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**Influence of Systemic Hypothermia on the Myocardial Oxygen Tension during
Extracorporeal Circulation: comparative study in german landrace pigs**

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Abstract

During extracorporeal circulation (ECC) controlled hypothermia is a common method of myocardial protection due to a reduction of the myocardial oxygen consumption. Although the beneficial aspects of hypothermia on the myocardial metabolism have been widely demonstrated the effect of hypothermia on the myocardial oxygen tension (P_{myO_2}) is unclear. For this reason the P_{myO_2} of German Landrace pigs (male, three months of age) during ECC was analysed under mild hypothermia (32 °C, n=6 pigs) and under normothermia (n=10 pigs, control group) within a time period of 23 min (1400 sec). Flexible invasive Clark type microcatheters were used to measure the P_{myO_2} in the beating heart. During normothermal ECC a continuous P_{myO_2} increase from 36.5 ± 15.8 mmHg to 52.6 ± 27.2 mmHg (+44.1%) after 1400 sec was measured (p=0.02). In contrast, mild hypothermia caused a continuous P_{myO_2} decrease from initially 46.9 ± 17.5 mmHg to 36.7 ± 20.8 mmHg (-21.8%, p<0.013) in the test period. Electrocardiography revealed no signs of ischemia or arrhythmia during normo- and hypothermic ECC. It seems obvious that mild hypothermia results in a reduction of the oxygen transfer to the myocardial cells and that this effect outweighs the beneficial effects of hypothermia in the myocardium which are related to reduced oxygen consumption. However, in mild hypothermia oxygen supply to the myocardium remained sufficient for normal myocardial function.

Keywords: hypothermia, pig, microcirculation, myocardial oxygen tension

Introduction

Induced hypothermia is well established for myocardial and cerebral protection during open heart surgery or cardioplegic arrest [1-3]. Recently hypothermia was also utilized in patients with acute myocardial infarction, during percutaneous coronary interventions and in the treatment of stroke [4-8].

The beneficial effect of hypothermia is due to a significant reduction of the cellular metabolism and of the cellular oxygen consumption respectively with decreasing body temperature [9]. But hypothermia is also known to cause effects which can higher the risk of ischemia. With decreasing body temperature the blood flow is reduced [10], the blood fluidity decreased [11-14] and a dysfunction of the microcirculation can be observed [15-17]. Additionally hypothermia results a left shift of the oxygen dissociation curve thus reducing the oxygen transfer to the tissues [18].

As left ventricular myocardial necrosis caused by ischemia is the most common reason for postoperative death or depressed cardiac performance [19,20], it is important to know how mild hypothermia influences the P_{myO_2} . However, up to now the correlation between mild hypothermia and the P_{myO_2} of a beating heart is unclear. Therefore, the study was aimed to measure the P_{myO_2} in the unloaded beating heart of pigs during extracorporeal circulation and mild hypothermia, whereby the target temperature was set at 32 °C.

Materials and Methods

Animal housing and care, study groups

The experiments were approved by the Animal Care and Use Committee of Saxony and performed at the animal facility of the University of Technology, Dresden (Germany) with juvenile male pigs (n = 16, age: 3 months) from the breed German Landrace (body weight 32.7±4.8 kg). Pigs were used because of their numerous similarities to humans, including minimal pre-existing coronary collaterals as well as similar coronary anatomy and physiology [21].

The animals were randomly divided into two study groups with 6 pigs representing group H for the tests under hypothermia and 10 pigs representing group N for the tests under normothermia. Until tests started the animals were housed in groups of at least five in an environmentally controlled room (12/12 light/dark-rhythm, 15–24°C, 55±10% relative

humidity) and cared according to the guidelines of the European Societies of Laboratory Animal Sciences.

The manuscript was written in accordance with the ethical guidelines of *Clinical Hemorheology and Micocirculation* [22].

Anaesthesia

Anaesthesia was started by intravenous (i.v.) administration of azaperone (6 mg/kg body weight, Stresnil™, Janssen-Cilag), ketamine (0.1 ml/kg body weight, Ketamin™ 10%, Sanofi-Ceva), xylazine (0.3 mg/kg body weight, Rompun™ TS, Bayer Vital), diazepam (0.35 mg/kg body weight, Faustan™, Arzneimittelwerk Dresden) and atropine (0.01 mg/kg body weight, Atropinum Sulfuricum™, Eifelfango). For the maintenance of anaesthesia, fentanyl (8 µg/kg body weight and hour i.v., Fentanyl, Curamed) and thiopental (2-3 mg/kg body weight i.v., Trapanal™, Byk Gulden) were applied. Additionally, lactated Ringer solution (B. Braun, Melsungen, Germany) was continuously infused (5 ml/min, i.v.). After confirmation of surgical tolerance, relaxation was induced by pancuroniumbromide (4 mg/kg body weight i.v., Pancuronium™, Curamed) and subsequently, an endotracheal tubus was placed for artificial respiration (50% O₂, 4.0–4.5 l/min, 16–19 breaths/min). Until tests started, the arterial oxygen tension (PaO₂) was kept stable within a range of 130–150 mmHg, the arterial carbon dioxide tension (PaCO₂) within 40–45 mmHg and the pH at 7.4. These parameters and the systolic and diastolic blood pressures (SBP, DBP) were measured via a catheter in the A. carotis communis dextra. In addition, a Swan-Ganz catheter was placed via the V. jugularis interna dextra to continuously measure the cardiac output (CO), the venous oxygen tension (PvO₂), the mixed venous oxygen saturation (SvO₂) and the central venous pressure (CVP) within the pulmonary artery. Additionally the hematocrit (Hct), the hemoglobin concentration (Hb) and the arterial oxygen saturation (SaO₂) were measured after blood sampling (1 ml blood) in intervals of ten minutes. To control heart activity an ECG was used.

Measurement of pO₂

For PmyO₂ measurement flexible microprobe catheters of the Clark type with an outer diameter of 470 µm and a sensitive area near the catheter tip of 7.38 mm² were used (Integra NeuroSciences, Andover, England). The pigs were placed with their back on an operating table covered with a heater mat (37 °C) to prevent a decrease of body temperature. The thorax was opened and disposable, flexible microprobe catheters (Licox, C1.2, Integra Neurosciences, Andover, UK) for PmyO₂ measurement were inserted 4.8±1.1 mm in the myocardium using an introducing cannula (one microprobe catheter in the midmyocardium of the left and right ventricle each; see Fig. 1). A flexible temperature microprobe in the left ventricle myocardium permitted the correction of PmyO₂ for local tissue temperature. Insertion depth was validated by necropsy after the death of the animal. To avoid any influence of organ/tissue movement on PmyO₂ and temperature measurements, the probes were fixed by sutures before connecting them to a digital monitor (Licox CMP, Integra Neurosciences) displaying PmyO₂ values in mmHg together with a graphical trend. The surface of the heart was kept wet by a first gauze layer saturated with isotonic NaCl-solution and secondly by a sterile blanket put on top of the gauze and covering the opened thorax to prevent cooling and a loss of humidity. The skin wound at the left leg was closed by sutures. PmyO₂ measurements were performed for 1400 sec. They were started only 30 min after microprobe insertion to allow PmyO₂ stabilization after microprobe insertion which is known to cause an initial PmyO₂ decrease [23]. Details of the measurement method have been published elsewhere [23-26].

Extracorporeal circulation and hypothermia induction

After cannulation and administration of heparin (Heparin-Natrium 25000 ratiopharm, Ratiopharm, Ulm, Germany) extracorporeal circulation (ECC) was established in all pigs via the aorta ascendens and the right atrial appendage using a heart-lung machine (Stöckert SIII,

Stöckert, Munich, Germany). ECC rate was adjusted in dependence of the CO between 2.5 and 4.3 l/min for each individual pig. The heart was completely unloaded.

For hypothermia induction the oxygenator temperature and the blood temperature respectively were initially lowered to 26°C until after about 500 sec rectal temperature reached 34°C. Thereafter the oxygenator temperature was set at 32°C in order to keep a stable rectal temperature of 32°C.

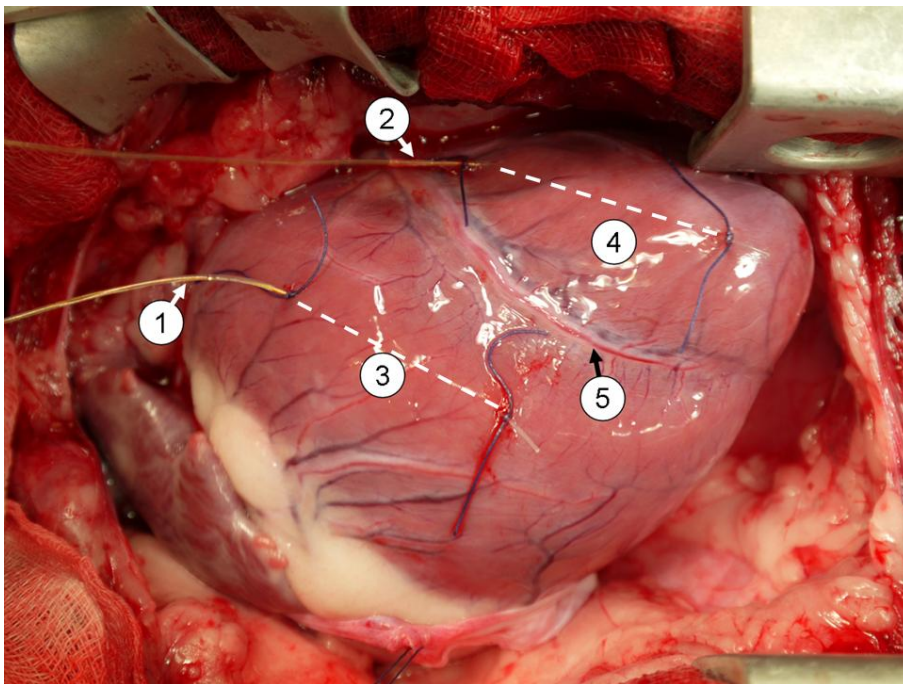


Fig. 1: Sternal view on the heart of a pig with implanted flexible microcatheter probes (1, 2) in the right (3) and left (4) ventricular myocardium, (5) Ramus interventricularis paraconalis, dashed lines mark the position of the sensors in the myocardium.

Statistical analysis

Results are shown as mean value \pm standard deviation. The statistical method used was paired student t-test for two tailed problems. A probability of less than $p \leq 0.05$ was considered significant.

Results

There were no signs of ischemia or arrhythmia in the ECG during normo- and hypothermal ECC. During the whole test period heart rate, haemoglobin concentration, haematocrit, SBP, DBP, CVP, CO, PvO₂, PaO₂, PaCO₂, SvO₂, SaO₂, pH, of the animals from both experimental groups showed no significant differences.

Normothermic ECC

In group N rectal temperature ($37.1\pm 0.8^{\circ}\text{C}$), blood temperature and the temperature of the myocardium ($36.9\pm 0.6^{\circ}\text{C}$) were stable and unchanged during the test period of 1400 sec. However, PmyO₂ significantly increased of from $36.5\pm 15.8\text{mmHg}$ to $52.6\pm 27.2\text{mmHg}$ (+44.1%, $p<0.05$) within this time (Fig. 2).

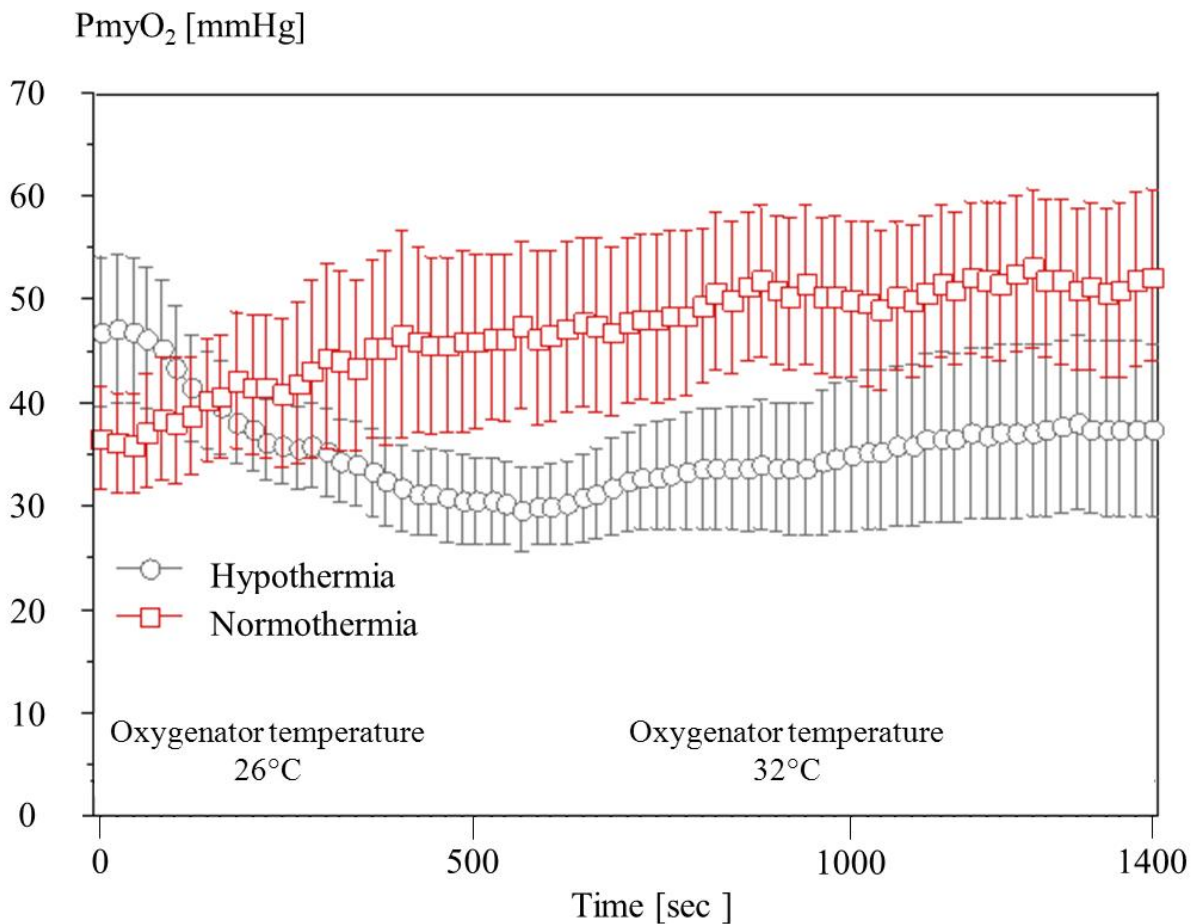


Fig 2: PmyoO₂ in pigs with extracorporeal circulation under hypothermic (32°C, n=6) and normothermic (37°C, n=10) conditions; each dot represents the average PmyoO₂ over 10 seconds. mean values ± standard deviation

Hypothermic ECC

Hypothermia caused a significant decrease ($p < 0.013$) of the PmyoO₂ from 46.9 ± 17.5 mmHg to 36.7 ± 20.7 mmHg (Fig. 2). Thus a temperature drop of 4°C resulted in a mean PmyoO₂ decrease of 10.2 mmHg or 21.8% respectively. However, temperature values developed differently in the animals of group H (Fig. 3). In three pigs within the first 500 sec after hypothermia induction myocardial temperature decreased to 32°C and stabilized at this level. In the other three animals hypothermia resulted in an initial reduction of the myocardial temperature much lower than 32°C before target temperature was reached. In these animals the maximal PmyoO₂ decrease was 27.4 ± 10.5 mmHg (see Fig. 4) and thus markedly greater than in the other animals of group H (8.5 ± 5.3 mmHg).

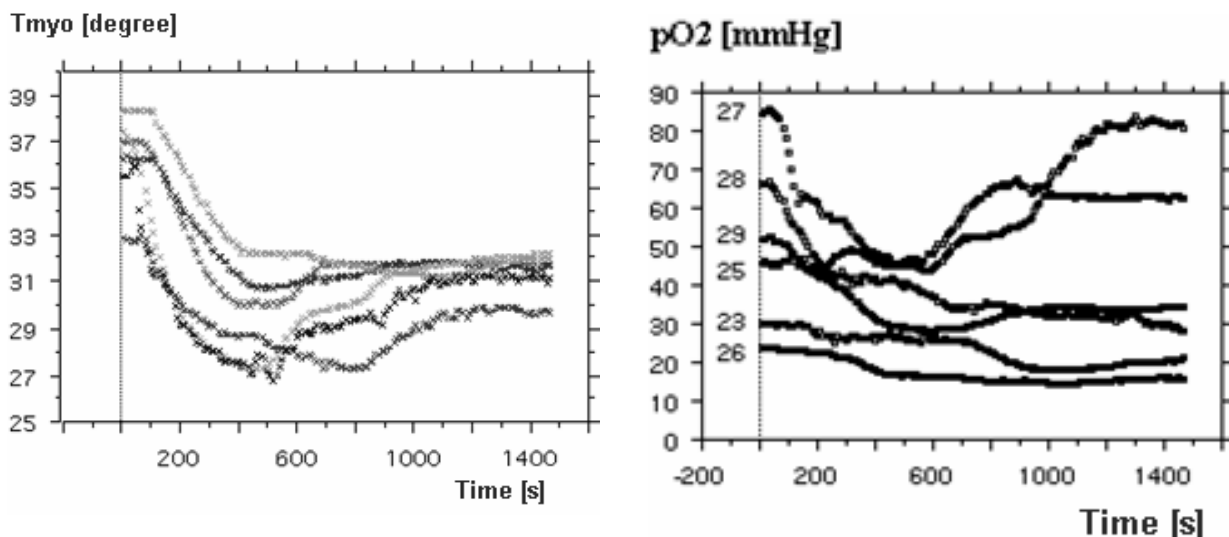


Fig. 3: Temperature of the myocardium (Tmyo) and myocardial oxygen tension (PmyoO₂) in pigs with extracorporeal circulation under hypothermic (32°C, n=6) conditions

The course of the temperature decrease in the rectum, the blood and the myocardium also initially differed in each animal within the test period (Fig. 4) and reached comparable levels only after about 720 sec.

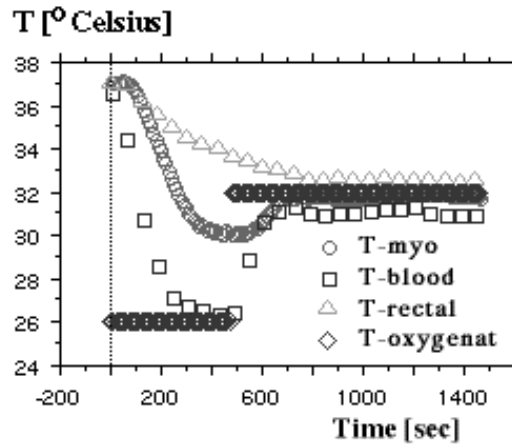


Fig. 3: Temperature of the myocardium (T-myo), the blood (T-blood), the rectum (T-rectal), and the oxygenator in a pig with extracorporeal circulation under hypothermic (32°C, n=6) conditions.

Discussion

Hypothermia with a target rectal temperature of 32°C was accompanied with a significant PmyO₂ decrease of 10.2 mmHg or 21.8% respectively. This effect must have been due to hypothermia since identical experimental conditions under normothermia caused a PmyO₂ increase. Later was most likely the result of the vasodilative activity of the narcotics [27, 28] and the muscle relaxant [29].

The above findings nicely correspond to measurements of cerebral oxygen tension (pO₂) in hypothermic rats [30]. Induced hypothermia of 32°C led to a significant drop of the cerebral pO₂ from 28±16 mmHg to 16±12 mmHg. In addition, Kamler and co-workers could show that during hypothermal ECC in Syrian gold hamsters the microvascular perfusion decreased significantly [15]. Knappe and co-workers found in mice that systemic hypothermia of 34°C

and 30°C aggravated the initial microcirculatory dysfunction in comparison to normothermal local soft tissue trauma [16].

Likewise to a clinical setting of hypothermal cardiac surgery cooling was controlled by measuring rectal temperature. A notable discrepancy in the hypothermic pigs between the rectal, blood and myocardial temperature was measured. Thereby the rectal temperature was at the same time always higher than the blood- and myocardial temperature. This led in some pigs to an initial decrease of the myocardial temperature much lower than 32°C resulting in a significant drop of the P_{myO_2} (-27.4 ± 10.5 mmHg) compared to those animals in which the myocardial temperature did not fall below 32°C (P_{myO_2} 8.5 ± 5.3 mmHg). This in mind it might be reasonable to measure myocardial temperature instead of rectal temperature to avoid myocardial ischemia during the initial cooling process.

By reducing the systemic temperature oxygen consumption decreased by 8 % per 1°C because of the decreasing speed of enzymatic reactions [31]. At a body temperature of 33°C oxygen consumption is 70% lower compared to baseline levels [31,32]. At the same time the number of oxygen carriers increases [33] most likely due to the effect of reduced plasmatic volume and simultaneous stimulation of erythrocytes released by the spleen [34] leading to an increase of the haematocrit. This should result in an increase of P_{myO_2} even if blood flow is unchanged. However,

- heart rate, cardiac output and coronary blood flow are diminished [10].
- a left shift of the oxygen dissociation curve [18] is apparent, which is despite a facilitated binding of oxygen to haemoglobin due to a reduced dissociation of oxygen at lower pO_2 levels.
- blood viscosity is notably elevated because of an increasing plasma viscosity [13] with decreasing temperature. In addition, higher shear forces are necessary to compensate for stronger erythrocyte aggregation thus lowering the ability of oxygen transfer to

tissue [11, 35, 36] and also a rigidification of erythrocytes is present, which may complicate the passage of erythrocytes through capillaries [12, 14].

These factors have a negative impact on oxygen transport to tissues [13,37]. Whether PmyO₂ in a beating heart during hypothermia is increasing due to lower oxygen consumption or decreases due to a diminished oxygen transport to the tissue was unclear up to now. In this study with healthy pigs in a clinical setting similar to open heart surgery hypothermia of 32°C led to a PmyO₂ decrease of 10.2 mmHg or 21.8% respectively compared to baseline values. The parameters which were used to control the integrity of the circulatory system (haemoglobin concentration, haematocrit, SBP, DBP, CVP, CO, PvO₂, PaO₂, PaCO₂, SvO₂, SaO₂, pH) stayed within the reference range [24, 26, 38] and no signs of ischemia or arrhythmia in the ECG during normo- and hypothermal ECC occurred.

Conclusion

While in normothermal conditions a continuous increase of PmyO₂ occurred during ECC, under hypothermal conditions a significant PmyO₂ decrease was observed. Thus reduction of oxygen transport to myocardial tissue in combination with a right shift of the oxygen dissociation curve had a higher impact on the PmyO₂ than the reduced myocardial oxygen consumption due to the lowered tissue temperature.

ECG revealed no signs of ischemia under both experimental conditions. Thus a beneficial effect of hypothermia on the PmyO₂ of the left ventricle in an unloaded heart on ECC in healthy pigs could not be demonstrated.

Further studies are in preparation to analyse whether this observation holds true even for an unloaded fibrillating heart on ECC. This could have an impact for surgeons operating on a fibrillating heart in avoiding hypothermal conditions.

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