Hibernating mitochondria, the cool key to cellular protection and transplant optimization

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General discussion

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BRIEF SUMMARY

This thesis examines the cellular threats resulting from forced hypothermia against the background of organ protection in transplantation and major surgery. Moreover, it explores the special features of hibernating animals to avoid these hazards.

In deceased donor organ transplantation, conquering the cellular damage arising from ischemia remains one of the major challenges. Since the start of organ transplantation, forced hypothermia has remained the cornerstone in the prevention of ischemic damage. However, forced hypothermia is also an important stressor by itself. In **chapter 2** we evaluated how lowering of temperature affects mitochondrial function and the production of oxidative damage in isolated mitochondria, cells and perfused kidneys. We found that while lowering of temperature strongly inhibits the mitochondrial energy production, it is less effective in lowering ROS production. Additionally, cooling induced a loss of anti-oxidant enzyme activity. Consequently, cooling results in a progressive discrepancy between energy and ROS production, explaining the deleterious effects of hypothermia in transplantation procedures. As hibernating animals show protection to ischemia, in **chapter 3** we compared the effects of forced hypothermic conditions between a human epithelial kidney cell and an epithelial kidney cell of a hibernator (hamster). We showed human cells to be highly sensitive to hypothermia, in terms of loss of mitochondrial activity and decreased survival, whereas cooled hamster cells showed sustained mitochondrial membrane potential and therewith mitochondrial activity and ATP production, with almost complete survival as result, even during prolonged periods of hypothermia. Based on these results, in **chapter 4** we analysed mitochondrial behavior of two hibernator-derived cell lines in comparison with two non-hibernator cell lines. Similar to findings in chapter 3, forced hypothermia in non-hibernator cells resulted in cell death, rooted in mitochondrial dysfunction, with subsequent energy (ATP) depletion, induction of ROS damage and cell death by ferroptosis. In contrast, both hibernator-derived cell lines showed maintenance of adequate ATP levels throughout cooling and a superior oxidant defense, thus avoiding ROS accumulation and ferroptosis, underlying their superior cell survival. In **chapter 5** we examined the effects of hypothermia on DNA stability in cultured cells and static cooled kidneys from a non-hibernator (respective rat and pig). Cooling induced a time and temperature dependent increase in single and double DNA strands breaks in both cells and static cold stored kidneys, which was strongly associated with excess ROS production. Additionally, the cooling-induced ATP depletion precluded DNA repair following rewarming. Interestingly, pretreatment with dopamine and the chromanol derivative SUL-121 prevented cooling-induced DNA strand breaks. As dopamine previously has been shown to protect from cooling[1], via increased

levels of the gasotransmitter H_2S, we reviewed mitochondrial protective effects for the three gasotransmitters CO, NO and H_2S in **chapter 6**. Among a plethora of protective properties of all three small molecules, H_2S contributes to maintenance of mitochondrial function, activates ROS scavenging pathways and acts as a potent ROS scavenger by itself. Additionally, H_2S is suggested to play an important role in hibernation. In **chapter 7** we demonstrated in a normothermic perfusion model that high concentrations of H_2S safely induce a hibernation-like hypometabolic state via mitochondrial depression in a human sized porcine kidney, suggesting that H_2S serves as a potential alternative for cold preservation. To evaluate long-term effects of temperature in a clinical setting, in **chapter 8** we investigated the relation of temperature management parameters with in hospital and five-year survival of nearly six thousand patients who underwent routine cardiovascular artery bypass grafting (CABG). Survival analysis showed that cooling at 32°C was associated with optimal short- and long-term survival, especially in patients with specific risk profiles such as elderly and patients with low kidney function, whereas deeper cooling was associated with decreased survival rates.

CELLS AS A BASIS

Why do normal cells die in hypothermia?

The presented work shows new insights in cellular and mitochondrial effects of hypothermia, elucidated pathways responsible for hypothermic associated cell death and suggested new hibernation derived strategies for hypothermic cell survival.

As shown in *chapter 2-5*, non-hibernator derived cells are vulnerable to cold-induced stress. Although the survival time at 4°C differs per cell line, all non-hibernator derived cell lines used in this thesis were sensitive to cooling. Different underlying mechanisms of cell death can be suggested, such as the traditionally dichotomous and well-known view on necrosis and apoptosis, comprising of unregulated and massive cell death governed by external factors *versus* internally organized and ATP-dependent cell death, respectively. Additionally, also more recently discovered pathways, collectively represented by regulated necrosis (RN), could play a role in ischemia-reperfusion injury (IRI) and hypothermia[3]. Among RN, we identified ferroptotic cell death, mediated by the iron-dependent accumulation of oxidatively damaged phospholipids[4], to play an important role in hypothermia induced cell death.

We showed two main detrimental effects of hypothermia: decreased ATP levels and increased ROS production. Several mechanisms are known to contribute to energy depletion and excessive ROS production, such as a decreased glycolysis flux[5], attenuated fatty acid oxidation[6] and increased ROS production from peroxisomes[7]. In this thesis, we showed that mitochondria play a central role.

We found that lowering of temperature caused a strong decrease in oxygen consumption, together with mitochondrial permeability transition pore (mPTP) opening and accordingly a failure to maintain a normal mitochondrial membrane potential (MMP). Together, this ultimately leads to mitochondrial failure, calcium overload and apoptosis[8]. Thereby, in hypothermic circumstances mitochondria lose their important function as ATP producers. As a result, a plethora of energy-demanding functions are impeded, among which protein synthesis and plasma membrane Na^+/K^+ transport, but also the ATP-dependent apoptosis. Because of the lack of ATP during cooling, apoptosis is suggested to be particularly present during rewarming/reperfusion rather than during the hypothermia/ischemia[9].

Although most cellular processes are less active due to the forced hypothermia, a certain amount of enzyme activity is needed also, or especially, in cold circumstances. For example, Na^+/K^+ transporters maintaining a healthy plasma membrane potential or enzymes neutralizing toxic products. Failure of these vital processes ultimately affects cellular function and homeostasis, leading to necrosis. Indeed, longer cold-exposure times are associated with necrosis, as

reflected in cells[9] and delayed graft function (DGF) of transplanted organs exposed to longer cold-ischemic times[10].

At the same time, we found that hypothermia causes ROS-induced cellular damage, which persisted after rewarming. Although the absolute amounts of ROS produced at low temperature decreased, it falls behind the decrease in oxygen consumption, leading to a relative discrepancy in lowering the activity of the mitochondria and the production of ROS. This could be explained by a difference in activity between electron chain complexes. While cooling seems to nearly inactivate the final complexes of the electron transport chain (IV and V), as indicated by the low MMP and oxygen consumption, we hypothesize the complexes I-III to be still relatively more active, leading to ROS production. Or in other words, complex I and III, the complexes known to be the primary source of ROS, can have a relatively smaller decrease in activity in hypothermia compared to complexes IV and V, inducing the production of free radicals[11,12].

Altogether, after cold exposure, especially <10°C, a time-dependent accumulation of ROS induced damage was observed, leading to ferroptosis[4,13,14] and DNA damage. Additionally, our experiments indicate that both ATP depletion and ROS damage are adding up, increasing the cellular impact of cooling. Due to hypothermia and ATP depletion, synthesis of the important antioxidant glutathione (GSH) is decreased[15], leading to a reduced scavenging capacity of cooled cells because of GSH depletion[16]. In addition to the decreased ROS scavenging, the lack of ATP decreases the synthesis and activity of repair enzymes, including DNA repair systems such as poly ADP-ribose polymerase (PARP), which amplifies the cellular risk of hypothermia.

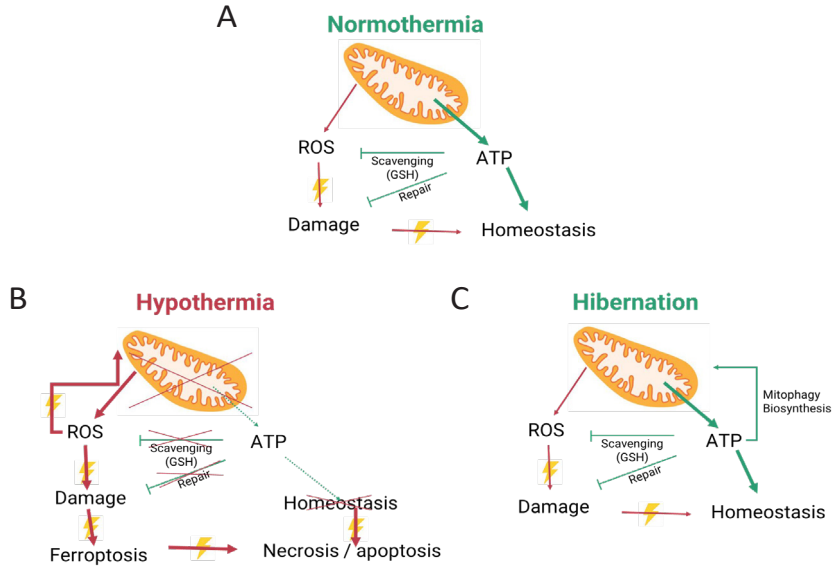

Figure 1. *Schematic overview of the effects of hypothermia to mitochondria.*

Why hibernating cells resist hypothermia

In contrast to the non-hibernating cell lines, hibernator-derived cells maintain sufficient ATP levels in hypothermic circumstances. We showed that, despite exposure to forced hypothermia, mitochondrial OXPHOS and glycolysis remain functional and, together or separately, ensure adequate ATP production, which provides sufficient energy to maintain vital cellular processes, including Na^+/K^+ pumps[17], GSH synthesis and repair systems. Indeed, also during natural hibernation antioxidant capacity was maintained in hibernating animals[18]. In addition, induced pluripotent stem cells (iPSCs) from a hibernator showed improved protein quality control during hypothermia[19].

The energy substrates driving energy production during hypothermia are not yet fully understood. Hibernating animals shift from glucose to fatty acid oxidation for energy supply[20,21]. As depletion of nutrients did not affect cell survival of cooled hibernator-derived cells, survival seems to rely on intracellularly stored energy. However, no increase in autophagy was observed. Interestingly, unlike during forced hypothermia, normothermic hibernator-derived cells deprived of nutrients showed massive cell death. Therefore, we propose energy saving as another important factor ensuring survival of hibernator cells during forced hypothermia.

In line, energy saving in times of food shortage is suggested to be the main reason for hibernation[22]. On the cellular level this would need the inhibition of as many of the energy consuming processes as possible. This could be supported by the low temperature, slowing down all processes. However, it would be crucial to keep the essential processes active and lower only the non-essential processes. To accomplish such, comprehensive management would be needed. Indeed, epigenetic changes[23], RNA regulation[24] and posttranslational modifications to proteins[25] consistent with advanced regulatory mechanisms are found in hibernation through different hibernation phases.

Are hibernator mitochondria fundamentally different from non-hibernator mitochondria?

Morphology

In *chapter 2*, we found morphological differences between hibernator and non-hibernator derived mitochondria. While cooling induced a DRP1 dependent change from an elongated to a dispersed mitochondrial network in non-hibernators, mitochondria of a hamster showed a comparable fission-like mitochondrial network in both normothermia and forced hypothermia.

To examine whether these mitochondrial morphological differences are characteristic for hibernators, we examined mitochondrial morphology in hamster and compared it to rat, as non-hibernator control. Electron microscopy (EM) pictures of kidney tubules were taken in summer euthermic, torpid and aroused hamster.

In normal and aroused conditions, hamster mitochondria showed a more fused network, whereas during torpor the mitochondria changed to a more dispersed network (figure 2A). By analyzing the perimeter of mitochondria, we indeed found smaller mitochondria during torpor compared to summer euthermic and aroused hamsters (figure 2B). When analyzing the 33% biggest mitochondria, to rule out an effect of the transversal cut mitochondria, a significant decreased mitochondrial perimeter is observed during torpor compared to summer euthermic and arousal (figure 2C, $p < 0.001$, ANOVA). Interestingly, the non-hibernating rat control shows a mitochondrial perimeter in between the hibernating and non-hibernating hamster. So, in line with *chapter 2*, we found a shift towards fission in kidney tubule mitochondria during torpor in hamster, which restored to normal during arousal. Although it remains unclear how to interpret these morphological differences, it could be a mechanism to protect the mitochondria from damage. Traditionally, a hyperconnected network is thought to support mitochondrial function by allowing even distribution of mtDNA, mitochondrial components and metabolites[26]. However, in stress conditions a hyperconnected network would allow damaged and dysfunctional parts to spread out over the mitochondria, disrupting an efficient mitophagy. The constantly dispersed mitochondrial network of hibernation derived cells can be seen as an adaptation to cellular stress, providing a highly efficient

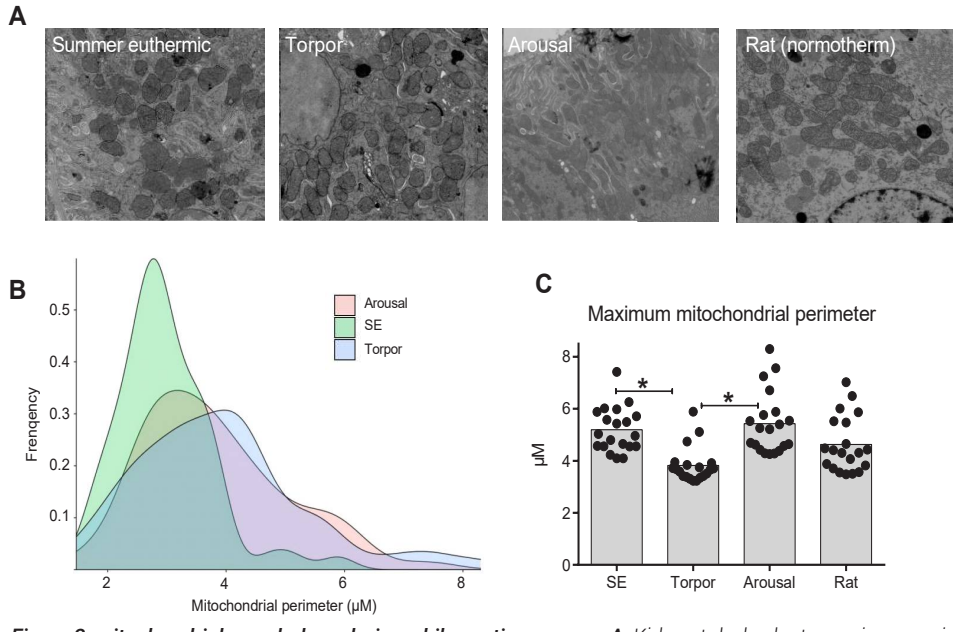

Figure 2, mitochondrial morphology during a hibernation season. A: *Kidney tubule electron microscopic images of 3 different hibernation seasons and a normothermic rat.* ***B:*** *Density plot of the perimeter of 60 randomly choosen mitochondria.* ***C:*** *The perimeter of the 20 largest mitochondria. * = $p < 0.001$, ANOVA.*

morphology for mitophagy to prune damaged parts of the network and limit ROS production. However, this self-cleaning system requires an increased biosynthesis of mitochondria, which is energy-consuming. Interestingly, as described in detail[27], the fission/fusion machinery has shown to be very complex and both fission and fusion are linked to improved and decreased mitochondrial function.

Genome

To our current knowledge, mtDNA does not fundamentally differ between hibernators and non-hibernators. Since most mitochondrial enzymes are nuclear encoded, we also examined nuclear encoded differences. In line, also nuclear DNA is suggested to not differ fundamentally between hibernators and non-hibernators. However, transcriptional differences are found in a variety of pathways during torpor, among which several mitochondrial related pathways. For example, upregulation of mitogenises by the respectively mitochondrial and nuclear encoded mitogenesis markers PGC1α and NRF1is describedd[18,28]. An improved mitogenesis would be in line with the self-cleaning system as proposed earlier. Changes in gene profiles are also described during seasonal changes[29,30] and are different among organs[31], both illustrating the complexity of the regulating mechanisms, complicating the search for a 'hibernation gene' or pharmacologic targets. Nevertheless, new gene mapping techniques have associated gene profiles to seasonal onset of hibernation[32], suggesting a role for genetics in hibernation.

In line with the found mitochondrial related transcriptional changes, enzyme profiles adapt during hibernation in order to optimize energy production during the physiological extremes. For example, during torpor bouts several ROS scavenging enzymes are upregulated[33] and glutamate dehydrogenase is dephosphorylated[34], promoting the oxidation of amino acids to fuel the Krebs cycle. Additionally, downregulation of all ETC complexes was found in hearts of hibernating arctic ground squirrels[33], although increased mitochondrial expression, in particular of COX1, were found in hibernating kidneys of the thirteen-lined squirrel[35]. Interestingly, also upregulation of peptides with a strong sequence similarity to its human analogue are identified to play a role in cytoprotection in mammalian hibernation, such as S-humanin[36].

Summarizing, no clear differences are found between mitochondria of hibernators compared to non-hibernators[37]. Therefore, we propose that adapted regulatory processes are key in hibernation, rather than fundamental differences in DNA or proteins. As described earlier, by actively limiting non-essential ATP consuming processes, a strong reduced mitochondrial activity is sufficient to maintain normal ATP levels.

What is hibernation on the cellular level (compared to forced hypothermia)?

At the cellular level, we suggest energy saving to be pivotal for hibernation. Indeed, torpor bouts in hibernating animals feature a strong metabolic depression, with metabolism lowered to only 1-30% compared to normal[38]. Interestingly, mitochondria of hibernators isolated during torpor showed lower maximum oxygen consumption rates compared to aroused isolated mitochondria[39], which suggests that the drop in metabolism results from a regulated reduction of mitochondrial activity, instead of being an effect of a lower temperature. In other words, in hibernation, hypothermia is a consequence of hypometabolism, not the driving force. In line, larger animals such as the black bear show a strong decrease in oxygen consumption, with only a small reduction in body temperature[40]. In contrast, in organ transplantation lowering temperature is the driving force leading to hypometabolism.

Forcing hypothermia upon a cell disrupts its homeostasis, activating an energy-demanding stress response, resulting in ATP depletion. Indeed, in line with this theory, our data in *chapter 2-4* and existing literature[41,42], forced hypothermia induced ATP depletion in cells as well as organs.

Therefore, it may be hypothesized that inhibition of energy-demanding processes prior to hypothermia may prevent the rapid, cooling induced ATP depletion. Together with a low but sufficient ATP production during hypothermia, hibernation derived cell survival can be explained.

WHOLE ORGANS AS A TARGET

Can natural hibernation be induced in a whole organ?

The ability to hibernate, albeit in various forms, range over a phylogenetically wide range of mammals[43]. In line, natural hibernation should be inducible in almost all cells, as we suggest that hibernation is mostly based on special regulatory pathways instead of fundamental changes in key molecules such as proteins or DNA. Since an organ is a combination of different cell types working together, it is suggested that induction of hibernation in organs is feasible. However, as the regulation of natural hibernation is very complex and the molecular and biochemical mechanisms underlying the natural shutdown of metabolic activities remain largely unknown, induction of a real torpor is difficult to achieve nowadays. On top, as organs consist of multiple cell types, the molecular pathways of different cell types need to be elucidated before natural hibernation can be induced.

However, in contrast to the induction of a natural hibernation, several pharmacological therapies are known to induce a hibernation-like hypometabolic state, with promising effects. For example 5'-AMP[44] and H_2S[45] induce a

hypometabolic state, in both cells as well as whole organisms. Molecules that induce a cryoprotective state, such as catecholamines, have also been described. However, although catecholamines have been shown to be protective in cells[1], translation to kidney transplantation had not yet been successful[46].

An analogy of a hibernation-like technique to off-set ischemia effects, which is currently being tested in clinical practice, albeit unfortunately without success yet[47], is known as (ischemic or anesthetic) preconditioning: a single or repetitive period of short-term sublethal organ ischemia enhances the resistance against profound ischemic injury[48]. Although the underlying mechanisms are not yet fully understood, several important pathways are described, among which priming the mPTP[49], mitochondrial biogenesis, and metabolic depression[50]. Hibernation and preconditioning are suggested to be different concepts[51], however, similar pathways are involved. Indeed, preconditioning showed genetic 'reprogramming' inducing a coordinated decrease in metabolic activity[52], potentially by suppressing non-essential biochemical processes. Also, like hibernators, preconditioning induced a shift towards glycolysis[53]. Additionally, preconditioning induced a mitophagy dependent clearance of damaged mitochondria, limiting ROS production during IRI[54], advocating the earlier described self-cleaning fission morphology in hamster. Altogether, preconditioning shows important similarities with hibernation; a coordinated lowering of metabolism and reductions in ROS damage.

In summary, although induction of natural hibernation is conceptually feasible, knowledge gaps must be bridged before safe torpor can be induced in non-hibernating animals. However, pharmacological induction of hibernation-like states and preconditioning are promising strategies to mimic hibernation-like protection during IRI and hypothermia.

H_2S induced hibernation-like state in organ transplantation?

As shown in *chapter 6 and 7*, H_2S is linked to a variety of protective mechanisms in IRI, such as the inhibition of apoptosis and modulation of the inflammation response. In hypoxic hamster cells, H_2S protects against oxidative damage by activating anti-oxidant proteins in a NRF2 dependent fashion[55]. Since H_2S easily evaporates, slow-releasing donors such as the non-targeted slow-releasing donor GYY4137 and the mitochondrial targeted H_2S donor AP39 were synthesized, to effectuate a stable H_2S concentration. These stable low concentrations of H_2S have been shown protective in kidney[56] and heart[57] transplantation.

In higher concentrations, H_2S is known to induce a hypometabolic state through reversible inhibition of complex IV (cytochrome c oxidase) of the ETC[45]. Whereas H_2S was previously only successful in small rodents, we showed in human-sized kidneys a safe and reversible reduction in respiration using a constant H_2S infusion. In the short time span of these experiments, we induced a drug-dependent hypometabolic state in absence of organ damage, without a decrease in ATP or accumulation of ROS.

In kidney transplantation, all procedures need to be performed as fast as possible to reduce ischemic damage. Especially extraction and transplantation times, both warm ischemic periods, are influencing post-transplant renal function. While hypothermia is difficult to induce in a fast way during surgery, since H_2S is very fast-acting, infusion with H_2S can help shorten the warm ischemic time. This reduces the time pressure, providing the surgical team with an extended timeframe.

To examine the possibility of a long-term infusion with H_2S to induce hypometabolism, a normothermic porcine kidney was perfused for 20 min with a constant level of 100 ppm H_2S, corrected for the flow. As showed in figure 3A, this infusion induced a stable state of a low metabolism, consisting of approximately 25% oxygen consumption relative to normal. After the infusion was stopped, metabolism stayed low for another 10 min and subsequently restored to just above normal values , in the next 30 min, showing a first order kinetic (figure 3B).

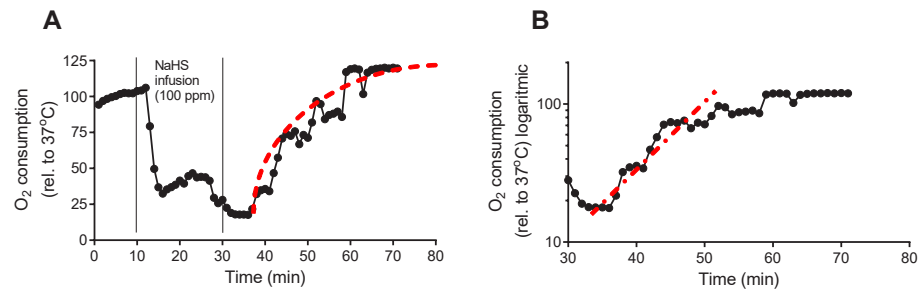

Figure 3, constant H_2S infusion induced a stable decreased kidney metabolism. A: *Metabolism expressed as oxygen consumption relative to normal (the average of the first 10 min) in a normothermic porcine perfusion set-up.* ***B:*** *Post infusion period, Y-axis expressed as semi-logarithmic.*

INTO THE CLINICS

CABG in relation to organ transplant

An interesting different model to study temperature effects on ischemic damage is cardio-pulmonary bypass (CPB) assisted coronary artery bypass grafting (CABG). In CABG procedures, cardiac arrest is realized by exposing the heart to an ice-cold, potassium rich cardioplegic solution. Meanwhile, the CPB machinery is taking care of the body perfusion by managing oxygenation, temperature control and pump function. As we showed in *chapter 8*, body temperatures during CPB vary in a range of 20 to 37 °C. Together, CABG surgery is creating a situation consisting of a deep hypothermic and hypoxic heart together with a stressed and moderate

hypothermic but oxygen-rich corpus.
We found mild hypothermia (32°C) as targeted body temperature to be associated with the best survival compared to normothermia or deeper hypothermia (<32°C). In relation to organ transplantation, this raises the question whether donors should be cooled, for example to 32°C. Indeed, cooling in DBD donors to 33/34°C lowered the odds ratio for delayed graft function significantly[58]. However, the effects on DGF did not translate into improved graft survival[59]. Based on our CABG data, it may be speculated that deeper cooling of organ donors to 32°C may increase the benefits for graft survival. However, proper management and prevention of potential side-effects such as coagulopathies are key factors for its effective clinical usage.

Ideal temperature (and corresponding technique) in organ transplant
Clarifying the optimal preservation temperature and technique is one of the greatest challenges in organ transplantation nowadays. Roughly, we can distinguish two contrasting main techniques: preservation of the normal physiology as closely as possible by normothermic perfusion[60] or decreasing metabolic activity as much as possible, mostly by forced hypothermia near ice-cold or even lower using 'supercooling' techniques[61]. A third main technique attempts to combine the best of both worlds using subnormothermic temperatures (around room temperature, 22°C) and corresponding metabolic activity, bridging reduced energy needs with sufficient metabolic activity[62]. In addition, various techniques such as the addition of oxygen[42], oxygen carriers[63], siRNAs[64], stem cells[65] or pharmacological modulators[66] are being tested in combination with different temperature strategies. Based on the temperature dependent discrepancy between energy and ROS production, as described above, we suggest that the near-physiological situation of normothermic, ischemic free transplantation represents the best technique in terms of optimal organ preservation. Such techniques have recently been developed[67]. However, ischemia free techniques are very time consuming and uses a lot of resources. Especially in organ transplantation, particularly now that extended criteria donors are needed more and more, it can be difficult to meet the time required for these new ischemia-free transplant techniques. Therefore, at least for the years ahead, it is conceivable that the need of hypothermic or subnormothermic remains. Since lowering temperature rapidly decreases mitochondrial function and a sufficient amount of metabolic activity is required for survival, we suggest, in a typical Dutch way of 'polderen', the middle mode of subnormothermic temperatures to be the most suitable for organ transplant preservation in the near future.

CONCLUSION AND FUTURE PERSPECTIVES

Hibernating species have the remarkable ability to preserve their organs during the physiological extremes of the repetitive torpor/arousal cycles, whereas non-hibernating species exposed to similar conditions suffer excessive organ damage. This thesis highlights that mitochondrial function plays a crucial role in both cell death in non-hibernating species and cell survival in hibernating species. We speculate two important principles are driving survival during hypometabolism: energy savings from a strict limitation of non essential cellular processes and maintenance of essential cellular processes.

From a scientific perspective, the key experiment would be to transplant mitochondria from a hibernator into a non-hibernator cell and vice versa, which would enable the distinction between nuclear regulation or mitochondrial characteristics as key mechanisms of survival. The understanding of the exact mechanisms and pathways of a regulated hypometabolism and rewarming will gain insides in preventing organ damage. As analogue of hibernation, preconditioning is a very interesting and promising strategy to induce hibernation-like pathways in non-hibernating organs. Also, the relatively new cellular phenotype of quiescence would be an interesting topic to examine in hibernation. Eventually unraveling the regulatory mechanisms and elucidating pharmacological targets will bring us closer to an induced torpor in human organs.

From a clinical perspective, improved organ preservation techniques would be of great importance in a broad medical spectrum, ranging from organ transplantation to major surgery or acute trauma care.
The ability to rapidly induce a safe hypometabolic state, for example immediately after the no-touch period just before an organ donation procedure, will protect the valuable organs from IRI while giving the medical staff more time to prepare and perform the donation procedure, transport and transplantation. Additionally, as most organs nowadays are preserved using machine perfusion, a more controlled 'organ anesthesia' would be of great interest.
Altogether, hibernation represents a unique model of organ preservation with major clinical potential. Elucidating the important role of mitochondria and other hibernation related pathways might help to the discovery of new pharmacological targets, ultimately bringing us closer to a hibernation-like hypometabolic state in organs.

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Chapter 9 • General discussion

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ABBREVIATIONS

ATP	adenosine triphosphate
CABG	cardiovascular artery bypass grafting
CPB	cardio-pulmonary bypass
DGF	delayed graft function
EM	electron microscopy
ETC	electron transport chain
GSH	glutathione
iPSCs	induced pluripotent stem cells
IRI	ischemia-reperfusion injury
MMP	mitochondrial membrane potential0
mPTP	mitochondrial permeability transition pore
mtDNA	mitochondrial DNA
OXPHOS	oxidative phosphorylation
PARP	poly ADP-ribose polymerase
Ppm	parts per milion
RN	regulated necrosis
RNA	ribonucleic acid
ROS	reactive oxygen species
siRNAs	small interfering RNA