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

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General introduction

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HIBERNATION

Nature offers an interesting mechanism of cellular protection against ischemia and reperfusion injury (IRI): hibernation. Hibernation comprises energy-saving mechanisms used to survive periods of food shortage and is found in almost all mammalian orders[1–3].

While differences between species are described, hibernation generally consists of repetitive cycles of hypometabolism called torpor, interspersed with phases of fast recoveries to normal physiology called arousals (figure 1). During torpor, hibernating animals show a strong decrease in metabolism resulting in a minimization of most physiological processes such as body temperature, heart rate and respiratory rate. Basal metabolic rates can be depressed to 2-4%[2]. During arousals, in 1-3 hours all physiological parameters, including body temperature, are restored to normal[4]. In small rodents such as the arctic ground squirrel and hamsters, these torpor bouts lasts days to weeks, whereas arousals last several hours to a day. In contrast to these animals displaying so called deep hibernation, several small species, including mice, show the shorter daily torpor with bouts lasting up to 8 hours to save energy during food shortage[5,6].

Interestingly, despite the hypothermia, hypoxia, hypo-metabolism, fast rewarming and reperfusion, hibernating animals survive the repetitive hibernation-induced IRI without organ damage[8,9]. Remarkably, even outside the hibernation season, hibernators showed resistance to IRI in a variety of organs[10–14]. Therefore, hibernation is a promising strategy to decrease cellular damage caused by ischemia, hypometabolism or hypothermia in for example organ transplantation or major surgery.

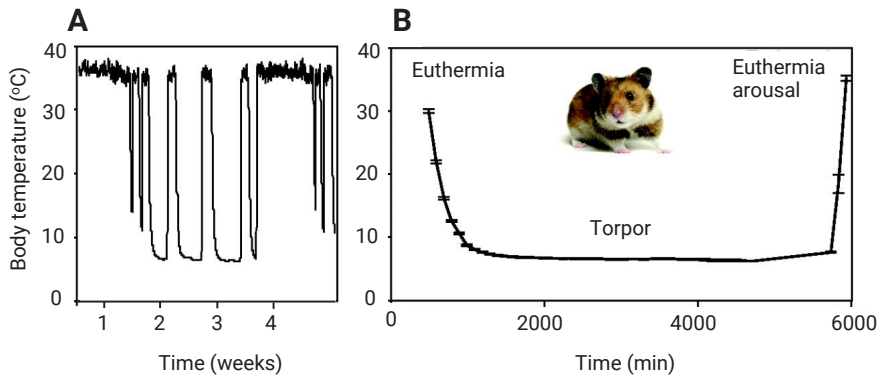

Figure 1. *Example of body temperature during hibernation. (Syrian hamster, data from Talaei et al.[7]).*

THE CRITICAL ROLE FOR MITOCHONDRIA IN ISCHEMIA, HYPOTHERMIA AND HIBERNATION

Mitochondria, 'the powerhouses of the cell,' represent the main source of energy, but also modulate important regulatory and signaling processes. In the process called oxidative phosphorylation, mitochondria oxidize substrates in their electron transport chain (ETC, consisting of five complexes) to create a proton gradient, which in turn is used to drive adenosine triphosphate (ATP) synthesis. However, besides the important function as energy supplier, mitochondria fulfill critical roles in the synthesis of metabolites, regulation of redox potential and Ca^{2+} homeostasis, thermogenesis and apoptosis.

Ischemia leads to a cascade of events, among which mitochondrial failure[15]. The lack of oxygen leads to a dysfunctional ETC, decreasing ATP production and inducing anaerobic metabolism with lactic acid production and acidosis as consequence. Lowering pH in turn impairs cellular enzyme function, further contributing to mitochondrial dysfunction and lowering ATP. Due to the insufficient ATP production, essential cell processes may fail, such as protein synthesis and the maintenance of membrane potential by the ATP dependent Na^+/K^+ transporter. Impairment of the latter allows disruption of the electrolyte homeostasis, ultimately leading to sodium and calcium accumulation followed by cell swelling and opening of mitochondrial permeability transition pores (mPTP). Moreover, the disrupted ETC produces excessive amounts of reactive oxygen species (ROS), damaging surrounding proteins or nucleotides. Together with the loss of mitochondrial protein quality control, this leads to damage to and dysfunction of critical enzymes, including those of the ETC. Collectively, the above changes are commonly denoted as mitochondrial failure.

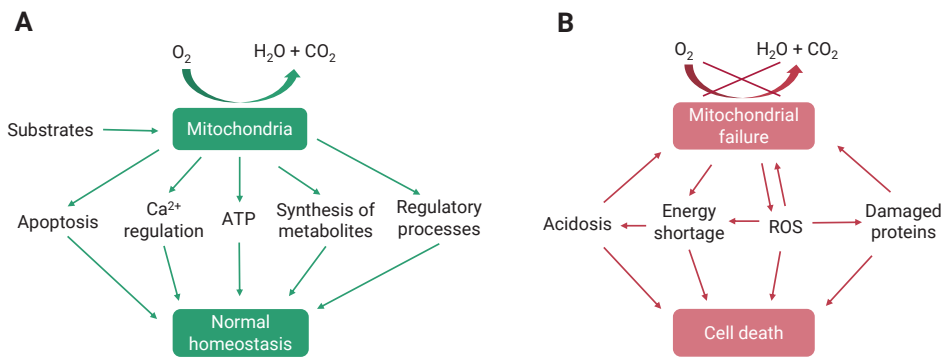

Figure 2. *Roles of mitochondria in normal (A) and ischemic/hypothermic conditions (B).*

Interestingly, most of the processes evoked by ischemia are observed in hypothermia as well. The ETC is disrupted, lowering mitochondrial function and subsequently ATP production meanwhile enhancing ROS production. Therefore, we inferred that hypothermia can be considered as a special form of IRI.

ORGAN TRANSPLANTATION

Organ transplantation is a lifesaving therapy for patients suffering from end-stage organ failure. Unfortunately, there is a long waiting list for organs suitable for transplantation. Most organs are retrieved from deceased donors. In living donor procedures, such as for liver or kidney, patients are selected for optimal organ function, procedures are planned and conditions are optimized, leading to very short ischemic times and optimal graft function[16,17].

In contrast to living donation, the circumstances for donation after death are suboptimal for organ preservation. During deceased donor transplant procedures, after death has been diagnosed and a short no-touch period has passed, the organs are procured, transported and eventually implanted into the recipient. During this process, lasting up to 36h, organs are exposed to prolonged ischemia and subsequent reperfusion injury, which is detrimental to organ quality. Since the start of organ transplantation, the cornerstone of reducing IRI remains the traditional philosophy to induce a forced hypometabolic state in the donor organ by cooling with ice. Creating this forced hypometabolic state decreases energy needs, eventually limiting ischemic damage. However, as described, hypothermia itself is a known factor inducing mitochondrial failure (with corresponding ATP depletion and ROS production). In addition, IRI still occurs during hypothermia, as longer cold ischemic times are associated with delayed graft function and lower survival rates[18–21]. To mitigate the adverse effects of hypothermia, cold ischemic times are shortened as much as possible, increasing the pressure on the surgical and logistic teams.

Due to the increasing need of transplants and the shortage of suitable donor organs, so-called suboptimal donors are increasingly used over the last decades. As donation after brain death (DBD) is associated with the highest survival rates, the so-called standard criteria DBD is known as the best donor pool for organs and often referred to as ideal donors. To extend the donor pool, expanded criteria donors (ECD) and donation after circulatory death (DCD) are increasingly used [22].

In contrast to the standard DBD, ECD are >60 year of age or a donor 50 to 59 years of age with at least two of the following three features: history of hypertension, terminal serum creatinine > 1.5 mg/dL (133 mmol/L), or cerebrovascular cause of death[23], which are all associated with lower quality organs. Whereas in DBD donors organ perfusion is remained until organ procurement, DCD donation inevitably

exposes the future donor organs to marginal perfusion before procurement. Therefore, organs from ECD or DCD donors start with a lower spare capacity and are more prone to damage throughout the transplantation procedure. Indeed, ECD organs showed lower allograft survival and increased delayed graft function[24,25]. In addition to poorer allograft outcome, grafts from ECD kidneys are associated with increased treatment cost and resource use, primarily resulting from longer length of hospital stay, increased requirement for dialysis after transplantation and a greater number of readmissions[23,24]. Thus, improving the preservation technique would limit adverse effects of ECD, in turn extending the donor pool (figure 3).

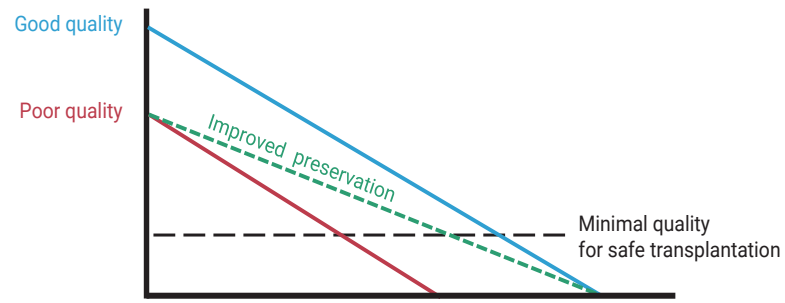

Figure 3. *Visual representation of time and organ quality effecting the success rate of transplantation, illustrating the need for improved preservation techniques.*

HIBERNATING MITOCHONDRIA AS NEW PRESERVATION TECHNIQUE?

Although cold ischemia has safely lengthened the time between explantation and implantation, cold ischemia is detrimental for donor organs and improvements in organ preservation are needed to optimize and increase the donor pool. In addition, improved preservation techniques can facilitate the transplantation procedure, limiting time pressure on the surgical team.

As hibernation shows protective mechanisms to both hypothermia and IRI, understanding these protecting mechanisms of hibernators will lead to new insights into cellular protection. Revealing the mechanisms that induce torpor will help to mimic hibernation in organs, creating new preservation techniques. Also, fast pharmacological induction of a hibernation-like state widens the timeframe in the surgical and logistic procedures.

Ultimately, increased knowledge about hypothermic and hypoxic cellular stress and mechanisms to mitigate the impact of such stress conditions will mitigate

organ damage not only in organ transplants, but also in a variety of other clinical situations, such as major surgery, trauma care, or circulatory arrest. Revealing these protective mechanisms can help to mimic these features by inducement of hibernation-like states organs of non-hibernators in a variety of fields, such as transplantation.

AIM OF THIS THESIS

This thesis hypothesizes that hypothermia can be seen as a special form of ischemia-reperfusion injury. As mitochondria fulfill an important role in cell survival and induction of damage and death, we speculate that cellular protection during organ preservation should affect cell metabolism through mitochondrial pathways.

Therefore, **this thesis aims to elucidate the effects of hypothermia on mitochondrial function and to explore mechanisms that protect mitochondria from hypothermic injury during natural hibernation, with the ultimate aim to mitigate acute organ injury during transplantation.** To this end, mitochondrial activity was evaluated and compared with radical damage in different models, i.e. a cellular cooling and rewarming model (comparing hibernation and non-hibernation derived cell lines), isolated perfused kidneys and a major surgery model.

Chapter 2 investigated how lowering of temperature affects mitochondrial function and the production of oxidative damage in isolated non-hibernation derived mitochondria, cells and perfused kidneys. As we found hypothermia to induce cellular damage and hibernating animals are suggested to resist hypothermia, **chapter 3** compared the effects of forced hypothermic conditions between a human epithelial kidney cell and an epithelial kidney cell of a hibernator (hamster). Based on these results, mitochondrial behavior of two hibernator-derived cell lines in comparison with two non-hibernator cell lines were analysed in **chapter 4**. In order to examine the effects of the increased ROS levels induced by hypothermia on DNA, **chapter 5** looked into the effects of hypothermia on DNA stability in cultured cells and static cooled kidneys from a non-hibernator (respective rat and pig). As literature suggest gasotransmitters to act on ischemic damage via mitochondrial pathways, mitochondrial protective effects were reviewed for the three gasotransmitters CO, NO and H_2S in **chapter 6**. Among a plethora of protective properties of these molecules, H_2S contributes to maintenance of mitochondrial function, activates scavenging pathways and acts as a potent scavenger. Additionally, H_2S is suggested to play an important role in hibernation. To this end, **chapter 7** demonstrated in a normothermic perfusion model that high concentrations of H_2S safely induced a hibernation-like hypometabolic state 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, **chapter 8** describes 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). Lastly, **chapter 9** discusses the data obtained in this thesis and provide future perspectives.

REFERENCES

1. Melvin RG, Andrews MT. Torpor induction in mammals: recent discoveries fueling new ideas. Trends Endocrinol Metab. 2009;20(10):490-498. doi:10.1016/j.tem.2009.09.005
2. Carey H V, Andrews MT, Martin SL. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. Physiol Rev. 2003;83(4):1153-1181. doi:10.1152/physrev.00008.2003
3. Geiser F. Hibernation. Curr Biol. 2013;23(5):R188-R193. doi:10.1016/j.cub.2013.01.062
4. Hampton M, Nelson BT, Andrews MT. Circulation and metabolic rates in a natural hibernator: An integrative physiological model. Am J Physiol - Regul Integr Comp Physiol. 2010;299(6):R1478-R1488. doi:10.1152/ajpregu.00273.2010
5. Renninger M, Sprau L, Geiser F. White mouse pups can use torpor for energy conservation. J Comp Physiol B. 2020;190(3):253-259. doi:10.1007/s00360-020-01263-8
6. Schubert KA, Boerema AS, Vaanholt LM, De Boer SF, Strijkstraand AM, Daan S. Daily torpor in mice: High foraging costs trigger energy-saving hypothermia. Biol Lett. 2010;6(1):132-135. doi:10.1098/rsbl.2009.0569
7. Talaei F, Hylkema MN, Bouma HR, et al. Reversible remodeling of lung tissue during hibernation in the Syrian hamster. J Exp Biol. 2011;214(8):1276-1282. doi:10.1242/jeb.052704
8. Zancanaro C, Malatesta M, Mannello F, Vogel P, Fakan S. The kidney during hibernation and arousal from hibernation. A natural model of organ preservation during cold ischaemia and reperfusion. Nephrol Dial Transplant. 1999;14(8):1982-1990. doi:10.1093/ndt/14.8.1982
9. Jani A, Martin SL, Jain S, Keys D, Edelstein CL. Renal adaptation during hibernation. Am J Physiol - Ren Physiol. 2013;305(11):F1521. doi:10.1152/ajprenal.00675.2012
10. Bhowmick S, Moore JT, Kirschner DL, Drew KL. Arctic ground squirrel hippocampus tolerates oxygen glucose deprivation independent of hibernation season even when not hibernating and after ATP depletion, acidosis, and glutamate efflux. J Neurochem. 2017;142(1):160-170. doi:10.1111/jnc.13996
11. Bogren LK, Olson JM, Carpluk J, Moore JM, Drew KL. Resistance to Systemic Inflammation and Multi Organ Damage after Global Ischemia/Reperfusion in the Arctic Ground Squirrel. Karhausen J, ed. PLoS One. 2014;9(4):e94225. doi:10.1371/journal.pone.0094225
12. Lindell SL, Klahn SL, Piazza TM, et al. Natural resistance to liver cold ischemia-reperfusion injury associated with the hibernation phenotype. Am J Physiol Liver Physiol. 2005;288(3):G473-G480. doi:10.1152/ajpgi.00223.2004
13. Otis JP, Pike AC, Torrealba JR, Carey H V. Hibernation reduces cellular damage caused by warm hepatic ischemia–reperfusion in ground squirrels. J Comp Physiol B. 2017;187(4):639-648. doi:10.1007/s00360-017-1056-y
14. Dar WA, Sullivan E, Bynon JS, Eltzschig H, Ju C. Ischaemia reperfusion injury in liver transplantation: Cellular and molecular mechanisms. Liver Int. April 2019:liv.14091. doi:10.1111/liv.14091
15. Kuznetsov A V., Javadov S, Margreiter R, Grimm M, Hagenbuchner J, Ausserlechner MJ. The role of mitochondria in the mechanisms of cardiac ischemia-reperfusion injury. Antioxidants. 2019;8(10). doi:10.3390/antiox8100454
16. Hong SK, Suh KS, Kim KA, et al. Pure Laparoscopic Versus Open Left Hepatectomy Including the Middle Hepatic Vein for Living Donor Liver Transplantation. Liver Transplant. 2020;26(3):370-378. doi:10.1002/lt.25697
17. Brunotte M, Rademacher S, Weber J, et al. Robotic assisted nephrectomy for living kidney donation (RANLD) with use of multiple locking clips or ligatures for renal vascular closure. Ann Transl Med. 2020;8(6):305-305. doi:10.21037/atm.2020.02.97
18. Gorayeb-Polacchini FS, Caldas HC, Fernandes-Charpiot IMM, Ferreira-Baptista MAS, Gauch CR, Abbud-Filho M. Impact of Cold Ischemia Time on Kidney Transplant: A Mate Kidney Analysis. Transplant Proc. 2020. doi:10.1016/j.transproceed.2019.12.052

19. Mikhalski D, Wissing KM, Ghisdal L, et al. Cold ischemia is a major determinant of acute rejection and renal graft survival in the modern era of immunosuppression. Transplantation. 2008;85(7 SUPPL.). doi:10.1097/TP.0b013e318169c29e
20. Pérez Valdivia MA, Gentil MA, Toro M, et al. Impact of cold ischemia time on initial graft function and survival rates in renal transplants from deceased donors performed in Andalusia. Transplant Proc. 2011;43(6):2174-2176. doi:10.1016/j.transproceed.2011.06.047
21. Salinas SJF, Pérez RE, López MC, Moreno Madrigal LG, Hernández Rivera JCH. Impact of Cold Ischemia Time in Clinical Outcomes in Deceased Donor Renal Transplant. Transplant Proc. March 2020. doi:10.1016/j.transproceed.2020.02.010
22. Rijkse E, IJzermans JN, Minnee RC. Machine perfusion in abdominal organ transplantation: Current use in the Netherlands. World J Transplant. 2020;10(1):15-28. doi:10.5500/wjt.v10.i1.15
23. Merion RM, Ashby VB, Wolfe RA, et al. Deceased-donor characteristics and the survival benefit of kidney transplantation. J Am Med Assoc. 2005;294(21):2726-2733. doi:10.1001/jama.294.21.2726
24. Port FK, Bragg-Gresham JL, Metzger RA, et al. Donor characteristics associated with reduced graft survival: An approach to expanding the pool of kidney donors. Transplantation. 2002;74(9):1281-1286. doi:10.1097/00007890-200211150-00014
25. Querard A-H, Foucher Y, Combescure C, et al. Comparison of survival outcomes between Expanded Criteria Donor and Standard Criteria Donor kidney transplant recipients: a systematic review and meta-analysis. Transpl Int. 2016;29(4):403-415. doi:10.1111/tri.12736

ABBREVIATIONS

ATP	adenosine triphosphate
CABG	coronary artery bypass grafting
DBD	donation after brain death
DCD	donation after circulatory death
ECD	expanded criteria donors
ETC	electron transport chain
IRI	ischemia-reperfusion injury
mPTP	mitochondrial permeability transition pore
ROS	reactive oxygen species