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Does lung ischemia and reperfusion have an impact on coronary flow? A quantitative coronary blood-flow analysis with inflammatory cytokine profile[†]

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Abstract

OBJECTIVE: Ischemia-reperfusion (IR) injury remains a major cause of early morbidity and mortality after lung transplantation with poorly documented extrapulmonary repercussions. To determine the hemodynamic effect due to lung IR injury, we performed a quantitative coronary blood-flow analysis in a swine model of *in situ* lung ischemia and reperfusion.

METHODS: In 14 healthy pigs, blood flow was measured in the ascending aorta, left anterior descending (LAD), circumflex (Cx), right coronary artery (RCA), right common carotid artery (RCCA), and left internal mammary artery (LIMA), along with left- and right-ventricular pressures (LVP and RVP), aortic pressure (AoP), and pulmonary artery pressure (PAP). Cardiac Troponin (cTn), interleukin 6 and 10 (IL-6 and IL-10), and tumor necrosis factor A (TNF-A) were measured in coronary sinus blood samples. The experimental (IR) group (n = 10) underwent 60 min of lung ischemia followed by 60 min of reperfusion by clamping and releasing the left pulmonary hilum. Simultaneous measurements of all parameters were made at baseline and during IR. The control group (n = 4) had similar measurements without lung IR.

RESULTS: In the IR group, total coronary flow (TCF = LAD + Cx + RCA blood-flow) decreased precipitously and significantly from baseline ($113 \pm 41 \text{ ml min}^{-1}$) during IR ($p < 0.05$), with the lowest value observed at 60 min of reperfusion (-37.1% , $p < 0.003$). Baseline cTn ($0.08 \pm 0.02 \text{ ng ml}^{-1}$) increased during IR and peaked at 45 min of reperfusion ($+138\%$, $p < 0.001$). Baseline IL-6 ($9.2 \pm 2.17 \text{ pg ml}^{-1}$) increased during IR and peaked at 60 min of reperfusion ($+228\%$, $p < 0.0001$). Significant LVP drop at 5 min of ischemia ($p < 0.05$) was followed by a slow return to baseline at 45 min of ischemia. A second LVP drop occurred at reperfusion ($p < 0.05$) and persisted. Conversely, RVP increased throughout ischemia ($p < 0.05$) and returned toward baseline during reperfusion. Coronary blood flow and hemodynamic profile remained unchanged in the control group. IL-10 and TNF-A remained below the measurable range for both the groups.

CONCLUSIONS: *In situ* lung IR has a marked negative impact on coronary blood flow, hemodynamics, and inflammatory profile. In addition, to the best of our knowledge, this is the first study where coronary blood flow is directly measured during lung IR, revealing the associated increased cardiac risk.

Keywords: Lung • Ischemia • Reperfusion • Coronary flow • Cytokines

INTRODUCTION

Although patients undergoing non-cardiac thoracic surgery and lung transplantation undergo preoperative evaluation of their cardiac function in an effort to minimize postoperative adverse - cardiac-related - events, such events are not infrequent. The extent and necessary scope of the preoperative cardiac

evaluation are variable. Incorporation of aggressive preoperative testing including treadmill stress test, dobutamine stress echo, nuclear stress test, and coronary angiography leading to corrective cardiac interventions does not completely mitigate the risk of postoperative cardiac events [1]. Previously recognized cardiac risk indices and preoperative evaluation algorithms for patients undergoing non-cardiac thoracic surgical procedures [2–5] need to be recalibrated to better address this difficult issue [6].

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This study aimed to investigate the impact of the *in situ* lung ischemia and subsequent reperfusion in the healthy heart by directly measuring the flow in each coronary artery, along with resultant hemodynamic and inflammatory profile changes.

MATERIALS AND METHODS

Fourteen healthy, adult, male Landrace pigs with an average body weight of 69 ± 5 kg were randomly assigned to two groups: ischemic or control. Random allocation was achieved using 14 identical envelopes. Ten contained cards that read 'ischemic' and four contained cards that read 'control'. Prior to each procedure, a random envelope was opened, designating the assignment of that animal to the appropriate study group.

In the study group ($n = 10$), the animals underwent 1 h of *in situ* left-lung ischemia followed by 1 h of reperfusion by clamping and releasing the left pulmonary hilum, respectively. The control group ($n = 4$) underwent the same surgical manipulation as the study group except for left-lung ischemia and reperfusion.

Anesthesia

The animals were sedated with a Telazol/xylazine/ glucose mixture. After endotracheal intubation, anesthesia was maintained by isoflurane mixed with oxygen and room air (Siemens Servo Ventilator 900C, Siemens – Elema, Sweden). Electrocardiography (ECG) electrodes, saturation of peripheral oxygen (SpO_2) monitor, and a temperature probe were placed and recorded. Blood gases were periodically checked. Body temperature, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), and pH remained within the normal ranges throughout the course of each experiment.

Surgical procedure

Left (LCCA) and right common carotid arteries (RCCA) and left and right jugular veins were dissected free. Introducer sheaths were placed into the LCCA, the left and right jugular veins, and the right femoral artery and secured. A 6-French (F) pigtail catheter filled with 0.9% normal saline was introduced into the LCCA sheath and advanced into the left ventricle (LV) to measure left-ventricular pressure (LVP). A 5-F pigtail catheter filled with 0.9% normal saline was introduced into the right jugular vein sheath and advanced into the right ventricle (RV) to measure right-ventricular pressure (RVP). Central venous pressure (CVP) was monitored throughout the course of the experiment through the side arm of the right jugular vein sheath. A Swan–Ganz catheter was introduced into the left jugular vein sheath and advanced into the pulmonary artery (PA) to measure pulmonary artery pressure (PAP). A 6-F pigtail catheter filled with 0.9% normal saline was introduced into the right femoral sheath and advanced into the aorta to measure aortic pressure (AoP). The aortic, LV, and RV pigtail catheters and the Swan-Ganz catheter were connected to pressure transducers. A perivascular flow probe (MediStim, Maple Grove, MN, USA) was placed around the circumferentially dissected RCCA to measure RCCA flow (RCCAF).

After median sternotomy and pericardiotomy, the ascending aorta along with the left anterior descending (LAD), circumflex artery (Cx), right coronary artery (RCA), and left internal

Figure 1: Quantitative coronary flow analysis model with six blood flows simultaneously measured. LAD: left anterior descending, Cx: circumflex artery, RCA: right coronary artery, RCCA: right common carotid artery, LIMA: left internal mammary artery.

mammary artery (LIMA) were circumferentially dissected free on the beating heart, and perivascular flow probes (MediStim, Maple Grove, MN, USA) were placed around them to measure blood flow (Fig. 1). A 15-F retrograde cardioplegia catheter (DLP Retrograde Cannula; Medtronic, Minneapolis, MN, USA) was inserted into the right atrium and advanced and secured into the coronary sinus (CS), for CS blood sampling. The left azygos vein was ligated to prevent it from draining into the CS.

A left pleural incision was made and the left hilum was dissected and prepared for clamping to induce left-lung ischemia for 60 min. At the end of that time, left-lung reperfusion was permitted by releasing the left hilar clamp (Fig. 2).

Simultaneous recordings of all the aforementioned parameters were made at baseline and at 5, 15, 30, 45, and 60 min of ischemia and also at 5, 15, 30, 45, and 60 min of reperfusion. At each time point that recordings were made, blood was drawn from the CS to measure cardiac Troponin (cTn), interleukin 6 (IL-6), interleukin 10 (IL-10), and tumor necrosis factor A (TNF-A). After the final recordings at 60 min of reperfusion, the animals were sacrificed by injection of Sleepaway euthanasia solution (Fort Dodge Animal Health, Iowa, USA).

Hemodynamic monitoring

The ECG, LVP, RVP, AoP, and PAP and the six arterial flows were simultaneously displayed in real time and stored for offline analysis to two multichannel pressure and flow monitors (VeriQ model 4122, MediStim, Maple Grove, MN, USA). The Doppler principle was applied for real-time flow measurements (transit-time flow measurements, TTF) sampled at 0.3–1 kHz. TT was measured for each pulse, with the difference in TT between the pulses going upstream and downstream being proportional to the volume passing through the flow probe. Maximum, minimum, and mean values of flow were displayed in real time and stored for offline analysis. The pressure channels were sampled at 250

Hz. Here again, the maximum, minimum, and mean values were displayed in real time and stored for off-line analysis.

Biochemical analysis

Enzyme-linked immuno-sandwich assays (ELISAs) were performed on cell-free plasma samples collected from each animal at baseline and at each time point over the course of lung ischemia and lung reperfusion. The assays were performed according to manufacturers' recommendations with commercially available kits, including standards and samples in duplicate. The IL-6,

IL-10, and TNF- α kits were purchased from R&D Systems (Minneapolis, MN, USA). The cTn assay was purchased from Life Diagnostics (Westchester, PA, USA). Data were acquired on a Spectra Max Plus-384 plate reader and analyzed with the manufacturer's included software (Sunnyvale, CA, USA).

Statistical analysis and regulatory matters

Descriptive statistics for all measurements are reported as mean $\pm$ SD or as a proportion. Within-group changes of measurements from baseline to 60 min of reperfusion were tested using paired *t*-tests, whereas the change between groups was compared using repeated measurements analysis of variance (ANOVA). All statistical tests were two-sided, with the alpha level set at 0.05 for statistical significance. SAS9.1 was the software used for statistical analysis.

This study conformed to the 'Position of the American Heart Association on Research Animal Use', adopted by the American Heart Association (AHA) on 11 November 1984 and was approved by the Mayo Clinic Institutional Animal Care and Use Committee.

RESULTS

Hemodynamic data

Total coronary flow (TCF) was calculated as the sum of LAD flow (LADF) plus Cx flow (CxF) plus RCA flow (RCAF) for each time point of the study ($TCF = LADF + CxF + RCAF$). Cardiac output (CO) was calculated as the sum of aortic flow (AoF) plus TCF for each time point of the study ($CO = AoF + TCF$). CVP remained constant throughout the course of each experiment. No difference in baseline hemodynamic profile was observed between the study group ($n = 10$) and the control group ($n = 4$).

Study group. The hemodynamic changes for the study group compared with baseline are presented in Table 1. Significant CO drop following induction of left-lung ischemia was observed at 5 and 15 min of ischemia, followed by a subsequent significant increase at 30 and 45 min of ischemia. A

Figure 2: In our model of *in situ* lung ischemia-reperfusion the left pulmonary hilum is clamped with the use of a flexible clamp to induce left lung ischemia. Release of the clamp without further surgical manipulations results in the initiation of reperfusion. RV: right ventricle, LV: left ventricle.

Table 1: Hemodynamic changes for the study group (except coronary flow) ($n = 10$)

	CO	HR	RCCAF	LIMAF	LVP-S	LVP-M	RVP-S	RVP-M	AoP-S	AoP-M	PAP-S	PAP-M
B	2.97 $\pm$ 0.81	81 $\pm$ 10	390 $\pm$ 87	72 $\pm$ 19	83 $\pm$ 16	37 $\pm$ 9	30 $\pm$ 8	14 $\pm$ 3	84 $\pm$ 12	61 $\pm$ 12	30 $\pm$ 7	22 $\pm$ 5
I-5	2.70 $\pm$ 0.84	82 $\pm$ 11 ¹	335 $\pm$ 90	56 $\pm$ 19	80 $\pm$ 19	35 $\pm$ 11*	36 $\pm$ 10	17 $\pm$ 4	79 $\pm$ 16*	57 $\pm$ 13*	34 $\pm$ 11	24 $\pm$ 7
I-15	2.67 $\pm$ 0.57	81 $\pm$ 12*	324 $\pm$ 78	54 $\pm$ 17	78 $\pm$ 20	36 $\pm$ 9*	34 $\pm$ 9	17 $\pm$ 3	76 $\pm$ 15	55 $\pm$ 14	35 $\pm$ 8	25 $\pm$ 6
I-30	2.93 $\pm$ 1.04*	84 $\pm$ 12*	333 $\pm$ 85	56 $\pm$ 20	79 $\pm$ 17	36 $\pm$ 9*	33 $\pm$ 8	17 $\pm$ 3	77 $\pm$ 15	56 $\pm$ 12*	34 $\pm$ 8	23 $\pm$ 6
I-45	2.87 $\pm$ 1.31*	83 $\pm$ 10*	335 $\pm$ 112	59 $\pm$ 27*	82 $\pm$ 24*	35 $\pm$ 9*	35 $\pm$ 7	17 $\pm$ 3	82 $\pm$ 22*	56 $\pm$ 16*	35 $\pm$ 8	23 $\pm$ 5
I-60	2.63 $\pm$ 1.04	86 $\pm$ 15*	303 $\pm$ 84	50 $\pm$ 18	72 $\pm$ 12	34 $\pm$ 9*	32 $\pm$ 8	16 $\pm$ 3	71 $\pm$ 14	51 $\pm$ 12	34 $\pm$ 7	22 $\pm$ 5
R-5	2.86 $\pm$ 0.95*	85 $\pm$ 14*	318 $\pm$ 58	54 $\pm$ 17	74 $\pm$ 9	34 $\pm$ 7*	30 $\pm$ 8*	14 $\pm$ 3*	76 $\pm$ 9	52 $\pm$ 6	32 $\pm$ 12*	18 $\pm$ 5
R-15	2.88 $\pm$ 0.85*	86 $\pm$ 12*	308 $\pm$ 52	55 $\pm$ 12	72 $\pm$ 11	33 $\pm$ 7*	27 $\pm$ 7	13 $\pm$ 3*	69 $\pm$ 10	49 $\pm$ 8	27 $\pm$ 7*	18 $\pm$ 5
R-30	2.78 $\pm$ 0.79	84 $\pm$ 12*	286 $\pm$ 53	49 $\pm$ 11	69 $\pm$ 10	32 $\pm$ 7	27 $\pm$ 8	14 $\pm$ 3*	68 $\pm$ 10	47 $\pm$ 8	27 $\pm$ 5*	18 $\pm$ 5
R-45	2.65 $\pm$ 0.75	84 $\pm$ 12*	275 $\pm$ 57	47 $\pm$ 12	67 $\pm$ 12	31 $\pm$ 7	28 $\pm$ 8*	14 $\pm$ 3*	64 $\pm$ 13	46 $\pm$ 9	26 $\pm$ 6*	17 $\pm$ 4
R-60	2.56 $\pm$ 0.72	83 $\pm$ 11*	255 $\pm$ 57	43 $\pm$ 11	64 $\pm$ 12	29 $\pm$ 7	27 $\pm$ 7	14 $\pm$ 3*	61 $\pm$ 12	43 $\pm$ 9	27 $\pm$ 7*	19 $\pm$ 5

Data show mean $\pm$ SD. B: baseline, I: ischemia, R: reperfusion, CO: cardiac output, HR: heart rate, RCCAF: right common carotid artery flow, LIMAF: left internal mammary artery flow, LVP-S: systolic left ventricular pressure, LVP-M: mean left ventricular pressure, RVP-S: systolic right ventricular pressure, RVP-M: mean right ventricular pressure, AoP-S: systolic aortic pressure, AoP-M: mean aortic pressure, PAP-S: systolic pulmonary pressure, PAP-M: mean pulmonary pressure.

*No significant change ($p < 0.05$) compared to baseline (B).

Figure 3: Blood flow and pressure measurements during *in situ* left lung ischemia and reperfusion. Plots show mean $\pm$ SD for each time point. I: Ischemia, R: reperfusion (I and R time in minutes), TCF: total coronary flow, LAD: left anterior descending, Cx: circumflex artery, RCA: right coronary artery, CO: cardiac output, RCCA: right common carotid artery, LIMA: left internal mammary artery, LVP-S: systolic left ventricular pressure, LVP-M: mean left ventricular pressure, RVP-S: systolic right ventricular pressure, RVP-M: mean right ventricular pressure.

second significant CO drop at 60 min of ischemia was followed again by a subsequent significant increase at 5 and 15 min of reperfusion. A third significant CO drop at 30 min of reperfusion persisted (Fig. 3(e)).

TCF changes for the study group compared with baseline are presented in Table 2. In contrast with the CO, TCF

dropped significantly from baseline throughout the course of the study, with the lowest value observed at 60 min of reperfusion (Fig. 3(a)). LADF, CxF, and RCAF along with RCCAF and LIMA flow had the same pattern of decrease as seen with TCF throughout the course of the study (Fig. 3(b), (c), (d), and (f), respectively).

Table 2: Coronary flow changes for the study group ($n = 10$)

	TCF ml/min	%	<i>p</i> value	LADF ml/min	%	<i>p</i> value	CxF ml/min	%	<i>p</i> value	RCAF ml/min	%	<i>p</i> value
B	113 ± 41	100		41 ± 18	100		26 ± 14	100		46 ± 15	100	
I-5	86 ± 40	-23.9	<0.001	32 ± 17	-22	<0.0001	21 ± 16	-19.2	0.0026	33 ± 13	-28.3	0.0004
I-15	78 ± 27	-31	<0.001	31 ± 17	-24.4	0.0022	17 ± 7	-34.6	0.0015	30 ± 9	-34.8	0.001
I-30	88 ± 40	-22.1	<0.001	32 ± 14	-22	0.0016	22 ± 16	-15.4	0.08	34 ± 13	-26.1	0.036
I-45	91 ± 50	-19.5	0.034	32 ± 13	-22	0.011	24 ± 22	-7.7	0.13	35 ± 17	-23.9	0.069
I-60	78 ± 39	-31	0.006	28 ± 10	-31.7	0.031	20 ± 18	-23.1	0.003	30 ± 14	-34.8	0.009
R-5	80 ± 34	-29.2	0.019	29 ± 10	-29.3	0.039	21 ± 16	-19.2	0.0026	30 ± 11	-34.8	0.012
R-15	86 ± 39	-23.9	0.04	30 ± 11	-26.8	0.039	21 ± 15	-19.2	0.002	35 ± 19	-23.9	0.07
R-30	77 ± 38	-31.9	0.003	27 ± 10	-34.1	0.003	20 ± 17	-23.1	0.003	30 ± 15	-34.8	0.0057
R-45	73 ± 37	-35.4	0.003	26 ± 9	-36.6	0.003	19 ± 17	-26.9	0.002	28 ± 14	-39.1	0.0017
R-60	71 ± 36	-37.1	0.003	26 ± 10	-36.6	0.003	18 ± 17	-30.8	0.0019	27 ± 13	-41.3	0.0014

Data show mean ± SD. %: percentage of change from baseline, I: ischemia, R: reperfusion, B: baseline, TCF: total coronary flow, LADF: left anterior descending flow, CxF: circumflex artery flow, RCAF: right coronary artery flow.

Significant left-ventricular systolic pressure (LVP-S) drop at 5 min of ischemia was followed by a slow return to baseline at 45 min of ischemia. A second significant drop at 60 min of ischemia persisted. Left-ventricular mean pressure (LVP-M) remained unaffected throughout ischemia and for the first 15 min of reperfusion, and then dropped significantly at 30 min of reperfusion until the end of the study (Fig. 3(g)). Right-ventricular systolic pressure (RVP-S) increased significantly throughout ischemia and returned toward baseline at 5 min of reperfusion. A further significant RVP-S drop occurred at 15, 30, and 60 min of reperfusion. Right-ventricular mean pressure (RVP-M) increased significantly throughout ischemia and returned toward baseline throughout reperfusion (Fig. 3(h)).

Control group. No hemodynamic changes were observed between baseline and each time point of the study for the entire control group (Table 3).

Inflammatory cytokine profile

Inflammatory cytokine profile changes are summarized in Table 4. For both the study and control groups, IL-10 and TNF- α remained below the measurable range for all the time points of the study. Differences in baseline values between the study and the control group were observed for cTn and IL-6, although absolute values were very low. To assist with interpretation of the inflammatory cytokine profile analysis, Δ cTn and Δ IL-6 were calculated for each time point of the study as follows:

Δ cTn = Study cTn - Control cTn; Δ IL-6 = Study IL-6 - Control IL-6.

Both Δ cTn and Δ IL-6 increased from baseline and throughout the course of ischemia and reperfusion (Fig. 4(a)) with the synchronous decrease in the TCF (Fig. 4(b) and (c), respectively).

DISCUSSION

Direct measurement of coronary blood flow in healthy coronary arteries was used in this study to reveal the physiologic and hemodynamic effect on the heart that is associated with extracardiac *in situ* ischemia and reperfusion of the lung. In our

quantitative coronary-flow-analysis model, TTF is applied for flow measurements. During coronary artery bypass graft surgery (CABG), TTF measurements offer an objective evaluation of the peripheral anastomoses, making this methodology the gold standard of flow measurement for arterial and venous grafts. The predictive value of TTF measurements on the postoperative outcome and especially on mortality post CABG has been documented [7].

It has been previously shown that changes in the inflammatory profile are observed following myocardial ischemia and reperfusion and following lung ischemia and reperfusion [8–10]. In addition, in cardiac surgery patients, the postoperative cTn release is associated with short and midterm all-cause mortality [8].

The injury and resultant negative impact of lung ischemia and reperfusion on the pulmonary tissue during lung transplantation has been well documented and it is associated with significant morbidity [9]. Significant elevation of IL-6 in the bronchoalveolar lavage during the first few hours after reperfusion of the pulmonary graft is directly related to the development of primary graft dysfunction [10]. After myocardial ischemia and reperfusion, IL-6 is also associated with changes of the mean diastolic coronary flow and with alterations in myocardial microvascular integrity, and may be used as a predictor of myocardial dysfunction [11].

The present study shows that *in situ* lung ischemia and lung reperfusion both result in significant reduction of coronary flow in all coronary arteries along with a drop in both the LVP and the RVP. Further, arterial flow in extracardiac vessels (CCA and in LIMA) is reduced in a similar pattern as is seen in the change in flow to coronary circulation.

A possible pathophysiologic mechanism that could explain the reduced hemodynamics and coronary flow during *in situ* lung ischemia is the following: hilar clamping leads to reduced atrial filling, as a portion of circulating volume remains sequestered in the ischemic lung resulting in reduced LV filling leading to reduced CO and reduced coronary flow. Even though the left atrial pressure was not measured in our experiments, the significantly reduced LVP-S measured at 5 min of *in situ* left-lung ischemia is supportive of the aforementioned possible mechanism.

During ischemia, the auto regulatory homeostatic mechanisms of the healthy animal lead to partial recovery, as observed by

Table 3: Hemodynamic changes for the control group ($n = 4$)

	CO	HR	LADF	CxF	RCAF	TCF	RCCAF	LIMAF	LVP-S	LVP-M	RVP-S	RVP-M	AoP-S	AoP-M	PAP-S	PAP-M
B	2.75 ± 0.79	79 ± 11	42 ± 16	25 ± 15	44 ± 17	111 ± 42	410 ± 78	74 ± 17	84 ± 17	36 ± 9	30 ± 7	14 ± 4	86 ± 10	62 ± 13	31 ± 8	23 ± 6
5	2.74 ± 0.77*	78 ± 10*	40 ± 17*	26 ± 13*	43 ± 18*	109 ± 41*	412 ± 82*	74 ± 17*	83 ± 17*	37 ± 8*	30 ± 8*	14 ± 3*	86 ± 8*	62 ± 13*	31 ± 8*	23 ± 6*
15	2.75 ± 0.81*	78 ± 11*	42 ± 16*	26 ± 15*	44 ± 16*	112 ± 42*	410 ± 81*	73 ± 18*	84 ± 18*	36 ± 8*	29 ± 8*	14 ± 3*	84 ± 9*	61 ± 11*	30 ± 8*	23 ± 5*
30	2.76 ± 0.82*	80 ± 12*	41 ± 16*	24 ± 12*	42 ± 16*	107 ± 41*	408 ± 77*	71 ± 19*	82 ± 16*	35 ± 9*	30 ± 9*	13 ± 4*	85 ± 9*	62 ± 11*	31 ± 7*	22 ± 6*
45	2.73 ± 0.79*	78 ± 10*	39 ± 15*	26 ± 14*	43 ± 15*	108 ± 42*	409 ± 80*	73 ± 19*	84 ± 17*	35 ± 7*	28 ± 8*	13 ± 3*	86 ± 9*	63 ± 12*	30 ± 7*	23 ± 6*
60	2.74 ± 0.81*	78 ± 9*	41 ± 17*	27 ± 13*	45 ± 15*	113 ± 43*	408 ± 82*	70 ± 18*	84 ± 16*	36 ± 7*	29 ± 8*	14 ± 3*	86 ± 9*	62 ± 10*	30 ± 6*	23 ± 4*
1 h, 5	2.74 ± 0.80*	76 ± 11*	40 ± 14*	25 ± 16*	45 ± 16*	110 ± 41*	410 ± 79*	72 ± 17*	82 ± 17*	37 ± 8*	30 ± 7*	14 ± 4*	84 ± 10*	61 ± 11*	31 ± 7*	22 ± 6*
1 h, 15	2.76 ± 0.78*	77 ± 9*	38 ± 17*	24 ± 15*	43 ± 17*	105 ± 42*	402 ± 83*	74 ± 18*	81 ± 18*	36 ± 9*	30 ± 7*	15 ± 3*	83 ± 10*	61 ± 12*	32 ± 8*	23 ± 5*
1 h, 30	2.74 ± 0.81*	76 ± 10*	39 ± 16*	26 ± 14*	44 ± 16*	109 ± 42*	406 ± 81 *	71 ± 18*	83 ± 19*	36 ± 7*	29 ± 7*	14 ± 3*	82 ± 9*	61 ± 12*	30 ± 8*	23 ± 6*
1 h, 45	2.76 ± 0.80*	76 ± 11*	41 ± 15*	25 ± 15*	45 ± 14*	111 ± 41*	403 ± 78*	70 ± 17*	84 ± 18*	37 ± 9*	29 ± 8*	13 ± 3*	83 ± 8*	62 ± 10*	30 ± 7*	23 ± 6*
2h	2.75 ± 0.78*	77 ± 10*	42 ± 17*	26 ± 14*	44 ± 15*	112 ± 42*	405 ± 81 *	72 ± 19*	82 ± 17*	37 ± 8*	30 ± 8*	14 ± 4*	83 ± 10*	62 ± 11**	31 ± 7*	23 ± 5*

Data show mean ± SD. B: baseline, time: 5,15, 30,45 and 60 isin minutes, h: hour, CO: cardiac output, HR: heart rate, LADF: left anterior descending flow, CxF: circumflex artery flow, RCAF: right coronary artery flow, TCF: total coronary flow, RCCAF: right common carotid artery flow, LIMAF: left internal mammary artery flow, LVP-S: systolic left ventricular pressure, LVP-M: mean left ventricular pressure, RVP-S: systolic right ventricular pressure, RVP-M: mean right ventricular pressure, AoP-S: systolic aortic pressure, AoP-M: mean aortic pressure, PAP-S: systolic pulmonary pressure, PAP-M: mean pulmonary pressure.

* No significant change ($p < 0.05$) compared to baseline (B).

Table 4: Inflammatory cytokine profile changes

	cTn Study	p -value*	Control	p -value**	IL-6 Study	p -value*	Control	p -value**	AcTn AcTn	p -value*	DIL-6 DIL-6	p -value*
B	0.11 ± 0.07		0.03 ± 0.01	<0.001	9.2 ± 2.17		0	<0.0001	0.08 ± 0.02		9.2 ± 2.17	
-5	0.13 ± 0.08	0.014	0.03 ± 0.02	<0.001	11.0 ± 4.36*	0.44	0	<0.0001	0.10 ± 0.03	NS	11.0 ± 4.36	NS
-15	0.14 ± 0.08	0.003	0.04 ± 0.02	<0.001	13.1 ± 5.51*	0.22	0	<0.0001	0.10 ± 0.03	NS	13.1 ± 5.51	NS
I-30	0.15 ± 0.09	0.001	0.04 ± 0.03	<0.001	17.24 ± 11.78	0.05	0.34 ± 0.17	<0.0001	0.11 ± 0.04	0.03	16.9 ± 0.08	0.005
I-45	0.17 ± 0.11	0.001	0.06 ± 0.03	<0.001	17.74 ± 11.98	0.014	1.14 ± 1.04	<0.0001	0.11 ± 0.04	0.03	16.6 ± 1.3	0.005
I-60	0.18 ± 0.09	0.003	0.07 ± 0.02	<0.001	21.53 ± 13.16	0.001	2.63 ± 1.69	<0.0001	0.11 ± 0.04	0.03	18.9 ± 2.2	<0.001
R-5	0.20 ± 0.11	0.003	0.07 ± 0.02	<0.001	20.48 ± 10.39	0.001	2.38 ± 1.32	<0.0001	0.13 ± 0.03	0.005	18.1 ± 1.9	<0.001
R-15	0.20 ± 0.09	<0.001	0.07 ± 0.03	<0.001	23.21 ± 14.83	<0.001	3.41 ± 2.05	<0.0001	0.13 ± 0.03	0.005	19.8 ± 4.0	<0.001
R-30	0.23 ± 0.12	<0.001	0.07 ± 0.02	<0.001	28.23 ± 12.75	<0.001	5.83 ± 3.60	<0.0001	0.16 ± 0.04	<0.001	22.4 ± 7.3	<0.001
R-45	0.26 ± 0.13	<0.001	0.07 ± 0.03	<0.001	30.38 ± 12.40	<0.001	5.48 ± 3.60	<0.0001	0.19 ± 0.04	<0.001	24.9 ± 7.9	<0.0001
R-60	0.25 ± 0.12	<0.001	0.08 ± 0.04	<0.001	38.50 ± 13.55	<0.001	8.3 ± 6.63	<0.0001	0.17 ± 0.05	<0.001	30.2 ± 8.1	<0.0001

Control cTn, DIL-6: Study IL-6 – Control IL-6, NS: non-statistically significant change.

* p -value for the difference between each time-point and baseline (B).

** p -value for the difference between the study and the control group for each time-point.

Figure 4: Cytokine measurements during *in situ* left lung ischemia and reperfusion. Plots show mean $\pm$ SD for each time point. I: Ischemia, R: reperfusion (I and R time in minutes), cTn: DcTn = Study cTn - Control cTn, IL-6: DIL-6 = Study IL-6 - Control IL-6, TCF: total coronary flow, LVP-S: systolic left ventricular pressure, AoP-S: systolic aortic pressure.

the slow increase of the LVP-S, CO, and coronary flow, as measured at 30 and 45 min of ischemia. It seems that this reactive response is time limited and is not enough for full recovery, as, at the 60 min of ischemia time point, both hemodynamics and coronary flow were significantly reduced again.

After releasing the pulmonary hilum to induce reperfusion, the sequestered volume of blood is reintroduced into the

circulation, resulting in increased atrial filling, increased LV filling, increased LVP-S, increased CO, and increased coronary flow, as measured at 5 and 15 min of reperfusion. Unfortunately, the previously sequestered blood volume releases also to circulation all the products of the anaerobic lung metabolism. This constitutes a secondary trauma (reperfusion injury) that results in a sustained decrease in

hemodynamics and coronary flow, as measured at 30, 45, and 60 min of reperfusion.

The combination of those negative effects of this 'remote', extracardiac ischemia and reperfusion on the coronary flow can have a potentially serious deleterious effect in patients undergoing non-cardiac thoracic surgical procedures. This would be expected to be magnified with even relatively small degrees of acquired coronary stenosis or obstruction. A further increased danger of potential cerebrovascular accident would be present in patients with any degree of carotid artery stenosis during lung ischemia and reperfusion.

In our study, LIMA blood flow was significantly reduced during both lung ischemia and reperfusion. The implications of the potential injury due to lung ischemia and reperfusion may, therefore, extend further to patients with repaired coronary artery disease, as the LIMA is currently the most commonly used conduit for coronary revascularization.

A potential limitation of our study is the prolonged duration of ischemia and reperfusion: 1 h each in our study model. It could be argued that the ischemic injury after 1 h is too severe. Temporary hilar clamping that may be necessary during a pulmonary resection, such as those requiring a pulmonary arterioplasty, is likely of a shorter duration. The typical warm ischemia period during reimplantation of a donor lung, however, is approximately 1–2 h [12]. Nevertheless, we can see that even after only 15 min of lung ischemia, there is a strong correlation between the significant TCF drop and the associated cTn and IL-6 increase. During the first 15 min of ischemia, a strong correlation of decreased coronary flow and increased cTn (R^2 : 0.95) and IL-6 (R^2 : 0.88) was observed (Fig. 4(d) and (e), respectively). This significant drop in the coronary flow occurs very soon after the induction of *in situ* lung ischemia and is highly correlated with the associated drop of both the LVP-S (R^2 : 0.96) and the AoP-S (R^2 : 0.97) (Fig. 4(f) and (g)), respectively.

The cardiovascular system usually responds as a system. This study shows that a local action (isolated, *in situ* lung ischemia) triggers, in a very short period of time, an important multidimensional systemic reaction. Given that, at least to some degree, in every lobectomy and pneumonectomy and especially during lung transplantation, there is lung ischemia, early detection of decreased systemic blood flow as manifested in a relative drop in systemic blood pressure should alert the surgical team to likely similar relative decreases occurring in coronary and cerebral blood flow. This would potentially allow for corrective action (administration of fluids and inotropes) to be initiated to support coronary and cerebral blood flow during this period. This could be especially important in patients with coronary or cerebrovascular pathology identified preoperatively. To our

knowledge, this is the first study where extracardiac 'remote' ischemia and reperfusion was induced, in pulmonary tissue, with the purpose of investigating the cardiac, hemodynamic, and inflammatory response under direct, coronary-flow measurements.

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REFERENCES

- [1] Jaroszewski D, Hul J, Chu D, Malaisrie C, Riffel A, Gordon H, Wang X, Bakaeen F. Utility of detailed preoperative cardiac testing and incidence of post-thoracotomy myocardial infarction. *J Thorac Cardiovasc Surg* 2008;135:648–55.
- [2] Goldman L, Caldera D, Nussbaum S, Southwick F, Krogstad D, Murray B. Multifactorial index of cardiac risk in noncardiac surgical procedures. *N Engl J Med* 1977;297:845–50.
- [3] Detsky A, Abrams H, Forbath N, Scott J, Hilliard J. Cardiac assessment for patients undergoing noncardiac surgery. A multifactorial clinical risk index. *Arch Intern Med* 1986;146:2131–4.
- [4] Larsen S, Olesen K, Jacobsen E, Nielsen H, Nielsen A, Pietersen A. Prediction of cardiac risk in non-cardiac surgery. *Eur Heart J* 1987;8: 179–85.
- [5] Cagirici U, Nalbantgil S, Cakan A, Turhan K. A new algorithm for pre-operative cardiac assessment. *Tex Heart Inst J* 2005;32:159–62.
- [6] Brunelli A, Varela G, Salati M, Jimenez M, Pompili C, Novoa N, Sabbatini A. Recalibration of the revised cardiac risk index in lung resection candidates. *Ann Thoracic Surg* 2010;90:199–203.
- [7] Kieser T, Rose S, Kowalewski R, Belenkie I. Transit-time flow predicts outcomes in coronary artery bypass graft patients: a series of 1000 consecutive arterial grafts. *Eur J Cardiothorac Surg* 2010;38:155–62.
- [8] Lurati Buse G, Koller M, Grapow M, Bolliger D, Seeberger M, Filipovic M. The prognostic value of troponin release after adult cardiac surgery—a meta analysis. *Eur J Cardiothorac Surg* 2010;37:399–406.
- [9] Pierre AF, Keshavjee S. Lung transplantation: donor and recipient critical care aspects. *Curr Opin Crit Care* 2005;11:339–44.
- [10] Moreno I, Vicente R, Ramos F, Vicente J, Barbera M. Determination of interleukin-6 in lung transplantation: association with primary graft dysfunction. *Transplant Proc* 2007;39:2425–6.
- [11] Bramos D, Ikonomidis I, Tsirikos N, Kottis G, Kostopoulou V, Pamboukas C, Papadopoulos E, Venetsanou K, Giatrakos N, Yang G, Nihoyannopoulos P, Toumanidis S. The association of coronary flow changes and inflammatory indices to ischemia-reperfusion microvascular damage and left ventricular remodeling. *Basic Res Cardiol* 2008;103:345–55.
- [12] Puri V, Patterson GA. Adult lung transplantation: technical considerations. *Semin Thorac Cardiovasc Surg* 2008;20:152–64.