Cardiac dysfunction and inefficiency after substrate-enriched warm blood cardioplegia

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Abstract

Objective: The study assessed the outcome after prolonged warm continuous antegrade blood cardioplegia (WCBC) with substrate enrichment, in terms of mechanical performance and mechanoenergetic efficiency. Methods: WCBC was given for 3 h to three groups of pigs on cardiopulmonary bypass; WCBC alone (n = 7), WCBC + glucose and insulin (+GIK, n = 7) and WCBC + l-glutamine (+GLN, n = 7). Cardiac systolic and diastolic function, pressure–volume area (PVA) and myocardial oxygen consumption (MVO2) were assessed before, and twice after WCBC using pressure-conductance catheter, coronary flow-probes and O2-content difference. Results: In the WCBC, +GIK and +GLN groups respectively, the following parameters decreased after WCBC compared to baseline: left ventricular developed pressure by 26, 19 and 25% (P < 0.001); dp/dtmax by 36, 37 and 34% (P < 0.001); preload recruitable stroke work by 35, 41 and 28% (P < 0.001); mechanoenergetic efficiency (PVA/MVO2) by 44, 41 and 22% (P < 0.001). End-diastolic stiffness increased early after WCBC in the WCBC and +GLN groups, while it was unchanged in the +GIK group (P = 0.032). Conclusion: Despite continuous aerobic conditions and additional substrates, post-WCBC cardiac contractile function and mechanoenergetic efficiency was severely depressed. The results demonstrate the hazards of sustained normothermic hyperkalemic perfusion. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Warm blood cardioplegia; Left ventricle; GIK; Glutamine; Mechanoenergetic efficiency

1. Introduction

Warm continuous blood cardioplegia (WCBC) is reported to provide superior protection of the myocardium during coronary artery bypass surgery and has been suggested for myocardial protection in procedures requiring long cross-clamp times [1]. However, clinical [2] and recent experimental results indicate that the use of WCBC might not be straightforward. Continuous warm potassium-induced aerobic arrest has been reported to induce post-cardioplegic dysfunction, possibly due to significant oedema [3], calcium accumulation [4] and/or metabolic alterations similar to the post-ischaemic state [5].

From our earlier works we know that during WCBC the heart tends to prefer fatty acids (FFA) as its main fuel [6,7]; however, after WCBC, the contribution of glucose and FFA oxidation to ATP production was more balanced, with enhanced turnover rates of substrates, and mechanical dysfunction [7]. These findings may partly explain a concomitant increase in myocardial oxygen consumption (MVO2) and reduced mechanoenergetic efficiency after WCBC [8]. To improve myocardial protection during cardiac surgery, several investigators have proposed a substrate-enriched cardioplegic solution [9,10], especially during ongoing, or preceding ischaemia. In the aerobic WCBC setting, substrate-enriched WCBC may be advocated for two reasons. First, by achieving a reduced rate of FFA oxidation, and thus improve mechanoenergetic efficiency by a reduction in MVO2. Second, the reduced post-WCBC cardiac function [3,7] has several similarities to post-ischaemic dysfunction (stunning) [5], and protective regimens proven effective following an ischaemic insult may thus be functional even in adequately perfused hearts.

The aim of the present study was to assess whether the suboptimal protective effect of WCBC [7] could be improved. We chose l-glutamine and glucose-insulin-potassium (GIK) as additives to the cardioplegic solution. l-glutamine was used since it may act as a precursor for glutamate, and is safer in terms of dosage and side effects [11]. The main rationale for using glutamine/glutamate is the role of glutamate in the malate-aspartate shuttle regulating the NAD+/NADH balance, and thereby enhancing...
glycolysis [10]. Additionally, a relative shortage of glutamate has been observed early after coronary surgery [10], and the addition of glutamate to blood cardioplegia improves ATP conservation in the human myocardium [10]. The rationale for GIK treatment is to increase the rate of ATP production from glycolysis for restoration of Ca<sup>2+</sup>-homeostasis, to replenish the glycogen stores, inhibit uptake, overload and use of fatty acids, and counteract the systemic neuroendocrine response [12]. This was investigated in an earlier described model from our laboratory [6,7].

2. Materials and methods

Animals were treated in compliance with the European Convention on Animal Care. The experimental protocol was approved by the institutional committee of the Norwegian Experimental Animal Board.

Locally bred domestic pigs of either sex were used, weighing 41–52 kg (mean 44.3). They were fasted overnight with free access to water. Intramuscular ketamine (20 mg/kg) and atropine 2 mg were used as premedication, and pentobarbital (10 mg/kg) and fentanyl (0.01 mg/kg) boluses as anaesthetic induction (i.v.). The pigs were tracheostomized and ventilated with an air/oxygen mixture on a volume-controlled respirator (FiO<sub>2</sub> = 0.5; Servo 900B, Siemens-Elema, Solna, Sweden). Continuous i.v. anaesthesia was administered: pentobarbital-Na (4 mg/kg per h), fentanyl (0.05 mg/kg per h) and midazolam (0.3 mg/kg per h).

2.1. Experimental setup

We used, in principle, the same experimental setup as described in detail previously [6,7], including a separate extracorporeal circuit for the heart to avoid systemic hyperkalemia. Coronary blood flow (CBF) was measured with ultrasonic transit-time probes (Medi-Stim AS, Oslo, Norway) on the left and the right main stem of the coronary arteries. The left hemiazygos vein was ligated. The blood was oxygenated using two separate membrane oxygenators (Dideco, Mirandola, Italy) and separate reservoirs; one baby sized set for the heart and one adult sized set for the rest of the body. Cardiopulmonary bypass was initiated after a ster-notomy with snared transatrial bicaval and left axillary artery cannulation with flow rates sufficient to give a systemic pressure of 50 ± 2 mmHg. The aorta and pulmonary artery were cross-clamped for 3 h and high-potassium warm blood cardioplegia was administered continuously through the aortic root cannula with a pressure of 75 mmHg [6]. During cross-clamping the left and right ventricles were vented.

The extracorporeal circuits were primed with homologous fresh blood from cross-matched donor pigs. The cardioplegic blood solution was prepared by mixing 200 ml St. Thomas’ solution no. 2 (Plegisol; Abbot Labs, Chicago, IL, USA) with 800 ml cross-matched blood from a donor pig. Potassium was added to a final [K<sup>+</sup>] at approximately 16 mmol/l (see Section 3). Substrate enrichment of the cardioplegic solution was infused separately into the cardioplegic line throughout the cardioplegic period. The glucose-insulin solution was prepared to deliver 1.0 g/kg per h of glucose and 0.17 IU/kg per h of insulin (Insulin Actrapid, Novo Nordisk, Denmark; total osmolarity at ~2600 mosmol/kg). The glutamine solution was prepared to deliver 0.375 μmol/kg per hour of glutamine (iso-osmotic). The activated clotting time (ACT; Hemochrom 400, Techidyne-Corp, Edison, NJ, USA) was kept above 500 s at all times during CPB. The temperature in the blood cardioplegia circuit was 37°C during the cardiac arrest period, whereas the temperature was lowered to 26°C in the systemic line. The systemic temperature was gradually elevated to 37°C over the last 30 min prior to declamping of the aorta and the pulmonary artery. The heart was the reperfused for 15–20 min and subsequently weaned from bypass over a period of 5–10 min.

2.2. Data acquisition

Mean arterial pressure (MAP) and cardioplegia delivery pressure were measured in the abdominal aorta and root of the ascending (clamped) aorta, respectively, with calibrated transducers (Transpac 3, Abbot Critical Care systems, Sligo, Ireland). Transit-time ultrasonic signals were converted to flow (ml/min) in a flow-computer (CM 4008, Medi-Stim). General haemodynamic parameters were automatically recorded and digitized at 0.25 Hz (LAB-View, National Instruments, Austin, TX, USA) and stored. Oxygen saturation was determined in arterial and coronary sinus blood (ABL3, Radiometer, Copenhagen, Denmark).

The conductance-catheter methodology has been extensively described earlier [13]. A 6-F, 12-electrode, dual field, pigtail combined microtip and conductance catheter for continuous measurements of left ventricular (LV) pressures and volumes, (Millar Instruments Inc., Houston, TX, USA) was introduced into the LV, along its long axis, via the left carotid artery. Positioning of the catheter was evaluated by palpation of the apex. The catheter was reinserted after weaning from CPB. A conductance conditioner (Leycom Sigma-5, Cardiodynamics BV, Leiden, the Netherlands) converted variations in intraventricular conductance throughout the heart cycle to time-varying volume. Intraventricular pressures and volumes were recorded at 250 Hz. Offset volume, i.e. conductance signals derived from the myocardial wall and right ventricle, was assessed from consecutive beats after injecting a bolus of 4 ml NaCl (10%) into the pulmonary artery [13].

2.3. Experimental design

Experiments were randomized to three groups (investigator blinded): WCBC alone, WCBC + glucose and insulin (+GIK) and WCBC + glutamine (+GLN). The seven pigs in the WCBC group (no substrate enrichment) served as
reference group. Metabolic and some mechanical data from these seven animals have been presented earlier [7], to answer questions related to earlier work from our laboratory [6]. However, new material, also from these animals is presented here. Baseline registrations of haemodynamic and pressure-conductance parameters along with blood sampling were performed about 15 min after the initial surgical preparation and before instituting CPB. Two sets of pressure-conductance data were obtained throughout 10-s intervals (10–15 beats). One set during stable preload, and one set during transient vena caval occlusion using a caval snare. Simultaneously were blood samples (haemoglobin and blood gases) drawn from arterial and coronary sinus catheters. After cross-clamping WCBC was given for 3 h in the three groups, as specified above. At approximately 30–40 min (‘early’) and 60–70 min (‘late’) after declamping, the same registrations as at baseline were performed.

2.4. Calculations

Calculation of conductance-derived parameters was performed using the Conduct-PC software (CPCW version V3.15, Cardiodynamics). The heart cycle was defined to start at the peak of the R-wave in the QRS-complex, identifying end-diastole. End-systole was defined at the maximum ratio between LV pressure and volume throughout one heart cycle.

The oxygen content in blood (ml O2/ml blood) was calculated according to the formula: [Hb(SO2)], where Hb is the haemoglobin concentration (g/ml), SO2 is blood saturation ratio of O2, the constant 1.39 is the oxygen binding capacity for haemoglobin (mlO2/g). Oxygen content was converted to Joules (J) by the factor: 20.2 J/mlO2. Myocardial oxygen consumption (MVO2, J beat-1/ml) was then calculated from arterial to coronary sinus O2-content difference times coronary flow, and divided by heart rate (HR).

Stroke work (SW, mmHg/ml) was calculated by iterative integration of the area of the pressure–volume loop, obtained from all sampled pressures and volumes during one heart cycle. The total pressure–volume area (PVA) is an index of total mechanical energy of the beating heart [14]. This area is the sum of SW and elastic potential energy (PE), the latter area delimited by the end-systolic and end-diastolic relationships (ESPVR and EDPVR) and of the isovolumic relaxation trajectory of the pressure–volume loop. We calculated PVA as (in J beat-1): PVA = [SW + (Pes(Ves - V0)/2) - (Pes(Ves - V0)/4)]1.33 × 10^-4 J mmHg -1 ml -1 [8]. Where Pes is end-systolic pressure (mmHg), Ves is end-systolic volume (ml), V0 is volume axis intercept of the linear ESPVR (Pes = Ees(Ves - V0)), where Ees is end-systolic elastance, Pes is end-diastolic pressure (mmHg) and Ved is end-diastolic volume (ml). Left ventricular total mechanical efficiency (%) was calculated from the ratio PVA/MVO2. The efficiency of transition of MVO2 to external useful work (SW) was calculated by the ratio SW/MVO2 (external efficiency, %). The efficiency of mechanical energy conversion was calculated by the ratio SW/PVA (%).

Left ventricular contractility was assessed by the concept of preload recruitable stroke work (PRSW) [15]. From beats recorded during transient vena caval occlusion, SW and Ved were fitted to the linear relationship: SW = M(Ved - V0). The slope, M, of this relationship is a load-independent index of left ventricular contractility, provided an unchanged x-intercept (V0). Shifts in V0 might limit the use of M as the sole indicator of PRSW [15]. Therefore, the individual SW–Ved relationships were used to calculate maximum recruitable SW (SWmax) by inserting experimental maximum Ved and time-dependent V0. To evaluate afterload, effective arterial elastance (Ea) was calculated from the ratio between Pes and conductance-derived stroke volume (SV) [16]. Time-constant of isovolumic relaxation (τ) was calculated according to Mirsky [21]. End-diastolic stiffness was quantified by non-linear exponential least squares regression of the end-diastolic pressures and volumes derived from consecutive beats during transient VCO according to the equation: Peds = ae[bP(V0)], with the resultant α (constant) and β (stiffness) coefficients.

2.5. Statistics

All data are presented as mean ± 1 standard deviation (SD). Variables were tested for normality of distribution (Shapiro–Wilks test), and all compiled. Data were analysed using analysis of variance for repeated measures (RANOVA). The effect of cardioplegic intervention per se was tested in an allover model including baseline with time, and time–group interaction, as predictors of difference. Any difference between groups in change from baseline after WCBC was tested in a model including delta values at early and late timepoints only (early-baseline, late-baseline), with between group, and time–group interaction, as predictors of difference. Some trends were also illustrated by within-groups paired t-test between early and baseline and adjusted for multiple comparisons (Bonferroni). Correlation was based on Pearson’s correlation coefficient (corrected for multiple comparisons). Three animals in the WCBC group and one animal in the +GIK group were not included in the final analysis (see Section 3). To see if this would have had an impact on mechanical and energetic outcome after cardioplegia they were given ‘worst ranks’ within respective groups, and analysed by the Kruskal–Wallis test. This did not change any of the results. Data analysis was performed using a statistical package (SPSS 10.0, SPSS Inc., USA). Differences were considered significant at P < 0.05.

3. Results

Of 29 pigs used in this study, 21 were successfully weaned from CPB without any use of supportive drugs, with seven pigs in each group. Four pigs were excluded before cardioplegia due to major surgical bleeding (two pigs) and perioperative infarction (two pigs). Another four pigs were excluded after cardioplegia due to resistant ventri-
cular fibrillation (three receiving WCBC only, and one receiving WCBC + GIK). Systemic venous \([K^+]\) was 4.4 ± 0.2 mmol/l, before and after cardioplegia. In the normothermic blood cardioplegic perfusate \([K^+]\) was 15.8 ± 0.6 mmol/l (all groups).

### 3.1. Haemodynamic and mechanical variables

Tables 1 and 2 summarize haemodynamic and mechanical parameters at baseline, and their change after 3 h of WCBC. As evident from these tables, four of 14 parameters were changed over time, while no differences in change, between groups, were detected.

Afterload, as expressed by arterial elastance \((E_a)\), was unchanged throughout experiments. Systolic contractile performance was considerably depressed after WCBC; \(dP/dt_{\text{max}}\) by 34\%, \(E_{\text{es}}\) by 32\%, \(M_e\) by 41\% and \(SW_{\text{max}}\) by 35\%; however, \(SW_{\text{max}}\) increased 9\% from early to late, when all animals were meaned \((P < 0.05)\). Both the \(SW/V_a\) and the ESPVR relationships seemed shifted to the left after WCBC in the \(+GLN\) group \((V_a\) and \(V_0\)); this was not significant when compared to the other groups. However, the high \(E_{\text{es}}\) values at baseline and the suggestively larger fall in this parameter after WCBC within the \(+GLN\) group, might explain the low PVA at baseline and the suggestively larger fall in this parameter after WCBC within the \(+GLN\) group, by a relative increase in end-systolic potential energy \([14]\). Some other parameters also seemed better preserved after cardioplegia within the \(+GLN\) group (MAP and SVcc), but when compared to the other groups this did not reach significance.

#### 3.2. Total mechanical energy and mechanoenergetic efficiency

Energy consumption \((MVO_2)\) was increased after WCBC; however, within groups, this was significant only in the \(+GLN\) group \((P = 0.021, \text{paired } t\)-test). Furthermore, external work performed \((SW\) by 37\%), and total mechanical energy generated \((PVA, \text{by } 24\%)\) were reduced after WCBC. At baseline, total efficiency was 36 ± 11, 37 ± 16 and 37 ± 9%, external efficiency was 19 ± 6, 20 ± 8 and 20 ± 5%, and efficiency of energy conversion \((SW/PVA)\) was 54 ± 4, 54 ± 8 and 62 ± 9% \((WCBC\) only, +GIK and +GLN, respectively). Absolute change in these ratios after WCBC are presented in Fig. 1. All parameters were significantly decreased vs. baseline \((total \text{efficiency by } 45\%, \text{external efficiency by } 50\%, \text{and } SW/PVA \text{by } 19\%), \text{all } P < 0.001\), and no between-group differences were detected. Independently, total efficiency was not reduced significantly in the \(+GLN\) group, but both external and mechanical energy conversion efficiencies were reduced significantly.

#### 3.3. Diastolic function

End-diastolic volume \((preload)\) was unchanged after WCBC, with no between group differences (Table 1). The time-constant of isovolumetric relaxation \((\tau)\) was

### Table 1

| Index          | Groups  | Baseline | Change from baseline after WCBC | \(P^*\)   
|----------------|---------|----------|---------------------------------|----------
|                |         |          | Early                          | Late     | vs. baseline | groups |
| HR (beats/min) | WCBC    | 91 ± 12  | +12 ± 16                        | +8 ± 19  | 0.01        | 0.86   |
|                | +GIK    | 91 ± 14  | +11 ± 15                        | +7 ± 15  |            |        |
|                | +GLN    | 91 ± 10  | +6 ± 12                         | +6 ± 11  |            |        |
| MAP (mmHg)     | WCBC    | 85 ± 15  | −24 ± 18                        | −28 ± 18 | <0.001     | 0.28   |
|                | +GIK    | 86 ± 7   | −13 ± 10                        | −17 ± 9  |            |        |
|                | +GLN    | 84 ± 12  | −13 ± 17                        | −15 ± 16 |            |        |
| LVDP (mmHg)    | WCBC    | 87 ± 9   | −22 ± 16                        | −24 ± 14 | <0.001     | 0.70   |
|                | +GIK    | 88 ± 8   | −16 ± 10                        | −18 ± 10 |            |        |
|                | +GLN    | 89 ± 10  | −21 ± 19                        | −23 ± 19 |            |        |
| \(V_a\) (ml)   | WCBC    | 53 ± 13  | −1 ± 4                          | −4 ± 8   | 0.15       | 0.65   |
|                | +GIK    | 54 ± 6   | −4 ± 13                         | −9 ± 15  |            |        |
|                | +GLN    | 54 ± 6   | −3 ± 12                         | −1 ± 8   |            |        |
| SV (ml)        | WCBC    | 31 ± 8   | −8 ± 4                          | −8 ± 4   | <0.001     | 0.46   |
|                | +GIK    | 29 ± 2   | −7 ± 6                          | −7 ± 6   |            |        |
|                | +GLN    | 31 ± 3   | −4 ± 6                          | −5 ± 6   |            |        |
| \(E_a\) (mmHg/ml) | WCBC    | 3.2 ± 0.7| +0.3 ± 0.8                      | +0.1 ± 0.7| 0.13       | 0.35   |
|                | +GIK    | 3.4 ± 0.5| +0.6 ± 0.8                      | +0.4 ± 0.7|            |        |
|                | +GLN    | 3.1 ± 0.4| −0.05 ± 0.6                     | −0.1 ± 0.7|            |        |
| MVO\(_2\) (l/beat) | WCBC    | 1.80 ± 0.46| +0.47 ± 0.64                     | +0.48 ± 0.82| 0.004     | 0.84   |
|                | +GIK    | 1.87 ± 0.74| +0.37 ± 0.83                     | +0.31 ± 0.99|            |        |
|                | +GLN    | 2.00 ± 0.18| +0.56 ± 0.39                     | +0.54 ± 0.40|            |        |

* Data are expressed as mean ± SD. \(^*\)RANOVA: vs. baseline is probability for a change from baseline irrespective of group; groups is probability for a difference in change after WCBC between groups (baseline and time excluded). No significant interactions were detected. \(E_a\), arterial elastance; +GIK and +GLN, WCBC+substrate enrichment – see Section 2; HR, heart rate, LVDP, left ventricular developed pressure; MAP, mean arterial pressure; MVO\(_2\), myocardial oxygen consumption; SV, stroke volume; \(V_a\), end-diastolic volume.
32.4 ± 0.9 ms at baseline (mean of all groups), and increased to 37.5 ± 1.4 early and 35.8 ± 1.0 late after WCBC (P = 0.002). No between-groups differences were detected. End-diastolic pressure (P\textsubscript{ed}) was 7 ± 3, 8 ± 2 and 8 ± 2 mmHg at baseline (WCBC only, +GIK and +GLN, respectively), and increased after cardioplegia (Fig. 2). Although no significant differences in change from baseline between groups were detected, the P\textsubscript{ed} seemed less increased in the +GIK group. Left ventricular end-diastolic stiffness (β) increased on an average after cardioplegia; however, as shown in Fig. 2, change vs. baseline at early vs. late after cardioplegia was different between groups. This may indicate that LV stiffness was minimally affected in the +GIK group early after WCBC, and that all groups were more or less similar at end of protocol. Correlation between β and PVA/MVO\textsubscript{2} was evaluated on a linear basis after logarithmic transformation (best fit). For the WCBC and +GLN groups, significant correlations were found: r = −0.75, P < 0.001 and r = −0.60, P = 0.012, respectively. The correlation was not significant in the +GIK group (r = −0.41, P = 0.18).

4. Discussion

Warm continuous blood cardioplegia is particularly attractive as a potentially superior protective regimen in high-risk surgical candidates with cardiac failure or ongoing ischaemia, facing complicated long-lasting procedures. These patients are threatened by post-operative low-output failure, the major reason for post-operative death in this group of patients. In this setting, the failure of WCBC to preserve systolic, diastolic and mechanoenergetic function in the present experimental study is particularly worrisome. Furthermore, no significant improvement in post-cardiopul- gic function was seen in the groups receiving substrate-enhanced WCBC.

Systolic function was reduced close to 40% after 3 h of WCBC, despite the fact that the hearts were from healthy pigs. Also, a clear reduction in active and passive diastolic function was observed. Whether inotropic interventions like adrenergic drugs could reverse this dysfunction was not assessed, and the contractile reserve of the LV after WCBC is thus unknown. However, clear mechanoenergetic inefficiency was observed, and inotropic drugs would most probably induce a more profound mismatch between mechanical and metabolic energy, by increasing non-mechanical energy expenditure [8,14].

The exact mechanism for the deteriorated cardiac function after WCBC was not directly addressed in the present study. It is, however, known that depolarized diastolic arrest induces a net influx of Ca\textsuperscript{2+}, leading to what has been termed K\textsuperscript{+}-induced Ca\textsuperscript{2+}-overload, when [K\textsuperscript{+}] between 15 and 20 mmol/l is used [4]. This Ca\textsuperscript{2+}-overload might furthermore lead to proteolysis in the contractile apparatus (troponin I and α-actinin) by Ca\textsuperscript{2+}-activated proteases [17]. These deterior-
ating processes may possibly be more pronounced during normothermia, since this is the temperature-optimum for biological processes. After observation of twitching with WCBC [K⁺] around 10 mmol/l, a [K⁺] at approximately 16 mmol/l was used to ensure a reproducible cardiac arrest. In man, [K⁺] is usually lowered for maintenance of cardiac arrest during WCBC to avoid systemic hyperkalemia. This was unnecessary in our model with an isolated cardioplegia circuit, effectively isolating the heart from the systemic circulation, keeping systemic [K⁺] within normal range after WCBC. However, compared to human hearts receiving WCBC, hearts in our pig model may have been subdued to a relatively higher constant [K⁺]. This may have enhanced the proposed detrimental effects of Ca²⁺ accumulation more than in human hearts protected with WCBC.

Furthermore, constant myocardial perfusion during WCBC may lead to oedema [3], with reduced LV contractile performance through restrictive mechanisms [18]. Myocardial oedema has also been associated with an increased energy requirement [19]. In the present study an inverse correlation between β and mechanoenertic efficiency was observed for the WCBC and +GLN groups. The poorer correlation in the +GIK group suggests that increments in β (if β is interpreted as increased oedema) is not enough to explain decreased performance after WCBC, since both Mₑ and mechanoenetric efficiency may be reduced irrespective of β. Furthermore, increased end-diastolic stiffness might as well be a result of Ca²⁺-induced contracture and myofilament derangement [17,20] as

Fig. 1. Absolute change (Δ), relative to baseline, in indexes of left ventricular mechanoenetric efficiency, after weaning from WCBC and cardiopulmonary bypass. Upper: Total efficiency (PVA/MVO₂), middle: external efficiency (SW/MVO₂) and lower: efficiency of mechanical energy conversion (SW/PVA). No significant interactions were observed. P-value: probability for a difference in change after WCBC between groups. All were significantly reduced compared to baseline (P < 0.001, irrespective of group). Bars are means, error bars are SD.

Fig. 2. Absolute change (Δ), relative to baseline, after weaning from WCBC and cardiopulmonary bypass. (Upper) End-diastolic pressure (Pₑd), no significant differences in change between groups (P-value), but Pₑd was allover increased compared to baseline (P < 0.001). (Lower) End-diastolic stiffness (β); *P-value indicates a significant time–group interaction, thus, a different development in change between groups from early to late. Bars are means, error bars are SD.
oedema, which likely explains the all over increased β late after WCBC.

Another possible mechanism for post-WCBC mechanical dysfunction may be a heterogeneous delivery of the WCBC perfusate, thus leaving areas of the myocardium insufficiently perfused and relatively ischaemic. Inhomogeneous delivery of the cardioplegic perfusate has been shown to depend on perfusion pressure, and any developed oedema during long periods of cardiac arrest [21]. To maintain adequate distribution, and overcome any increase in coronary resistance (oedema), a perfusion pressure of 75 mmHg was applied [6,21]. Also, no release of lactate to the coronary sinus was seen during the experiments (Elvenes OP, Korvald C, Myrmel T, Sorhie DG, unpublished results). Scattered ischaemia is thus not the most likely explanation for post-WCBC dysfunction in this study.

Cellular metabolic alterations that explain the relatively increased post-cardioplegic energy demand were not assessed in detail, needing preferably an isolated heart model [14]. The energy-consuming processes in the heart have been described thoroughly earlier, and consist mainly of basal metabolism (e.g. ionic gradients, protein synthesis), excitation–contraction (EC)-coupling and generation of PVA [14]. The classic ‘oxygen-waste’, as seen during inotropic stimulation, is an increased MVO2 at comparable mechanical energy, as a result of increased energy demand by EC-coupling [14]. In the present study, contractility was depressed, afterload unchanged and HR only slightly increased after WCBC. This makes an increased MVO2 due to EC-coupling less probable. On the contrary, less PVA was generated at similar or moderately increased levels of MVO2, which is more suggestive for a reduced contractile efficiency after WCBC, a possible effect of myofilamental derangement. Also, post-WCBC regeneration of ionic and protein homeostasis, along with an increased rate of fatty acid oxidation [8], could contribute to an increased basal metabolic MVO2. However, augmented fatty acid oxidation, after WCBC, seems to be of minor importance [7].

The post-WCBC total mechanoenergetic inefficiency in the +GLN group tended to be less affected compared to the other two groups (PVA/MVO2). However, this group was equally inefficient when the external efficiency was considered, and tended to have a more severely reduced efficiency of mechanical energy conversion. Thus, glutamine did not preserve cardiac function better, compared to GIK, and the increased amount of end-systolic potential energy generated in this group did not improve external work, and was likely lost in wall-stress and heat [14].

Numerous studies have shown beneficial effects of adding substrates during reperfusion or cardioplegia after ischaemia [10,12]. Additionally, GIK has been found to prevent diastolic contracture after ischaemia, and could therefore potentially reduce Ca2⁺-overload [20]. In the present study, enrichment with L-glutamine or GIK did not protect from contractile or diastolic dysfunction (low-output failure) and mechanoenergetic inefficiency after warm cardioplegic arrest, while GIK seemingly had an effect on end-diastolic stiffness. The lack of effect of substrate enrichment may suggest that metabolic derangements are of lesser importance in the development of post-cardioplegic contractile dysfunction, and should thus strengthen the prolonged depolarization as a deteriorating factor. The effect on end-diastolic stiffness induced in the +GIK group could be explained by a higher osmolarity of the cardioplegic solution with reduced oedema [22]. The effect of substrate-enriched WCBC during, or after, significant ischaemia was not covered by the present study, and should not be depreciated by the present results alone.

Some methodological considerations need mentioning. Firstly, the severity of post-WCBC mechanic depression and inefficiency observed was most probably an effect of arrest time. Less severe depression should be expected with a shorter cardioplegic period [3]. Secondly, we have no isochronous control group (on CPB, normokalaemic warm blood perfusion), which limits the possibility to rule out the effect of CPB only. Thirdly, the model was designed to isolate the heart and thus specifically address the effect of long-lasting WCBC on the heart per se, and not necessarily mimic the clinical situation. This setup stops the natural removal of cell aggregates and bioactive substances from the isolated cardiac circle during WCBC perfusion. This might have had an additional influence on post-WCBC dysfunction. The temperature difference between the isolated heart cycle and the systemic circulation (37 vs. 26°C), is also different from the clinical situation, and was applied to reduce the systemic effects of extracorporeal circulation per se.

There are similarities between reversible post-ischaemic dysfunction (stunning) and post-WCBC dysfunction (MVO2 up, LV function down), however, one should notice also their dissimilarities. Stunning is elicited by ischaemia, and the subsequent damage of oxygen radicals and Ca2⁺-overload [17], while post-WCBC dysfunction is probably elicited by a combination of K⁺-induced Ca2⁺-overload and oedema. As contracture probably is a more prominent feature in stunned hearts [23], this may explain the disparity in efficiency of energy conversion (SW/PVA), where in stunning it is increased (Korvald C, Elvenes OP, Aghajani E, Myhre ESP, Myrmel T, unpublished results), and as shown in the present work decreased after WCBC.

In summary, cardiac performance and mechanoenergetic efficiency were considerably reduced 3 h after WCBC in this experimental model. Our data point towards a central role of sustained depolarized arrest as a key factor in post-cardioplegic dysfunction, in line with an earlier report on isolated ventricular porcine myocytes [24]. Substrate enrichment failed to exert positive effects. The post-cardioplegic contractile dysfunction and mechanoenergetic inefficiency is most probably explained by derangement of the LV contractile properties, and prevention of this dysfunction would might be achieved by elimination of potassium induced depolarized arrest during long-lasting cardiac
procedures. Alternatives exist, such as hyperpolarized arrest [4], or short lasting β-blockers [25].

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References


Appendix A. Editorial Comment

In this issue of the European Journal of Cardio-thoracic Surgery, Korvald et al. [1] report an unexpected finding by showing that warm continuous blood cardioplegia for 3 h significantly reduces myocardial contractile function in pig heart. This observation is unexpected, because depressed contractile function after cardioplegic arrest has generally been attributed to the presence of ischaemia. This ischaemia should be completely avoided by the continuous infusion of oxygenated, warm blood cardioplegia. Thus, contractile function deteriorated despite the absence of ischaemia. Furthermore, this dysfunction could not be prevented by substrate enrichment with either amino acids or glucose-insulin-potassium (GIK). Korvald et al. [1] conclude that there may be a heterogeneous delivery of the warm contin-
uous blood cardioplegia, thus leaving areas of the myocardium insufficiently perfused and relative ischaemic, or that the prolonged depolarization may be responsible for this observation by altering calcium homeostasis.

These observations by Korvald et al. [1] may be of great clinical importance. Clinical reports demonstrating impaired recovery of function after cardioplegic arrest underline its relevance in humans [2]. Thus, the study refreshes an old but still unresolved controversy, i.e. what is the best strategy for myocardial protection of the human heart?

More than a decade ago, the discussion about strategies of myocardial protection revolved around the use of either crystalloid or blood cardioplegia. Today, additional ‘players have been introduced to the game’, among them the so-called warm heart surgery, by using continuous warm infusions of blood cardioplegia [3]. As with all new cardioplegic solutions, it is imperative that they all need to be tested experimentally with respect to safety and efficacy. In addition, a firm delivery protocol has to be developed (as has been done with cold intermittent blood cardioplegia) which might be different from one cardioplegic solution to the other. Myocardial edema might very well be the result of continuous perfusion of a flaccid heart, but could also be due to an inadequate osmolarity of the solution used for continuous perfusion.

The results by Korvald et al. [1] support the notion that repetitive contractile activity is an important feature for heart function. The loss of this activity results in immediate and dramatic changes of function (after re-establishment of contraction) and gene expression [1,4,5]. It is interesting to note in this context, that it appears difficult to maintain myocytes in culture without inducing significant changes in gene expression and function. Thus, it might be reasonable to conclude that repetitive contractile activity of the heart is not only important to support the body with an adequate circulation of blood but also as a stimulus to support the heart’s own function.

Interfering with the heart’s function by imposing cardioplegic arrest (ischaemic or non-ischaemic) may result in temporary or permanent depression of function once contraction resumes. In ischaemically arrested hearts, ischaemia tolerance may be extended, by using hypothermia or cardioplegia or, as mostly applied, both. It is easily understood that if the ischaemia tolerance is exceeded, injury occurs and function remains depressed. However, it is not clear why contractile function is impaired after continuous warm blood cardioplegia, when no ischaemia is present. Korvald et al. [1] tried to maintain function using substrate supplementation with either amino acids or GIK. They based their strategies on two rationales. First, that glutamate supplementation would result in support of the malate aspartate shuttle, transporting amino acids and reducing equivalents across the mitochondrial membrane. Second, that GIK would promote anaerobic ATP-production which in turn may support calcium homeostasis. However, these rationales may apply for the setting of ischaemic, cardioplegic arrest but may not be valid for the setting of continuous warm blood cardioplegia (which seems to be supported by the observed lack of a protective effect). In order to fully understand this argument, it is important to review some basics of myocardial substrate metabolism.

The heart is a metabolic omnivore [6]. The energy that is needed for ATP production is derived from the oxidation of fatty acids, glucose, lactate, ketone bodies, and even amino acids. In addition, the heart is able to utilize some of its endogenous substrates, glycogen and triglycerides. Under normoxic conditions, up to 90% of ATP production is derived from the oxidation of fatty acids. Although glucose oxidation contributes only minor amounts of energy for total ATP production, glucose is considered an essential fuel for the heart, since contractile function seizes in its absence [6].

In reperfused hearts, fatty acid oxidation rapidly normalizes but glucose oxidation remains depressed, as does contractile function [7]. In patients undergoing coronary artery revascularization, a positive correlation between enhanced glucose uptake and return of contractile function could be demonstrated [8]. Another observation associated with ischaemic cardioplegic arrest of the heart is the loss of glutamate from the mitochondrial pool [9,10]. Based on these observations, substrate enrichment of cardioplegic solution or during reperfusion with glutamate [9,11,12] or supporting glucose metabolism by GIK [11,13,14] appears reasonable and has been successfully performed to enhance post-cardioplegic recovery of function. However, despite intense investigation in this field, and many positive observations (including our own [15]), the exact mechanisms are not fully understood. For example, it is not yet clear, whether the supplemented glutamate actually reaches the site of origin, i.e. the mitochondrial matrix. Lewandowski et al. [16] observed an impairment in the function of the malate–aspartate shuttle after ischaemia in the isolated rabbit heart. While the clinical relevance of this is not clear, the studies not demonstrating an effect of amino acid supplementation are rare [17,18]. However, there is no rationale for supplementation of continuous cardioplegia with glutamate, where no loss of glutamate has been demonstrated.

The studies on GIK are just as controversial (see Ref. [14] for review), although the majority of studies support its efficacy during or after acute phases of ischaemia. Again, the exact mechanisms are not known. However, most of these suggested mechanisms relate to the post-ischaemic myocardium, where insulin’s spectrum of actions may be different. For example, we demonstrated a positive inotropic effect after ischaemia in the isolated rat heart, which was not present under normoxic conditions [19]. Insulin has been suggested to affect calcium homeostasis [20]. This assumption may explain the lowest end-diastolic pressures in the GIK group in the study by Korvald et al. [1].

Korvald et al. [1] suggest that the impairment in function may be due to the hyperkalemic arrest. The elevated potas-
sium may lead to an increase in intracellular calcium causing end-diastolic and systolic dysfunction. If this mechanism applies, addition of GIK bears the potential to worsen rather than improve diastolic function since insulin increases potassium uptake by both directly activating Na+/K+-ATPase and by co-transport with glucose. As with glutamate, the theoretic benefit of adding GIK to warm continuous blood cardioplegia appears limited. The results of Korvald et al. [1] and the results of Hynninen et al. [21], who ineffectively used insulin supplementation under similar conditions in the clinical setting, support our argument.

In summary, Korvald et al. [1] demonstrate a potentially clinically significant, adverse effect of continuous warm blood cardioplegia, which was unexpected in its magnitude. The mechanism for this effect is not clear. Metabolic interventions under these conditions in the conventional way appear inappropriate which is supported by the lack of an effect in practice. If the suggested mechanism by Korvald et al. [1], of an intracellular increase in calcium is true, addition of drugs inhibiting this calcium overload may prevent this effect.

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