Online Supplementary material to

**Left ventricular diastolic dysfunction during acute myocardial infarction: effect of mild hypothermia.**

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**Methods**
The experimental protocol was approved by the local bioethics committee of Vienna, Austria (BMWF-66010/0103-II/10b/2009), and conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

**Experimental model**
19 overnight-fasted landrace pigs (70±2 kg) were sedated with 0.25 mg/kg midazolam and 10 mg/kg ketamine. Anaesthesia was introduced with 7 mg/kg ketamine and 7 µg/kg fentanyl. After tracheotomy, sheaths were introduced into both common carotid arteries (9F and 10F), both internal jugular veins (6F and 8F) and the left-sided femoral vein (14F). Anaesthesia was maintained with 1 % sevoflurane, 30 µg/kg/h fentanyl, 1 mg/kg/h midazolam, and 1.7 mg/kg/h rocuronium. The respirator was set to 50% oxygen, 5 mmHg positive end-expiratory pressure, and 10 ml/kg tidal volume. The respiration rate was adjusted to keep end-expiratory carbon dioxide partial pressure between 35 and 40 mmHg. Under fluoroscopic guidance, a Swan-Ganz catheter was positioned in the pulmonary artery (Edwards Lifesciences CCO and Vigilance I, Edwards Lifesciences, Irvine, CA,
USA), and a pacing probe was placed in the right atrium. A pressure-conductance catheter (5F, 12 electrodes, 7 mm spacing, Millar Instruments, Houston, Texas, USA) connected to a signal processing unit (MPVS Ultra, Millar Instruments, Houston, Texas, USA) was placed in the left ventricle. The conductance catheter position was optimized to obtain stable pressure-volume loops. A valvuloplasty catheter (20 ml, Osypka, Rheinfelden, Germany) was placed in the descending aorta, and an intravascular cooling catheter (Accutrol™ Catheter 14F, Philips Healthcare, Vienna, Austria) in the inferior vena cava. Aortic pressure was derived from a carotid artery sheath sidearm.

To prevent venous thrombosis, a bolus of 150 IU/kg heparin was given, followed by a continuous infusion of 75 IU/kg/h. A balanced crystalloid infusion (Elo-Mel Isoton, Fresenius) at room temperature was administered at a fixed rate of 10 ml/kg/h throughout the protocol. Body temperature was maintained at 38 °C by the intravascular device (InnerCool RTx Endovascular System, Philips Healthcare, Vienna, Austria).

**Experimental protocol**

After instrumentation, all animals were allowed to stabilize for 30 min. At baseline conditions, blood samples were drawn from the aorta and pulmonary artery, and steady-state haemodynamics were acquired over three respiratory cycles at spontaneous heart rate and during right atrial pacing at 100, 120, 140, 160, and if possible, at 180 bpm. To vary loading conditions, the aortic balloon catheter was then inflated briefly three times at spontaneous heart rate. After baseline measurements, the aortic balloon catheter was removed, and a custom bent 4F diagnostic coronary catheter (Cordis, Bridgewater, NJ, USA) was positioned in the proximal left circumflex coronary artery (LCX). Myocardial infarction with subsequent LV dysfunction was then
induced by coronary microembolisation (CME): repetitive slow (1 min) injections of 500,000 polystyrene microspheres (Polybead Microspheres 45µm, Polysciences Inc., Eppelheim, Germany) into the LCX were administered, until cardiac power output (W, mean aortic pressure × cardiac output / 451) was reduced by more than 40 %. The LCX-position was repeatedly verified via coronary angiography. The coronary catheter was then removed and the aortic balloon catheter was repositioned. Three animals developed ventricular fibrillation during microsphere injections that could not be treated successfully, and were excluded from subsequent analysis. The remaining pigs were assigned sequentially 1:1 to either MH (n=8, 33 °C) or normothermia (NT, n=8, 38 °C).

Measurements outlined above were repeated immediately (CME 0h), and at 2h, 4h and 6h after CME (CME 2h, CME 4h and CME 6h, respectively). In MH, cooling was started after the CME 0h measurement. Finally, a thoracotomy was performed, the angiography of the LCX artery was repeated, and the heart was inspected to exclude any coronary damage after selective catheterization of the LCX artery. Animals were sacrificed by an 80 mmol potassium chloride bolus.

At each time point reported, volumetric conductance data were calibrated by assessing cardiac output (CO, slope factor alpha) and hypertonic (10 %) saline infusion (parallel conductance) as described before.

Before measurements at CME 0h, 2h, 4h and 6h, a bolus of 4.8 mmol magnesium (Magnesium Gluconicum, Lannacher GmbH, Austria) was administered to prevent arrhythmia. Arising episodes of atrial or ventricular tachycardia were terminated by short bursts of overdrive pacing. Ventricular fibrillation occurred in one normothermic animal at 4:30h after CME and in one hypothermic animal at 0:30h after CME and was successfully treated by single defibrillation (200 J biphasic, Responder 2000, General Electric, Fairfield, CT, USA).
**Blood samples**

Blood samples were processed immediately after withdrawal using a blood gas analyser (ABL 600, Radiometer Copenhagen, Denmark) equipped for the measurement of oxygen, carbon dioxide, pH, haemoglobin, sodium, potassium, glucose and lactate. Troponin T levels were assessed in serum obtained at CME 6h (Roche Diagnostics GmbH, Vienna, Austria).

**Data analysis**

Data analysis has been described before.\(^8\)\(^{-12}\) Haemodynamic and conductance data were analysed off-line by CircLab software (custom made by P. Steendijk). End-diastole was defined as the time point of zero crossing of dP/dt before its rapid upstroke. End-systole was defined as the time point of maximum pressure/volume ratio. Systemic vascular resistance (SVR) was derived from the difference of mean aortic and central venous pressure divided by CO. Whole body oxygen consumption was calculated as the difference of aortic and pulmonary artery oxygen content multiplied by CO.

The LV isovolumic relaxation constant tau (\(\tau\)) was calculated based on the method of Glantz (for detailed explanation of calculations please see \(^8\)). As LV relaxation depends on loading conditions and LV end-systolic pressure we used data acquired during aortic balloon catheter inflation to calculate values for \(\tau\) at an end-systolic pressure of 100 mmHg (linear regression). Similarly, dP/dtmin at an end-systolic pressure of 100 mmHg was calculated.

Pressure-volume relations were generated from data acquired during aortic balloon catheter inflation. Linear regressions were performed between end-systolic pressure and volume (end-systolic pressure-volume relationship, ESPVR). To provide a single measure of systolic function derived from the ESPVR, the end-systolic volume
corresponding to an end-systolic pressure of 100 mmHg was determined. End-diastolic pressure and volume data were fit by an exponential regression to obtain the EDPVR: \( LVP_{ed} = C + A \cdot e^{B \cdot LV_{ved}} \). The curve fit parameters \( A \) (mmHg), \( B \) (ml\(^{-1}\)) and \( C \) (mmHg) were calculated for each time point. The resulting equations were used to calculate end-diastolic volumes for given end-diastolic pressures at each time point during the protocol.

Statistics

All data are presented as mean±SEM. To compare data within one group over time, a 1-way ANOVA for repeated measurements was used. Data between groups at CME 6h were analysed by Student’s unpaired t-test. Data between groups over the time were analysed by 2-way ANOVA for repeated measurements. Pressure-volume relationships were compared by analysis of covariance (ANCOVA). Post-hoc testing was performed by Tukey’s test. A p-value < 0.05 indicated significant differences.
Diastolic time intervals

Figure 1:

The duration of diastole was shortened by right atrial pacing (A and B). At a given heart rate, diastole was shorter during MH at CME 6h (B). The ratio of the diastolic time interval and tau fell with increasing heart rates (C and D). At CME 6h in NT, this fall was slightly more pronounced, indicating slowed relaxation after CME (C). With additive MH, the ratio became substantially shorter (D), reflecting the prolongation of contraction and more slowed active relaxation at 33°C. However, at the spontaneous heart rate of less than 70 bpm at CME 6h in MH, the ratio of diastolic duration and tau was still >4. Given the monoexponential fit of tau, this allowed for a >98% decay of LV pressure during diastole. Spontaneous bradycardia thus compensated for delayed active relaxation during MH.
Response of cardiac output to right atrial pacing

**Figure 2:**

Right atrial pacing increased cardiac output at baseline by a maximum of $2.5\pm0.3$ l/min in NT. This increase was smaller at CME 6h ($0.8\pm0.3$ l/min, $p<0.05$ vs baseline). A similar change was seen in MH (baseline $2.4\pm0.6$, CME 6h: $1.3\pm0.4$ l/min, $p=0.10$ vs baseline, $p=0.41$ vs NT at CME 6h).