeostasis and this should be kept in mind when attempting at changing bodily conditions drastically. In particular, the fact that most homeostatic control mechanisms are negative feedback mechanisms should be stressed. The net effect of such systems is that the output of the system decreases the original stimulus (e.g., lowering ambient temperature) thereby causing the variable (e.g., body temperature) to change in a direction opposite to that of the initial change in order to return to its "ideal" value.

In the following chapters we shall consider selected mechanisms which could conceivably lead to a controlled, reversible human hypometabolic state. A general caveat to the reader is that a large amount of speculation is enclosed due to lack of complete scientific data and the obvious ethical, regulatory and technical problems involved in human experimentation.

2.2 Mechanisms

2.2.1 Mechanism 1 – Changing body temperature

2.2.1.1 - Resetting the body temperature set-point

Hibernating animals retain the ability to sense and defend body temperature; however, when they enter torpor their hypothalamic set-point for body temperature regulation is gradually lowered (Heller, 1979). Resetting of the body temperature set point in the human should help getting a hyopometabolic state. It is generally accepted that the set-point is reset during fever, therefore it is regulatable. Unfortunately, the mechanism by which the set-point is determined is still a mystery (Cooper 2002). Possible targets are "cold" and "warm" thermosensitive neurones in the hypothalamus; their firing rate could be affected by thermoregulatory neurotransmitters such as catecholamines, serotonin, dopamine, GABA, glutamate, acetylcholine, or nitric oxide (Gertsberger, 1999). All of the molecules listed above have many important effects on different body systems other than affecting temperature; therefore, their use in humans should be very prudent.

2.2.1.2 – Lowering body temperature

Lowering of ambient temperature is a key factor to induce hibernation and in general decrease in body temperature parallels the fall in metabolic rate in hibernators (Geiser 2004). The decrease in body temperature is apparently a key factor to reduce the rate of metabolic and enzymatic activities on a purely thermodynamic drive; however, during entry into torpor metabolic rates drop rapidly even before a significant drop in body temperature (Ortmann and Heldmaier, 2000) suggesting that low temperature alone cannot explain this phenomenon.

When a human is exposed to low environmental temperatures, body temperature begins to fall and hypothermia ensues; the homeostatic mechanism of shivering fails at body temperature of 30-32 °C, the heart fibrillates at about 28 °C and ventilation stops at about 23 °C (Ivanov, 2000). Albeit deep hypothermia is regularly achieved during major surgery requiring extracorporeal circulation, this is clearly a very special condition unsuitable to get long-lasting hypometabolism in healthy people. As

shivering is inhibited during entry into hibernation, it could be conceived that blocking shivering would aid reaching hypothermia in the human; a human model of deep hypothermia has actually been proposed (Giesbrecht et al, 1997). By using the drug meperidine – a strong inhibitor of shivering- Giesbrecht et al were able to reach a core temperature of 35°C for tens of minutes before rewarming; they could not prolong and deepen hypothermia due to ethical reasons.

2.2.1.3. Ischaemic preconditioning

The organs of summer-active hibernators are sensitive to hypothermia the same way as those of nonhibernating species. Recent research has shown that before true hibernation begins, animals go through a number of cycles where metabolic rate and body temperature drop briefly before entering hibernation. This could be a natural form of hypothermia preconditioning. Interestingly, studies in non hibernators have shown that one or more short periods of ischaemia results in a substantial improvement in the ability of cells and organs to tolerate a subsequent, longer period of ischaemia (Cohen, 2000; Bonventre, 2002). Short periods of interrupted blood flow could therefore trigger the initiation of protective responses, but the effects of such a treatment at the whole body level ere not known.

2.2.1.4. Protecting from freezing

Some hibernators (non mammals and mammals) are able to survive body temperature as low as –3 °C. They adopt the strategy of supercooling to resist freezing (Lee and Costanzo 1998). Frogs are able to produce and introduce in the bloodstream low molecular weight, cryoprotective carbohydrates (specially glycerol) before cooling (they are echtotherm animals). However, supercooling can be dangerous because it is a metastable condition where ice nucleation starts with ease. Interestingly, it has been found (Lee et al, 2000) that in rats infusion of the long-lasting volume expander hetastach was able to improve survival during hypothermia (22°C).

2.2.2 Mechanism 2 - Changing metabolism

During torpor bouts, hibernating species are able to down regulate their cellular metabolic activity to a new hypometabolic steady state (up to $1/100^{th}$ of basal metabolic rate) without damage during

the prolonged cold exposure as well as during the transitions between warm and cold and vice-versa. That means that a novel regulation of metabolic and physiological pathways is needed to allow survival.

Basically, ***a new balance between the ATP demand and the ATP supply must be established*** (Boutilier, 2001). Consequently, during hibernation mitochondrial functions are drastically reduced, even though not completely arrested. In this state they preferentially utilize lipid reserves – especially unsaturated and polyunsaturated fatty acids (Geiser, 1990; Geiser et al., 1994) - as energy source, while the utilization of carbohydrate is drastically reduced (reviews in Hoffman, 1964; Lyman et al., 1982; Wang, 1987; French, 1988; Heldmaier et al., 1999). On this basis, it could be hypothesised that a shift from carbohydrate to lipid utilization could be useful to promote a hypometabolic state. To this aim, hormones such as the leptin, responsible for fatty acid mobilization (Rousseau et al., 2003), could represent a key factor. Moreover, during hibernation, several mitochondrial functions undergo modifications, in particular the enzymes responsible for fatty acid transport and utilization (e.g. acyl-CoA oxidase, pyruvate dehydrogenase kinase) increase (Kabine et al., 2003; Hittel and Storey, 2001, 2002a,b; Buck et al., 2002), although the mitochondrial proton conductance remains unchanged (Barger et al., 2003). Changes in mitochondrial enzyme activity similar to those reported in hibernating animals have been observed in non-hibernators under conditions favouring a shift from carbohydrate to fatty acid oxidation. For example, pyruvate dehydrogenase kinase levels increase in humans fed on a isocaloric but high-fat diet (Peters et al., 2001), and acetyl-CoA carboxylase decreases during starvation, when lipid becomes the main fuel source. Therefore, the change in metabolic fueling could facilitate torpor entrance. However, the activation/disactivation of mitochondrial functions in hibernators appears as a quite complex phenomenon in which numerous and various factors seem to be involved. In fact, not only do many mitochondrial enzyme activities undergo modification, but calcium ions are responsible for the inactivation of the intramitochondrial ATPase, thereby preventing the exhaustion of cellular ATP in de-energized mitochondria (Bronnikov et al., 1990) and the intracellular pH also

plays a regulatory role (Malan et al., 1985, 1988; Malan and Mioskowski, 1988). Therefore, the induction of a reversible down-regulation/inactivation of mitochondrial functions would imply a coordinated regulation of different factors, making difficult this possibility. In addition, the drastic reduction in ATP production should be accompanied by a contemporary counterbalancing decrease in ATP consumption. In hibernating animals all metabolic activities undergo a more or less profound depression: protein synthesis rate is drastically reduced (Whitten and Klain, 1968; Derij and Shtark1985; Bocharova et al., 1992; Frerichs et al., 1998; Giacometti et al., 1989; Koebel et al., 1991) as well as the transcriptional activity (van Breukelen and Martin, 2002). The mechanisms responsible for the natural hypometabolic state identified so far are mostly based on the **reversible phosphorylation of several regulatory enzymes** (Morano et al., 1992; MacDonald and Storey, 1999, Arendt et al., 2003; Storey, 1987), as well as on the differential enzyme control at different body temperatures (Storey, 1997; MacDonald and Storey, 1998; van Breukelen and Martin, 2001). These mechanisms appear again very complex and based on the coordinated functional modifications of different factors.

It seems that the metabolic pathways involved in the hibernating process are so numerous and the knowledge of the metabolic modifications needed for entering natural torpor is so limited and fragmentary that at present it is impossible to identify one or a few key pathways to be targeted for inducing a reversible hypometabolic state.

It may be interesting to remember in this context that several newborn mammals (inclusive of the human newborn) actually respond to hypoxia by a decrease in metabolic rate accompanied by depressed thermogenesis and this is probably a regulated response (Mortola, 1999). However, the mechanisms involved are unknown. In the adult human, acute hypoxia disturbs the normal circadian patterns and, specifically, depress those of body temperature (Bosco et al, 2003), but chronic hypoxia is obviously harmful. On the other side, low ambient oxygen *per se* does not seem to induce hibernation in mammals; instead, it is effective in determining hibernation of amphibia (Boutilier, 2001) and reptiles (Jackson, 2002).

2.2.3 Mechanism 3 - Regulating gene expression

Some seasonal hibernators can enter the hibernation condition even in the absence of external input from environmental cues; this provides evidence that the ability to hibernate is driven by a molecular genetic mechanism rather than being an acute response to e.g., low ambient temperature. We could therefore hypothesize interventions at the gene expression/transcription level to obtain hypometabolism in the human.

2.2.3.1 Genes related to hypometabolic states.

Humans are not hibernators (unfortunately! somebody would add). As a consequence, the idea of making them hibernate has some features strikingly characteristic of science fiction. It is obvious that all interventions are subjected to ethical reasons. It should, however, be considered that, at least in some cases, the genes expressed by hibernators for specific functions are present in our genome and expressed during fetal or neonatal life, like it is the case of pancreatic lipase (Terada and Nakanuma, 1995) which in newborns and hibernators is expressed in tissues other than pancreas.

The simplest and more attractive possibility would be represented by the expression of HIT (Hibernation Induction Trigger, Vybiral and Jansky, 1997) a protein only partially sequenced and reputed to initiate the cascade effect finally leading to hypometabolic state. The implications of the expression of HIT or the use of HIT-like substances are discussed in the section *Modulation of cell nuclear activity.*

We could hypothesize, for instance, to force the expression of a particular factor involved in the induction of a hypometabolic state, or, more simply, to achieve the goal of increasing fuel storage. As it happens in hibernators, lipid accumulation could be achieved by a careful balance of increased plasma insulin in the presence of unaltered glucagon levels.

According to the "sliding set-point" hypothesis (Mrosovsky and Fisher 1970), adipose tissue mass is controlled by a hypothalamic "lipostat" that senses body lipid content and initiates compensatory changes in appetite and energy expenditure to maintain a seasonally appropriate level of adiposity,

leading to fat gain during late summer and autumn and loss during winter. Molecular support for a lipostat has come from the recent cloning of the *leptin (lep)* gene, which was first identified as the gene that is defective in obese ob/ob mice (Zhang et al. 1994). In humans, mice, and rats, blood concentrations of leptin, a 16 kD protein, are proportional to total body fat, and leptin production is hypothesized to function as a peripheral signaling component of the lipostat, with high levels causing decreased food intake and increased energy expenditure and low levels resulting in greater hunger and energy conservation (Stephens and Caro 1998). As mentioned above, pancreatic lipase would then intervene to increase fatty acids liberation, since this enzyme can work also at near-zero temperatures.

Another interesting opportunity would be to express α2-macroglobulin, a protein playing an important role in preventing blood clotting (Srere et al., 1995). During induced torpor, in fact, blood circulation would be severely slowed down, and the risk of impairing microcirculation is high. The presence of this protein has been shown to enhance survival in hibernators.

As suggested by these few examples, it comes out clearly that even if ethically accepted and technically feasible, induction of torpor (or hypometabolic state) in humans involves not only the expression of one (or few) key genes but needs to take into consideration all the cascade effects on different systems. Heart, brain control, circulation, all the systems involved in homeostasis need to be carefully considered.

2.2.3.2 Involved mechanisms

Regulation of gene expression is a field recently given new life by the discovery of a new, alternative mechanism involved. So far, gene expression and gene regulation have been strictly intertwined, and the possibility for a gene to be expressed or repressed was thought to be only linked to the presence, in the vicinity of the gene itself, of several factors responsible for the switching on/off of the gene. These factors have been recognized to be proteins with specific activities, and also the configuration of chromatin structure is known to play a role in silencing genes simply by modifying its folding.

However, it has been recently discovered that a mechanism present in worms works perfectly also in mammalian cells. RNA interference, in fact, is more and more involved in gene expression. This mechanism is based on the regulation of a single gene via the destruction of its product. The intervention of specially featured small RNAs occurs in the cytoplasm, i.e. far away from the site of transcription. Why is this important?

These findings imply the possibility of controlling a gene (and hence the production of a protein) AFTER the message for its production has been sent to the "factory". This consequently implies the possibility of intervening in a simpler way to tamper with genes.

For instance, if one wants to obtain the disappearance of a single factor from the cell, it is possible to design the appropriate set of RNAs to do this. Note that the regulation, so far, is limited to blocking proteins, not to inducing protein synthesis. In practice, we can speculate of modulating gene expression only by deleting protein factors, we cannot make the cell produce a specific protein. The fact of working in negative is already extremely important.

Likewise, some genes related to hibernation *sensu lato* are definitely present also in our genome, possibly within silent DNA, i.e. non transcribing, non expressed genes. A definite possibility exist that by silencing the protein(s) interfering with the expression of such genes, a cascade effect could be achieved leading to a hypometabolic state.

The previous considerations involve the gene control at the organism level. In other words, the expression (or inhibition) of hibernation-related genes will be either induced at the level of all the tissues or at the level of only some key tissues, chosen for their role in modulating the metabolic state. There is, however, another way which could be followed: **gene therapy**. This can be accomplished basically by two different methods. In the first, a particular gene of interest could be introduced into cells of a specific tissue in order to have that tissue express the same genes of hibernators. As for the second, **stem cells** could be used. Stem cells, with their totipotency or multipotency capabilities can be modified in order to express the genes of interest and then be inserted into the organism to modify a certain organ or tissue. The major point concerns the use of

autologous or heterologous stem cells. The first type of cells will have the advantage of not interfering with the host immune system, but, given the present difficulties in obtaining stem cells from humans, the yield will be low. The advantage of heterologous cells, on the contrary, will be on the amount of cells obtainable; in principle there will be no limitations, but the inserted cells will cause an immune response.

2.2.4 Mechanism 4 – Modulating cell nuclear activity

A more accessible strategy to get a human hypometabolic state could be based on the interference with primary cell functions responsible for the regulation of the whole cell metabolism, for example at the nuclear level. It has been in fact demonstrated that, in hibernating animals, cell nuclei undergo modifications of constituents involved in RNA transcription and splicing (Zancanaro et al., 1993; Malatesta et al., 1994a,b, 1995, 1999, 2000, 2001, 2003; Tamburini et al., 1996), probably facilitating the transitions involved in the euthermia-hibernation-arousal cycle.

There is clear evidence for depression in the cold and reactivation during arousal for protein translation and protein synthesis is affected by hibernation at both the levels of initiation and elongation. Taken together, these data indicate a large role for temperature in depressing gene expression at both the transcriptional and translational levels. Instead of being uniquely adapted to express genes in the cold, hibernators, in concordance with metabolic demands, depress protein synthesis. However, hibernators employ mechanisms to preserve mRNA pools that could aid in the resumption of gene expression during the interbout arousal for the replenishment of protein pools.

The cell nucleus presides over many activities, among which are DNA replication and transcription. These activities are under strict enzymatic control in order to maintain and express the genome. The nature of the extracellular signalling agent(s) involved in coordinating the deep modification of nuclear activities in hibernation is still unknown. The hibernation induction trigger (HIT), an elusive protein factor present in the blood of hibernators has been suggested as

responsible for the hibernation cascade, starting possibly at the nuclear level. HIT has never been completely characterized, and there are also contradictory results as to its efficacy. Nevertheless, in principle, a HIT-like molecule (i.e. a trigger) must exist. Some recent papers have demonstrated that DADLE, a peptide of the enkephalin family, is capable of inducing a hypometabolic state in explanted organs as well as in cultured cells. This peptide does not provoke apoptosis and it is not toxic at the concentrations utilized. Our recent results indicate that the preservation of morpho-functional characteristics is evident in cultured cells, and we can hypothesize that also tissues will be kept in good condition. The induction of a hypometabolic state should give as a first response a decreased replication and proliferation, as well as transcription.

At least four scenarios should be tested by the use of DADLE:

1. Since transcription is temperature dependent, the use of DADLE to induce hypometabolic stasis in parallel with hypothermia, would establish a feedback so that transcription is additionally decreased.
2. We know that, in particular conditions, DADLE is able to induce the formation of Heterogeneous Ectopic RNP-derived Structures (HERDS, Biggiogera and Pellicciari, 2000) which represent a storage point the cells can utilize (at the end of the hibernation periods) to restart rapidly transcription and splicing. Noteworthy, these structures created in vitro by the peptide, disappear rapidly once the trigger is removed from the culture medium.
3. An hibernating animal, in addition, needs to have the immediate control over a rapid protein synthesis; this can only be accomplished if the organism is capable of storing also messenger RNAs (mRNAs) with a long half-life.
4. Finally, the expression of protective factors, such as heat shock protein or stress protein, can be advisable, especially in view of their potentiality to prevent direct damage to the nascent or stored mRNA

DADLE should be used to induce all the above molecular changes in target organs such as hypothalamus, liver and muscle.

3. Clinical applications

In this section an outline of possible clinical application of the four above-described potential mechanisms leading to hypometabolism is given, taking in to account statements made at point 2.1 as well as paying special attention to the eventual exploitation in manned spaceflight.

In general terms, induction, maintenance, control and termination of hypometabolism should be performed at maximal efficiency and minimal risk for crew; therefore, strategies, technologies and tools are to be implemented in order to ensure optimal hypometabolism and prevention/mitigation of risks. Since no protocol of regulated hypometabolism is implemented in the clinical setting to date, a great deal of preclinical research should be envisaged prior to human application.

3.1 Monitoring the hypometabolic subject

Whatever the mechanism chosen for the induction of a hypometabolic state in humans, continuous monitoring of several parameters is needed to ensure both survival during the hibernation-like state and complete restitution to the normal activity.

A list of parameters to be monitored should include the following:

Body temperature (core [ear canal] and skin), electrocardiogram (ECG), electroencephalogram (EEG), pulse, blood pressure (possibly central) and flow, haematocrit, blood pH, plasma ions (Ca, K, Na, Mg), plasma key metabolites (glucose, ketone bodies, etc), clotting function, blood and tissue O_2 and CO_2, respiratory rate and depth, gas analysis and flow, urine production (possibly composition), electromyogram (EMG), tremor, body composition.

Ideally, all the above parameters should be measured non-invasively. The relevant technology is already available for some of them (e.g., body temperature, ECG, EEG, pulse) or under development (e.g., evaluation of haematocrit, blood glucose, tissue O_2 and pH by means of near-infrared [NIR] spectroscopy, Saptari and Youcef-Toumy, 2004; Klaessens et al, 2003).

Alternatively or in conjunction with non-invasive measurements, miniaturized (portable or implantable) biosensors could be used (Lakard et al, 2003; Albers et al, 2003; Mansuri, 2003).

3.2 Mechanism-related problems

Each of the four possible mechanisms illustrated in Section 2.2 presents specific clinical aspects to be dealt with. First, we should distinguish mechanisms of permanent conditioning and mechanisms of non-permanent (transient) conditioning.

3.2.1 Mechanisms of permanent conditioning

Regulation of gene expression might be considered as a permanent modification of the organism in the sense that one or more genes should be either introduced or forced to express in the whole organism, or in a tissue or organ of interest. Were these genes available, the basic technology to insert them in the recipient is already available as is the RNA interference procedure.

The consequences of this approach, even if limited to selected parts of the whole organism, could nevertheless be extended to the entire body. In fact, the expression of even a single gene in a single organ can have dramatic systemic effects via a cascade of events reaching targets far from the site of expression. Therefore, preliminary, careful animal experimentation should be at least carried out using this mechanism in order to check for safety and feasibility.

Provided that such basic problems are solved, specific problems could ensue. Let us consider, for example, the expression of HIT in humans; in hibernators, this trigger needs for some external favourable conditions to be expressed e.g., day/night altered rhythms, food scarcity, lowering temperature. These conditions may be difficult to extend to humans; consequently, the required gene expression needs to be accompanied by a molecular clock triggering its initiation and arrest. It is not difficult to imagine a gene promoter ignited by a blood signal. The body would then start to hibernate and revive only when the absence of signal will trigger the gene terminator.

Moreover, an environment which could favour the hypometabolic state (absence of external stimuli, absence of light:dark cycle, constant low temperature) in the first phase and the awakening in the second is required.

Finally, the fact that permanent changes are to be introduced in the individual's genes complement should be considered: the presence of foreign genes could lead to unexpected long-term consequences or modify the individual's ability to cope with the ambient.

3.2.2 Mechanisms of non-permanent conditioning

Changing body temperature, changing metabolism and modulating nuclear activity can be envisaged as mechanisms leading to a non-permanent conditioning of the subject.

3.2.2.1 *Resetting the body temperature set point*

This will probably be accomplished by pharmacological treatment. The clinical consequences of a regulated, controlled resetting of the set point yielding a lower body temperature cannot be anticipated with ease; in clinical practice the opposite event is known (i.d., fever). Administration of drug(s) should be chronic in order to ensure permanent hypothermia/hypometabolism. According to the degree of body temperature lowering to be achieved and/or the drug employed (crossing or not the brain-blood barrier) the oral, intravenous or intracerebroventricular way of administration should be employed.

3.2.2.2 *Lowering body temperature*

Extrinsic lowering of body temperature could be obtained by wearing a liquid cooling garment; this seems more practical than ambient (intravehicle) temperature conditioning; a more invasive approach would be flushing the peritoneal cavity with a coolant in a way similar to peritoneal dialysis. The joined effects of low temperature and low heart rate could promote blood clotting phenomena which could be prevented by the administration of cryoprotective and/or anticoagulant

substances. These substances could be administered automatically in relation to the variations of the blood parameters.

3.2.2.3 *Ischaemic preconditioning*

This could be obtained by (1) lowering the oxygen content of inhaled air, (2) reducing blood flow to key organs or (3) a combination of 1 and 2. Lowering the oxygen content of inhaled air is relatively simple and easily amenable to automation. Reducing blood flow to organs is more complicated and will probably require invasive procedures.

3.2.2.4 *Protecting from freezing*

Cryoprotective substances could be infused in the circulation; for example, glycerol has been widely used in the clinical setting as a treatment of cerebral oedema resulting from acute ischaemic stroke, intraocular hypertension (glaucoma), intracranial hypertension, postural syncope; however, it has also relevant osmotic effects.

3.2.2.5 *Changing metabolism*

The reversible phosphorylation of several regulatory enzymes, which is apparently relevant to inducing hypometabolism in hibernating animals, requires the development of specific drugs or gene therapy to be affected. This field is being extensively investigated because abnormal phosphorylation is a cause or consequence of many diseases. Unfortunately, the reversible phosphorylation of proteins regulates almost all aspects of cell life.

3.2.2.5 *Modulating cell nuclear activity*

Substances able to reversibly inhibit nuclear functions (e.g. opioids) could allow to down regulate the activity of the whole cell. Cell metabolism is in fact capable of balancing RNA production and protein synthesis. This natural mechanism would then allow to obtain a cascade effect just by acting on a single target. Of course, side effects are expected from opioids at the level of central nervous system during and after administration; specific attention should be paid to prevent/treat them. Opioids act at very low concentrations, therefore they could easily be administered from a small reservoir by means of available portable pumps.

3.3 Space-related problems

Manned interplanetary missions are demanding in terms of careful medical care during flight and beyond; this is also the case for “hibernating” astronauts. Moreover, special issues should be addressed with hypometabolic subjects. A sketch of the more obvious problems follows.

First, a reliable telemetry system should be provided to ensue continuous monitoring of astronauts; second, an expert system should be implemented aboard to start proper countermeasures in case of urgent abnormal event since telemetry data will reach the ground control in tens of minutes at the best; third, methods and equipment are to be developed to store, deliver and preserve the drugs to be used.

4. References

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