


CHARLES UNIVERSITY
First Faculty of Medicine

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Hemodynamic adaptation mechanisms of heart failure to percutaneous venoarterial extracorporeal circulatory support

Mechanismy adaptace hemodynamiky při uplatnění perkutánní venoarteriální mimotělní podpory oběhu u srdečního selhání

Summary of dissertation thesis

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty

Contents

Abstract

Introduction: Venoarterial extracorporeal membrane oxygenation (VA ECMO) is widely used in the treatment of circulatory failure, but repeatedly, its negative effects on the left ventricle (LV) have been observed. The purpose of this study is to assess the influence of extracorporeal blood flow (EBF) on systemic hemodynamic changes and LV performance parameters during VA ECMO therapy of decompensated heart failure.

Methods: Porcine models of low-output chronic and acute heart failure were developed by long-term fast cardiac pacing and coronary hypoxemia, respectively. Profound signs of circulatory decompensation were defined by reduced cardiac output and tissue hypoperfusion. Subsequently, under total anesthesia and artificial ventilation, VA ECMO was introduced. LV performance and organ specific parameters were recorded at different levels of EBF using an LV pressure-volume loop analysis, arterial flow probes on carotid and subclavian arteries, and transcutaneous probes positioned to measure cerebral and forelimb regional tissue oxygen saturations.

Results: Conditions of severely decompensated heart failure led to systemic hypotension, low tissue and mixed venous oxygen saturations, and increase in LV end-diastolic pressure. By increasing the EBF from minimal flow to 5 L/min, we observed a gradual increase of LV peak pressure, reduced arterial flow pulsatility, and an improvement in organ perfusion. On the other hand, cardiac performance parameters revealed higher demands put on LV function: LV end-systolic volume and end-diastolic pressure and volume all significantly increased (all $P < 0.001$). Consequently, the LV stroke work increased ($P < 0.05$) but LV ejection fraction did not. Also, the isovolumetric contractility index did not change significantly.

Conclusions: In decompensated chronic and acute heart failure, excessive VA ECMO flow increases demands on left ventricular workload and can be potentially harmful. To protect the myocardium, VA ECMO flow should be adjusted with respect to not only systemic perfusion, but also to LV parameters.

Abstrakt

Úvod: Venoarteriální mimotělní membránová oxygenace (VA ECMO) je široce využívaná metoda v léčbě závažného oběhového selhání, avšak opakovaně byl pozorován také její negativní vliv na levou komoru srdeční (LV). Cílem práce je zhodnotit vliv průtoku mimotělní podporou (EBF, extracorporeal blood flow) na systémovou hemodynamiku během VA ECMO terapie dekompensovaného srdečního selhání.
Metody: Biomodel (prase domácí) srdečního selhání s nízkým srdečním výdejem byl vytvořen dlouhodobou rychlou stimulací myokardu (chronické srdeční selhání) a koronární hypoxemií (akutní srdeční selhání). Známky dekompensovaného srdečního selhání byly definovány sníženým srdečním výdejem a tkáňovou hypoperfusí. Následně bylo v celkové anestezii zavedeno VA ECMO a během různých průtoků mimotělní podporou byla pomocí PV diagramu hodnocena práce levé komory. Orgánově specifické parametry byly monitorovány regionální tkáňovou oxygenací a měřením průtoku krve v karotické a subklaviální arterii.
Výsledky: Závažné dekompensované srdeční selhání vedlo k systémové hypotensi, nízké tkáňové oxygenaci, nízké saturaci smíšené venosní krve a ke zvýšení end-diastolického tlaku v levé komoře. Zvyšováním EBF od minimálního průtoku až na 5 l/min došlo k výraznému zvýšení systolického tlaku v levé komoře, snížení pulsatility v tepnách a zlepšení orgánové perfuse. Na druhou stranu ale byly zjištěny zvýšené nároky na funkci levé komory: významně se zvýšily end-systolický a end-diastolický objem a end-diastolický tlak (pro všechny $P < 0.001$). V důsledku toho vzrostla práce levé komory ($P < 0.05$), avšak ejekční frakce se neměnila. Významně se nezměnil ani isovolumický index kontraktility.
Závěr: U modelu dekompensovaného chronického i akutního srdečního selhání se při vyšších průtocích VA ECMO zvyšují nároky na práci levé komory. Vysoký průtok VA ECMO tak může být potenciálně nebezpečný a pro ochranu myokardu by měl být průtok mimotělní podporou nastaven nejen s ohledem na systémovou perfusi, ale také na parametry levé komory.

Introduction

Patients suffering of heart failure (HF) require intensive and highly specialized management from the onset of disease. Combination of life style, medication and implantable electrical devices is considered conventional therapy. Despite of full supportive treatment, patient's hemodynamic status can change abruptly into circulatory decompensation and cause severe prognosis (Jackson et al. 2000).

Serious HF decompensation represents a medical emergency and its management strategies include oxygen therapy, diuretics to reduce intravascular volume, nitrates with vasodilating effect in volume overload, or vasopressors such as norepinephrine or dobutamine to raise blood pressure and redistribute cardiac output (CO) to vital organs. However, these are potentially hazardous due to generation of increased ventricular afterload and myocardial oxygen demands and can further worsen peripheral tissue perfusion (Werdan et al. 2014). Added burden on the heart with increased cardiac work may then cause myocardial ischemia (Fuhrman et al. 1999). Immediate correction of underlying etiology remain the ultimate treatment for severe shock states (Millane et al. 2000).

In situations of inadequate oxygen delivery, we need to support circulation, gas exchange, or both. Extracorporeal life support (ECLS) is a treatment modality that provides prolonged blood circulation and gas exchange and can partially support or fully substitute functions of heart and lungs in patients with severe but potentially reversible cardiopulmonary failure refractory to conventional therapy. This can provide time for treatment of underlying disease (Abrams et al. 2014, Brogan et al. 2017). The system consists of intravascular cannulas connected to tubing set which is attached to mechanical pump. The extracorporeal circuit is then closed in a loop with a gas exchange unit, also called the artificial lung. Thus, the functions of heart pump and lungs are transferred outside the body until the native organs recover. Due to this typical setting, ECLS is also referred to extracorporeal membrane oxygenation (ECMO) and went through thorough research and

development in the last decades (Brogan et al. 2017).

ECMO hemodynamics

The extracorporeal and native circulations in venoarterial ECMO settings are connected in parallel. If some degree of CO is preserved, i.e. the extracorporeal bypass is partial, both heart and ECMO are pumping blood into the aorta – the reinfused blood mixes in the aorta with the blood ejected from LV (which before passed through the lungs). The mixing site depends on the ratio of native CO and the extracorporeal blood flow (EBF) and the position of cannula tip. Especially in concomitant lung disease with poor oxygenation, this has important consequences on coronary perfusion (Kinsella et al. 1992, Kamimura et al. 1999).

ECMO supports systemic circulation by taking over part of cardiac workload, but it does not automatically reduce stroke work (SW) of the heart (Fuhrman et al. 1999). Instead, reinfusion of blood from extracorporeal circuit increases systemic afterload. Especially with high EBF this increase becomes significant and LV ejection is competing with higher aortic pressure (Shen et al. 2001, Shen et al. 2001). The impairment of cardiac performance with increased EBF during ECMO has been well documented in several experimental and clinical studies (Seo et al. 1991, Aissaoui et al. 2012, Burkhoff et al. 2015, Broome and Donker 2016, Truby et al. 2017).

$$SW = \int_{Vs}^{Vd} P \, \mathrm{d}V$$

When impaired contractility reduces LV ejection against increased afterload, the ventricle retain blood (Figure 1). Thereby LV end-diastolic pressure and wall tension rises which relates to sarcomere stretch throughout the myocardium. The contractility force will increase according to the Frank-Starling law, unless it becomes exhausted. In this setting, coronary perfusion may not keep pace with myocardial metabolic demands (MVO_2) and initiates a vicious cycle. Further hemodynamic complications of ECMO like LV dilation or pulmonary edema were described but their risk factors remain unclear (Barbone et al. 2011, Soleimani and Pae 2012, Boulate et al. 2013).

$$MVO_2 \approx constant * (PE + SW)$$

On the opposite site, right atrial pressure is reduced by draining blood into the venous cannula, decreasing ventricular preload (unpreloading of LV). This by itself, improves organs perfusion at any aortic pressure. Draining the right heart should also help to reduce pulmonary artery pressure (Fuhrman et al. 1999).

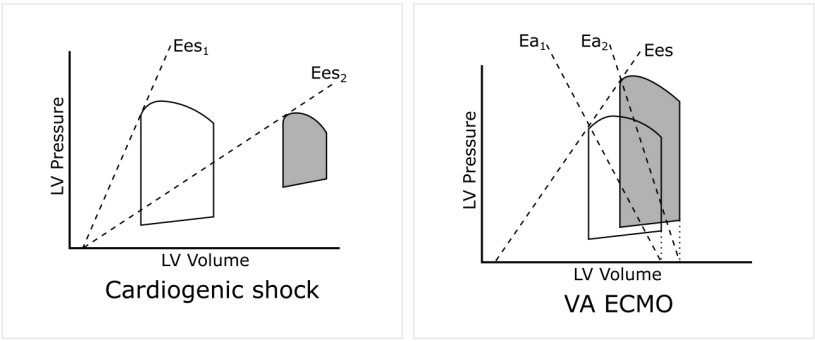

Figure 1. Pressure-volume parameters. *Left* – normal PV loop (white) and PV loop in cardiogenic shock (gray); slope of ESPVR (Ees) is severely reduced, LV EDV and EDP are increased, SV reduced. *Right* - changes of normal PV loop (white) by effects of VA ECMO (gray). Increased afterload ($Ea_1 < Ea_2$) leads to reduced SV, and increased LV EDP and EDV.

According to current opinions, decreasing the venoarterial (VA ECMO) support to the minimal EBF necessary for tissue perfusion is advisable in situations of decompensated HF (Seo et al. 1991), but the optimal level of EBF remain unknown. Furthermore, relieving the overloaded LV seems to alleviate the risks of lung fluid accumulation and progression to pulmonary edema (Soleimani and Pae 2012, Donker et al. 2019). Therefore, monitoring and better understanding of heart hemodynamics during ECLS might improve prognosis (Soleimani and Pae 2012, Truby et al. 2017, Na et al. 2019).

In clinical practice mechanical circulatory supports are often being applied to circulatory decompensation which develops on grounds

of previously already present chronic heart disease. In such situations the adaptation mechanisms are fully developed and can play an important role in inconsistency of outcomes observed according to the "acuteness or chronicity" of underlying cardiac disease (Tarzia et al. 2015). Thus, appropriate experimental models are important to properly test hemodynamics of VA ECMO support.

This thesis will focus on effects of ECLS during decompensation of HF and will discuss available methods of systemic perfusion and ventricular hemodynamic assessment. Summary of our experimental results is further complemented by attached documents.

Hypotheses

1. Venoarterial extracorporeal membrane oxygenation causes significant changes to ventricular hemodynamics.
2. Certain extracorporeal blood flow can provide sufficient systemic perfusion and minimalize adverse effects on work of the left ventricle.

Aims

Based on the analyses stated above, I specified and defined the following goals:

1. Heart failure models development
    1.1. Develop experimental models of acute and chronic heart failure
    1.2. Allow for titratable severity of heart failure
    1.3. Aim for low mortality and reproducibility
2. Hemodynamic effects of VA ECMO
    2.1. Apply VA ECMO with variable extracorporeal flow to models of heart failure
    2.2. Assess effects of extracorporeal flow on organ perfusion
    2.3. Assess effects of extracorporeal flow on heart hemodynamics and ventricular work

Methodology

Experimental work included development of variety of HF models and their titration into circulatory decompensation. All necessary instrumentation and an ECMO circuit were introduced. Then, a standardized ramp protocol with increasing EBF was delivered to test hemodynamics during a full spectrum of rates of extracorporeal circulatory support.

Model of chronic HF

Tachycardia-induced cardiomyopathy as a form of dilated cardiomyopathy was generated by long-term fast cardiac pacing (Spinale et al. 1990, Nikolaidis et al. 2001, Gupta and Figueredo 2014).

Due to suitable anatomy five healthy crossbred female swine (Sus scrofa domestica) with initial weights of 37-46 kg were included. After 1 day of fasting, under general anesthesia and mechanical ventilation, a heart pacing unit was implanted (Figure 2). A single pacing lead with active fixation was inserted transvenously by fluoroscopic guidance in the apical part of right ventricle and subcutaneously tunneled to connect through an in-house modified "Y" connecting part to a cardiac pacemaker which then was buried into a dorsal subcutaneous pocket. The "Y" connection allowed a convergent conduction of both pacemaker outputs together to the single pacing lead. These arrangements provide a wide range of high rate pacing frequencies and proved to prevent device-related complications.

After recovery from the surgical procedure, a rapid ventricular pacing was started. According to previous publications (Chow et al. 1990, Hendrick et al. 1990, Tomita et al. 1991) and our own experience, the rapid pacing protocol was defined and started at a pacing rate of 200 beats/min. The frequency was then escalated to 220 beats/min after one week, to 240 beats/min the following week, and sustained or was adjusted to individual HF progression.

Due to interindividual differences in response to fast pacing, time needed to produce chronic HF with profound signs of decompensation varied from 4 to 8 weeks. Dilation of all heart chambers, severe systolic dysfunction, and valve regurgitations were apparent and correlated with low cardiac index and low venous blood oxygen

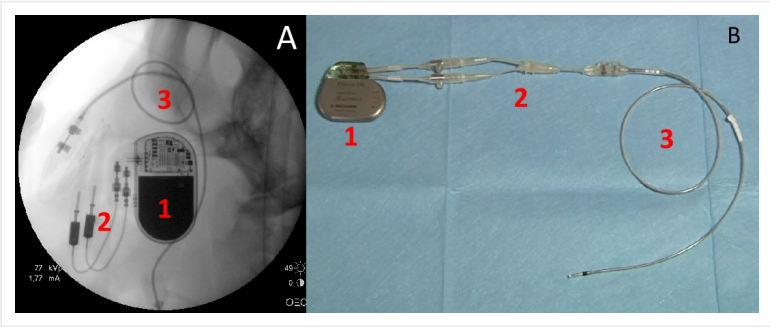

Figure 2. Heart pacing unit – X-ray (A) and photography (B) of dual-chamber pacemaker (Effecta, Biotronik SE & Co. KG, Germany, 1), "Y" shaped adapter (2) and ventricular pacing lead (3).

saturation. At that point ventricular pacing was discontinued for further hemodynamic studies and all animals were examined in sinus rhythm.

Models of acute HF

Two porcine models of acute HF were utilized for the measurements. 1) induced by regional coronary hypoxemia and 2) induced by global coronary hypoxemia.

Ad 1) in five swine the largest branch of left main coronary artery (left anterior descending artery or left circumflex artery) was identified by coronary angiogram and with the use of two coronary guide wires, a balloon catheter and an over-the-wire perfusion catheter were advanced. Distally to the balloon, venous blood from the pre-oxygenator part of the ECMO circuit was pumped and solely perfused the corresponding portion of myocardium at a flow of approximately 40 mL/min.

Ad 2) sixteen female swine, mean body weight 45 kg, were mechanically ventilated and a femoral VA ECMO circuit was inserted percutaneously with the outflow cannula advanced to the descending aorta. Reinfused was well oxygenated blood but at a low EBF of 1 L/min. Then, by changing the ventilator settings, severe desaturation of the blood in left-sided heart chambers was maintained. This caused tissue hypoxia in all organs perfused by LV ejection, including

the coronary arteries, while the ECMO supported other organs (resembling the clinical condition of selective cyanosis – the "Harlequin syndrome"). The resulting global myocardial hypoxia rapidly lowered contractility, ejection fraction of both ventricles, and arterial blood pressure leading to cardiogenic shock.

Hemodynamic monitoring

In all these models, right carotid and subclavian arteries were surgically exposed and ultrasound flow probes attached enabling detection of blood flow velocities. For both arteries the pulsatility index (PI) was calculated as the difference between the peak systolic and minimum diastolic velocities divided by the mean velocity and as such represents the variability of arterial flow during cardiac cycle.

A balloon Swan-Ganz catheter was placed to the pulmonary artery allowing thermodilution-derived cardiac output, mixed venous oxygen saturation (SvO_2), pulmonary artery, and pulmonary wedge pressure assessments. Regional tissue oxygenation (rSO_2) was monitored by near-infrared spectroscopy with sensors placed on forehead and right forelimb region representing brain and peripheral tissue oxygen saturation levels (Wolf et al. 2007). Intracardiac and transthoracic echocardiography probes were used for 2D and color Doppler imaging.

LV parameters and SW analysis

Through the aortic valve, a pressure-volume catheter was positioned in the LV cavity. Measured LV parameters included end-diastolic pressure and volume (EDP and EDV), end-systolic volume (ESV), LV peak pressure (LVPP), stroke work (SW; defined as LV pressure integral with respect to volume), and maximal positive change of LV pressure, defined as first time derivative of LV pressure (dP/dt_{max}). When normalized to EDV, $dP/dt_{max}/EDV$ ratio represents a preload independent index of LV contractility (Glower et al. 1985, Little 1985, Kass et al. 1987, Burkhoff et al. 2005, Walley 2016). Additional calculated parameters were stroke volume (SV) and left ventricular ejection fraction (EF).

Under conditions of previously developed profound HF with tissue hypoperfusion, extracorporeal circulation was maintained by a

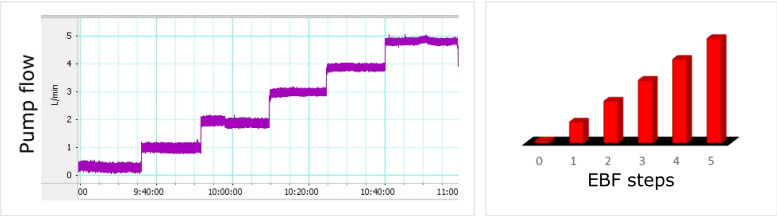

Figure 3. Experimental ECMO protocol. Example of real measured ECMO flow over a single stepwise ECMO protocol run (left) and diagram of corresponding categorized levels of EBF (right).

femoral VA ECMO system. By changing the ECMO pump rotation speed, the EBF was set according to standardized ramp protocol (Figure 3). EBF was gradually increased by increments of 1 L/min every 5-15 minutes from minimal flow to 5 L/min, and these stepwise categories with constant EBF were referred to as EBF 0, 1, 2, 3, 4, and 5. At each step, animals were allowed to stabilize to a steady state condition in which hemodynamic parameters including PV loop data were recorded.

Statistical analysis

All data sets were tested for normality and are expressed as mean ± standard error of mean (SEM). Comparisons between different levels of EBF were analyzed by using the Friedman test with Dunn's multiple comparison. Linear regression with Pearson correlation and scatter plots were used for value comparisons in between models. In all experiments, a two-sided P-value < 0.05 was considered statistically significant.

Results

Characteristics of HF models

The chronic HF model was represented by the tachycardia-induced cardiomyopathy caused by artificial ventricular pacing. At the end of the pacing protocol, physical examination revealed severe clinical signs of chronic HF in all animals.

Initial mean heart rate of sinus rhythm was 100 ± 19 beats/min. Chest X-rays showed dilated heart shadow. On echocardiography dilation of all heart chambers, severe systolic dysfunction of both ventricles, and significant mitral and tricuspid regurgitations were apparent. The LV wall was judged non-hypertrophic with a thickness of 7-10 mm and dyssynchrony of LV contraction was obvious.

CO in resting state was 2.9 ± 0.4 L/min and SvO_2 62 ± 8% corresponded with inadequate tissue oxygen delivery. Arterial flow in the carotid artery was 211 ± 72 mL/min and in the subclavian artery was 103 ± 49 mL/min. Similarly, cerebral regional tissue saturation recorded was only 57 ± 6%, and it was even lower on the right forelimb, at 37 ± 6%.

The pressure-volume loop obtained from the PV catheter illustrates the hemodynamic measures and work produced during each cardiac cycle (Figure 6). LV peak pressure was reduced, but EDP remained low. The measured volumes of the left ventricular chamber were reflective of its dilation and systolic dysfunction. EDV was increased to 189 ± 26 mL, ESV to 139 ± 17 mL. The SV was reduced to 51 ± 20 mL and the EF 25 ± 7%.

By analyzing the pressure-volume loops, LV stroke work (SW) was calculated to 1434 ± 941 mmHg*mL. In addition, a preload independent index of LV contractility can be represented by a $dP/dt_{max}/EDV$ ratio, which was averaged to 2.2 ± 0.8 mmHg/s/mL.

An autopsy confirmed cardiomegaly with heart weight of 471 ± 127 g, which formed 0.7% of body weight. No shunt or other cardiac anomaly was found in any of the animals.

Both acute HF models resulted in extensive myocardial injury sufficient to cause cardiogenic shock. Low cardiac output of 2.81 ± 0.34 or 1.7 ± 0.7 L/min was measured together with increased heart rate

of 94 ± 4 or 106 ± 3 beats/min; EF was reduced to 43 ± 3 or $22 \pm 7\%$, and LVPP to 60 ± 7 or 64 ± 22 mmHg for regional or global coronary hypoxemia, respectively.

Effects of EBF on chronic HF

With stepwise increase of EBF from minimal to maximal flow (from EBF 0 to EBF 5), we observed gradual increase in mean aortic blood pressure by 79% (Table 1 and Figures 4 and 5). Similarly, arterial blood flow increased with every increase of EBF. In carotid artery it changed by 127% and in subclavian artery by 187%. Interestingly, baseline PI was considerably higher in subclavian than in carotid artery but its reduction associated with EBF was also higher in the subclavian.

Baseline SvO_2 was $62 \pm 8\%$ which corresponds with inadequate tissue oxygen delivery in our model. It increased to $77 \pm 3\%$ with EBF 1 and reached $> 80\%$ with all higher EBF steps. With increasing of EBF, the average value of CVP did gradually fall, but not under 7 mmHg, avoiding ECMO underfilling.

Similarly, the regional tissue oxygenations both cerebral and periferal were low at baseline, but increased promptly with increase of EBF. Cerebral rSO_2 changed by 30% and forelimb rSO_2 by 108%.

LVPP increased by 49% and EDP increased by 114%. Every escalation of EBF emphasized LV dilation. ESV increased severely by 20% and EDV by 15%. On the other hand, SV and EF changed only with less significant mean differences and both reached highest values at EBF 3 L/min. Left ventricular SW was calculated from measured pressure-volume loops and exhibited significant flow-dependent increase from EBF 0 to 4. However, preload independent index of LV contractility ($dP/dt_{max}/EDV$ ratio) showed no consistent change during the ECMO protocol.

Effects of EBF on acute HF

When the stepwise ramp protocol was applied to the model of acute heart failure induced by regional coronary hypoxemia, changes in hemodynamic parameters were similar, but still differed in some details compared to observations in the chronic HF model (see Figure 6).

LVPP increased significantly, although there was only a numerical increase in EDP. Also, the LV volumes demonstrated dependence on the EBF - ESV increased, but EDV did not change significantly. Finally, SW increased from 2096 ± 342 to 2884 ± 412 mmHg*mL (for EBF 1 to 5).

Additional calculated parameters showed myocardial contractility change with increasing EBF - both left ventricular stroke volume and ejection fraction decreased significantly and led to reduction in calculated cardiac output.

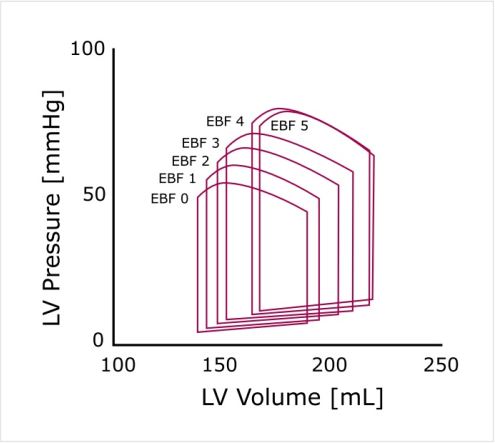

Figure 4. Schematic mean PV loop changes by effects of increasing VA ECMO flow. The left ventricular work parameters in a porcine model of chronic heart failure reveal a significant dependence on VA ECMO flow (EBF in L/min).

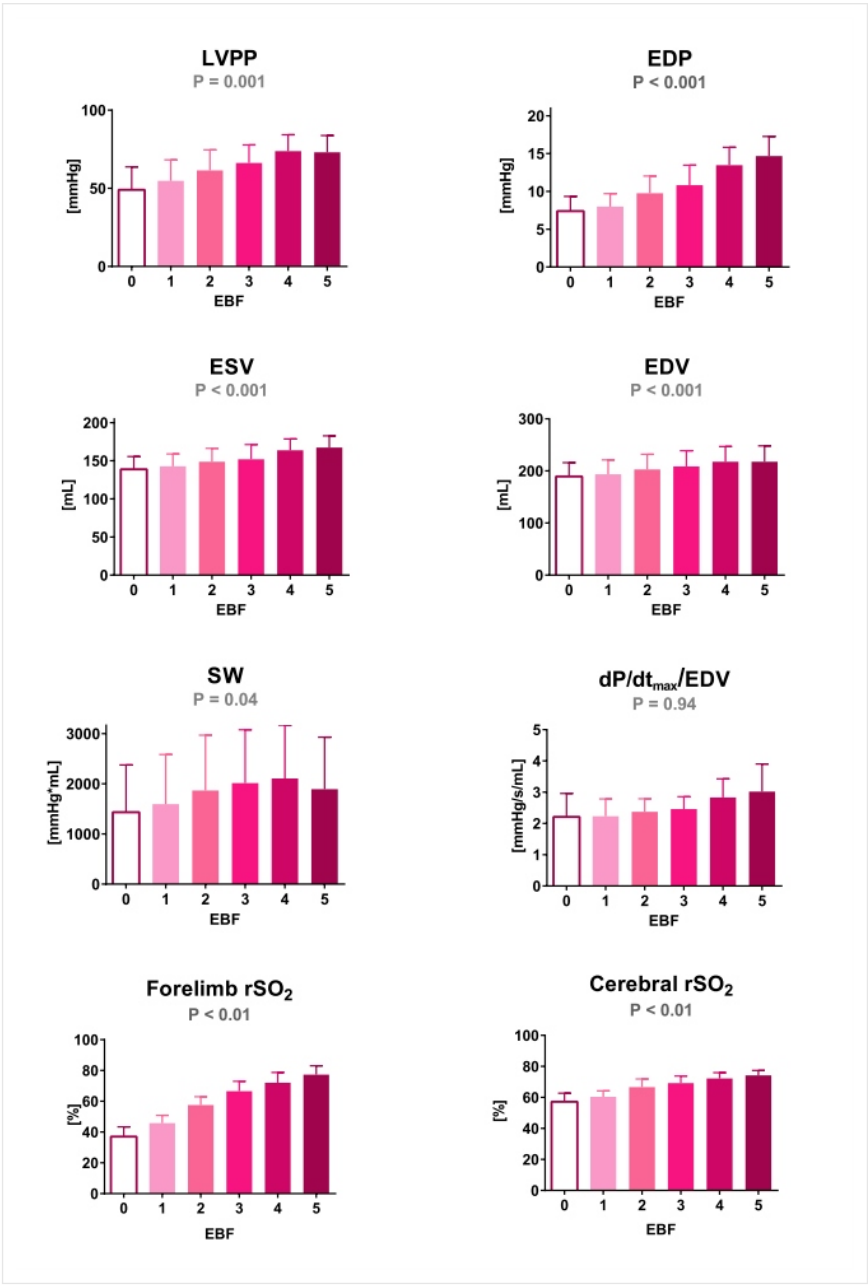

Figure 5. Effects of VA ECMO blood flow (EBF in L/min) on perfusion and LV hemodynamic parameters in a porcine model of chronic HF.

		VA ECMO blood flow in chronic HF model							Relative
Parameter	***Units***	**EBF 0**	**EBF 1**	**EBF 2**	**EBF 3**	**EBF 4**	**EBF 5**	**P**	EBF 0-5
Ventricular hemodynamics:									
LVPP	*mmHg*	49 ± 15	55 ± 13	61 ± 13	66 ± 12	$74 \pm 10*$	$73 \pm 11*$	0.001	49%
EDP	*mmHg*	7 ± 2	8 ± 2	10 ± 2	11 ± 3	$13 \pm 2*$	$15 \pm 3*$	< 0.001	114%
ESV	*mL*	139 ± 17	143 ± 16	148 ± 18	152 ± 19	$164 \pm 15*$	$167 \pm 15*$	< 0.001	20%
EDV	*mL*	189 ± 26	194 ± 27	203 ± 29	209 ± 30	$217 \pm 29*$	$218 \pm 30*$	< 0.001	15%
SV	*mL*	51 ± 20	51 ± 20	56 ± 20	59 ± 20	55 ± 21	52 ± 21	0.03	2%
EF	*%*	25 ± 7	24 ± 6	26 ± 7	27 ± 7	23 ± 6	21 ± 6	0.18	-16%
HR	*beats/min*	101 ± 22	96 ± 19	93 ± 17	90 ± 13	90 ± 14	86 ± 14	0.34	-15%
SW	*mmHg*mL*	1434 ± 941	1595 ± 987	1867 ± 1102	2014 ± 1062	$2105 \pm 1060*$	1892 ± 1036	0.04	32%
dP/dtmax/EDV	*mmHg/s/mL*	2.2 ± 0.8	2.2 ± 0.6	2.4 ± 0.4	2.5 ± 0.4	2.8 ± 0.6	3 ± 0.9	0.94	36%
Perfusion parameters:									
Carotid flow	*mL/min*	211 ± 72	291 ± 62	314 ± 57	356 ± 57	$447 \pm 64*$	$479 \pm 58*$	< 0.001	127%
Car. pulsatility index		1.43 ± 0.12	0.91 ± 0.2	0.75 ± 0.19	0.64 ± 0.21	$0.44 \pm 0.19*$	$0.34 \pm 0.15*$	< 0.01	-76%
Subclavian flow	*mL/min*	103 ± 49	128 ± 44	158 ± 40	208 ± 47	$266 \pm 47*$	$296 \pm 54*$	< 0.001	187%
Sbcl. pulsatility index		5.7 ± 1.9	3 ± 1	2.2 ± 0.8	1.5 ± 0.6	$1.1 \pm 0.5*$	$0.8 \pm 0.5*$	< 0.001	-86%
Cerebral rSO_2	*%*	57 ± 6	60 ± 4	67 ± 5	69 ± 5	$72 \pm 4*$	$74 \pm 3*$	< 0.001	30%
Forelimb rSO_2	*%*	37 ± 6	46 ± 5	58 ± 5	67 ± 6	$72 \pm 7*$	$77 \pm 6*$	< 0.001	108%
SvO_2	*%*	62 ± 8	77 ± 3	81 ± 3	86 ± 4	$89 \pm 4*$	$89 \pm 4*$	< 0.001	44%
CVP	*mmHg*	14 ± 2	11 ± 2	10 ± 2	$8 \pm 2*$	$9 \pm 2*$	$8 \pm 2*$	0.001	-43%

Table 1. Hemodynamic and pressure-volume characteristics on chronic HF model. For each step of increasing extracorporeal blood flow (EBF in L/min), hemodynamic values are expressed as mean ± SEM. Values significantly different from EBF 0 are marked with *.

Discussion

VA ECMO is being used as an ultimate method in cases of severe circulatory decompensation, but multiple clinical and experimental studies have documented adverse changes in LV function with the increased EBF (Soleimani and Pae 2012, Burkhoff et al. 2015, Ostadal et al. 2015, Brogan et al. 2017) for both cardiac (Truby et al. 2017) and respiratory (Tanke et al. 2005) compromised patients. The incidences of these complications vary widely between 12 to 68% (Cheng et al. 2014, Truby et al. 2017, Kim et al. 2019, Na et al. 2019) and are still believed to be underreported (Cheng et al. 2014, Truby et al. 2017).

The goal of our experimental work was to assess the response of hemodynamic parameters and LV workload to different levels of EBF. To mimic severe circulatory failure, appropriate models of both acute and chronic HF were first developed. Then ECMO was introduced and defined protocols of stepwise flow rates were applied to test its impacts.

HF experimental models

It has been shown that in predisposed hearts long-lasting incessant tachyarrhythmias can lead to systolic dysfunction and dilation with decreased CO. This condition of TIC was firstly described in 1913 (Gossage and Braxton Hicks 1913), widely used in experiments since 1962 (Whipple et al. 1962), and is nowadays a well-known disorder.

Similar effects of circulatory suppression were observed by introduction of high rate cardiac pacing in animal models. In a porcine model, atrial or ventricular heart rate over 200 beats/min (which is above physiological rate in exercise or stress) is potent to induce end-stage HF in a period of weeks (progressive phase) with characteristics of TIC (Spinale et al. 1992, Shinbane et al. 1997). These findings correspond well to decompensated cardiomyopathy and the animal model of TIC offers multiple advantages for HF testing and treatment innovations. It offers to easily control the severity of HF progression with titration of the pacing protocol and, importantly, the HF persists also after cessation of the rapid pacing (chronic phase) (Moe et

al. 1988, Cruz et al. 1990, Tomita et al. 1991, Shinbane et al. 1997, Takagaki et al. 2002, Umana et al. 2003, Schmitto et al. 2011).

In attached Document 2 (Hala et al. 2018) we present a protocol with a video demonstration to produce a TIC by long-term fast cardiac pacing in swine.

Hemodynamics of failing circulation was denoted by arterial hypotension, and due to poor contractility and low SV, CO was reduced to approximately 50% of a healthy animal's expected value (Wyler et al. 1979, Tranquilli et al. 1982). Functional reflection is then the failing circulation and tissue hypoperfusion. The forelimb and cerebral regional tissue oxygen saturation as well as the regional blood flow in subclavian and carotid artery suggests centralization of the blood circulation. Their low values show severely reduced tissue perfusion in peripheral as well as in vital organs, which was confirmed by low SvO_2. The low tissue perfusion was in general concordance with the measurements of low CO.

Hemodynamics and mechanical work of LV during each cardiac cycle were well documented by the PV diagram instant measurements. Poor myocardial strength was denoted by maximum LV peak pressure during systole and the $dP/dt_{max}/EDV$ ratio, a preload independent index of LV contractility. LV chamber volumes were enlarged during the whole cycle, thus the image of dilated cardiomyopathy. The LV filling pressure remains low, most likely due to high compliance of the LV thin myocardial wall (Little 2005).

Additionally, two models of acute HF were used for the presented experiments – a model of regional coronary hypoxemia and a model of global coronary hypoxemia. Both led to myocardial hypoxia, contractility dysfunction, and progression to acute circulatory decompensation. As it has been previously reported that despite the proximal placement of reinfusion cannula, the coronary arterial flow is predominantly derived from the LV ejection (Kinsella et al. 1992), the amount of oxygen delivery to the myocardium is easily titrated by the oxygen saturation and coronary blood flow in the perfused region to reach conditions of cardiogenic shock.

In contrast to other frequently used approaches to induce acute HF, like coronary artery ligation or embolization with subsequent

myocardial infarction which are associated with a high acute mortality rate, the concept of regional or global coronary hypoxemia allowed reversibility of the developed HF. Although severe acute cardiogenic shock was successfully induced in all animals with CO reduction to $< 50\%$, there was no need for defibrillation and zero mortality. In both acute HF models, regional myocardial hypoxia led to resultant ESV and EDV of far smaller dimensions (46% for ESV, 58% for EDV) and lower LV pressures compared to chronic HF.

EBF effects on chronic HF

With initiation of the extracorporeal circulation and stepwise increase of EBF, progressive dilation of LV was observed. Affected were both the EDV and ESV and this dilation was strongly pronounced between EBF 0 to 4; beyond this flow rate, LV did not further dilate.

LV pressures demonstrated upward trends as well. LVPP increased by 49% but still remained abnormally low despite high EBF. Also EDP increased by over 114% leading to high preload and high wall tension, possibly opposing coronary perfusion (Kato et al. 1996). Similar ventricular pressure trends were described by (Seo et al. 1991) with the conclusion that VA ECMO flow should be kept as low as possible, in view of undesirable hemodynamic effects on both ventricles. Likewise, the measures of LV ejection showed increase from EBF 1 to 3, but with higher EBF, their mean values decreased.

SW, defined as the area of PV loop and representing the instant LV workload, was expected to be proportional to LVPP and SV (Glower et al. 1985, Burkhoff et al. 2005). In our measurements, demands on the LV work showed significant increase by 40% from EBF 0 to 4. In fact, SW is well respecting the trend of LVPP and SV.

The ratio of $dP/dt_{max}/EDV$ continued to increase but did not meet statistical significance. For isovolumetric phase of contraction, this could imply that LV did not lose its ability to contract. The combination of increasing SW and not declining $dP/dt_{max}/EDV$ ratio demonstrate increased demands on LV work, placed by the high afterload, and this increase in LV work occurs concurrently with LV dilation (Burkhoff et al. 2005).

The regional arterial flows, venous and tissue saturations demon-

strated significant increases with respect to EBF (Hala et al. 2016). Low initial value of SvO_2 improved already at level of EBF 1, suggesting sufficient systemic perfusion with only minor extracorporeal support. Not surprisingly, with every higher EBF step, average SvO_2 and carotid arterial flow rose, but the pulsatility index gradually decreased, demonstrating loss of aortic pulse pressure and the dominance of ECMO blood flow in systemic circulation (Belohlavek et al. 2012, Hala et al. 2016).

Overlook of our results confirms that with stepwise increase of the EBF, arterial blood flow in carotid and subclavian artery increase in a manner respecting the increase of mean aortic blood pressure. As both pulsatile systemic and non-pulsatile extracorporeal circulations are concomitantly meeting in the thoracic aorta, with increasing of EBF, the relative contribution of LV ejection to arterial flow is decreasing.

EBF effects on acute HF

In comparison to chronic HF study, baseline LV volumes were far smaller (46% for ESV, 58% for EDV) and the effect of faster EBF was different – by increasing EBF, ESV increased by 31%, but EDV only by 10%. Likewise, increase of LVPP was more pronounced (by 67%), but changes of EDP were only mild. In both experiments, SW followed the same trend, reaching the highest value at EBF 4, and HR declined with every increment of EBF.

In the acute model induced by regional myocardial hypoxemia supported by stepwise VA ECMO, trend of LV EF continued to decline. This was observed neither in the chronic HF model, nor in the model of global myocardial hypoxia. Interestingly, in the latter experiment, this trend was not observed regardless of pulsatile or non-pulsatile ECMO flows. One possible explanation of LV EF trends is that in the ECMO protocol, high extracorporeal flows improve the coronary perfusion with oxygen rich blood, in contrast to the acute models, where a major portion of LV myocardium is perfused by constant flow of oxygen-poor blood, stunned, and therefore cannot keep pace and eject against increasing afterload. Long-term adaptation to chronic HF conditions with humoral activation during TIC model induction could be another possible explanation.

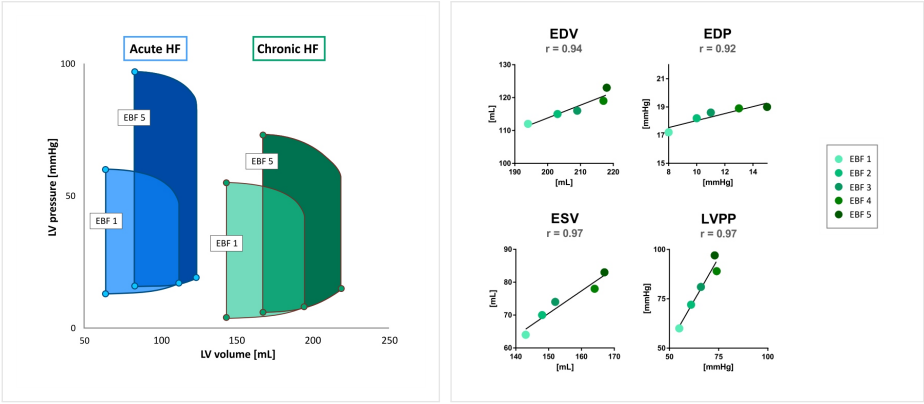

Figure 6. Effect of increasing EBF on acute (blue) and chronic (green) heart failure. Left – averaged PV loops for EBF 1 and EBF 5 are demonstrating the change of LV parameters increasing EBF. Right – scatter plots of LV pressure-volume parameters, increasing EBF effects on chronic (horizontal axes) and on hypoxic acute heart failure (vertical axes). In each graph, both axis (horizontal and vertical) have identical scales. For all, $P < 0.05$.

In Figure 6, PV characteristics of regional myocardial hypoxic model are plotted against the chronic TIC model. Values of EDV, ESV, EDP, and LVPP for corresponding EBF steps reveal linear relations with dissimilar slopes ($r = 0.94$, $r = 0.97$, $r = 0.92$, and $r = 0.97$, respectively, all $P < 0.05$). When summarized, in the chronic HF model, EDP was lower at baseline but increased more with higher EBF, and end-diastolic dilation was more pronounced. EF and SV were proved to decline only in the case of acute regional hypoxic HF.

In another model of acute HF generated by hypoxic myocardial perfusion, Shen et al. (2001) reported a decline of dP/dtmax and of LVPP associated with VA ECMO flow, but in their settings all of the coronary vascular bed received constantly hypoxemic blood. Later, findings of EBF effects were also confirmed by computer modeling (Broome and Donker 2016).

Correlation of tissue saturation and perfusion

The perfusion and regional tissue oxygenation of forelimb and

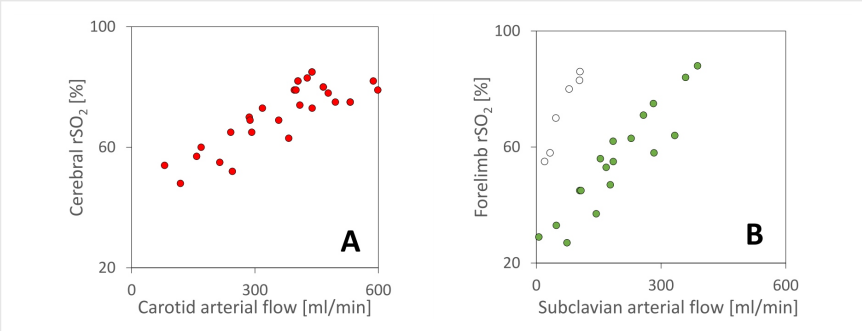

Figure 7. Correlation of carotid (A) and subclavian (B) arterial flow and corresponding regional tissue oxygenation. Correlation coefficients r = 0.75 (for A) and r = 0.94 (B), P < 0.001 for both; omitting one outlying subject – in white color.

brain are visualized on Figure 7 and demonstrate a linear relationship. Carotid flow correlated with brain oxygenation (r = 0.75) and subclavian flow correlated with corresponding forelimb oxygenation (r = 0.94). As both methods can be considered reliable and reproducible (Wolf et al. 2007, Ito et al. 2012, Ostadal et al. 2014), the well demonstrated linearity confirms a relation of saturation and perfusion in both central and peripheral organs. Notably, as the relation slopes are steeper for forelimb, this is proposing a stronger autoregulation of the brain perfusion and centralization of CO.

Our results of cerebral perfusion are similar to clinical study reported by Liem et al. (1995). In infants undergoing VA ECMO for cardiorespiratory failure, they evidenced increased total cerebral blood flow accompanied by increased cerebral blood volume and also loss of pulsatility assessed by transcranial Doppler ultrasound. On contrary, different observations were reported by Stolar and Reyes (1988) in healthy lambs – two hours of high flow VA ECMO narrowed pulse pressure, but caused no significant changes of carotid flow. The use of healthy animals, absence of increase in mean arterial pressure or cannulas' position can explain this discrepancy.

Conclusions

Large animal models of acute and chronic forms of heart failure were successfully developed. The design allowed for wide range of titration and in all experiments extracorporeal circulatory support was then applied to decompensated states of circulatory failure. Thanks to a constant methodology of heart failure models preparation, highly reproducible measurements were obtained.

A set of hemodynamic monitoring tools were used to assess left ventricular workload and systemic perfusion. Particularly pressure-volume loop analyses, regional tissue saturation, and invasive arterial flow measurements were used to describe circulatory status.

Effects of different levels of circulatory support were tested by a stepwise protocol and main changes caused by extracorporeal flow increments were observed – with already mild flows, the regional tissue saturations and systemic flow improved, but on the other hand ventricular dilation, increases in pressures, and thus increased left ventricular work were observed with every step-up of extracorporeal flow. Overall, similar trends were seen in both acute and chronic heart failure models, but small differences like differences in end-diastolic pressures or myocardial contractility deserve to be pointed out.

Based on obtained results, we can confirm both our hypotheses were correct. Venoarterial extracorporeal membrane oxygenation causes significant changes to hemodynamics by putting higher demands on the left ventricular work. Though, this might be accompanied by sufficient coronary perfusion.

Although experiments were performed on severely compromised circulation, systemic perfusion parameters improved already with low to mild extracorporeal blood flow support. The results imply that even submaximal flows can provide hemodynamic support and in matching settings, high rates of EBF may not only be unnecessary, but also harmful to the heart.

Considering the experimental results, we propose that to decrease the risk of LV overload, VA ECMO flow should be maintained at the lowest level securing adequate tissue perfusion.

List of attached documents

Document 1
Hala P., Mlcek M., Ostadal P., Janak D., Popkova M., Boucek T., Lacko S., Kudlicka J., Neuzil P., and Kittnar O. (2016). "Regional tissue oximetry reflects changes in arterial flow in porcine chronic heart failure treated with venoarterial extracorporeal membrane oxygenation." *Physiological Research* 65(Supplementum 5): S621-S631.

Document 2
Hala P., Mlcek M., Ostadal P., Janak D., Popkova M., Boucek T., Lacko S., Kudlicka J., Neuzil P., and Kittnar O. (2018). "Tachycardia-Induced Cardiomyopathy as a Chronic Heart Failure Model in Swine." *Journal of Visualized Experiments* 132(e57030).

Document 3
Hála P., Mlček M., Ošťádal P., Popková M., Janák D., Bouček T., Lacko S., Kudlička J., Neužil P., and Kittnar O. (2020). "Increasing venoarterial extracorporeal membrane oxygenation flow puts higher demands on left ventricular work in a porcine model of chronic heart failure." *Journal of Translational Medicine* 18(1).

Document 4
Ostadal P., Mlcek M., Kruger A., Hala P., Lacko S., Mates M., Vondrakova D., Svoboda T., Hrachovina M., Janotka M., Psotova H., Strunina S., Kittnar O., and Neuzil P. (2015). "Increasing venoarterial extracorporeal membrane oxygenation flow negatively affects left ventricular performance in a porcine model of cardiogenic shock." *Journal of Translational Medicine* 13: 266.

Document 5
Ostadal P., Mlcek M., Strunina S., Hrachovina M., Kruger A., Vondrakova D., Janotka M., Hala P., Kittnar O., and Neuzil P. (2016). "Novel porcine model of acute severe cardiogenic shock developed by upper-body hypoxia." *Physiological Research* 65(4): 711-715.

Document 6
Ostadal P., Mlcek M., Gorhan H., Simundic I., Strunina S., Hrachovina M., Kruger A., Vondrakova D., Janotka M., Hala P., Mates M., Ostadal M., Leiter J. C., Kittnar O., and Neuzil P. (2018). "Electrocardiogram-synchronized pulsatile extracorporeal life support preserves left ventricular function and coronary flow in a porcine model of cardiogenic shock." *PLoS One 13*(4): e0196321.

Document 7
Carr B. D., Poling C. J., Hala P., Caceres Quinones M., Prater A. R., McLeod J. S., Bartlett R. H., Rojas-Pena A., and Hirschl R. B. (2019). "A Model of Pediatric End-Stage Lung Failure in Small Lambs <20 kg." *ASAIO Journal*. DOI: 10.1097/MAT.0000000000001017