Impacts of warm ischemia on myocardial protein phosphorylation after normothermic ex vivo reperfusion of hearts donated after circulatory death in a rat DCD model

Ling Gao(1), Jeanette Villanueva(1), Aoife Doyle(1), Peter Macdonald(1,2)

1.Cardiac Physiology and Transplantation, Victor Chang Cardiac Research Institute, Sydney

2. Heart and Lung Transplant Unit, St Vincent's Hospital, Sydney

NSW, Australia

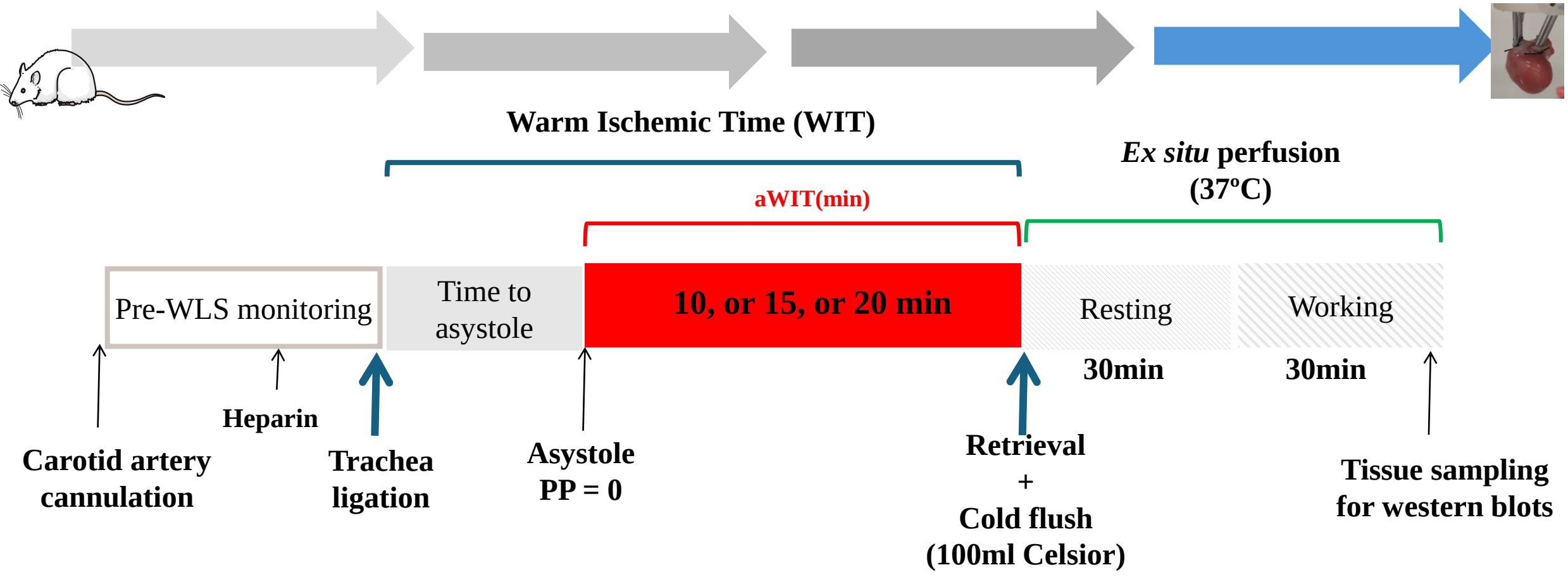
The authors have no conflicts to declare

Background and Aims

Background: The global demand for hearts donated after circulatory death (DCD) for transplantation is increasing in an effort to expand the donor pool. Consequently, there is a need to better understand the impacts of warm ischemia on DCD heart functional recovery and molecular signaling. This is critical in the development of optimal preservation strategies to reduce the risk of post-transplant primary graft dysfunction.

Aims of study: By using an established rat DCD model to investigate the impact of up to 20 minute asystolic warm ischemic time (aWIT) on the phosphorylation status of key elements in signaling pathways where mitochondria are a common downstream target by western blotting.

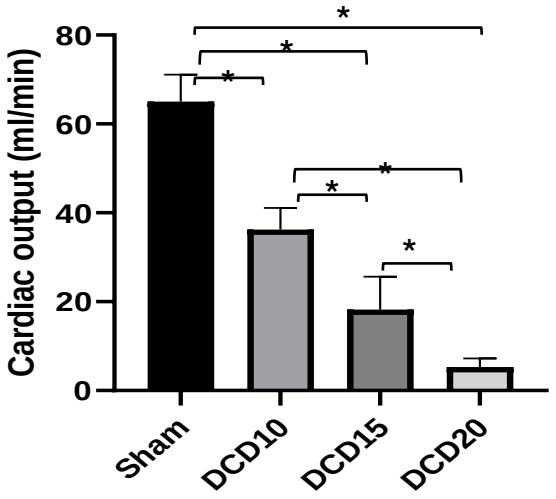
Rat DCD model & ex vivo perfusion of the retrieved DCD hearts



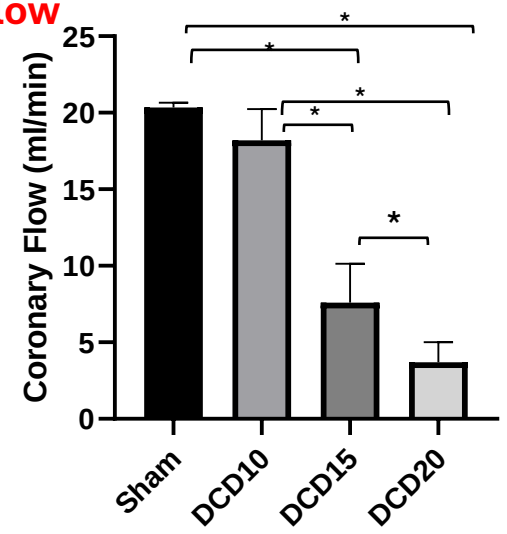
WLS *= Withdraw of Life Support.* ***PP*** *= pulse pressure .*
aWIT *= Asystolic warm ischemic time: Time from asystole to heart removal.*

Cardiac function of the retrieved hearts during *ex vivo* perfusion: Sham vs DCDs

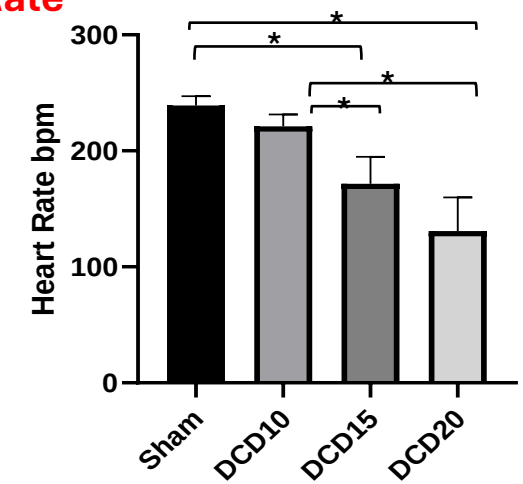
Cardiac Output



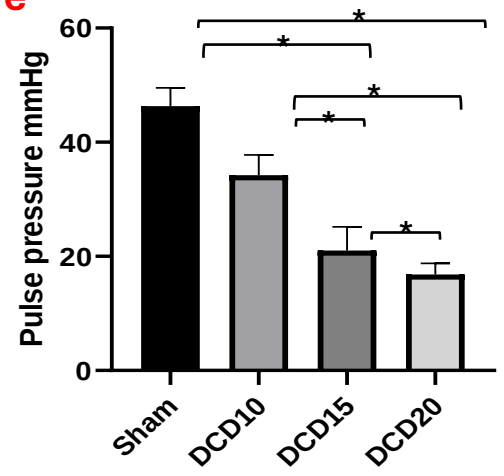
Coronary Flow



Heart Rate

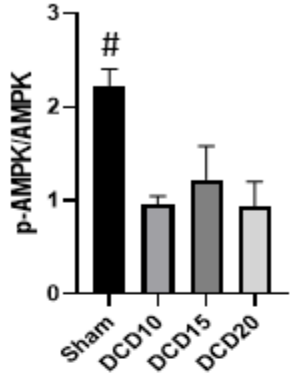


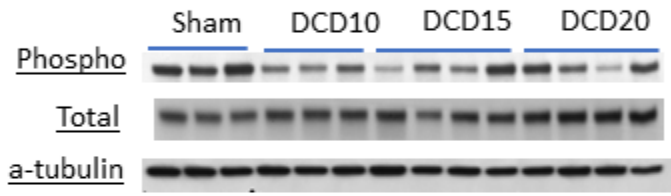
Pulse Pressure



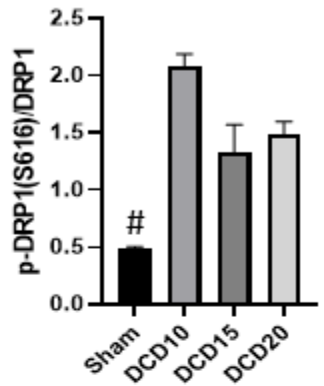
* $p < 0.05$

a

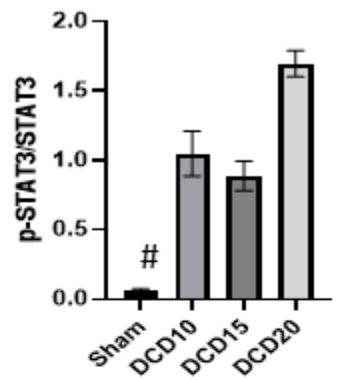


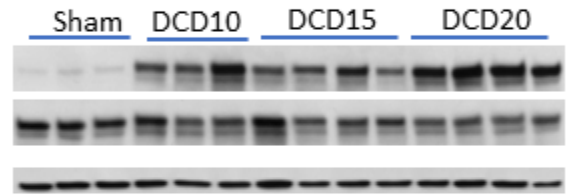


b



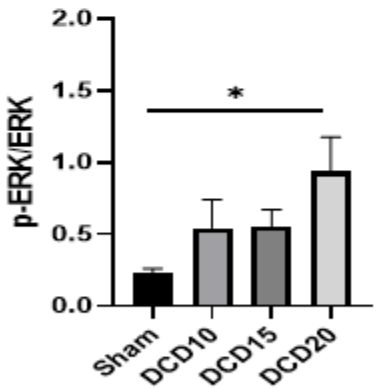
c

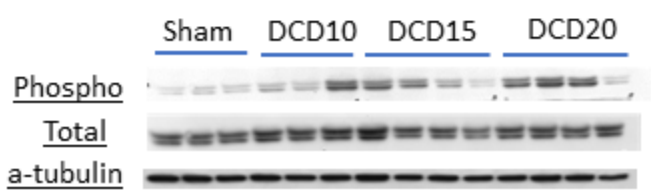




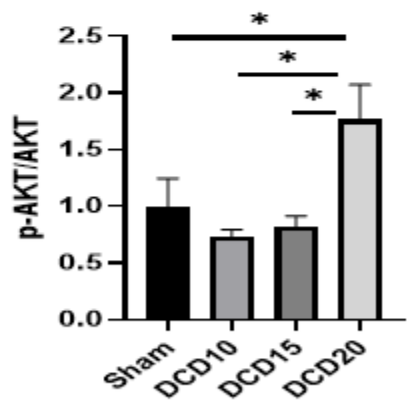
# p < 0.05 vs all DCDs

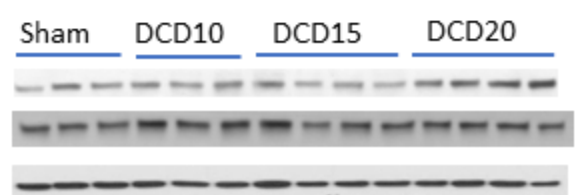
d





e





* p < 0.05

Results Summary

1. **DCD hearts had significantly reduced cardiac functional recovery after reperfusion:**
   - Cardiac output in hearts retrieved after 20 minute aWIT recovered only 8% to that of Sham controls (5.3±2.0 v 63±4.4 ml/min, $p < 0.05$);

2. **Western blots of hearts after 1hr ex vivo reperfusion:**
   - Decreased phosphorylation of AMP kinase were shown in all DCD hearts ($p < 0.05$), while phosphorylation of Drp1S616 and Stat3 were increased significantly ($p < 0.05$).

   - Phosphorylation of Akt and Erk were increased in DCD hearts but only in DCD20 group ($p < 0.05$ vs Sham).

Conclusions

1. Our results showed that hearts exposed to aWIT up to 20min had significant decreases of AMPK phosphorylation, accompanied by significantly increased phosphorylation of Drp1S616, a key promotor of mitochondrial fission activated through phosphorylation.

2. Our findings may suggest the involvement of mitochondrial fission-mediated mPTP opening in DCD hearts during retrieval. Studies to explore this as a potential target of pharmacological conditioning against warm ischemia reperfusion injury in DCD hearts are well deserved.