Succinate accumulation accounts for greater transplant reperfusion injury induced by warm compared to cold ischaemia in mouse, pig and man

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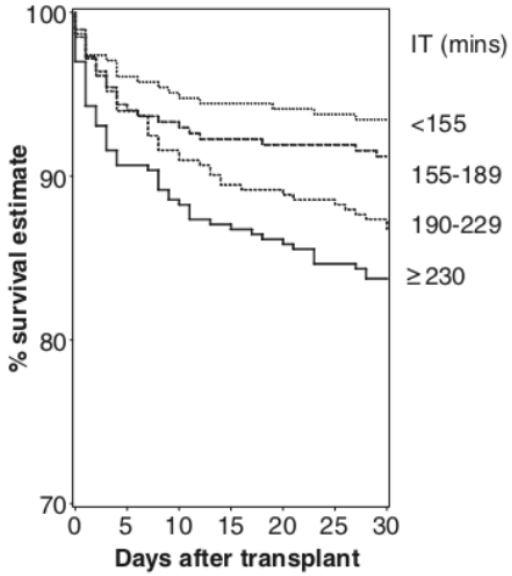
Ischaemia Reperfusion (IR) Injury in Transplantation

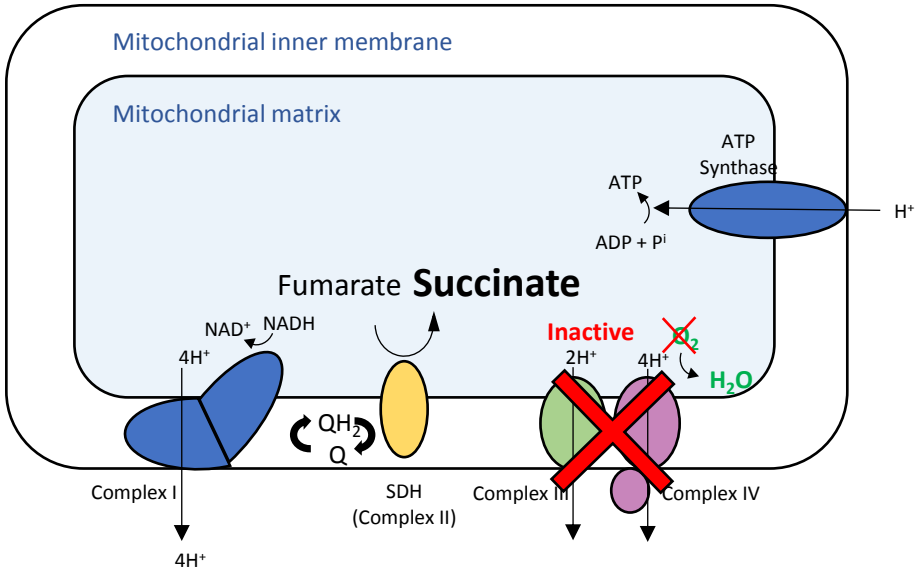
- IR injury is inevitable in transplantation
- There has been a rapid expansion in the use of DCD organs
- Warm ischaemia is more detrimental than cold ischaemia
- Mechanism poorly understood
- Unique opportunity for therapeutic intervention



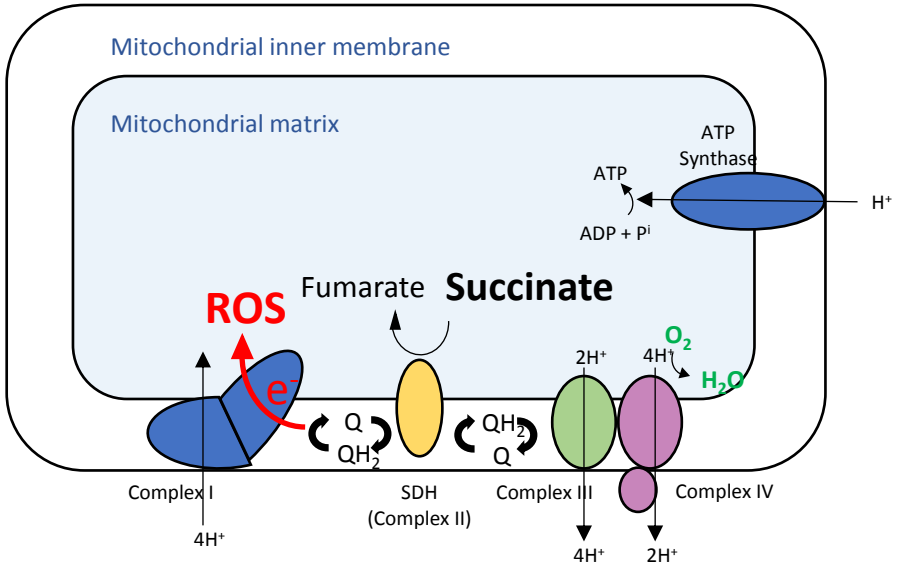
Banner *et al. Transplantation*. 86(4):542-547. 2008

Role of Mitochondria in IR Injury

Ischaemia



Reperfusion



- Succinate is key source of reactive oxygen species (ROS) in IR injury
- ROS cause tissue damage in IR injury

Chouchani *et al*. *Nature*. 2014

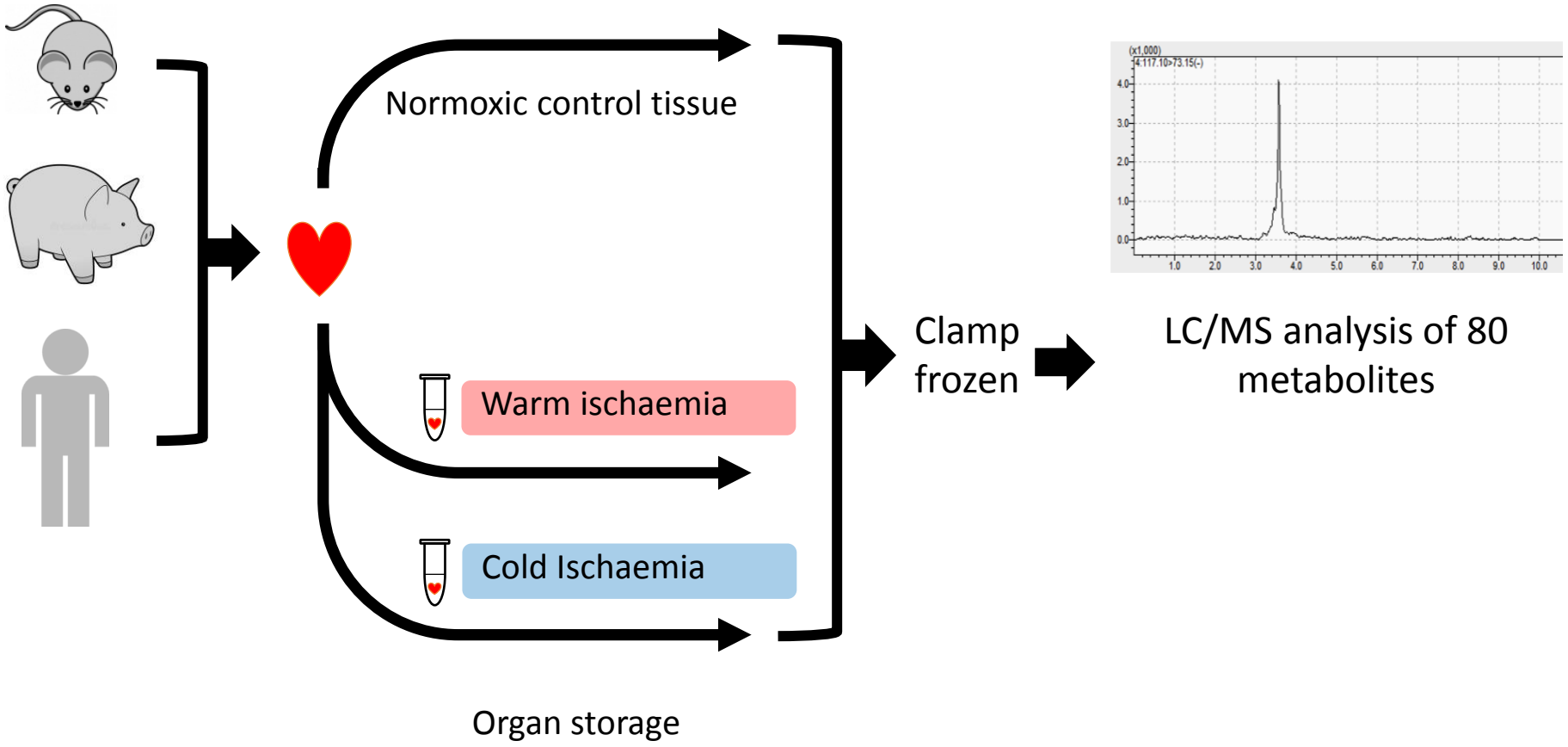
Aims

1. Characterise and compare the metabolic signature of cold and warm ischaemia

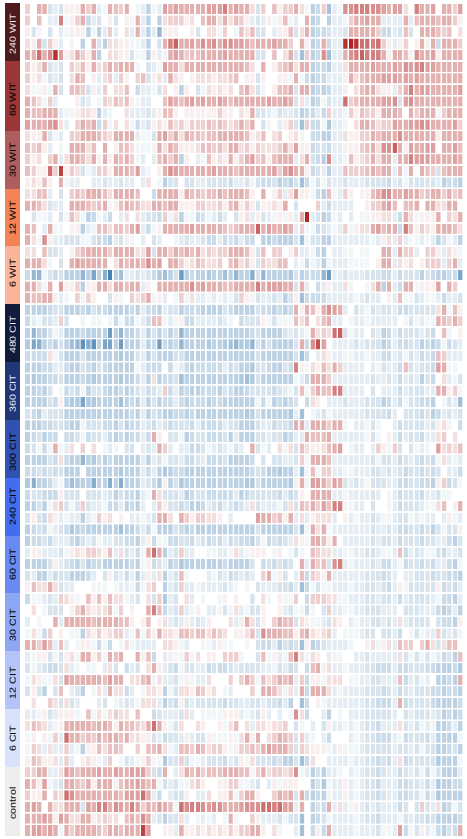
2. Translate findings from mouse to pig to human

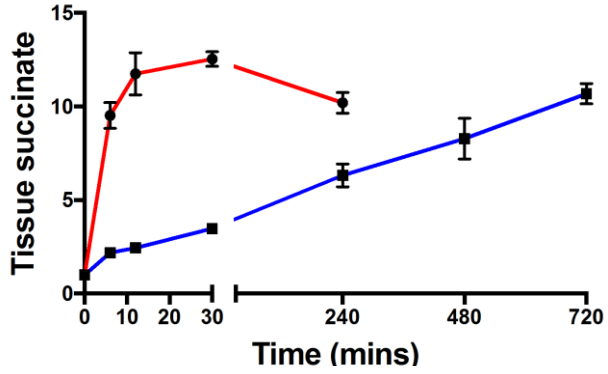
3. Demonstrate the efficacy of manipulating the metabolic pathways during ischaemia

Methods: Metabolic Profile of Ischaemia

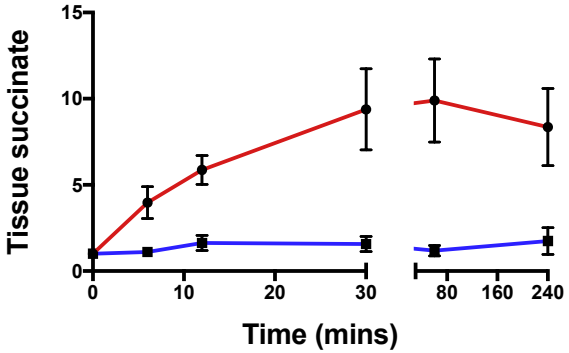


Methods: Metabolic Profile of Ischaemia

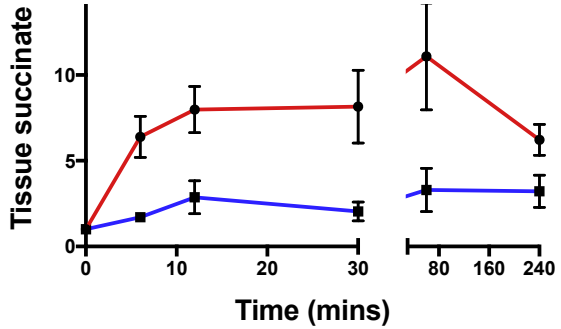




p < 0.0001
Two-way ANOVA
(n = 5-8)



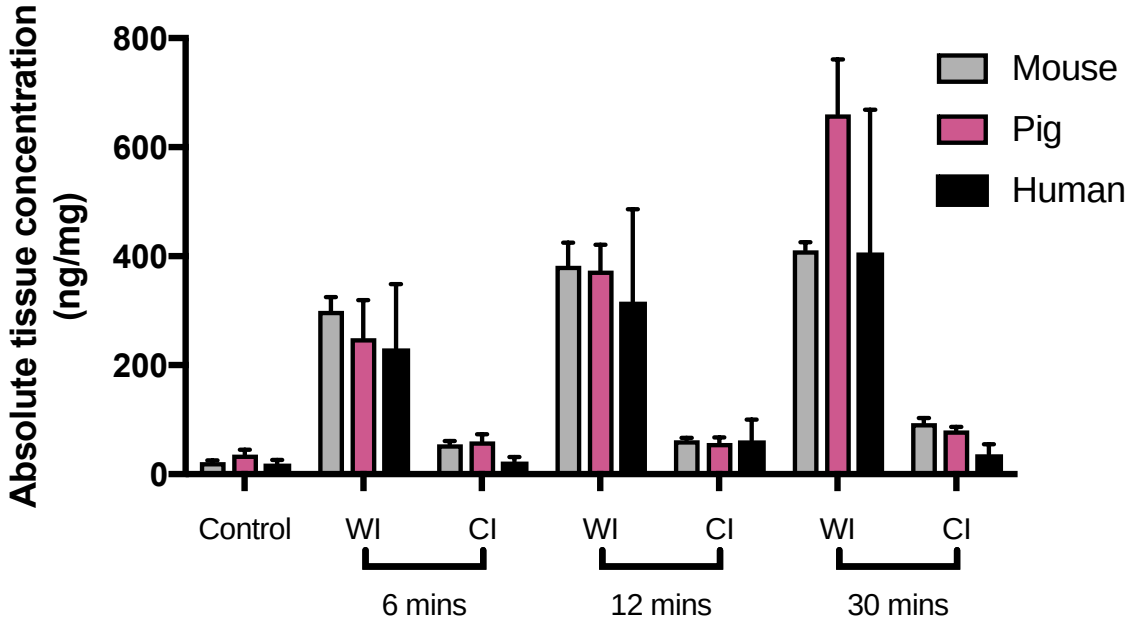
p = 0.0006
Two-way ANOVA
(n = 4)



p = 0.006
Two-way ANOVA
(n = 4)

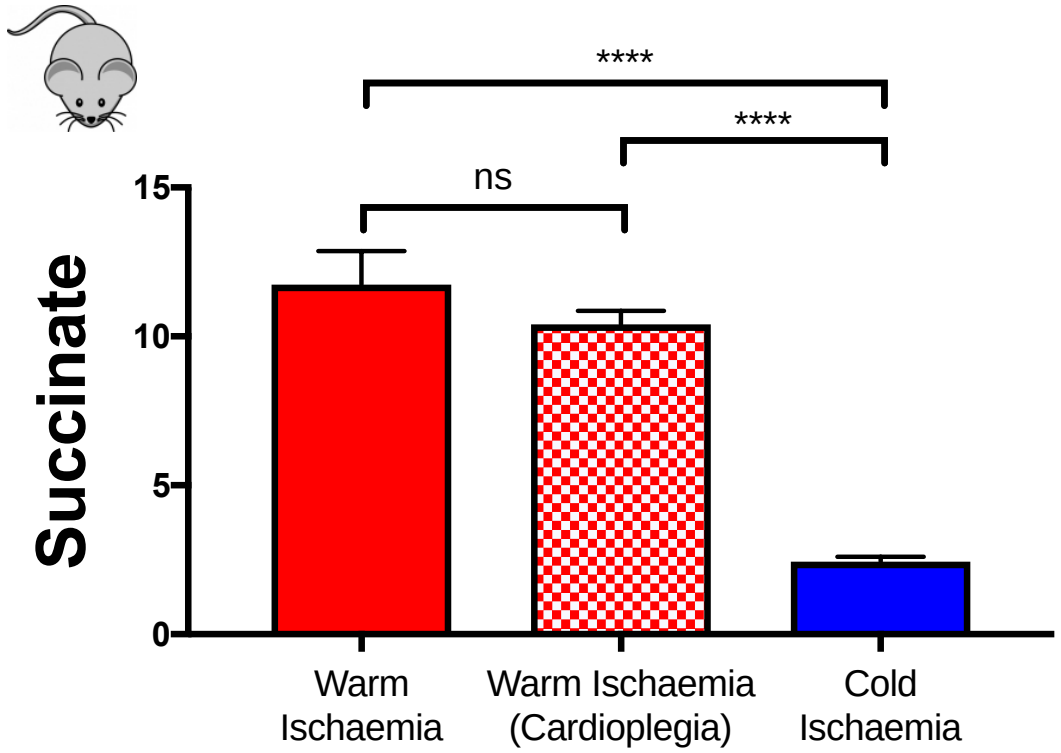
Warm Ischaemia
Cold Ischaemia

Succinate Accumulation During Ischaemia



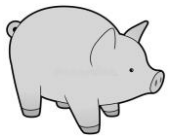
- Succinate accumulates more rapidly during warm ischaemia
- Succinate accumulation is highly conserved metabolic process

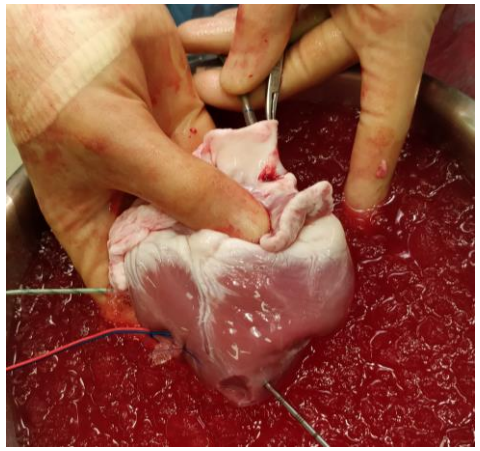
Cardiac Contraction and Succinate Accumulation

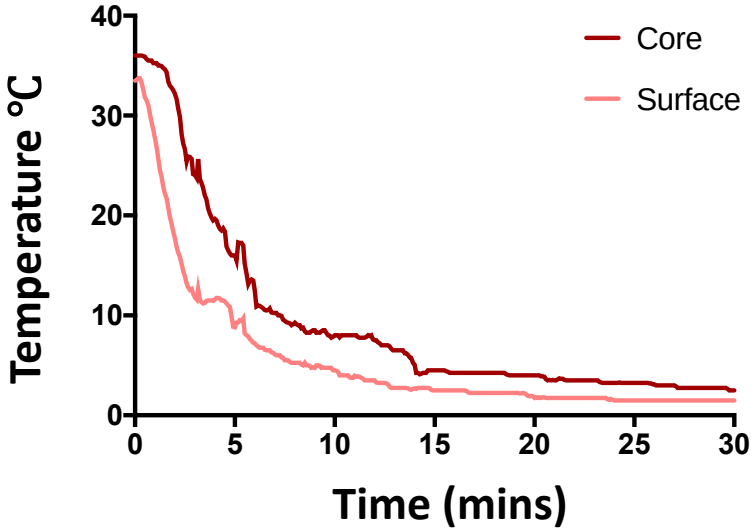


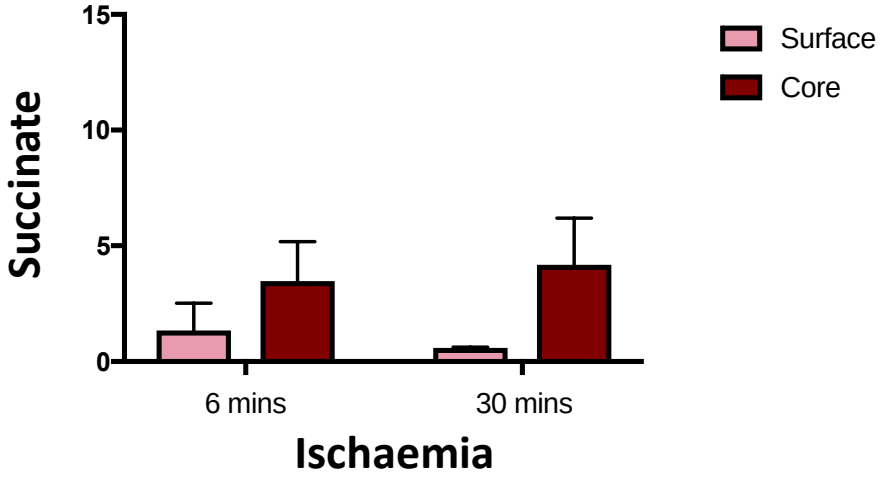
- Cardiac contraction accounts for a significant proportion of energy requirements
- Temperature directly affects cardiac contraction
- Succinate accumulation is significantly greater during warm ischaemia irrespective of cardiac contraction

Succinate Accumulation in the Pig Heart



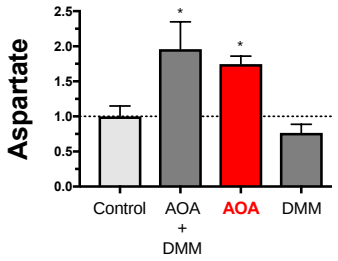






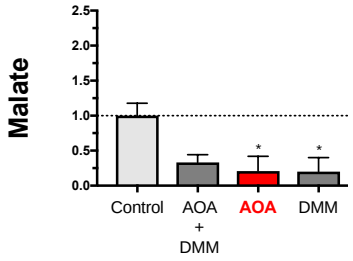
- Succinate accumulates during ischaemia in the pig heart
- Succinate accumulation is dependent on temperature

Inhibition of the Malate / Aspartate Shuttle



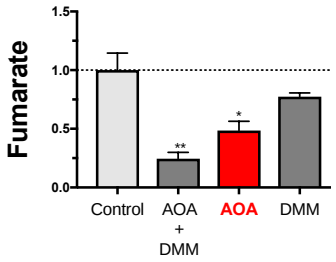
AOA

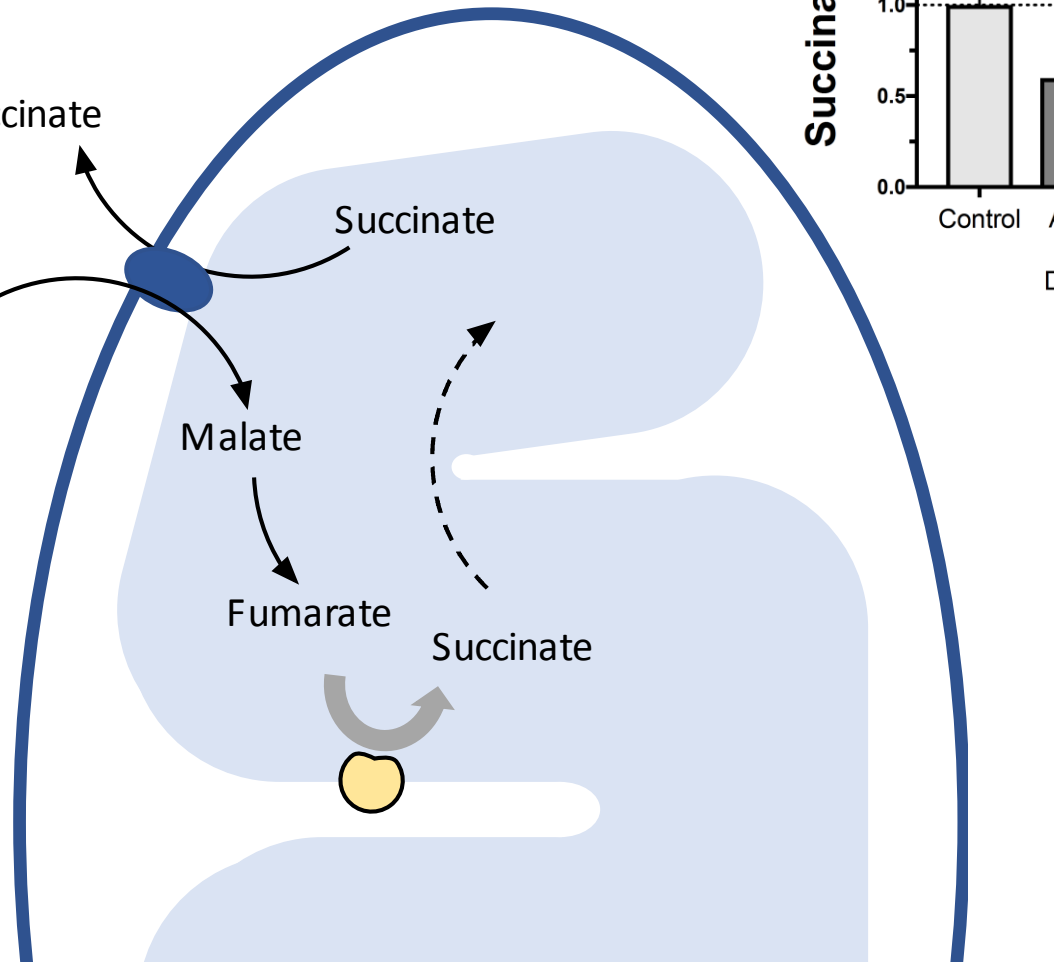
Oxaloacetate

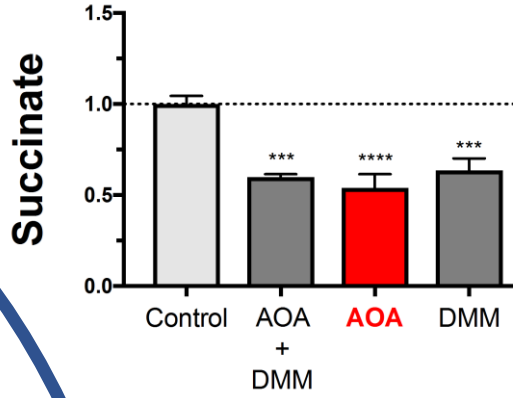


NADH

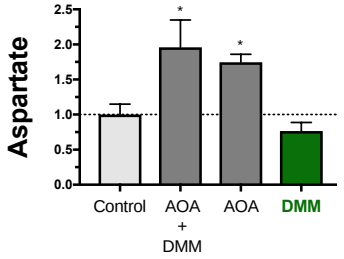
NAD+



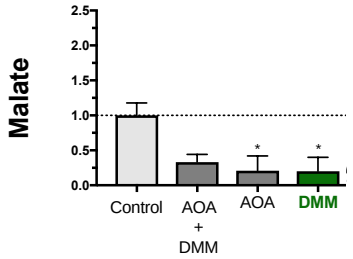


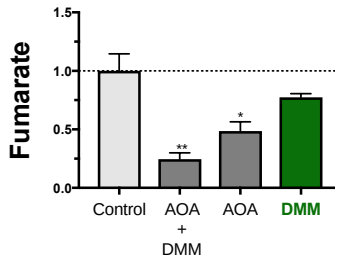


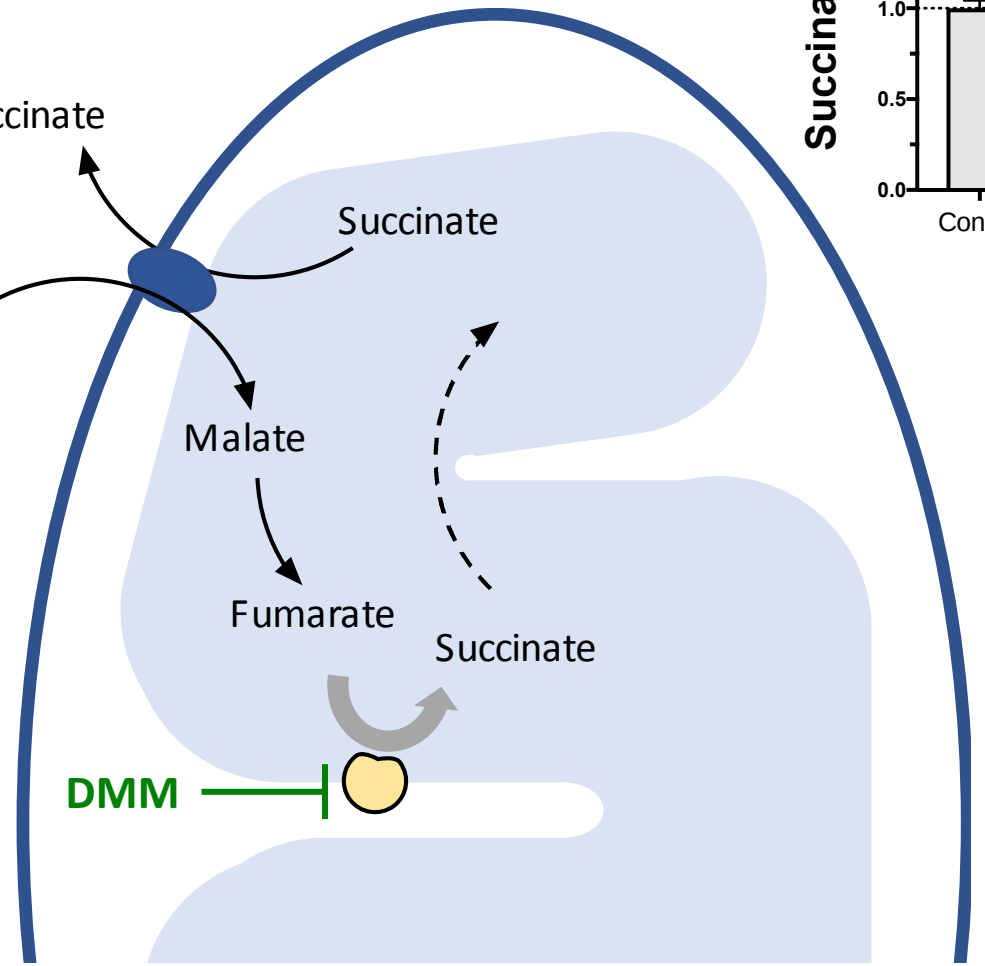
Competitive Inhibition of Succinate Dehydrogenase

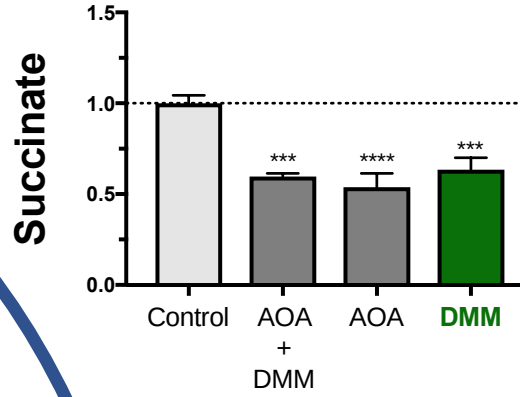


Oxaloacetate

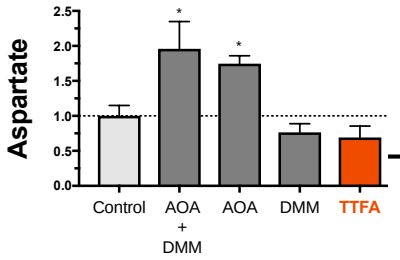




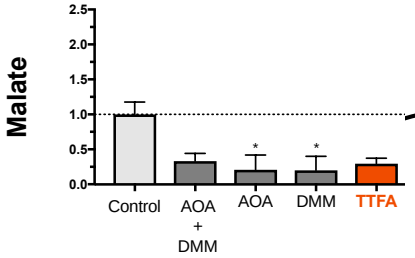




Direct Inhibition of Succinate Dehydrogenase (SDH)

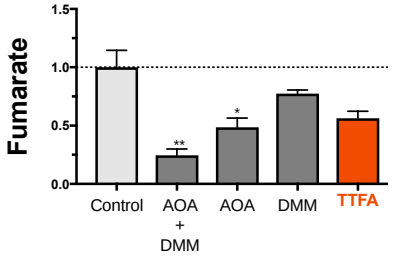


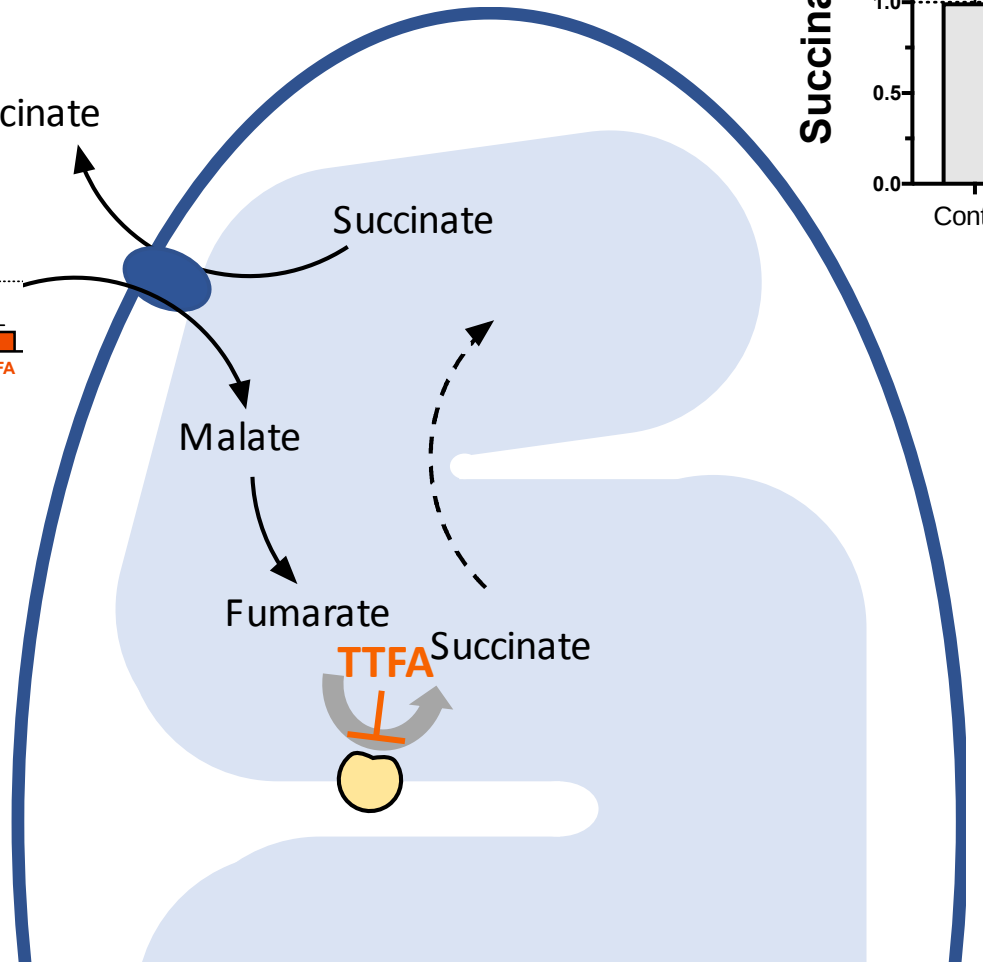
Oxaloacetate

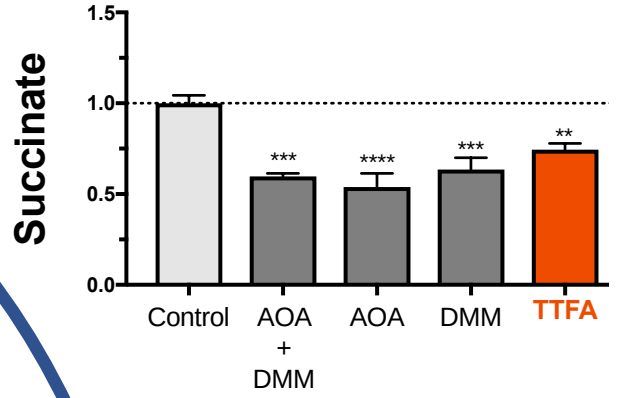


NADH

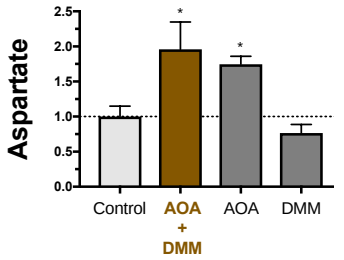
NAD+





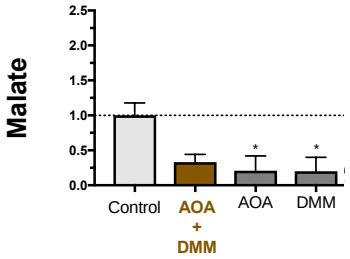


Combined Inhibition of the MAS and SDH



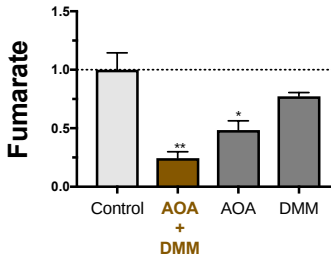
AOA

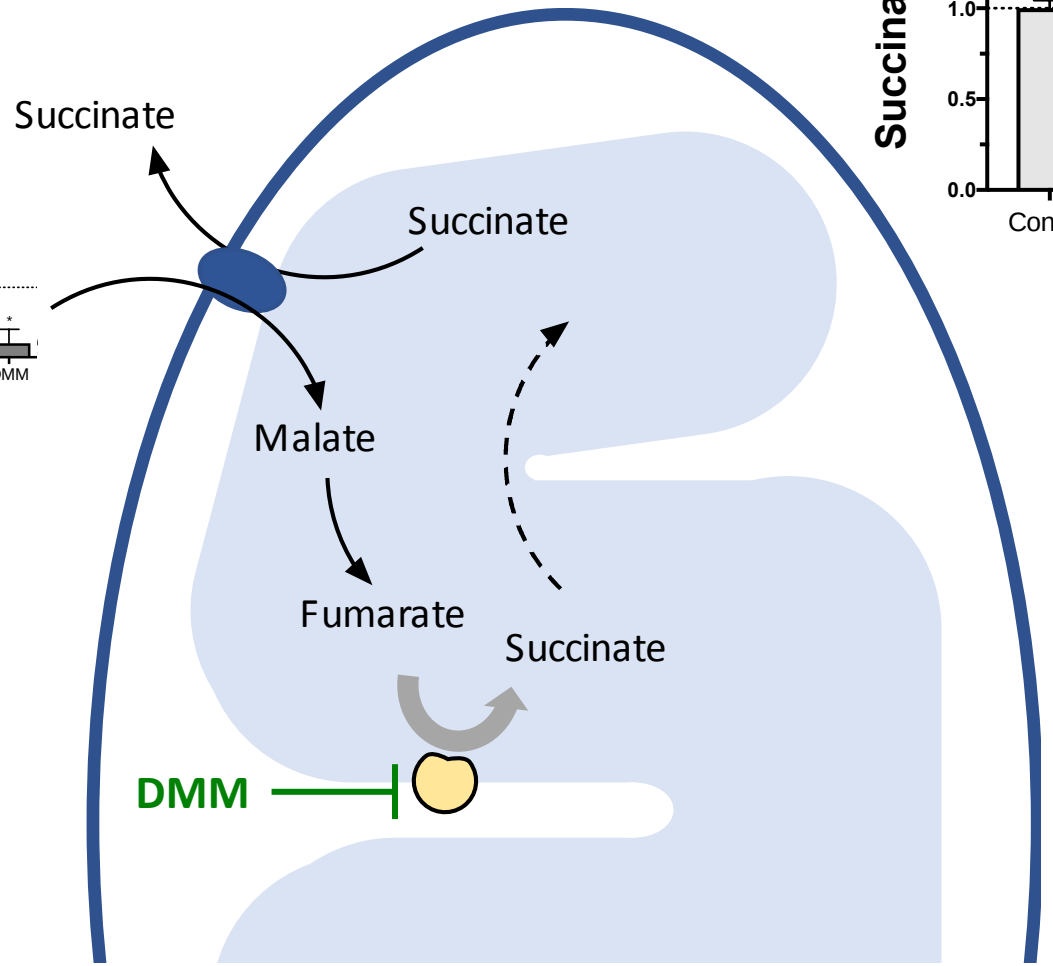
Oxaloacetate

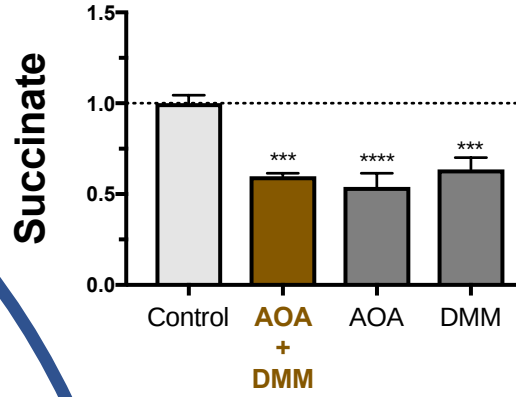


NADH

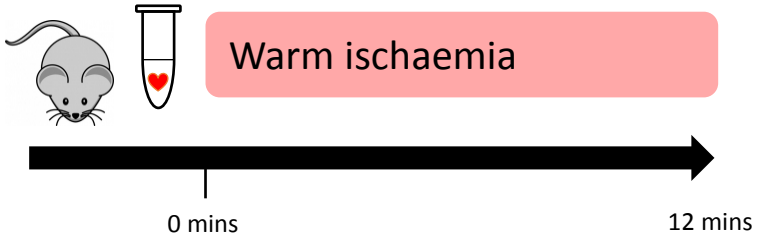
NAD+

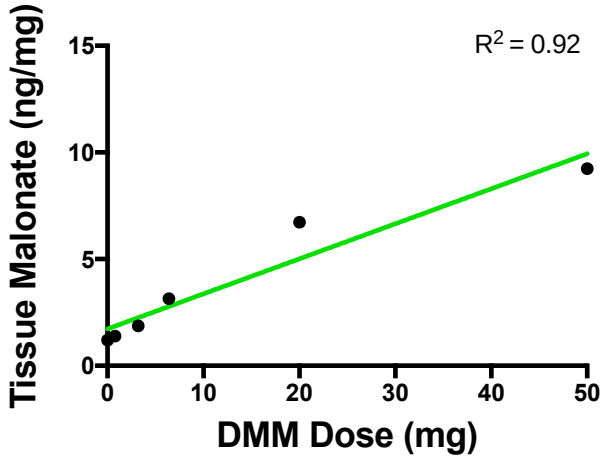


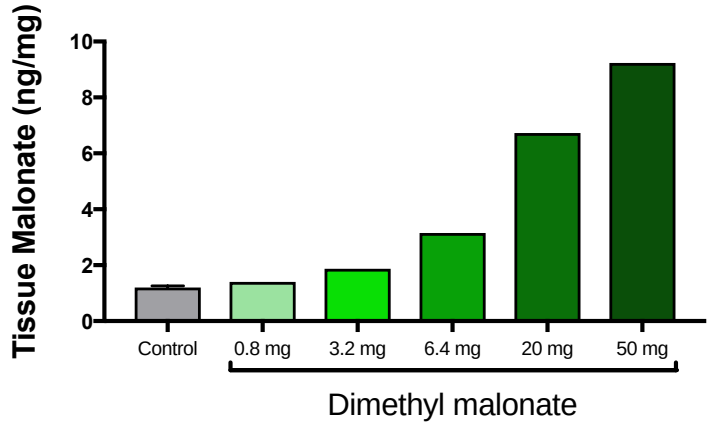


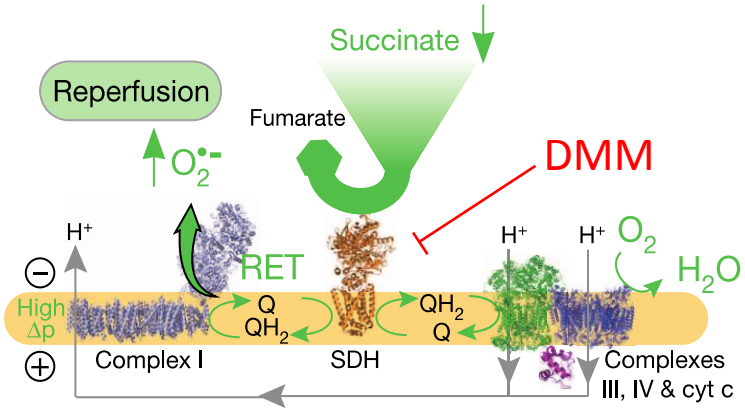


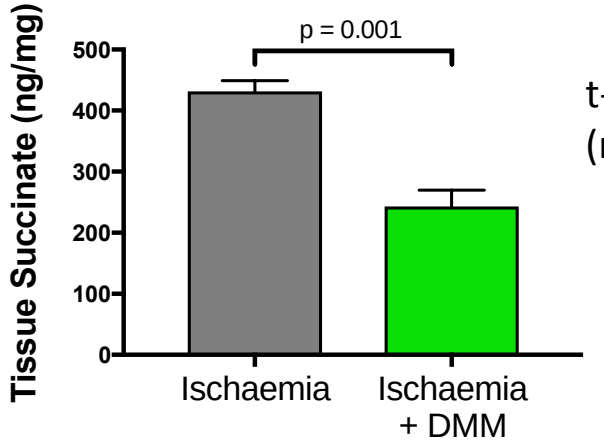
Pharmacodynamics of DMM Inhibition







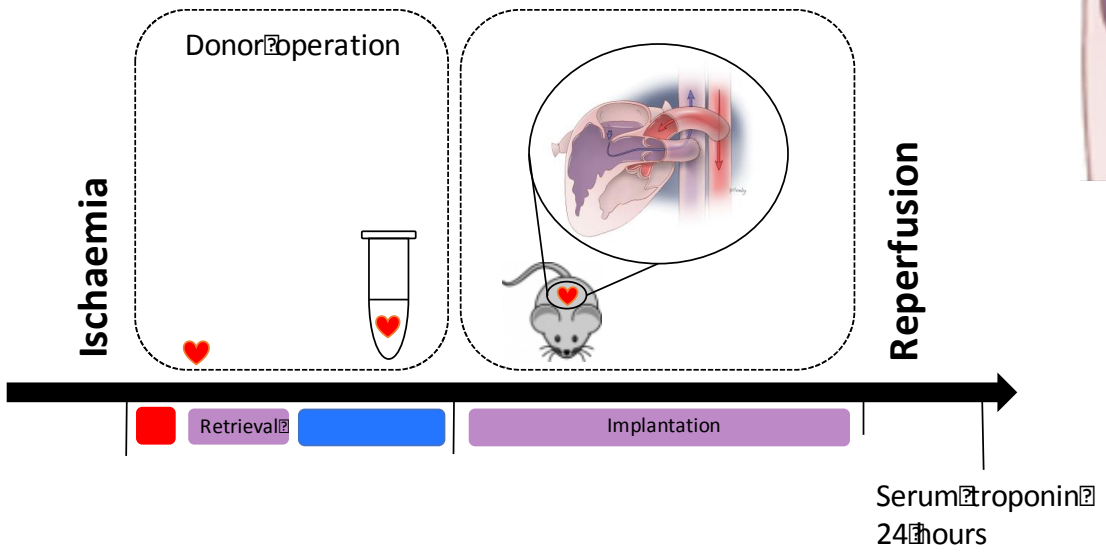


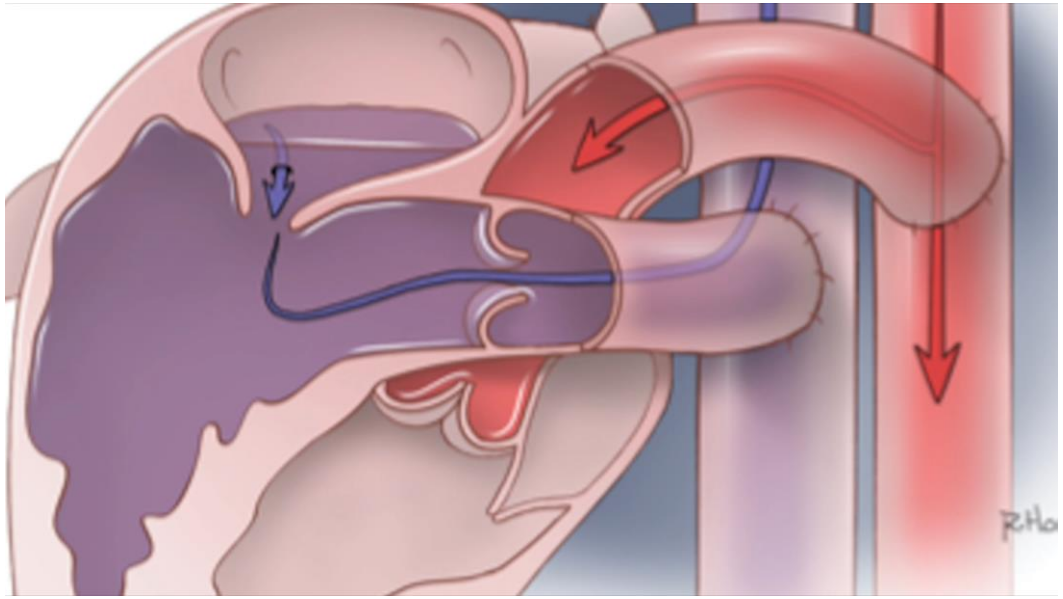


t-test
(n = 4)

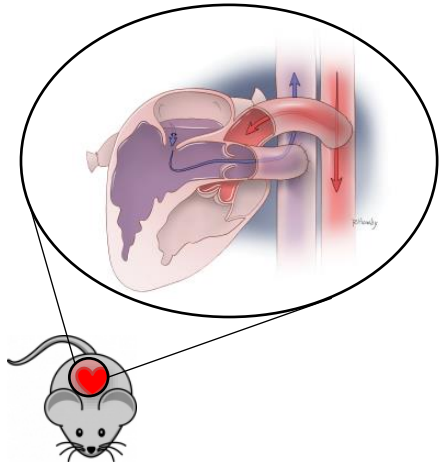
- DMM reduces succinate accumulation during warm ischaemia

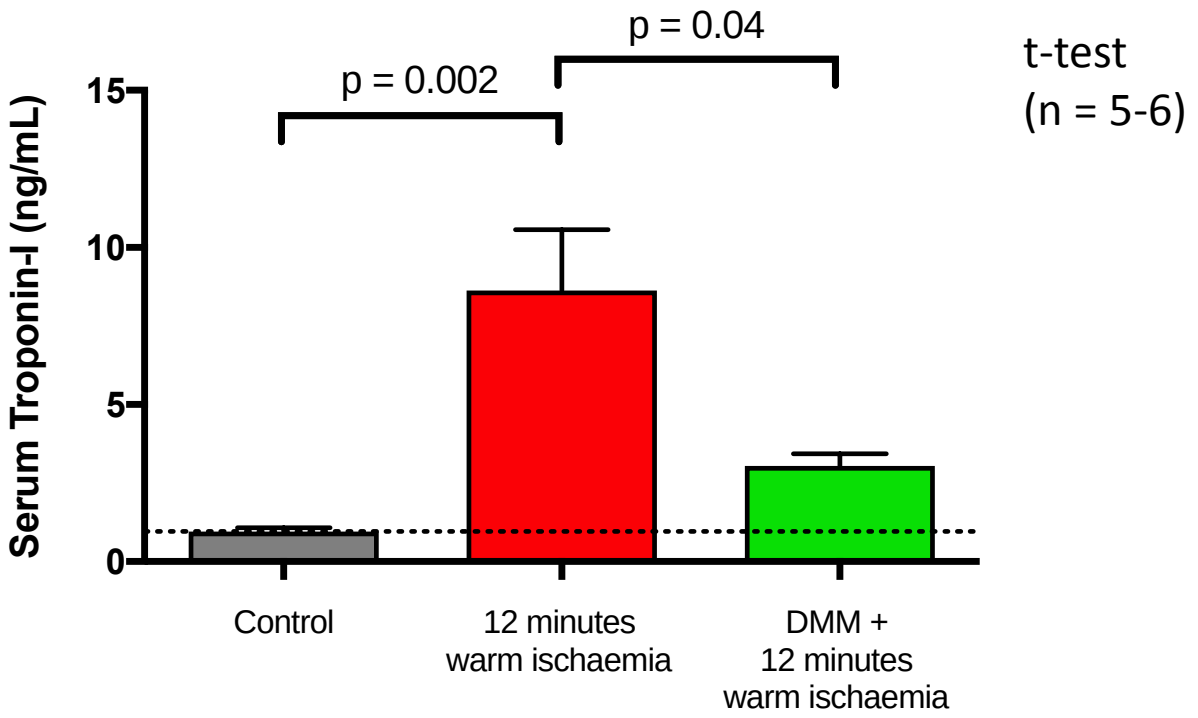
Methods: Heart Transplant Model





Efficacy of Inhibiting Succinate Accumulation





t-test
(n = 5-6)

- DMM ameliorates IR injury in transplant reperfusion model

Conclusions

- Succinate is a key metabolite in IR injury in animals and man
- Pathway is amenable to pharmacological intervention
- Ameliorating succinate accumulation is a promising therapeutic approach in both DBD and DCD transplantation
- Potential applications to other IR-related diseases including:
  - Myocardial infarction
  - Cerebrovascular accident
  - Limb ischaemia