Ischemic preconditioning reduces unloaded myocardial oxygen consumption in an in-vivo sheep model

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Received 5 September 2001; accepted 21 January 2002

Abstract

Objective: Ischemic preconditioning (IP) describes the adaptation of the myocardium to ischemic stress preceded by short periods of ischemia and reperfusion. However, its cardioprotective mechanisms are not completely understood. We assessed the effect of IP on ventricular energetics in an in-vivo sheep model. Methods: IP was performed in six sheep by three 5 min aortic cross-clamping periods interspersed with 5 min of reperfusion during cardiopulmonary bypass and with six sheep as time-matched controls. Global myocardial ischemia was subsequently achieved by 30 min aortic cross-clamping with left ventricular unloading during normothermic cardiopulmonary bypass. Weaning from cardiopulmonary bypass was performed 40 min after reperfusion. At baseline, after treatment (IP or time-matched cardiopulmonary bypass), and up to 100 min after reperfusion, left ventricular pressure–volume loops were measured using a conductance catheter during a right heart bypass preparation. Contractility, diastolic function, and ventriculo–arterial coupling were evaluated. Ventricular energetics \[\text{the relation between myocardial oxygen consumption (MVO\textsubscript{2}) and systolic pressure–volume area (PVA)}\] was also evaluated. A right heart bypass was instituted to control the preload and to decompress the right ventricle completely, thereby eliminating parallel conductance variation and minimizing the contribution of the right ventricle to MVO\textsubscript{2}. Results: IP reduced unloaded MVO\textsubscript{2} (PVA-independent MVO\textsubscript{2}). Contractility, diastolic function, and ventriculo–arterial coupling in the IP group were better preserved than in the control group after ischemia-reperfusion. Conclusions: IP reduces unloaded MVO\textsubscript{2}, and preserves contractility, diastolic function, and ventriculo–arterial coupling after 30 min global myocardial ischemia in an in-vivo sheep model.

Keywords: Ischemia; Oxygen consumption; Preconditioning; Reperfusion; Ventricular function

1. Introduction

Ischemic preconditioning (IP) describes the adaptation of the myocardium to ischemic stress by short preceding periods of ischemia and reperfusion [1]. Initially, the cardioprotective effect of IP was reported as a reduction in infarct size in an open-chest anesthetized dog preparation [1], and the same protective effect has since been demonstrated in a variety of species both in in-vitro and in-vivo studies [2]. IP has also been shown to protect against arrhythmias [3] and contractile dysfunction [4,5]. The cardioprotective mechanisms of IP are not completely understood yet [2]. A better preservation of ATP in myocardium during the subsequent sustained ischemia has been reported as a possible mechanism [6,7]. Alterations of myocardial oxygen consumption (MVO\textsubscript{2}) after IP and during sustained ischemia may play an important role [8–10].

IP has been demonstrated in several animal species, and...
more than 2000 papers have been published in this field. However, most studies used small animals, and relatively few studies have been performed using an in-vivo large animal model [11–13], which might be closer to the clinical situation. We developed an in-vivo sheep right heart bypass (RHB) model, and assessed the effect of IP on left ventricular (LV) function and energetics.

2. Methods

All animals were treated in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1996). The study was approved by the Ethics Committee of the Katholieke Universiteit Leuven.

2.1. Preparation

Twelve sheep were randomly divided in two groups: the control group (group C, n=6, weight 57.5±3.3 kg) and the IP group (group IP, n=6, weight 54.5±3.5 kg). All sheep were premedicated with ketamine hydrochloride (10–20 mg / kg) intramuscularly and anesthetized with isoflurane (1–2%, v/v). The animal was placed in right lateral decubitus position, and ventilated mechanically after intubation.

A left lateral thoracotomy was performed through an incision at the third intercostal space. The heart was suspended in a pericardial cradle, and the hemiazygous vein was ligated. The heart–lung machine consisted of a centrifugal pump and a membrane oxygenator, primed with Ringer’s solution. Blood transfusion was not performed. After heparin (400 U/kg) was administered intravenously, a normothermic (37°C) cardiopulmonary bypass (CPB) was instituted with the venous return from the superior and inferior venae cavae, and arterial outflow through the left carotid artery. After CPB was instituted, mechanical ventilation was stopped, a second arterial outflow into the main pulmonary artery was constructed, and a RHB preparation was then established as previously described [14–16]. With this system, the flow can be directed to the aorta (CPB) or to the pulmonary artery (RHB) by clamping one of the arterial outflow cannulas (Fig. 1). The tapes around the superior and inferior venae cavae were snared to direct the systemic venous blood return into a reservoir, and the oxygenated blood was pumped back to the main pulmonary artery for RHB. The coronary venous return was drained by a right ventricular cannula, and the flow was measured by an in-line ultrasonic flow probe (Transonic Systems, New York, NY, USA). The aortic flow was measured by a 20Fr ultrasonic flow probe (Transonic Systems) around the ascending aorta. The pump flow was measured by an in-line ultrasonic flow probe (Transonic Systems).

To measure the LV volume, a 7F 12-electrode conductance catheter (CD Leycom, Zoetermeer, The Netherlands) was inserted into the left ventricle through the apex. The catheter was attached to a signal generator/processor (CD Leycom Sigma 5 DF). A Mikro-tip catheter pressure transducer (Millar Instruments, Houston, TX, USA) was inserted into the left ventricle to measure the LV pressure, and then the LV pressure–volume loops were measured. Two fluid-filled pressure lines were also inserted into the descending aorta and the left atrium for pressure measurement.

A RHB was instituted to control the LV venous return and to completely decompress the right ventricle, thereby eliminating parallel conductance variation and minimizing the contribution of the right ventricle to MVO₂. The volume signal of the conductance catheter was calibrated by the aortic flow and the coronary flow. The parallel
conductance volume was calculated by the hypertonic saline technique [17].

2.2. Experimental protocol

The experimental protocol is shown in Fig. 2. All assessments were performed under normothermic (37 °C) extracorporeal circulation (CPB or RHB). Arterial oxygen tension was maintained between 100 and 300 mmHg throughout the experiment. The flow was maintained minimally at 80 ml/kg per min and the mean arterial pressure was maintained above 60 mmHg by adjusting the flow during CPB. The RHB bypass flow was adjusted to keep the balance between the arterial pressure and the left atrial pressure.

IP was performed by three 5 min aortic cross-clamping periods interspersed with 5 min of reperfusion during CPB. In group C, 30 min CPB was performed (time-matched controls). Global myocardial ischemia was subsequently achieved by 30 min aortic cross-clamping with LV unloading during normothermic CPB (37 °C). LV venting was performed during aortic cross-clamping and early after reperfusion.

All signals (electrocardiogram, pressures, flows and volume) were continuously monitored with a cardiovascular setup consisting of a combination of transducers, amplifiers, and data acquisition software (Hugo Sachs Elektronik, March-Hugstetten, Germany). Signals were online digitized at 200 Hz, and recorded on a personal computer.

All animals were disconnected from CPB 40 min after reperfusion without inotropic or vaso-active agents. Conventional hemodynamic variables and LV function were assessed at baseline, immediately after treatment (IP or time-matched CPB), 20 min after treatment, and 40 min, 70 min, 100 min after reperfusion. Ventricular energetic parameters were assessed at baseline, after treatment, and from 80 to 100 min of after reperfusion. The heart rate was kept at 120 beats per min by right atrial pacing with an external pacemaker during the assessment period.

2.3. Conventional hemodynamic variables and left ventricular function

Conventional hemodynamic variables (mean aortic pressure, left atrial pressure, and cardiac output) and LV function (LV pressure–volume relationship) were assessed during RHB without inotropic or vaso-active agents at baseline, immediately after treatment, 20 min after treatment, and 40, 70, 100 min after reperfusion. Cardiac output was calculated from the aortic flow and the coronary flow. The multiple LV pressure–volume loops were obtained during transient preload reduction by reducing the RHB flow. The digitized data were analyzed by computer algorithms using a C-language-type program. LV contractility was evaluated by the end-systolic pressure–volume ($P_{es}$–$V_{es}$) relation [18] and the stroke work–end-diastolic volume (SW–$V_{ed}$) relation (preload recruitable stroke work) [19]. The $P_{es}$–$V_{es}$ relation was fitted by linear regression to obtain the slope $(E_a)$ as described by Kono et al. [20] and $V_{es}$ associated with $P_{es}$ of 100 mmHg ($V_{100,es}$). SW was plotted against $V_{ed}$ to obtain the slope $(M_{sw})$ and $V_{es}$ associated with SW of 2000 mmHg ml ($V_{2000,sw}$) of the SW–$V_{ed}$ relation. The end-diastolic point was defined as the point of the upstroke of the first derivative of the LV pressure (dP/dt). The time constant of isovolumic LV pressure relaxation ($\tau$) was also calculated as parameter of diastolic function [21]. The ventriculo–arterial coupling between the left ventricle and the arterial system was indexed by the ratio of the ventricular to arterial elastances ($E_v/E_a$) [22]. The effective arterial elastance ($E_a$) was calculated as: $P_{es}/(V_{ed}–V_{es})$ using the

Fig. 2. Experimental protocol. All assessments were performed under normothermic (37 °C) extracorporeal circulation (RHB or CPB). IP was performed by three 5 min aortic cross-clamping periods interspersed with 5 min of reperfusion. In group C, 30 min CPB was performed (time-matched controls). Global myocardial ischemia was subsequently achieved by 30 min aortic cross-clamping with LV unloading during normothermic CPB (37 °C). LV venting was performed during the aortic cross-clamping and early after reperfusion. Vertical arrows indicate the measurement points of conventional hemodynamic variables, and contractility, diastolic function, and ventriculo–arterial coupling. Horizontal arrows indicate the measurement periods of ventricular energetics.
2.4. Ventricular energetic parameters

Multiple steady-state pressure–volume loops, coronary flow, and arterio–venous oxygen difference data were obtained at various preload volumes for the assessment of ventricular energetics during RHB without inotropic or vaso-active agents at baseline, after treatment, and during 80 to 100 min after reperfusion. Five steady-state pressure–volume loops were measured for the analysis at each point. LV energetic parameters were assessed by the analysis of the relation between LV MVO and PV A as described by Suga [18]. Unloaded MVO₂ (PVA-independent MVO₂, the y-axis intercept of the MVO₂–PVA relation) and contractile efficiency (the reciprocal of the slope of the MVO₂–PVA relation) were then calculated. MVO₂ was calculated from the product of the coronary flow in ml/min and coronary arterio–venous oxygen difference in % (v/v). This quantity was divided by heart rate to yield oxygen consumption per beat, MVO₂, in ml O₂/beat. It was normalized with respect to the LV weight to give MVO₂ in ml O₂/beat per 100 g of the left ventricle. The oxyhemoglobin percent saturations of the coronary arterial and venous blood were measured with a pH/blood gas analyzer. PVA was normalized with respect to the LV weight and expressed in mmHg ml/beats per 100 g of the left ventricle.

2.5. Triphenyl tetrazolium chloride staining and histological evaluation

The hearts were immediately excised at the end of the experiment. The ventricles were cut into slices of 5 mm thick, parallel to the base of the heart. Slices were then incubated at 37°C for 10 min with a triphenyl tetrazolium chloride solution to visualize macroscopic areas of myocardial necrosis [24]. Biopsies of the LV anterior wall, lateral wall, and septum were fixed by immersion in 6% neutral buffered formalin, embedded in paraffin, sectioned into 4-μm slices, and stained with hematoxylin–eosin. An experienced observer examined the histological sections in a blinded fashion. Each specimen was evaluated for the presence of coagulation necrosis of the cytoplasm of the myocytes, interstitial edema, polymorphonuclear infiltrate, and hemorrhage.

2.6. Statistics

The results are presented as mean±standard deviation. Two-way analysis of variance with repeated measures on one factor (time) was used for the variables at six points (conventional hemodynamic variables and LV function) or three points (ventricular energetics). The Student–Newman–Keuls test was used as post hoc test.

3. Results

3.1. Recovery after ischemia-reperfusion

Immediately after reperfusion, all sheep required defibrillation in group C, whereas one sheep recovered in sinus rhythm and five sheep required defibrillation in group IP. All sheep were easily defibrillated immediately using an electric defibrillator. Weaning from CPB was possible in all sheep after ischemia-reperfusion without inotropic or vaso-active agents.

3.2. Conventional hemodynamic variables and left ventricular function

The changes of conventional hemodynamic variables are provided in Table 1. Left atrial pressure in group IP was lower than that in group C 100 min after reperfusion (p<0.05), and cardiac output in group IP was statistically significantly higher than that in group C throughout the reperfusion period.

Representative multiple LV pressure–volume loops of both groups are shown in Fig. 3. Parameters of LV systolic function were evaluated as SW/PV A efficiency [23]. Unloaded MVO₂ (PV A-independent MVO₂, the y-axis intercept of the MVO₂–PVA relation) and contractile efficiency (the reciprocal of the slope of the MVO₂–PVA relation) were then calculated. The changes of conventional hemodynamic variables are provided in Table 1.
significant higher than those of group C 70 min and 100 min after reperfusion. Although \( M_{SW} \) 40 min after reperfusion of both groups is significantly lower than those before ischemia, \( M_{SW} \) of group IP improved gradually up to 100 min after reperfusion. The pressure–volume relations of group IP shifted significantly toward the left in the operating range compared with those of group C throughout the reperfusion period, manifested by the differences in \( V_{100,es} \) and \( V_{2000,sw} \). Although at 40 and 70 min after reperfusion \( \tau \) of both groups was significantly higher than at baseline, \( \tau \) of group IP improved gradually while that of group C worsened gradually after ischemia-reperfusion. \( \tau \) of group IP was lower than that of group C 100 min after reperfusion (\( p<0.05 \)).

Parameters of ventriculo–arterial coupling are provided in Table 3. Although \( E_{es}/E_{a} \) 40 min after reperfusion of both groups was statistically significantly lower than those of baseline, \( E_{es}/E_{a} \) of group IP improved gradually after reperfusion, and the difference between the two groups in \( E_{es}/E_{a} \) reached statistical significance 70 and 100 min after reperfusion. SW/PVA of group IP decreased immediately after IP (\( p<0.05 \)), and the difference between the two groups at this point just failed to reach significance (\( p=0.073 \)). Although SW/PVA throughout the reperfusion period of both groups was significantly lower than those of baseline, SW/PVA of group IP was significantly better than that of group C throughout the reperfusion period.

### 3.3. Ventricular energetics

Parameters of ventricular energetics are provided in Fig. 4. Unloaded MVO\(_2\) of group IP decreased after IP (from 0.0399±0.0066 to 0.0295±0.0060 ml O\(_2\)/beat/100 g LV,

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**Table 2**

Parameters of left ventricular function in group C and group IP

<table>
<thead>
<tr>
<th></th>
<th>( E_{es} ) (mmHg/ml)</th>
<th>( V_{100,es} ) (ml)</th>
<th>( M_{sw} ) (mmHg)</th>
<th>( V_{2000,sw} ) (ml)</th>
<th>( \tau ) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.30±1.81</td>
<td>23.8±6.0</td>
<td>84.6±11.1</td>
<td>46.7±4.6</td>
<td>28.2±2.0</td>
</tr>
<tr>
<td>Treated 1</td>
<td>5.26±2.28</td>
<td>32.1±18.9</td>
<td>92.5±28.8</td>
<td>49.0±5.6</td>
<td>29.3±3.8</td>
</tr>
<tr>
<td>Treated 2</td>
<td>5.72±2.86</td>
<td>25.0±15.2</td>
<td>85.2±16.3</td>
<td>47.3±6.1</td>
<td>28.5±2.9</td>
</tr>
<tr>
<td>40 min</td>
<td>3.20±1.10</td>
<td>46.7±26.7*</td>
<td>42.5±16.5*</td>
<td>87.2±18.7*</td>
<td>44.5±13.5*</td>
</tr>
<tr>
<td>70 min</td>
<td>3.12±1.74</td>
<td>57.5±24.1*</td>
<td>32.7±18.4*</td>
<td>109.4±54.4*</td>
<td>46.1±9.3*</td>
</tr>
<tr>
<td>100 min</td>
<td>2.98±1.39</td>
<td>58.4±33.1*</td>
<td>32.4±12.0*</td>
<td>113.3±41.9*</td>
<td>52.9±13.8*</td>
</tr>
<tr>
<td>Group IP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.23±1.52</td>
<td>21.0±6.8</td>
<td>85.0±20.4</td>
<td>43.5±5.3</td>
<td>29.4±2.6</td>
</tr>
<tr>
<td>Treated 1</td>
<td>4.99±2.85</td>
<td>28.2±10.5</td>
<td>76.5±23.9</td>
<td>47.1±6.6</td>
<td>33.5±3.4</td>
</tr>
<tr>
<td>Treated 2</td>
<td>5.22±1.61</td>
<td>21.5±5.0</td>
<td>88.3±13.7</td>
<td>43.8±5.5</td>
<td>30.1±2.9</td>
</tr>
<tr>
<td>40 min</td>
<td>4.21±0.90</td>
<td>30.0±6.4*</td>
<td>52.0±21.7*</td>
<td>60.5±12.9*</td>
<td>48.5±5.6*</td>
</tr>
<tr>
<td>70 min</td>
<td>6.41±2.02*</td>
<td>24.1±7.8*</td>
<td>81.9±29.8*</td>
<td>49.9±9.2*</td>
<td>40.4±6.4*</td>
</tr>
<tr>
<td>100 min</td>
<td>6.80±2.10*</td>
<td>22.6±7.2*</td>
<td>83.4±19.2*</td>
<td>51.2±4.5*</td>
<td>37.3±5.4*</td>
</tr>
</tbody>
</table>

\( E_{es} \), Slope of left ventricular end-systolic pressure–volume relation; \( V_{100,es} \), end-systolic volume associated with end-systolic pressure of 100 mmHg; \( M_{sw} \), slope of left ventricular stroke work–end-diastolic volume relation; \( V_{2000,sw} \), end-diastolic volume associated with stroke work of 2000 ml mmHg; \( \tau \), time constant of isovolumic relaxation; baseline, before treatment (IP or time-matched CPB); treated 1, immediately after treatment; treated 2, 20 min after treatment; 40 min, 40 min after reperfusion; 70 min, 70 min after reperfusion; 100 min, 100 min after reperfusion. Data are presented as mean±standard deviation; * \( p<0.05 \) versus group C; † \( p<0.05 \) versus baseline; ‡ \( p<0.05 \) versus 40 min.
Table 3
Parameters of ventriculo–arterial coupling in group C and group IP

<table>
<thead>
<tr>
<th></th>
<th>$E_a$ (mmHg/ml)</th>
<th>$E_{sv}/E_a$</th>
<th>SW/PVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.30±0.75</td>
<td>2.37±0.95</td>
<td>0.745±0.091</td>
</tr>
<tr>
<td>Treated 1</td>
<td>2.68±1.20</td>
<td>2.12±0.84</td>
<td>0.720±0.066</td>
</tr>
<tr>
<td>Treated 2</td>
<td>2.86±0.98</td>
<td>2.00±0.99</td>
<td>0.696±0.069</td>
</tr>
<tr>
<td>40 min</td>
<td>5.40±2.34</td>
<td>0.62±0.26</td>
<td>0.356±0.054</td>
</tr>
<tr>
<td>70 min</td>
<td>4.18±1.93</td>
<td>0.71±0.39</td>
<td>0.360±0.127</td>
</tr>
<tr>
<td>100 min</td>
<td>4.60±2.40</td>
<td>0.75±0.55</td>
<td>0.330±0.156</td>
</tr>
<tr>
<td>Group IP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.83±0.74</td>
<td>2.35±1.31</td>
<td>0.743±0.064</td>
</tr>
<tr>
<td>Treated 1</td>
<td>3.28±0.59</td>
<td>1.66±0.95</td>
<td>0.607±0.082</td>
</tr>
<tr>
<td>Treated 2</td>
<td>3.16±0.66</td>
<td>1.97±0.90</td>
<td>0.636±0.083</td>
</tr>
<tr>
<td>40 min</td>
<td>4.48±1.77</td>
<td>1.13±0.46</td>
<td>0.515±0.079</td>
</tr>
<tr>
<td>70 min</td>
<td>4.14±1.53</td>
<td>1.89±1.17*</td>
<td>0.590±0.083 *</td>
</tr>
<tr>
<td>100 min</td>
<td>3.98±1.11</td>
<td>1.98±1.03*</td>
<td>0.569±0.069 *</td>
</tr>
</tbody>
</table>

$E_a$, Effective arterial elastance; $E_{sv}/E_a$, ventriculoarterial coupling; SW, stroke work; PVA, systolic pressure–volume area; baseline, before treatment (IP or time-matched CPB); treated 1, immediately after treatment; treated 2, 20 min after treatment; 40 min, 40 min after reperfusion; 70 min, 70 min after reperfusion; 100 min, 100 min after reperfusion. Data are presented as mean±standard deviation; *, $p<0.05$ versus group C; †, $p<0.05$ versus baseline.

3.4. Triphenyl tetrazolium chloride staining and histological evaluation

In none of the hearts, macroscopic areas of necrosis were visible by the triphenyl tetrazolium chloride staining. Histologically, ischemic myocardial injury in all sheep of group C was more severe compared with group IP. In particular, moderate interstitial hemorrhage was invariably present in group C, whereas only rare and very mild in group IP. Polymorphonuclear infiltrate was associated with interstitial edema and also more pronounced in the former group. Coagulation necrosis of myocytes was only met twice in hearts of group C, but absent in IP hearts. Necrosis was limited to individual cells.

4. Discussion

The major findings of the present study are as follows: (1) unloaded $\text{MO}_2$ decreased after IP, and reverted to the baseline after reperfusion in the IP sheep, whereas unloaded $\text{MO}_2$ remained unchanged after time-matched CPB, and decreased after reperfusion in the control sheep; (2) contractile efficiency of the IP sheep did not change after IP and after reperfusion, whereas that of the control sheep did not change after time-matched CPB, and decreased after reperfusion; (3) contractility, diastolic function, and ventriculo–arterial coupling after reperfusion of the IP sheep were all better preserved than those of the control sheep, although SW/PVA efficiency was temporally worse immediately after IP; (4) IP diminished myocardial ischemic damage as histologically seen. To the best of our knowledge, this is the first study to demonstrate the
influence of IP on ventricular energetics, \( \text{MVO}_2 \)-PVA relation, in an in-vivo large animal model.

### 4.1. Ventricular energetics and ischemic preconditioning

IP has been demonstrated to induce adaptation of the myocardium to ischemic stress [1,6]. From the viewpoint of a better ATP preservation in myocardium during the subsequent sustained ischemia after IP, some reports directly investigated the effect of IP on \( \text{MVO}_2 \). Grund et al. reported that \( \text{MVO}_2 \) decreased after IP using open-chest pigs [8]. Xu et al. reported that the reduction of \( \text{MVO}_2 \) in the IP heart persisted during sustained ischemia by the measurement of tissue oxygenation using spectrophotometry in isolated rat hearts [9]. However, their assessments of \( \text{MVO}_2 \) were performed in a load-dependent manner, and influenced by ventricular mechanical activity. Ventricular energetics based on the concept of PVA has the advantage to enable the analysis of \( \text{MVO}_2 \) in a load-independent manner [18].

With respect to the \( \text{MVO}_2 \)-PVA framework, Suzuki et al. have reported a reduction in unloaded \( \text{MVO}_2 \) after IP using isolated canine hearts with a servo-pump system [10]. Contractile efficiency was unchanged after IP in their study. The present in-vivo study found effects of IP compatible with Suzuki et al.’s in vitro results. Furthermore, the assessment in the present study was performed not only after IP but also after sustained 30 min ischemia and reperfusion, although Suzuki et al.’s assessment was performed only after IP and not after the subsequent, sustained ischemia. In the present study, decreased unloaded \( \text{MVO}_2 \) reverted to the baseline after ischemia-reperfusion, whereas unloaded \( \text{MVO}_2 \) in the control sheep remarkably decreased after ischemia-reperfusion. Contractile efficiency in the IP sheep did not change throughout the experiment, whereas that in the control sheep decreased after ischemia-reperfusion. The reduction of unloaded \( \text{MVO}_2 \) after IP might be instrumental in the cardioprotective effect induced by IP on myocardium for subsequent sustained ischemic stress. The decrease of unloaded \( \text{MVO}_2 \) after 30 min ischemia in the control sheep might be an indication of a decreased number of viable myocardial cells. These results suggest that IP preserved the ventricular energetics after sustained 30 min ischemia.

#### 4.2. Significance of the reduction of unloaded myocardial oxygen consumption after ischemic preconditioning

Unloaded \( \text{MVO}_2 \), PVA-independent \( \text{MVO}_2 \), consists of non-mechanical energy expenditure for excitation–contraction (E–C) coupling and basal metabolism of myocardium [18]. E–C coupling might decrease after IP, because contractility also decreased after IP, even though the differences did not reach statistical significance. However, if only E–C coupling decreased, it is difficult to explain why the preconditioned hearts showed a superior post-ischemic cardiac function as compared to the control heart. We speculate that the reduction of unloaded \( \text{MVO}_2 \) after IP represented a reduction not only in E–C coupling but also in basal metabolism of myocardium. The reduction of basal myocardial metabolism induced by IP might lead to better preservation of ATP in myocardium during the subsequent sustained ischemia, and to preserved LV contractility, diastolic function, and ventriculo–arterial coupling after ischemia-reperfusion. Although many questions remain unanswered regarding the exact mechanism for the cardioprotective effect of IP, the reduction of \( \text{MVO}_2 \) after IP might be one of the most important mechanisms of IP-induced cardioprotection as demonstrated in this study.

### 4.3. Left ventricular function and ischemic preconditioning

In the present study, hearts underwent 30 min unloaded normothermic global ischemia, which caused severe myocardial damage as manifested by cardiac dysfunction and myocardial ischemic damage (histological findings) after ischemia-reperfusion in the control group. IP improved contractility, diastolic function, and ventriculo–arterial coupling after ischemia-reperfusion, and diminished myocardial ischemic damage. However, contractility and diastolic function immediately after IP temporarily worsened, even though the differences did not reach statistical significance. SW/PVA efficiency significantly decreased immediately after IP. The transient depression after IP has already been reported in previous studies [1,10]. It is still unknown whether this mild cardiac dysfunction after IP is instrumental in the cardioprotective effect of IP.

### 4.4. Right heart bypass model with a left ventricular conductance catheter

We developed an in-vivo sheep model to test the effect of IP on ventricular energetics and global post-ischemic myocardial dysfunction, with accurate measurements of the LV pressure–volume relationship by right ventricular unloading using a RHB model. Because the right ventricle was decompressed completely in this model, the parallel conductance variation and the contribution of the right ventricle to \( \text{MVO}_2 \) were minimized. A RHB model with a conductance catheter in the LV cavity is a useful model for the in-vivo assessment of ventricular energetics, which is usually performed in an isolated heart preparation with a servo-pump system [10,18]. A RHB model is an in-vivo model, which has the possibility of accurate and detailed hemodynamic measurements comparable with an in-vitro model. Autonomic reflexes can have an influence on the measurement of LV function and energetics in this in-vivo model. Autonomic blockade was not induced in this study.
because agents to induce this such as hexamethonium bromide may have some influence on the IP effect [25].

Although normothermic myocardial global ischemia is unlikely in the clinical setting, we wanted to study a model with significant ischemic damage not limited to pure myocardial stunning, a situation encountered after prolonged aortic cross-clamp times. Nevertheless, the application of these findings to the clinical situation requires some caution. First, the hearts used for this study were initially normal. Further study using failing or chronically ischemic hearts is warranted to manifest the therapeutic effects of IP on diseased hearts. Secondly, open-chest sheep under general anesthesia were used. The anesthetic management itself has the potential of substantially altering vascular responses. Furthermore, the influence of an extracorporeal circulation on cardiac function and vascular capacitance should be taken into consideration [26]. Nevertheless, the RHB model used in this study is useful for a detailed evaluation of in-vivo hemodynamics in a large animal model, possibly mimicking the clinical situation closer than in vitro studies or experiments performed in small rodents.

5. Conclusions

We developed a sheep RHB model with a conductance catheter in the LV cavity, which enabled the assessment of in-vivo LV function and energetics. In this model, IP reduced unloaded MVO₂, preserved LV contractility, diastolic function, and ventriculo–arterial coupling, and diminished histological myocardial ischemic damage after 30 min global myocardial ischemia. The reduction of MVO₂ after IP suggests an effect of IP on both E–C coupling and basal metabolism of myocardium. This reduction of MVO₂ after IP might be an important mechanism of IP-induced cardioprotection as demonstrated in this study.

Acknowledgements

This study was supported by a Research Grant of the Fund for Scientific Research—Flanders (Belgium) (F.W.O.-Vlaanderen, KAN2001-1.5.057.01).

References

