

Received: 2017.10.26
Accepted: 2018.01.08
Published: 2018.04.24

Oxygen Consumption of the Aerobically-Perfused Cardioplegic Donor Heart at Different Temperatures

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Data Interpretation D
Manuscript Preparation E
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Source of support: This work was supported by grants from the Hans-Gabriel and Alice Trolle-Wachtmeister's Foundation for Medical Research, Skåne University Hospital, and the Swedish Heart Lung Foundation

Background: The aim of this study was to investigate the oxygen consumption of explanted aerobically-perfused cardioplegic porcine hearts at different temperatures.


Material/Methods: Explanted hearts from 30 pigs weighing 50 kg were randomized into 5 groups. The hearts received continuous antegrade perfusion within a temperature-controlled sealed system. The perfusate consisted of an albumin-containing hyperoncotic cardioplegic nutrition solution with erythrocytes to a hematocrit of 10%. Five temperatures were studied: 37, 30, 22, 15, and 8°C. When the erythrocytes in the perfusate were fully saturated, the oxygenator was excluded from the circuit and blood gases were analyzed periodically until the erythrocytes had desaturated to less than 20%. Between 80% and 60% saturation the desaturation curves were linear in all groups and the oxygen consumption was calculated from this part of the curves.

Results: The weight of the hearts was 208 ± 4 g (mean \pm SEM, $n=30$). The oxygen consumption in mL/min/100 g heart tissue was (mean \pm SEM, $n=6$ in each group) 37°C: 1.10 ± 0.04 ; 30°C: 0.58 ± 0.02 ; 22°C: 0.33 ± 0.01 ; 15°C: 0.21 ± 0.01 ; and 8°C: 0.16 ± 0.02 .

Conclusions: The oxygen consumption of the cardioplegic perfused pig heart at normothermia was 1.1 mL/min/100 g and was reduced by 85% to 0.16 mL/min/100 g at 8°C.

MeSH Keywords: Heart • Metabolism • Organ Preservation • Oxygen Consumption • Perfusion

Full-text PDF: <https://www.annalsoftransplantation.com/abstract/index/idArt/907753>

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Background

The criterion standard for clinical preservation of donor hearts for transplantation consists of flushing the coronary vessels with a cold, crystalloid cardioplegic solution followed by static ischemic storage at 4°C [1]. Data from the International Society of Heart and Lung Transplantation (ISHLT) registry based on 19 948 heart transplantations between 2009 and June 2014 reveal an increase in hazard ratio for 1-year mortality from 1.0 (i.e., no increased risk) after 3 hours and 20 minutes of cold ischemia to 1.2, 1.4, and 1.8 after 4, 5, and 6 hours, respectively [2].

A logical way of extending the safe preservation time of donor hearts during cold cardioplegic storage is to omit hypoxia by aerobic perfusion. Recently a study was published [3] demonstrating safe orthotopic transplantation of porcine donor hearts harvested 24 hours after total brain and brain stem death (decapitation between cervical vertebra 2 and 3). The donor hearts were perfused at 8°C with a cardioplegic nutrition solution with oxygenated erythrocytes for 24 hours and then orthotopic heart transplantation was done in ten recipient pigs; all pigs had excellent heart function during the 24-hour follow-up period. In another study [4] intact endothelial and contractile function of coronary artery was shown after 8 hours of preservation-perfusion with the same method. A clinical study is now ongoing with this preservation method and the first patient was successfully transplanted on the 31st of October 2017.

During heart surgery using extracorporeal circulation, after aortic clamping, the heart is deprived of coronary blood flow except for non-coronary collateral blood flow from mediastinal arteries connecting with the coronary arteries [5]. This collateral blood flow cannot be measured, and therefore makes it impossible to know the exact oxygen consumption of the hearts *in situ*. However, during perfusion of the explanted whole heart there is no non-coronary collateral blood flow, and by using the method described in the present study an exact calculation of the oxygen consumption of the whole heart can be made. By knowing the oxygen consumption of the whole heart at different temperatures the amount of perfusion needed can be estimated and unnecessary perfusion can be avoided, minimizing hemolysis and myocardial edema that may occur during long-term perfusion of cardioplegic hearts.

The aim of the present study was to measure the oxygen consumption of perfusion-preserved cardioplegic porcine hearts at 37, 30, 22, 15, and 8°C.

Material and Methods

Thirty Swedish domestic pigs with a mean weight of 50 kg (range 48 to 55 kg) were used. All animals received care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Resources and published by the National Institute of Health (NIH, Publication No 86-23, revised 1996). The ethics committee of the University of Lund approved the study (No. M174-15).

Anesthesia and animal preparation

Anesthesia was induced by means of an intramuscular injection of ketamine 20 mg/kg body weight (Ketaminol Vet. Intervet, Boxmeer, Netherlands), xylazine 100 mg (Rompun Vet, Bayer, Solna, Sweden), and atropine 0.5 mg (Atropine, Mylan AB, Stockholm, Sweden). Fentanyl 4 µg/kg body weight (Fentanyl B. Braun, Melsungen, Germany) and midazolam 0.4 mg/kg body weight (Midazolam Panpharma, Panpharma S.A., Trittau, Germany) were given intravenously through an ear vein before tracheostomy. Anesthesia and muscle relaxation were maintained by continuous intravenous infusion of a mixture of ketamine (10 mg/h/kg body weight) and rocuronium (1.5 mg/h/kg) (Fresenius Kabi, Uppsala, Sweden).

Volume-controlled and pressure-regulated ventilation was used, with a minute volume of 100–150 mL/kg body weight and a frequency of 20 breaths/minute. Positive end-expiratory pressure was adjusted to 5 cm H₂O and the inspired oxygen fraction was 0.5. Aortic pressure was measured with a catheter placed in the ascending aorta.

A median sternotomy was performed and the heart exposed. Heparin 500 IU/kg body weight (Heparin, LEO Pharma, Malmö, Sweden) was given intravenously. A cardioplegic pin was placed in the ascending aorta and after clamping the aorta, cardiac arrest was induced with St. Thomas cardioplegic solution (Plegisol, Pfizer, Sollentuna, Sweden). The heart was explanted and mounted in the heart perfusion system and perfusion was started within 15 minutes. The pulmonary artery and the superior and inferior caval veins were cut short and left open for coronary sinus blood to freely leave the heart. A silicon tube with side holes was placed through the left atrium into the left ventricle to drain the left heart in case aortic valve insufficiency should occur during perfusion.

The perfusion system

The main components of the perfusion system are: 2 roller pumps, oxygenator, 2 heater-cooler units, a 3-liter zipper plastic bag and a water bath (Figure 1). One pump was used to

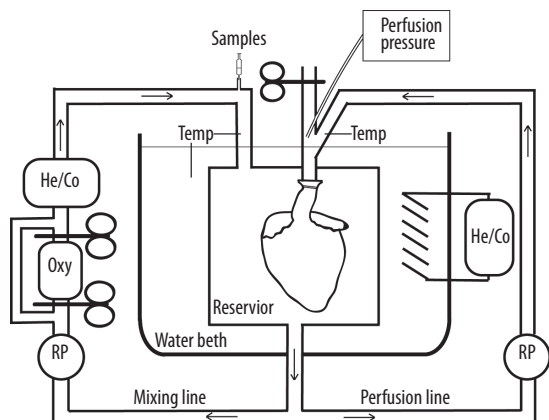


Figure 1. Schematic drawing of the perfusion system. He/Co – heater-cooler unit; oxy – oxygenator; RP – roller pump.

perfuse the heart and the other pump was used to mix the perfusate. The plastic bag containing the heart was submerged in a temperature-controlled water bath to maintain a stable temperature. All tubes were wrapped in thermo-insulating material to decrease temperature exchange with the environment. Temperatures were measured in 3 places: 2 needle probes penetrated the perfusion tube and the mixing tube, respectively, and a regular probe was placed in the water bath. Perfusion pressure in the aorta was measured and recorded by a computer system. The venous blood from the whole heart was emptied into the reservoir, and to obtain a complete mixing, 500 mL/min was circulated in a separate circuit. Blood gas samples were taken from the mixed blood as indicated in Figure 1.

The perfusion solution has been described previously [3]. It is an albumin-containing hyperoncotic potassium-cardioplegic nutrition-hormone solution with washed and leucocyte-filtered erythrocytes from a compatible pig blood donor. The hemoglobin concentration of the perfusion solution was approximately 30 g/L in all groups, giving a hematocrit of approximately 10%. In earlier experiments we have found that immersed cardioplegic porcine hearts can be adequately perfused with a perfusion pressure of 20 mm Hg [3,4]. Twenty mm Hg in perfusion pressure gives a coronary flow of around 140 mL/min in hearts from pigs weighing about 50 kg.

The perfusion flow was set to 140 mL/min and kept at this level throughout the experiment in all groups. The mean perfusion pressure was 18 mm Hg (range 16 to 21 mm Hg). Initially the oxygenator was supplied with 95% oxygen and 5% carbon dioxide until the erythrocytes in the perfusate were fully saturated. The oxygenator was then excluded from the system. The perfusion was continued until the oxygen saturation of the erythrocytes in the perfusate fell to less than 20% (Figure 2). The pO_2 -values were then less than 2 kPa (Figure 3).

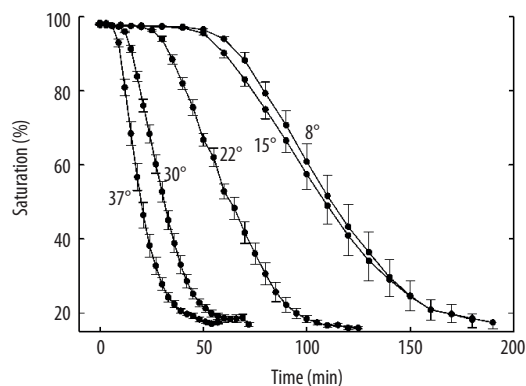


Figure 2. Oxygen saturation vs. time at the different perfusion temperatures. Mean \pm SEM, $n=6$.

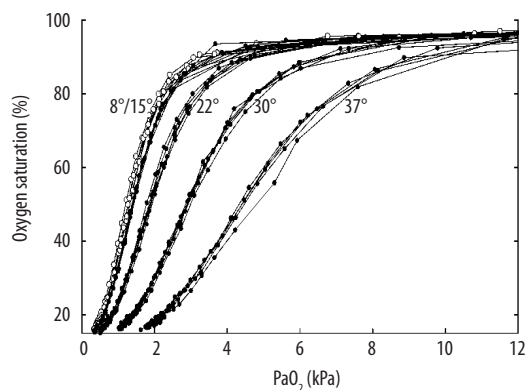


Figure 3. Oxygen saturation vs. oxygen partial pressure curves at different temperatures of the heart perfusate for all individual animals. To separate between 8°C and 15°C, 8°C is shown as open symbols.

Design of the study

The hearts were randomized into 5 groups, with each group exposed to one of the following temperatures: 37, 30, 22, 15, and 8°C. Blood samples were collected regularly for analysis in a blood gas machine (Radiometer ABL800 flex, Copenhagen, Denmark). Values for pH, PO_2 , and PCO_2 were corrected for temperature by the machine. The perfusate volume and blood sampling intervals in the different groups are shown in Table 1. Due to the low oxygen consumption at 8°C, a smaller perfusate volume was used to limit the experimental time to about 3 hours. Similarly, due to the higher oxygen consumption at 37°C, a greater perfusate volume was used to avoid the experimental time being too short. On the days on which the experiments were conducted, the mean atmospheric pressure was 101.45 ± 0.15 kPa, and the mean oxygen tension in the water-bath surrounding the plastic bag containing the heart was

Table 1. Perfusate volume and blood sampling interval used in the different groups.

Group temperature (°C)	Perfusate volume* (mL)	Blood sampling interval (min)
8	700	10
15	1000	10
22	1000	5
30	1000	3
37	1200	3

* To limit the experimental time to about three hours a smaller volume was used at 8°C and to avoid the experimental time at 37°C being too short, a larger volume was used at this temperature.

21.30±0.10 kPa. Calculation of oxygen consumption was done during the period in which the reduction in oxygen saturation of the erythrocytes in the perfusate was linear, which was between 80% and 60% (Figure 2).

Investigation of the airtightness of the perfusion system

As seen in Table 2, the PaO₂ values in the SO₂ interval between 80% and 60% were in the range of 7 to 1 kPa, whereas the ΔPO₂ within each group was in the range of 1.7 to 0.63 kPa. The PO₂ in the water-bath was around 21 kPa; thus, there was a possibility for leakage of oxygen from the water-bath into the perfusate if the plastic bag was not impermeable to oxygen. To investigate whether there was oxygen leakage into the perfusate during perfusion, separate experiments were performed. Using the same setup as described above, including

erythrocyte-mixed perfusate but without a heart, a mixed gas consisting of 93% nitrogen and 7% carbon dioxide was supplied to the oxygenator to remove the oxygen from the perfusate. After oxygen saturation had decreased to less than 20%, the oxygenator was bypassed. Blood samples were taken every 10 minutes for blood gas analysis. A constant oxygen leakage (evidenced as re-saturation of the perfusate) was found for each temperature. When the oxygen saturation lay between 60% and 80% the oxygen leakage at 37, 30, 22, 15, and 8°C was 0.082, 0.072, 0.066, 0.060, and 0.042 mL/min, respectively. The results were adjusted for this leakage when calculating the oxygen consumption of the heart.

Weight measurement of the heart

The weight of the heart, including the weight of the ascending aorta and the aortic cannula, was measured before the heart was mounted in the system (W1). After the experiment had been completed, the aorta including the cannula and the pulmonary artery, were excised from the heart. The weight of the excised material was measured (W2). The heart weight (W) was calculated as: W1–W2.

Data analysis

The formula used to calculate oxygen consumption (MVO₂), given as mL/min/100 g heart weight, was:

$$MVO_2 = (\Delta SO_2 \times Hb \times 1.34 + \Delta PO_2 \times 7.5 \times 0.03) \times 100 / W \times \text{volume/time},$$

where Δ is the difference between 2 consecutive samplings, SO₂ is hemoglobin oxygen saturation, Hb is hemoglobin concentration (g/L), PO₂ is the partial pressure of oxygen (kPa),

Table 2. Blood gases in the different groups obtained in the perfusate at 80% and 60% oxygen saturation.

Temp (°C)	sO ₂ (%)	pH	pO ₂ (kPa)	pCO ₂ (kPa)	Glucose (mmol/L)	Lactate (mmol/L)
37	79.0±1.6	7.355±0.007	6.91±0.22	6.02±0.15	6.6±0.0	0.3±0.1
	60.7±2.2	7.348±0.007	5.21±0.16	6.20±0.17	6.5±0.1	0.3±0.1
30	78.9±0.8	7.367±0.008	4.70±0.06	5.52±0.07	6.3±0.1	0.3±0.1
	60.8±1.0	7.360±0.008	3.42±0.05	5.67±0.08	6.3±0.1	0.3±0.0
22	78.2±1.6	7.355±0.007	3.08±0.12	5.05±0.02	6.4±0.1	0.3±0.0
	60.3±0.7	7.351±0.007	2.21±0.05	5.08±0.03	6.4±0.1	0.3±0.0
15	78.7±0.8	7.331±0.005	2.13±0.04	4.65±0.09	6.5±0.1	0.4±0.1
	60.8±0.7	7.327±0.006	1.51±0.03	4.70±0.10	6.5±0.1	0.4±0.1
8	79.4±1.2	7.230±0.007	2.40±0.08	5.88±0.08	6.4±0.1	0.2±0.0
	59.6±1.4	7.224±0.007	1.64±0.04	5.95±0.07	6.4±0.1	0.2±0.0

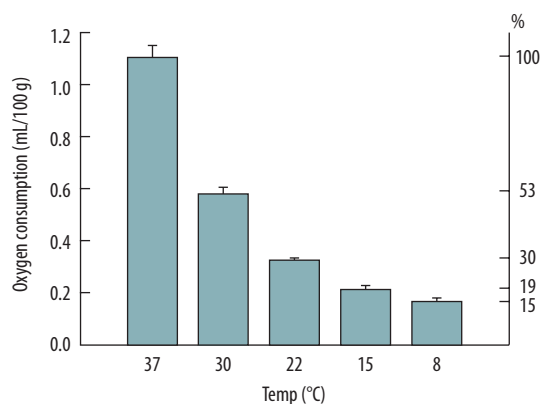


Figure 4. Oxygen consumption at different temperatures of isolated cardioplegic hearts expressed as mL/min/100 g heart tissue. Mean \pm SEM, n=6.

W is the heart weight (g), volume is the perfusate volume (L), and time is the time in minutes between 2 consecutive samplings [6,7]. Results were expressed as the mean \pm standard error of the mean, n=6 in each group.

Results

The weight of the hearts was 208 ± 4 g (n=30).

The oxygen consumption given as mL/min/100 g heart tissue was at $37^\circ\text{C}=1.10 \pm 0.04$; $30^\circ\text{C}=0.58 \pm 0.02$; $22^\circ\text{C}=0.33 \pm 0.01$; $15^\circ\text{C}=0.21 \pm 0.01$, and $8^\circ\text{C}=0.16 \pm 0.02$ (Figure 4).

As seen in Figure 2, the rate of erythrocyte desaturation decreased with decreasing temperature. In the 8°C group it took 180 minutes to reach 20% saturation, even though the perfusate volume was 700 mL, whereas the 37°C group reached 20% saturation after only 40 minutes, even with a perfusate volume of 1200 mL.

Discussion

The domestic pig is the most commonly used large animal in cardiopulmonary research today. Domestic pigs are bred and managed to reach a body weight of 100–110 kg within 150–200 days of life. Even though the commercial life span of domestic pigs is only 6 months in meat production, their natural life span may be 15–25 years depending upon the breed and environmental circumstances. Due to the rapid growth of domestic pigs, their metabolic needs are greater than those of humans; the metabolizable energy intake in kcal/day for the indicated body weights is: 3300 (10–20 kg), 6100 (20–50 kg), and 8400 (50–80 kg) [8]. With this high energy intake and rapid

growth, it is relevant to ask whether the oxygen consumption of domestic pig hearts is correspondingly high, since the clinical relevance of using pig hearts to study heart preservation is greater if such hearts have a metabolism similar to that of man. The pig heart anatomy differs slightly from that of the human heart. The left azygos vein in pigs drains its blood directly into the coronary sinus. To calculate the oxygen consumption based on the hemoglobin saturation in the coronary sinus, the left azygos vein must be ligated, otherwise a dilution of the coronary sinus blood with systemic vein blood will occur and make the calculation of the oxygen consumption uncertain.

Budrikis et al. [6] have studied the oxygen consumption of 60 kg domestic pigs at normothermia in a beating heart model with a mean cardiac output of 5.0 ± 0.24 L/min, a mean coronary flow of 246 ± 30 mL/min, and a mean left ventricular developed pressure of 145 ± 9 mm Hg; they found the mean oxygen consumption of the heart to be 5.6 ± 0.4 mL/min/100 g heart tissue. They calculated oxygen consumption based on the hemoglobin concentration and saturation in the ascending aorta and in the coronary sinus (with the left azygos vein ligated). Considering that the coronary sinus drains only around 85% of the whole heart, an estimated oxygen consumption in the range 6 to 7 mL/min/100 g seems reasonable based on adding 15% to the calculated value of 5.6 mL/min/100 g. In the present study, the oxygen consumption of the cardioplegic heart at 37°C was 1.1 mL/min/100 g, representing a reduction of 85% compared to a working normothermic porcine heart. At 8°C the oxygen consumption was only 0.16 mL/min/100 g, representing a 97% reduction compared to a working heart at normothermia [6].

In the study by Steen et al. [3] showing safe preservation of orthotopically transplanted porcine hearts after 24 hours of perfusion-preservation at 8°C , the hearts were perfused only 20% of the time in cycles of 15 minutes perfusion and 60 minutes non-perfusion; before start of each perfusion the SO_2 in the coronary sinus had fallen from 97% to 23–30%, i.e., during the non-perfusion phase the heart received oxygen from the de-saturating red cells within the capillaries of the heart. With the low oxygen consumption at 8°C , the perfusion can be interrupted for 1 hour without myocardial hypoxia occurring. Besides minimizing the perfusion trauma, this makes the method safer during transport.

Interestingly, the oxygen consumption of the cardioplegic human heart at 37°C has been found to be within the range of 0.6 to 1.5 mL O_2 /min/100 g, at 22°C it is 0.31 mL/min/100 g, and at 10 to 12°C it is 0.135 mL/min/100 g [9]. This is close to what we found in the present study, indicating that the oxygen consumption of the cardioplegic pig heart does not differ significantly from that of the human heart. The pig heart is of special interest since it is the leading candidate as a donor heart in xenotransplantation [10].

Conclusions

The oxygen consumption of the perfused cardioplegic porcine heart at normothermia is 1.1 mL/min/100 g, at 22°C it is 0.33 mL/min/100 g, and at 8°C it is reduced by 85% to 0.16 mL/min/100 g.

References:

1. Minasian SM, Galagudza MM, Dmitriev YV et al: Preservation of the donor heart: From basic science to clinical studies. *Interact Cardiovasc Thorac Surg*, 2015; 20: 510–19
2. The International Society for Heart and Lung Transplantation. <http://www.ishlt.org/registries/slides.asp?slides=heartLungRegistry>. Accessed October 25, 2017.
3. Steen S, Paskevicius A, Liao Q, Sjöberg T: Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours. *Scand Cardiovasc J*, 2016; 50: 193–200
4. Qin G, Sjöberg T, Liao Q et al: Intact endothelial and contractile function of coronary artery after 8 hours of heart preservation. *Scand Cardiovasc J*, 2016; 50L 362–66
5. Buckberg GD: A proposed 'solution' to the cardioplegic controversy. *J Thorac Cardiovasc Surg*, 1979; 77: 803–15
6. Budrikis A, Liao Q, Bolys R et al: Effects of cardioplegic flushing, storage and reperfusion, on coronary circulation in the pig. *Ann Thorac Surg*, 1999; 67: 1345–49
7. Grocott MPW, Martin DS, Levett DZH et al: Arterial blood gases and oxygen content in climbers on Mount Everest. *N Engl J Med*, 2009; 360: 140–49
8. Swindle MM: Swine in the laboratory. Surgery, anesthesia, imaging, and experimental techniques. Second edition. CRC Press, Taylor & Francis Group. Boca Raton, FL, USA. 2007
9. Barner HB: Historical aspects and current review of myocardial protection. In: Warm heart surgery. Salerno TA (ed.), Edward Arnold. London 1995
10. Mayr T, Bauer A, Reichart B, Guethoff S et al: Hemodynamic and perioperative management in two different preclinical pig-to-baboon cardiac xenotransplantation models. *Xenotransplantation*. 2017; 24(3)

Conflict of interest

None.