

# The Critical Role of Bioenergetics in Donor Cardiac Allograft Preservation

David A. Schipper<sup>1,3</sup> · Katherine M. Marsh<sup>1</sup> · Alice S. Ferng<sup>1,2</sup> · Dirk J. Duncker<sup>3</sup> · Jon D. Laman<sup>4</sup> · Zain Khalpey<sup>1,2,5,6,7</sup>

Received: 4 March 2016 / Accepted: 19 April 2016 / Published online: 10 May 2016  
© Springer Science+Business Media New York 2016

**Abstract** The traditional philosophy of *ex vivo* organ preservation has been to limit metabolic activity by storing organs in hypothermic, static conditions. This methodology cannot provide longevity of hearts for more than 4–6 h and is thereby insufficient to expand the number of available organs. Albeit at lower rate, the breakdown of ATP still occurs during hypothermia. Furthermore, cold static preservation does not prevent the permanent damage that occurs upon reperfusion known as ischemia-reperfusion (IR) injury. This damage is caused by increased reactive oxygen species (ROS) production in combination with mitochondrial permeability transition pore (mPTP) opening, highlighting the importance of mitochondria in ischemic storage. There has recently been a major paradigm shift in the field, with emerging research supporting changes in traditional storage approaches. Novel research suggests achieving metabolic homeostasis instead of

attempting to limit metabolic activity which reduces IR injury and improves graft preservation. Maintaining high ATP levels and circumventing cold organ storage would be a much more sophisticated standard for organ storage and should be the focus of future research in organ preservation. Given the link between mPTP, Ca<sup>2+</sup>, and ROS, managing Ca<sup>2+</sup> influx into the mitochondria during conditioning might be the next critical step towards preventing irreversible IR injury.

**Keywords** Bioenergetics · Preservation · Mitochondria · Reactive oxygen species · Transplant

## Abbreviations

IR injury	Ischemia-reperfusion injury
ROS	Reactive oxygen species
mtDNA	Mitochondrial DNA
UW solution	University of Wisconsin solution
HTK solution	Histidine-tryptophan-ketoglutarate solution
ATP	Adenosine triphosphate
AMP	Adenosine monophosphate
mPTP	Mitochondrial permeability transition pore

---

Editor-in-Chief Jennifer L. Hall oversaw the review of this article

---

✉ Zain Khalpey  
zkhalpey@surgery.arizona.edu

<sup>1</sup> Department of Surgery, Division of Cardiothoracic Surgery, University of Arizona College of Medicine, 1501 North Campbell Avenue, Room 4302, Tucson, AZ 85724, USA

<sup>2</sup> Department of Physiological Sciences, University of Arizona College of Medicine, Tucson, AZ, USA

<sup>3</sup> Division of Cardiology, Thoraxcenter Erasmus University Medical Center, Rotterdam, Netherlands

<sup>4</sup> Department of Neuroscience, University Medical Center Groningen, Groningen, Netherlands

<sup>5</sup> Department of Translational and Regenerative Medicine, University of Arizona College of Medicine, Tucson, AZ, USA

<sup>6</sup> Department of Medical Imaging, University of Arizona College of Medicine, Tucson, AZ, USA

<sup>7</sup> Department of Biomedical Engineering, University of Arizona College of Medicine, Tucson, AZ, USA

## Introduction

For patients with end-stage heart failure, heart transplant remains the preferred treatment and alternative options are limited. The increasing discrepancy between the number of hearts donated for transplant and the number of possible recipients on transplant lists is a global problem. Hypothermic *ex vivo* organ storage is used in the context of transplantation as an attempt to suspend metabolic activity and decrease energy usage, the accrual of toxic metabolites and damage to the graft [1]. However, the limitations of *ex vivo* storage conditions including classic organ preservation solutions persist, most notably with ischemia-reperfusion injury (IR injury).

Recently, there has been a major paradigm shift in the field, with emerging research supporting changes in traditional storage approaches. While inhibiting cellular processes in order to postpone energy loss is the classic approach to organ storage, novel research suggests that achieving metabolic homeostasis instead reduces IR injury and improves graft preservation. This metabolic equilibrium is obtained by controlling the supply and demand of energy via both oxidative phosphorylation and glycolysis. The goal of this novel approach is to attain preservation of bioenergetics, defined here as stable mitochondrial membrane polarization and preserved mitochondrial functionality. During IR injury, pro-apoptotic factors such as cytochrome C and reactive oxygen species (ROS) are released into the cytosol as a result of mitochondrial disruption. Apoptosis can be induced this way, or by the mitochondria directly, either of which implicates mitochondria in organ graft dysfunction.

In this review, we discuss the ideal milieu to maintain heart viability and the importance of controlling metabolic demand in order to retain mitochondrial integrity, which is essential in the inhibition of ROS and release of apoptotic factors into the cytosol upon reperfusion. We examine the role of bioenergetics in aspects of ex vivo donor heart preservation including organ storage solutions, storage time and mode, temperature norms, and IR injury. We suggest future directions accordingly. Lastly, we consider the effects of circulating mitochondrial DNA (mtDNA) on the immune response and the possibility of mtDNA as a biomarker for IR injury.

### Ischemia-Reperfusion Injury

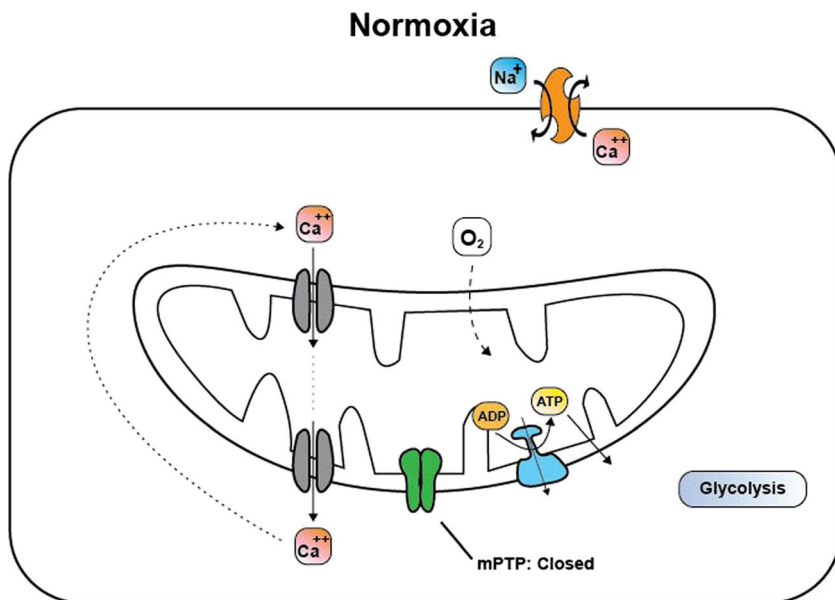
IR injury is one of the major problems in transplantation. Reperfusion injury occurs when blood supply returns to the tissue after a period of ischemia and is encountered in numerous clinical cardiac scenarios including open-heart surgery, percutaneous coronary intervention, and orthotopic heart transplantation. When myocardial blood flow is interrupted, acute myocardial damage can be limited by rapid restoration of blood flow and nutrient delivery. However, after prolonged ischemic time, reperfusion injury causes irreversible tissue damage due to increased ROS production in combination with mitochondrial permeability transition pore (mPTP) opening [2, 3].

ROS play an important role in both the cytosol and mitochondria of cells and are necessary for normal mitochondrial function (Fig. 1). Oxidative stress, which occurs due to an imbalance of ROS production and removal, causes macromolecular damage and is implicated in several disease states [4]. Under certain pathological conditions, ROS overproduction and resultant tissue damage can be stimulated by  $\text{Ca}^{2+}$  overload, which is paradoxical since  $\text{Ca}^{2+}$  also has numerous positive effects in the mitochondria [2].  $\text{Ca}^{2+}$  overload caused by

the loss of ATP, as in hypothermic ex vivo storage, is due to impaired function of the  $\text{Na}^+/\text{Ca}^{2+}$ -antiporter. In hypoxia, metabolism shifts to a glycolytic state, the tissue acidifies, and the  $\text{Na}^+/\text{H}^+$ -antiporter attempts to restore cellular pH (Fig. 2). The corollary increased sodium gradient drives the  $\text{Na}^+/\text{Ca}^{2+}$ -antiporter, which is unsuccessfully activated due to the lack of ATP.  $\text{Na}^+/\text{Ca}^{2+}$ -antiporter dysfunction leads to an increased intracellular and intramitochondrial  $\text{Ca}^{2+}$  concentration, ultimately causing ROS overproduction. In addition to the increase in ROS,  $\text{Ca}^{2+}$  is an important factor in propagating mitochondrial permeability transition pore (mPTP) opening.

$\text{Ca}^{2+}$ -induced mitochondrial  $\text{Ca}^{2+}$  overload in combination with certain pathological conditions leads to the persistent opening of mPTP. Ischemia is an example of one such pathological circumstance, with a lowered AMP/ATP ratio and depletion of adenine nucleotides. The mPTP is nonselective and allows all molecules up to 1.5 kDa to move freely across the outer and inner mitochondrial membranes. Upon opening, the mitochondrial membrane potential is no longer maintained (Fig. 3). This leads to the influx of water, mitochondrial swelling and rupture, and eventually the release of cytochrome C and other apoptotic factors from the inner mitochondrial membrane [2]. The mitochondrial membrane potential is lost, resulting in an uncoupling effect. Persistent opening of the mPTP will not only impede the mitochondria from producing ATP through oxidative phosphorylation, but it will also actively breakdown the ATP that is produced via glycolysis in an attempt to restore the cellular pH levels and concentration gradient across the mitochondrial membrane. In this concept, the low pH that occurs during prolonged ischemic periods thereby inhibits mPTP opening. Once reperfused and supplied with oxygen, the reverse occurs. The cell shifts to aerobic metabolism and pH increases, which opens the mPTP and further increases ROS levels. This mechanism accounts for the time delay observed in IR injury. Though acidic conditions can inhibit mPTP-opening, controlling pH alone cannot protect the tissue against mPTP-induced injury [5]. However, well-maintained mitochondrial function and prevention of pathological circumstances inhibit mPTP opening and ultimately reduce IR injury [6]. Lastly, during IR injury, there is a release of mtDNA upon mitochondrial rupture, which becomes freely circulating. Given this mechanism, mtDNA has recently been considered to be a possible biomarker for myocardial infarction [7–9]. In addition to the apoptosis induced by mitochondrial disruption, free mtDNA can add to the cardiomyocyte death rate [10, 11]. In vitro studies have shown a dose-dependent increase in cell death upon liver IR injury caused by mitochondrial damage-associated molecular pathways (DAMP) [12]. Upon tissue trauma, Toll-like receptor 9 (TLR9) recognizes the mtDNA that is released in the circulation after mitochondrial disruption as non-human material, as it strongly resembles bacterial

**Fig. 1** Normoxic state metabolism: mitochondria produce ATP in the presence of oxygen. A calcium equilibrium is maintained and the mPTP remains closed



DNA. As a response, the body initiates systemic inflammation [13]. Interestingly, high circulating levels of mtDNA along with increased TLR9 expression have been used in recent clinical studies as a predictor of mortality in critically ill intensive care unit (ICU) patients [14]. Furthermore, urinary mtDNA has been used as a biomarker of mitochondrial damage in acute kidney injury [15], and circulating mtDNA could play an important role in assessing cardiac graft damage from IR injury in a similar way. A recent study demonstrated that coronary artery bypass graft (CABG) surgery, a procedure in which cardiopulmonary bypass temporarily inhibits cardiac blood flow, leads to elevated free mtDNA levels in the blood [16]. These findings strongly support the proposition of imposing free

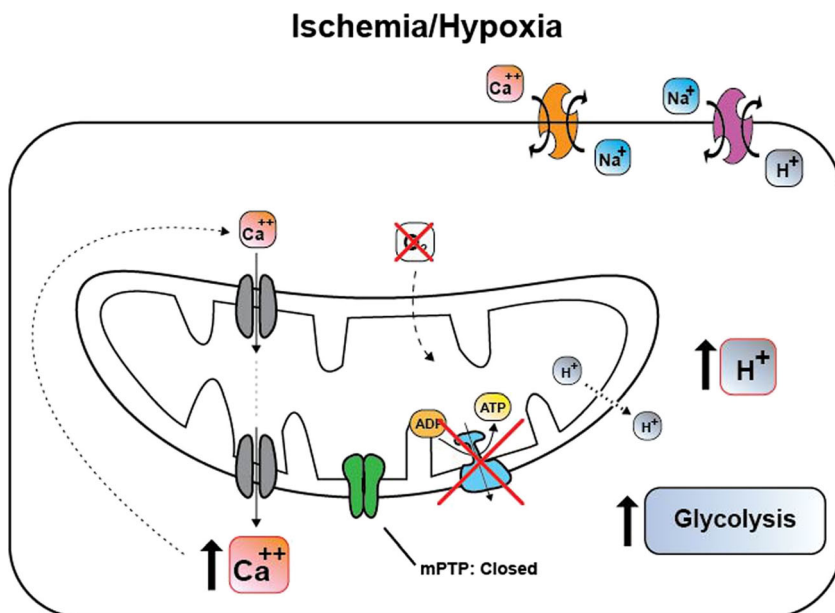
circulating mtDNA as a biomarker for IR injury in cardiac transplant.

Current transplant standards address mitochondrial function by resorting to hypothermic ex vivo storage and using ischemic pre-, post-, and remote conditioning with various solutions as discussed below [17].

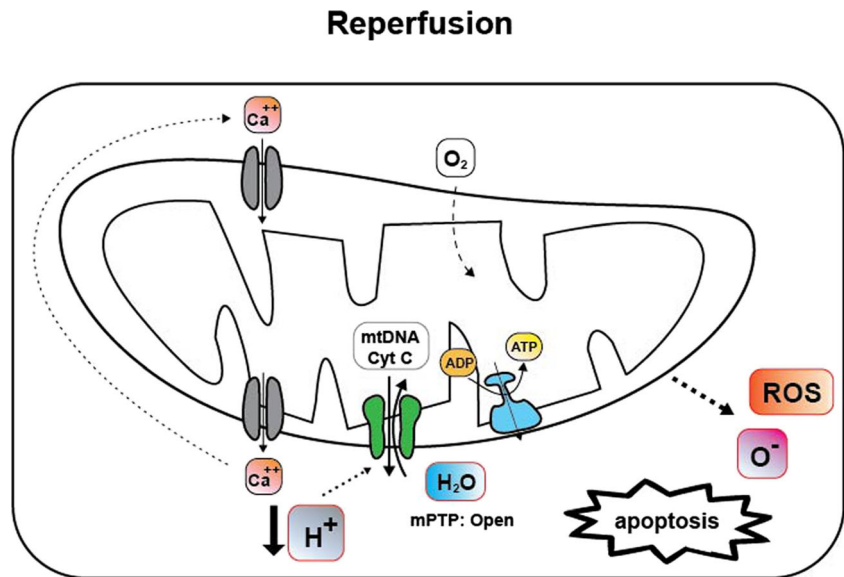
**Organ Storage Solutions**

Ex vivo storage of donor hearts is time limited, with effective allograft function highly dependent on using a storage solution. Historically, all ex vivo organ storage solutions have

**Fig. 2** Ischemic or hypoxic metabolism: lack of oxygen leads to an increase in glycolysis to maintain ATP levels and acidification of the cell occurs. The cascade of ionic changes to counteract cellular acidification generates calcium overflow in the cell and consequently in the mitochondria. At this stage, the mPTP is still closed, as the low pH is an inhibiting factor



**Fig. 3** Reperfusion metabolism: upon reperfusion, the mitochondrial ATP production restores, and ROS is generated. In addition, cellular environment returns to physiological pH. This sets an ideal circumstance for the mPTP to open, causing large molecules and water to enter the mitochondria. Once the mitochondrial inner membrane cannot swell bigger, the outer membrane ruptures and apoptotic factors are released into the cytosol



three common underlying concepts: (1) hypothermic arrest of metabolism, (2) maintaining viability of the tissue during a slowed metabolic state to abate cellular swelling, and (3) minimizing reperfusion injury caused by free radicals and the inflammatory response. To attain these goals, the ionic ingredients included in preservation fluids reduce the transmembrane  $K^+$  gradient, rapidly depolarize the myocardial cellular membrane, and stop cardiac electrical activity [18]. All existing organ storage solutions fall into one of two categories: intracellular ( $Na^+$  concentration less than 70 mmol/L and  $K^+$  concentration ranging between 30 and 125 mmol/L) and extracellular ( $Na^+$  concentration greater than or equal to 70 mmol/L and  $K^+$  concentration between 5 and 30 mmol/L) organ preservation solutions. Both types of solutions generally have comparable results on graft preservation, though this point has been debated [18, 19]. For an overview and detailed composition of selected heart preservation solutions, see Table 1.

Developed in the late 1960s, Euro-Collins was the first widely accepted preservation solution until F. Belzer introduced UW solution in the 1980s. UW solution remains the gold standard to which all new solutions continue to be compared still today, with Celsior as a notable contender [1, 20]. Of the many novel components of UW solution, glutathione is metabolically most relevant. Glutathione can act as a reducing agent and antioxidant, limiting the formation of lipid peroxides and other cytotoxic end products of oxygen metabolism [20, 21]. The basis of UW solution is lactobionic acid combined with raffinose, instead of chloride as used in Collins solution [22]. In other solutions, lactobionic acid was either replaced or used in combination with the potent buffer histidine, and the edema-preventing capacity observed in the UW solution was conserved or even further improved [23, 24]. Histidine is the first of three major components of another

preservation solution, HTK solution, added to prevent acidosis and promote adenosine triphosphate (ATP) production during ischemia [25, 26]. Ketoglutarate also acts to promote ATP production [27]. Ketoglutarate, tryptophan, and mannitol, all included in HTK solution and in some other solutions as well, possess antioxidant properties (Table 1) [20].

Though not part of HTK solution, another compound included in many organ preservation solutions (including UW solution) is adenosine. This endogenous purine nucleoside was originally added to replete ATP following ischemic storage via the purine salvage pathway and was later found to have multiple important functions [28]. Stimulation of  $A_{2A}$  receptors protects against the inflammatory response by inhibiting rolling, adhesion, and migration of inflammatory cells.  $A_{2A}$  stimulation also attenuates other inflammatory responses by inhibiting  $CD4^+$  cells,  $TNF-\alpha$ , superoxides, and interleukin release [29]. When organs are preconditioned with adenosine, activation of  $A_1$  and  $A_3$  adenosine receptors protects the heart against subsequent ischemia and reduces infarct size [30, 31]. Adenosine-mediated preconditioning also reduces ischemic acidosis and enhances post-ischemic recovery of  $[P_i]$ ,  $[Mg^{2+}]$ , and  $\Delta G_{ATP}$  unrelated to cytosolic (ATP) [32].

All the discussed solutions and additives focus on maintaining viability of the tissue during a slowed metabolic state. A paradigm shift is emerging in the field, with a new focus on providing organs with necessary metabolic nutrients instead of slowing metabolism. Somah solution attempts to meet the energy requirements of cardiomyocytes and coronary endothelium by preserving anaerobic metabolism and high-energy phosphates, while safeguarding the mitochondrial gradient [33]. Interestingly, the Somah solution is most effective at 21 °C [34], adding to the question whether conventional low ex vivo storage temperature is justified.

**Table 1** Composition of selected organ preservation solutions; all values are in mmol/L

Ingredient	Euro-Collins	UW solution	HTK	Celsior	Somah
Na <sup>+</sup>	10.00	25.00	15.00	100.00	125.00
K <sup>+</sup>	115.00	125.00	10.00	15.00	7.00
NaCl	10.00	–	15.00	–	125.00
KCl	108.00	–	9.00	15.00	7.00
MgCl <sub>2</sub>	–	–	4.00	13.00	0.50
NaCO <sub>3</sub> H	10.00	–	–	–	5.00
NaOH	–	25.00	–	100.00	–
KOH	–	100.00	–	–	–
MgSO <sub>4</sub>	–	5.00	–	–	0.50
Glucose	180.00	–	–	–	11.00
Adenosine	–	5.00	–	–	2.00
Lactobionate	–	100.00	–	80.00	–
Glutamic acid	–	–	–	20.00	–
Glutathione	–	3.00	–	3.00	1.50
Allopurinol	–	1.00	–	–	–
Mannitol	–	–	30.00	60.00	–
Raffinose	–	30.00	–	–	–
CaCl <sub>2</sub>	–	–	0.02	0.25	1.30
Phosphate	60.00	25.00	–	60.00	0.44
Insulin	–	40.00	–	–	1.00
Dexamethasone	–	16.00	–	–	–
Pantafraction HES	–	50.00	–	–	–
Penicillin G	–	200000.00	–	–	–
L-arginine	–	–	–	–	5.00
L-citrulline malate	–	–	–	–	1.00
Creatine orotate	–	–	–	–	0.50
Creatine monohydrate	–	–	–	–	2.00
L-carnosine	–	–	–	–	10.00
L-carnitine	–	–	–	–	10.00
Dichloroacetate	–	–	–	–	0.50
Ascorbic acid	–	–	–	–	1.00
Sodium phosphate	–	–	–	–	0.19
Histidine	–	–	18.00	30.00	–
pH (at 4 °C)	7.3	7.4	7.4	7.3	7.5

## Ex Vivo Storage

### Temperature

The heart normally relies heavily on oxidative phosphorylation and the Krebs' cycle for energy production. Once oxygen and other substrates are limited as during ex vivo storage, the cells shift to anaerobic metabolism. This shift to glycolysis can have deleterious effect on stored organs due to the overall loss in ATP. Decreasing the temperature of an organ from 37 to 0 °C results in a 12–13-fold decrease in metabolic rate, analogous to van 't Hoff's rule [35, 36]. This is the basis of the conventional cold storage preservation method that has been in use since the late 1960s—lowering organ temperature

in order to slow cellular processes and accretion of mitochondrial by-products such as ROS.

Though slowing the unavoidable buildup of ROS may be beneficial, there are limits to cold preservation and acceptable ex vivo storage times are organ-dependent. Heart preservation is currently limited to 4–6 h of cold ischemia time, with successful outcomes exponentially dropping as cold ischemia time increases past 6 h [37]. ATP production decreases dramatically during cold ischemic storage. This leads to loss of mitochondrial membrane potential, causes mitochondrial membrane permeabilization, and ultimately induces cell death [38]. Furthermore, prolonged cold ischemia is an independent risk factor for organ dysfunction upon transplant. These major limitations of hypothermic storage have led to a change in



perspective in organ solutions exploring subnormothermic storage [34, 39]. Recently, it has been shown that cardiac function can be preserved, and myocardial injury reduced during long-term storage of swine hearts at subnormothermic temperatures [34, 40]. Abandoning the hypothermic storage model has led to superior results, particularly with perfused and beating *ex vivo* hearts.

### Static vs. Perfused Storage

Prior to transplant, organs have traditionally been stored under static conditions, without any resemblance to the *in vivo* environment. This classic approach has been renovated with the advent of *ex vivo* mechanical perfusion. Mechanical perfusion simulates the *in vivo* environment with oxygen and substrate delivery, enabling organs to undergo continuous aerobic metabolism and washout of toxic metabolic by-products [41]. Hearts that are stored with machine perfusion exhibit lower lactate levels, increased adenosine monophosphate (AMP)/ATP ratio, and lower phosphocreatine levels compared to hearts in static cold storage [42, 43]. Lastly, mechanical perfusion may also decrease IR injury [44]. In a porcine model, perfused hearts show less mitochondrial injury after reperfusion on a Langendorff system [45]. On high-risk transplant procedures, the use of an organ care system (OCS™) that provides continuous heart perfusion has shown very promising results [46]. Currently, the OCS™, manufactured by TransMedics Inc., is the only commercially available device capable of *ex vivo* heart perfusion. Other limitations include the necessity to limit warm ischemia time. Failure to ensure rapid perfusion may exacerbate the  $\text{Ca}^{2+}$  overload caused by the loss of ATP. This loss of ATP will inevitably occur more quickly in an organ stored at warmer temperatures, further emphasizing the need for nutrient-rich storage solutions and/or blood. Lastly, as seen in the Langendorff model, continuous perfusion of hearts in the OCS™ has been associated with myocardial edema [47], a limitation that can be overcome by proper storage solution composition as well.

### Discussion

Despite continued research in organ transplantation, significant challenges remain. Many of these problems can be attributed to the lack of optimal *ex vivo* storage. The traditional approach of organ storage has been to slow cellular metabolism and all other cellular processes including cell death. Current standards call for *ex vivo* storage of hearts using existing organ storage solutions at hypothermic static conditions. While hypothermic conditions lower organ metabolism and therefore the need for energy, hypothermia itself can be damaging to the graft. Although this strategy may be successful for some organs, hearts require near-instant high ATP

levels once transplanted. Thus, the lack of ATP and shift to glycolytic metabolism during hypothermic storage is detrimental in hearts. Once reperfused and at baseline, beating hearts rely heavily on oxidative phosphorylation. Mitochondrial damage caused by persistent opening of the mPTP during reperfusion is therefore more damaging to the heart than to other organs. Given the link between the mPTP,  $\text{Ca}^{2+}$ , and ROS, managing  $\text{Ca}^{2+}$  influx into the mitochondria during conditioning might be the next critical step towards preventing irreversible IR injury.

Free circulating mtDNA has exciting potential as a biomarker for IR injury. During cold static preservation, it remains extremely difficult to judge the prognosis of the stored organ, as most injury is induced during reperfusion. Increased levels of free circulating mtDNA can be correlated directly to mitochondrial damage and cell death. Though metabolic assessment of the allograft is not currently performed during static storage, implementation of *ex vivo* graft perfusion would allow real-time assessment of organ condition using mtDNA as a potential biomarker.

Considering the significant damage caused by IR injury following traditional hypothermic storage, it is surprising that organ preservation strategies have barely changed for decades. The lack of innovation in organ preservation solutions and other storage standards may be because all the solutions have the same aim discussed above. Instead of trying to minimize metabolic demand, the recent novel approach is to stabilize and replenish precursors for the bioenergetic supply. As long as substrates are readily available and metabolic waste can be removed, there would theoretically be no reason for an organ to fail outside the body. Substrates can be provided by a storage solution or blood, and waste can be removed by utilizing *ex vivo* perfusion. *Ex vivo* perfusion systems at subnormothermia may emphasize normal mitochondrial ATP turnover and may have remarkably less ATP depletion and tissue necrosis and apoptosis than current storage methods and should therefore be closely investigated. Maintaining high ATP levels and circumventing cold organ storage would be a much more sophisticated standard for cardiac graft storage and should be the focus of future research in heart preservation.

### Conclusion and Future Directions

The classic approach of suspending cellular metabolism through hypothermia does not provide longevity of hearts for more than 4–6 h. The breakdown of ATP, albeit at a lower rate, still occurs in hypothermic conditions. More importantly, the mitochondria undergo permanent changes that result in active ATP dissipation and induce cell death. This limitation will continue to result in a large amount of discarded and underutilized organs for transplantation. Current organ preservation solutions aim to diminish the consequences of

improper mitochondrial protection by including antioxidants and impermeants to prevent tissue damage caused by ROS and edema, respectively. These additives improve graft outcomes to some extent, but the main target to increase organ longevity and functional durability has been overlooked. Preserving the graft's bioenergetic homeostasis can be achieved in multiple ways and is an excellent target for future studies of ex vivo heart preservation. This is demonstrated by the promising results of marginal human grafts transplanted after continuous perfusion, as well as static porcine heart preservation in Somah at various temperatures. Overall, there is a growing understanding of the consequences of permanent mPTP opening, the prevention of which is key to proper organ preservation until transplant. Along with the exploration of subnormothermic storage and use of ex vivo perfusion in order to optimize graft preservation, the role of mtDNA as a biomarker of metabolic organ integrity due to mitochondrial disruption is an exciting proposition.

The transplant gap for hearts continues to widen. It has become clear that classic cold static preservation approaches are insufficient to expand the amount of available organs. A combination of preservation solutions, techniques, and technologies will aid the field of cardiac transplant in the search of increasing the pool of organs suitable for transplantation. Not only will an improvement in graft longevity increase the amount of viable organs being transplanted into patients directly, it also has the ability to open new paths to rejuvenation of marginal organs.

**Acknowledgments** The authors would like to acknowledge the members of the Khalpey Laboratory for useful discussions.

#### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

## References

- Cameron, A. M., & Barandiaran Cornejo, J. F. (2015). Organ preservation review: history of organ preservation. *Current Opinion in Organ Transplantation*, 20(2), 146–151.
- Brookes, P. S., Yoon, Y., Robotham, J. L., Anders, M. W., & Sheu, S. S. (2004). Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *American Journal of Physiology. Cell Physiology*, 287(4), C817–C833.
- Halestrap, A. P., & Richardson, A. P. (2015). The mitochondrial permeability transition: a current perspective on its identity and role in ischaemia/reperfusion injury. *Journal of Molecular and Cellular Cardiology*, 78, 129–141.
- Ray, P. D., Huang, B. W., & Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cellular Signalling*, 24(5), 981–990.
- Crompton, M. (1999). The mitochondrial permeability transition pore and its role in cell death. *The Biochemical Journal*, 341(Pt 2), 233–249.
- Hernandez-Esquivel, L., Pavon, N., Buelna-Chontal, M., Gonzalez-Pacheco, H., Belmont, J., & Chavez, E. (2014). Citicoline (CDP-choline) protects myocardium from ischemia/reperfusion injury via inhibiting mitochondrial permeability transition. *Life Sciences*, 96(1–2), 53–58.
- Wang, L., Xie, L., Zhang, Q., Cai, X., Tang, Y., Wang, L., et al. (2015). Plasma nuclear and mitochondrial DNA levels in acute myocardial infarction patients. *Coronary Artery Disease*, 26(4), 296–300.
- Bliksoen, M., Mariero, L. H., Ohm, I. K., Haugen, F., Yndestad, A., Solheim, S., et al. (2012). Increased circulating mitochondrial DNA after myocardial infarction. *International Journal of Cardiology*, 158(1), 132–134.
- Sudakov, N. P., Popkova, T. P., Katyshev, A. I., Goldberg, O. A., Nikiforov, S. B., Pushkarev, B. G., et al. (2015). Level of blood cell-free circulating mitochondrial DNA as a novel biomarker of acute myocardial ischemia. *Biochemistry Biokhimiia*, 80(10), 1387–1392.
- Yue, R., Xia, X., Jiang, J., Yang, D., Han, Y., Chen, X., et al. (2015). Mitochondrial DNA oxidative damage contributes to cardiomyocyte ischemia/reperfusion-injury in rats: cardioprotective role of lycopene. *Journal of Cellular Physiology*, 230(9), 2128–2141.
- Bliksoen, M., Baysa, A., Eide, L., Bjoras, M., Suganthan, R., Vaage, J., et al. (2015). Mitochondrial DNA damage and repair during ischemia-reperfusion injury of the heart. *Journal of Molecular and Cellular Cardiology*, 78, 9–22.
- Hu, Q., Wood, C. R., Cimen, S., Venkatachalam, A. B., & Alwayn, I. P. (2015). Mitochondrial Damage-Associated Molecular Patterns (MTDs) are released during hepatic ischemia reperfusion and induce inflammatory responses. *PloS One*, 10(10), e0140105.
- Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., et al. (2010). Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*, 464(7285), 104–107.
- Krychtiuk, K. A., Ruhittel, S., Hohensinner, P. J., Koller, L., Kaun, C., Lenz, M., et al. (2015). Mitochondrial DNA and toll-like receptor-9 are associated with mortality in critically ill patients. *Critical Care Medicine*, 43(12), 2633–2641.
- Whitaker, R. M., Stallons, L. J., Kneff, J. E., Alge, J. L., Harmon, J. L., Rahn, J. J., et al. (2015). Urinary mitochondrial DNA is a biomarker of mitochondrial disruption and renal dysfunction in acute kidney injury. *Kidney International*.
- Qin, C., Liu, R., Gu, J., Li, Y., Qian, H., Shi, Y., et al. (2015). Variation of perioperative plasma mitochondrial DNA correlate with peak inflammatory cytokines caused by cardiac surgery with cardiopulmonary bypass. *Journal of Cardiothoracic Surgery*, 10, 85.
- Heusch, G. (2015). Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. *Circulation Research*, 116(4), 674–699.
- Jahania, M. S., Sanchez, J. A., Narayan, P., Lasley, R. D., & Mentzer, R. M., Jr. (1999). Heart preservation for transplantation: principles and strategies. *The Annals of Thoracic Surgery*, 68(5), 1983–1987.
- Chiang, C. H. (2001). Comparison of effectiveness of intracellular and extracellular preservation solution on attenuation in ischemic-reperfusion lung injury in rats. *Journal of the Formosan Medical Association*, 100(4), 233–239.
- Latchana, N., Peck, J. R., Whitson, B., & Black, S. M. (2014). Preservation solutions for cardiac and pulmonary donor grafts: a review of the current literature. *Journal of Thoracic Disease*, 6(8), 1143–1149.
- Jamieson, N. V., Lindell, S., Sundberg, R., Southard, J. H., & Belzer, F. O. (1988). An analysis of the components in UW solution

- using the isolated perfused rabbit liver. *Transplantation*, 46(4), 512–516.
22. Sumimoto, R., & Kamada, N. (1990). Lactobionate as the most important component in UW solution for liver preservation. *Transplantation Proceedings*, 22(5), 2198–2199.
  23. Sumimoto, R., Lindell, S. L., Southard, J. H., & Belzer, F. O. (1992). A comparison of histidine-lactobionate and UW solution in 48-hour dog liver preservation. *Transplantation*, 54(4), 610–614.
  24. Sumimoto, R., Kamada, N., Jamieson, N. V., Fukuda, Y., & Dohi, K. (1991). A comparison of a new solution combining histidine and lactobionate with UW solution and eurocollins for rat liver preservation. *Transplantation*, 51(3), 589–593.
  25. Kallerhoff, M., Blech, M., Gotz, L., Kehrer, G., Bretschneider, H. J., Helmchen, U., et al. (1990). A new method for conservative renal surgery—experimental and first clinical results. *Langenbecks Archiv für Chirurgie*, 375(6), 340–346.
  26. Saitoh, Y., Hashimoto, M., Ku, K., Kin, S., Nosaka, S., Masumura, S., et al. (2000). Heart preservation in HTK solution: role of coronary vasculature in recovery of cardiac function. *The Annals of Thoracic Surgery*, 69(1), 107–112.
  27. Tretter, L., & Adam-Vizi, V. (2005). Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 360(1464), 2335–2345.
  28. Lasley, R. D., & Mentzer, R. M., Jr. (1994). The role of adenosine in extended myocardial preservation with the University of Wisconsin solution. *The Journal of Thoracic and Cardiovascular Surgery*, 107(5), 1356–1363.
  29. Hasko, G., Linden, J., Cronstein, B., & Pacher, P. (2008). Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nature Reviews Drug Discovery*, 7(9), 759–770.
  30. Liu, G. S., Thomson, J., Van Winkle, D. M., Stanley, A. W., Olsson, R. A., & Downey, J. M. (1991). Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. *Circulation*, 84(1), 350–356.
  31. Thomson, J. D., Liu, G. S., Olsson, R. A., & Downey, J. M. (1992). Intravenous pretreatment with A1-selective adenosine analogues protects the heart against infarction. *Circulation*, 85(2), 659–665.
  32. Headrick, J. P. (1996). Ischemic preconditioning: bioenergetic and metabolic changes and the role of endogenous adenosine. *Journal of Molecular and Cellular Cardiology*, 28(6), 1227–1240.
  33. Thatte, H. S., Rousou, L., Hussaini, B. E., Lu, X. G., Treanor, P. R., & Khuri, S. F. (2009). Development and evaluation of a novel solution, Somah, for the procurement and preservation of beating and nonbeating donor hearts for transplantation. *Circulation*, 120(17), 1704–1713.
  34. Lowalekar, S. K., Cao, H., Lu, X. G., Treanor, P. R., Thatte, H. S. (2014). Subnormothermic preservation in Somah: a novel approach for enhanced functional resuscitation of donor hearts for transplant. *American Journal of Transplantation : Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*.
  35. Belzer, F. O., & Southard, J. H. (1988). Principles of solid-organ preservation by cold storage. *Transplantation*, 45(4), 673–676.
  36. Ardehali, A., Esmailian, F., Deng, M., Soltesz, E., Hsich, E., Naka, Y., et al. (2015). Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. *Lancet (London, England)*, 385(9987), 2577–2584.
  37. Bourge, R. C., Naftel, D. C., Costanzo-Nordin, M. R., Kirklin, J. K., Young, J. B., Kubo, S. H., et al. (1993). Pretransplantation risk factors for death after heart transplantation: a multiinstitutional study. The Transplant Cardiologists Research Database Group. *The Journal of Heart and Lung Transplantation : the Official Publication of the International Society for Heart Transplantation*, 12(4), 549–562.
  38. Kroemer, G., Galluzzi, L., & Brenner, C. (2007). Mitochondrial membrane permeabilization in cell death. *Physiological Reviews*, 87(1), 99–163.
  39. Jeevanandam, V. (2010). Improving donor organ function-cold to warm preservation. *World Journal of Surgery*, 34(4), 628–631.
  40. Yang, Y., Lin, H., Wen, Z., Huang, A., Huang, G., Hu, Y., et al. (2013). Keeping donor hearts in completely beating status with normothermic blood perfusion for transplants. *The Annals of Thoracic Surgery*, 95(6), 2028–2034.
  41. Michel, S. G., LaMuraglia, G. M., II, Madariaga, M. L., Titus, J. S., Selig, M. K., Farkash, E. A., et al. (2014). Preservation of donor hearts using hypothermic oxygenated perfusion. *Annals of Transplantation : Quarterly of the Polish Transplantation Society*, 19, 409–416.
  42. Van Caenegem, O., Beauloye, C., Vercautse, J., Horman, S., Bertrand, L., Bethuyn, N., et al. (2015). Hypothermic continuous machine perfusion improves metabolic preservation and functional recovery in heart grafts. *Transplant International : Official Journal of the European Society for Organ Transplantation*, 28(2), 224–231.
  43. Cobert, M. L., Merritt, M. E., West, L. M., Ayers, C., Jessen, M. E., & Peltz, M. (2014). Metabolic characteristics of human hearts preserved for 12 hours by static storage, antegrade perfusion, or retrograde coronary sinus perfusion. *The Journal of Thoracic and Cardiovascular Surgery*, 148(5), 2310–2315. e1.
  44. Bon, D., Delpech, P. O., Chatauret, N., Hauet, T., Badet, L., & Barrou, B. (2014). Does machine perfusion decrease ischemia reperfusion injury? *Progrès en Urologie*, 24(Suppl 1), S44–S50.
  45. Michel, S. G., LaMuraglia, G. M., Madariaga, M. L., Titus, J. S., Selig, M. K., Farkash, E. A., et al. (2015). Twelve-hour hypothermic machine perfusion for donor heart preservation leads to improved ultrastructural characteristics compared to conventional cold storage. *Annals of Transplantation : Quarterly of the Polish Transplantation Society*, 20, 461–468.
  46. Garcia Saez, D., Zych, B., Sabashnikov, A., Bowles, C. T., De Robertis, F., Mohite, P. N., et al. (2014). Evaluation of the organ care system in heart transplantation with an adverse donor/recipient profile. *The Annals of Thoracic Surgery*, 98(6), 2099–2105. discussion 105-6.
  47. Messer, S., Ardehali, A., & Tsui, S. (2015). Normothermic donor heart perfusion: current clinical experience and the future. *Transplant International : Official Journal of the European Society for Organ Transplantation*, 28(6), 634–642.