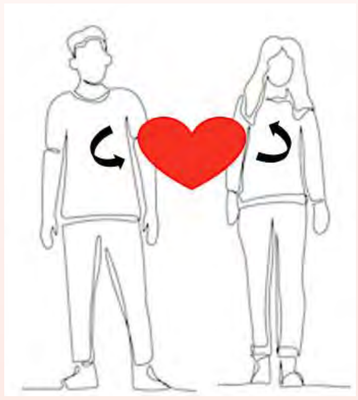

Sex differences in a rat model of heart transplantation with donation after circulatory death

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1. Abstract

Currently, there are still too few heart donors available. Donation after circulatory death (DCD) could be a good complement to conventional donation after brain death. However, warm ischemia is a problem in DCD donation, but the ischemic time after procurement can be circumvented with the Organ Care System. Likewise, sex differences in the DCD model have not been studied previously. The aim of this work was to characterize and investigate sex differences in rats in the DCD model. A model similar to the clinical DCD model was established. No significant differences were found between the sexes, but free fatty acid and norepinephrine levels tended to be higher in male rats than in female and ovariectomized rats. Based on these results, both male and female rats can donate heart grafts with the same clinical protocol. A model to study sex differences in DCD heart transplantation has been successfully established. Further experiments need to be performed to confirm these results.

2. Introduction

Heart transplantation remains the treatment of choice for people with heart failure, but the supply of donor organs is insufficient. Donation after circulatory death (DCD) could increase the number of available hearts [1]. Graft damage from warm ischemia is a concern, and further damage is caused by reperfusion, called ischemia-reperfusion injury (IRI)[2]. To reduce this ischemic damage, donor hearts are now transported using the Organ Care System (OCS). This is a device that perfuses the heart during transport and maintains it in a beating state.

Although beneficial effects have been attributed to the sex hormones estradiol and progesterone, related to ischemic tolerance and metabolism, this has not been examined in clinical trials in DCD heart transplantation[3].

During DCD, a catecholamine surge in the donor can cause an increase in heart rate and systolic aortic pressure. Catecholamines include epinephrine, norepinephrine, and dopamine. Increased catecholamine levels leads to an increase in circulating free fatty acid (FFA) [4]. Elevated concentrations of FFA exacerbate IRI during reperfusion [5]. Sex differences in myocardial metabolism of FFA and in expression of genes involved in lipid metabolism suggest that FFA metabolism may be handled differently in the context of DCD between males and females and therefore influence graft quality [6].

3. Aim

The aim of this work was to characterize and investigate a rat model for sex differences in DCD heart transplantation.

4. Methods and material

A DCD model was established to compare female, male, and ovarectomized (OVX) rats. Two randomized groups were made:
1. 0min ischemia: female (n=5), male (n=6), and OVX rats (n=4);
2. 22min ischemia: female (n=7), male (n=5), and OVX rats (n=6).
Following simulation of DCD in the rats and 22min ischemia, explanted hearts were connected to the perfusion system and perfused for one hour via the aorta with a Krebs-Henseleit buffer. In the 0min ischemia group, heart were explanted and directly perfused. Blood samples were collected during the ischemia period in the 22min group and buffer samples were collected during reperfusion in both groups. The figure below shows the flow of the protocol.

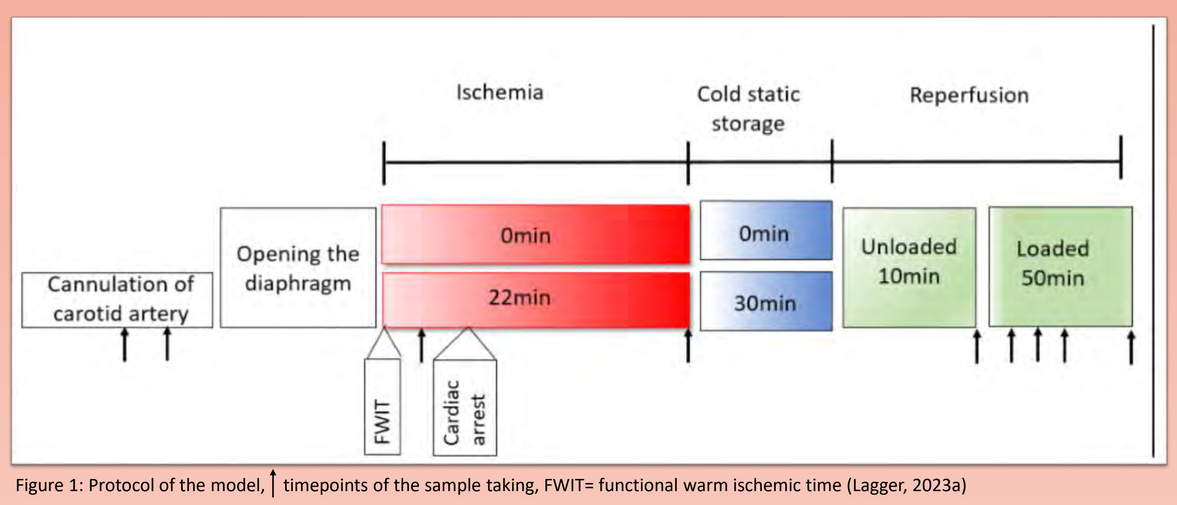

Figure 1: Protocol of the model, ↑ timepoints of the sample taking, FWIT= functional warm ischemic time (Lagger, 2023a)

5. Results

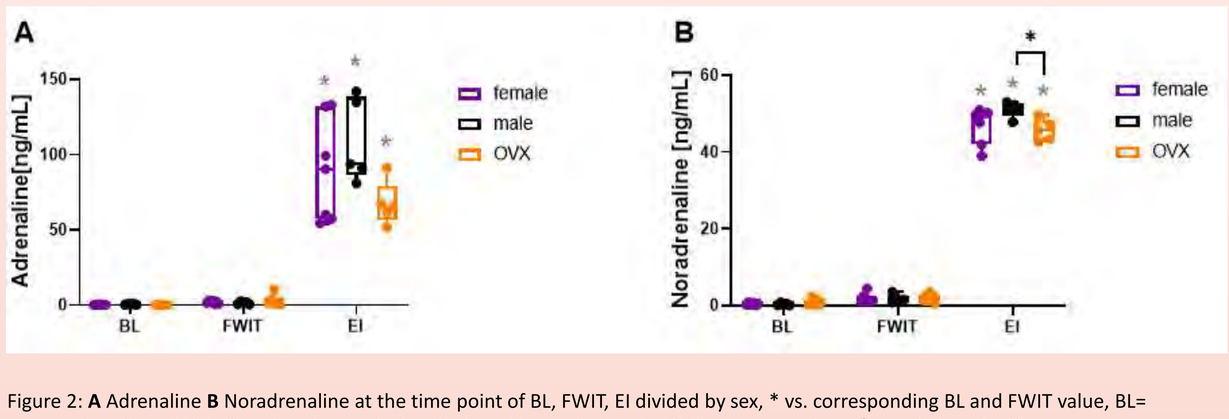

Figure 2: **A** Adrenaline **B** Noradrenaline at the time point of BL, FWIT, EI divided by sex, * vs. corresponding BL and FWIT value, BL= baseline, FWIT= functional warm ischemic time, EI= end-ischemic, OVX= ovarectomized, *= statistical significance, Adrenaline: BL and EI (female p=0.0036, male p=0.0237, OVX p=0.0129), FWIT and EI (female p=0.0018, male p=0.0237, OVX p= 0.0129), Noradrenaline: BL and EI (female p=0.0036, male p=0.0237, OVX p=0.0129), FWIT and EI (female p=0.0018, male p=0.0237, OVX p= 0.0129) (Lagger, 2023b)

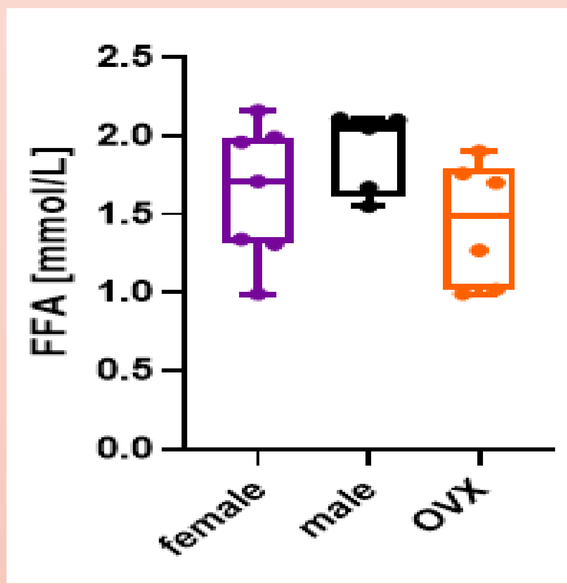

Figure 3: Free Fatty acid results divided by sex, OVX= ovarectomized (Lagger, 2023c)

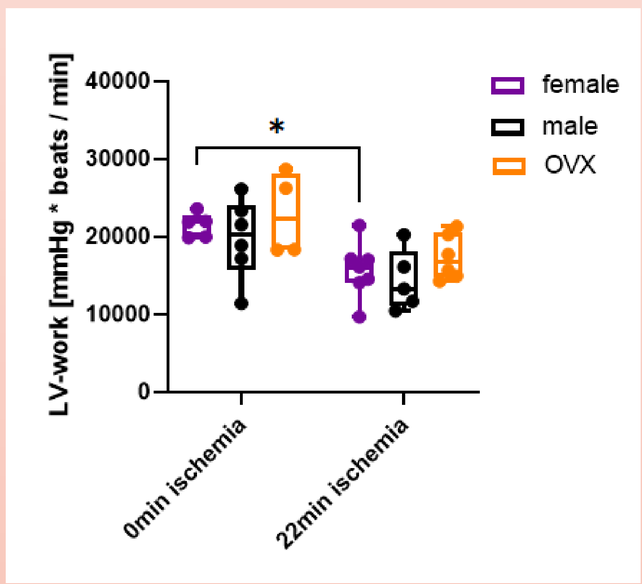

Figure 4: Lv-work at 60min of reperfusion divided by 0min ischemia and 22min ischemia and sex, OVX= ovarectomized, *= statistical significance, p=0.0303 (Lagger, 2023d)

Adrenaline and noradrenaline in figure 2 were significantly elevated at the end- ischemia vs. FWIT and BL. At end-ischemia, a tendency for higher levels in males compared with OVX was observed, but this reached statistical significance only for noradrenaline. Figure 3 shows the FFA levels of the three sex groups and the differences are not statistically significant. However, a tendency for increased FFA levels in males vs. females and ovariectomized females was observed. For LV-work in figure 4, although there was a tendency for decreases in all groups with 22min ischemia vs. no ischemia, this reached statistical significance only in female rats.

6. Discussion

It could be seen that for adrenaline and noradrenaline, there was a tendency for elevated levels in end-ischemia in the male rats. Interestingly, a similar, but non-significant pattern was observed for FFA. Increased catecholamines stimulate elevations in circulating FFA. This suggests that withdrawal of life supporting therapy induced increases in catecholamines may contribute to increased FFA observed at EI, with tendency for higher levels in males compared to both females and OVX rats. This is of particular importance, as it has been shown that pre-ischemic exposure to high levels of FFA decrease post-ischemic heart recovery [7]. Although ischemia tended to decrease leftvetricular-work, statistical significance was achieved only for female hearts, suggesting that differences in catecholamines and/or FFA were insufficient to alter functional recovery.

A rat model to investigate sex differences in DCD heart transplantation was successfully established. Using this model, cardiac functional and metabolic recovery was similar among male, female and OVX rats. Thus, cardiac grafts from male and female rats can be procured and treated with the same clinical protocol. Additional experiments are required to confirm these findings.

References

[1] Niederberger et al., 2017, p.9
[2] Yellon & Hausenloy, 2007, p.93
[3] Lagranha et al., 2010, p.2
[4] Ali et al., 2011, p.8
[5] Lopaschuk et al., 2010, p.2
[6] Ockner et al.,1979, p.1
[7] Niederberger et al., 2017, p.2

Figures

Figure 1 Lagger. (2023a). *Protocol of the model.* medi
Figure 2 Lagger. (2023b). *Catecholamine.* medi
Figure 3 Lagger. (2023c). *Free Fatty acid.* medi
Figure 4 Lagger. (2023d). *LV-work.* medi