Journal of Visualized Experiments

Post-myocardial infarction heart failure in closed-chest coronary occlusion/reperfusion model in Göttingen minipigs and Landrace pigs

--Manuscript Draft--

Article Type:	Invited Methods Article - Author Produced Video
Manuscript Number:	JoVE61901R2
Full Title:	Post-myocardial infarction heart failure in closed-chest coronary occlusion/reperfusion model in Göttingen minipigs and Landrace pigs
Corresponding Author:	Gábor B Brenner Semmelweis University Budapest, HUNGARY
Corresponding Author's Institution:	Semmelweis University
Corresponding Author E-Mail:	brenner.gabor@med.semmelweis-univ.hu
Order of Authors:	Gábor B Brenner
	Zoltán Giricz
	Rita Garamvölgyi
	András Makkos
	Zsófia Onódi
	Nabil V Sayour
	Tamás G Gergely
	Tamás Baranyai
	Örs Petneházy
	Dénes Kőrösi
	Gergő P. Szabó
	Hajnalka Vago
	Zsófia Dohy
	Csilla Czimbalmos
	Béla Merkely
	Swetlana Boldin-Adamsky
	Elena Feinstein
	Iván G Horváth
	Péter Ferdinandy
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Open Access (US$3000)
Please confirm that you have read and agree to the terms and conditions of the author license agreement that applies below:	I agree to the Author License Agreement
Please specify the section of the	Medicine

submitted manuscript.	
Please provide any comments to the journal here.	

TITLE:

Post-myocardial infarction heart failure in closed-chest coronary occlusion/reperfusion model in Göttingen minipigs and Landrace pigs

AUTHORS AND AFFILIATIONS:

Gábor B Brenner [1,2]*, Zoltán Giricz [1, 2]*, Rita Garamvölgyi[3, 4], András Makkos[1], Zsófia Onódi[1], Nabil V Sayour[1], Tamás G Gergely[1], Tamás Baranyai[1], Örs Petneházy[3,4], Dénes Kőrösi[3], Gergő P. Szabó[3], Hajnalka Vago[5], Zsófia Dohy[5], Csilla Czimbalmos[5], Béla Merkely[5], Swetlana Boldin-Adamsky[6], Elena Feinstein[6], Iván G Horváth[7], Péter Ferdinandy[1, 2#]

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary
[2]Pharmahungary Group, Szeged, Hungary
[3]Moritz Kaposi Somogy County Teaching Hospital, Diagnostic, Radiation Oncology, Research and Teaching Center, Kaposvár, Hungary
[4]Kaposvar University, Faculty of Agricultural and Environmental Sciences
[5]Heart and Vascular Center, Semmelweis University, Budapest, Hungary
[6]Quark Pharmaceuticals, Inc, Newark, CA, USA
[7]Heart Institute, Medical School, University of Pécs, Pécs, Hungary

*These authors contributed equally.

Email addresses of co-authors:

Gábor B Brenner	(brenner.gabor@med.semmelweis-univ.hu)
Zoltán Giricz	(giricz.zoltan@med.semmelweis-univ.hu)
Rita Garamvölgyi	(dr.garamvolgyi.rita@gmail.com)
András Makkos	(makkos.andras@med.semmelweis-univ.hu)
Zsófia Onódi	(onodi.zsofia@med.semmelweis-univ.hu)
Nabil V Sayour	(sayour.nabil@gmail.com)
Tamás Gergely	(tamas.gergely95@gmail.com)
Tamás Baranyai	(bartamas@gmail.com)
Örs Petneházy	(ors.petne@gmail.com)
Dénes Kőrösi	(korosidenes@gmail.com)
Gergő P. Szabó	(szabo.gergo@sic.medicopus.hu)
Hajnalka Vago	(vagoha@gmail.com)
Zsófia Dohy	(dohyzsofi@gmail.com)
Csilla Czimbalmos	(czimbalmos.csilla@med.semmelweis-univ.hu)
Béla Merkely	(titkarsag@kardio.sote.hu)
Iván G Horváth	(ivan.g.horvath@gmail.com)
Swetlana Boldin-Adamsky	(SAdamsky@quarkpharma.com)
Elena Feinstein	(EFeinstein@quarkpharma.com)

#Corresponding author:

Péter Ferdinandy (peter.ferdinandy@pharmahungary.com)

KEYWORDS:
infarct size, scar size, reperfusion, sus scrofa, swine, piglet, hog, miniature, right ventricle, cardioprotection, BARI score

SUMMARY:
The overall goal of the current study is to present the techniques of induction of myocardial infarction (MI) and post-myocardial infarction heart failure (post-MI HF) in closed-chest, adult Göttingen minipigs and the characterization of post-MI HF model in Göttingen minipigs as compared to Landrace pigs.

ABSTRACT:
The development of heart failure is the most powerful predictor of long-term mortality in patients surviving acute myocardial infarction (MI). There is an unmet clinical need for prevention and therapy of post-myocardial infarction heart failure (post-MI HF). Clinically relevant pig models of post-MI HF are prerequisites for final proof-of-concept studies before entering into clinical trials in drug and medical device development.

Here we aimed to characterize a closed-chest porcine model of post-MI HF in adult Göttingen minipigs with long-term follow-up including serial cardiac magnetic resonance imaging (CMRI) and to compare it with the commonly used Landrace pig model.

MI was induced by intraluminal balloon occlusion of the left anterior descending coronary artery for 120 min in Göttingen minipigs and for 90 min in Landrace pigs, followed by reperfusion. CMRI was performed to assess cardiac morphology and function at baseline in both breeds and at 3 and 6 months in Göttingen minipigs and at 2 months in Landrace pigs, respectively.

Scar sizes were comparable in the two breeds, but MI resulted in a significant decrease of left ventricular ejection fraction (LVEF) only in Göttingen minipigs, while Landrace pigs did not show a reduction of LVEF. Right ventricular (RV) ejection fraction increased in both breeds despite the negligible RV scar sizes. In contrast to the significant increase of left ventricular end-diastolic (LVED) mass in Landrace pigs at 2 months, Göttingen minipigs showed a slight increase in LVED mass only at 6 months.

In summary, this is the first characterization of post-MI HF in Göttingen minipigs in comparison to Landrace pigs, showing that the Göttingen minipig model reflects post-MI HF parameters comparable to the human pathology. We conclude that the Göttingen minipig model is superior to the Landrace pig model to study the development of post-MI HF.

INTRODUCTION:
Despite the decreasing mortality of acute myocardial infarction (MI), the incidence of post-myocardial infarction heart failure (post-MI HF) has not changed over time [1]. Heart failure (HF) is one of the most powerful predictors of death in MI patients [2]. To date, reperfusion therapy is the only available treatment option to limit myocardial infarct size and to reduce the risk of a

subsequent HF [3-5]. HF and other complications may occur as a consequence of reperfusion injury; therefore, there is still an unmet need for the development of cardioprotective therapies beyond timely reperfusion [6-8]. Numerous cardioprotective therapies effective even in large animal models have been described, but only remote ischemic conditioning (RIC) seemed to improve clinical outcomes of post-MI HF in a small clinical trial [9]. However, this encouraging result on the efficacy of RIC was questioned in a single-blind, randomized controlled trial (CONDI-2/ERIC-PPCI) performed at 33 centers across Europe in STEMI patients, where RIC failed to improve clinical outcomes [10]. Potential reasons for the failed translation of the preclinical data might be the use of suboptimal post-MI HF animal models with low clinical relevance [11].

Cardiovascular (patho)morphology and (patho)physiology of the pig models resemble human conditions; thus, it is widely used and accepted in translational cardiovascular research [12-14]. Pig breeds used in cardiovascular research belong to the very diverse domestic pig (Sus scrofa domestica) species that includes swine that vary in size, appearance, and genetic background [15,16]. Although post-MI HF has been researched in pigs extensively, no study was published with the aim of characterizing and comparing the effect of MI on the outcome of post-MI HF in Landrace pigs and Göttingen minipigs. The intensive growth rate of Landrace pigs may affect the cardiac morphofunctional outcomes; however, Göttingen minipigs with restricted growth patterns may overcome these concerns and can serve as a feasible model for long-term follow up in the assessment of post-MI HF. Moreover, a guideline on the relevance of rigor and reproducibility in preclinical studies on cardioprotection recommends the use of cardiac magnetic resonance imaging (CMRI) as a clinically relevant model for measurement of ventricular function in pigs [12].

To analyze the scientific interest on post-MI HF in pigs we performed literature search on PubMed using the following search string: *"(pig OR swine OR porcine OR sus-scrofa OR minipig OR mini-pig OR miniature-pig OR miniature-swine) AND (infarct* OR ischem* OR ischaem* OR reperfus*) AND (heart OR cardi* OR myocard*) AND (LAD OR left-anterior* OR LCX OR left-circumflex OR RCA) AND (heart-failure OR lvef OR ejection-fraction OR infarct-size OR infarction-size)"* and found that pig models of cardiac ischemia/reperfusion are frequently used to study MI and post-MI HF, but only 17% (71 out of 425 articles) of studies involved minipigs and 7% (30 out of 425 articles) used Göttingen minipigs. Only about 1% (5 out of 425) of studies used Göttingen minipigs and clinically relevant protocols with long-term follow-up (1-9 months of reperfusion) and CMRI to analyze cardiac function. The small number of clinically relevant studies highlights the translational gap between basic research and clinical trials. Therefore, a comprehensive characterization of the closed-chest post-MI HF models in Göttingen minipigs and Landrace pigs with repeated assessment of left and right ventricular function and anatomy using CMRI during long-term follow up is required. Here we aimed to focus on the technical feasibility and clinical relevance of two post-MI HF models to describe standardized and reproducible experimental protocols for post-MI HF studies that may be used to assess cardioprotective drug and/or medical device therapies.

The present study is the first one in the literature to characterize a clinically relevant model of post-MI HF using adult Göttingen minipigs and to compare morphological and cardiac left and

right ventricular functional parameters with that of the adolescent Landrace pigs.

PROTOCOL:

13 healthy and sexually mature female Göttingen minipigs (age between 12 and 14 months) and 10 healthy and sexually immature female Landrace pigs (age between 2 and 3 months) were housed in pig stalls conforming to the size recommendations of the most recent Guide for the Care and Use of Laboratory Animals DHEW and EU Guidelines 63/2010. Animals were not spayed. The temperature of the animal rooms was controlled, and animals were kept at a 12-hour light/dark cycle and vermin-free. Ad libitum feeding leads to overt weight gain in both Göttingen minipigs and Landrace pigs, therefore, pigs from both the breeds were fed with a restricted diet regimen. Göttingen minipigs were put on restricted diet as early as they arrived to the animal facility and for the whole study duration. Special Diet Services pig chow 180-220 g/meal/animal was given twice daily according to "Taking good care of Ellegaard Göttingen Minipigs" guideline (revision date: 13 March, 2013) in the first 2 days. Between day 3 and 12 animals were fed 50% Special Diet Services pig chow and 50% maintenance minipig diet. From day 14 until the end of the study animals were fed a maintenance minipig diet. Landrace pigs received pregnant sow chow, 1.5% of body weight given two times a day according to PIC Wean to Finish Manual 2008 and 2013. All the animals received food individually dispensed and food intake was monitored to avoid competition for chow. Animals with feeding difficulties were fed individually aided by tending personnel. All animals received tap water ad libitum. The experimental protocol of post-MI HF Göttingen minipigs and in Landrace pigs is shown in **Figure 1**.
[Place **Figure 1** here]

1. Baseline CMRI

1.1. Withdraw food from animals at least 12 hours before the start of anesthesia but secure access to water to prevent dehydration.

1.2. Anesthesia

1.2.1. Induce anesthesia of the animals with ketamine hydrochloride (12 mg/kg), xylazine (1 mg/kg), and atropine (0.04 mg/kg) as an intramuscular injection to the neck region.

1.2.2. Measure the body weight and length of animals. The calculation of the body surface areas (BSA) formulas were described by Itok et al. for Göttingen minipigs (BSA [m^2] = (7.98 × BW [kg]$^{2/3}$)/100) [17] and by Swindle et al. for Landrace pigs (BSA [m^2] = (7.34 × BW [kg]$^{0.656}$)/100) [18].

1.2.3. Intubate animals, maintain anesthesia with isoflurane (2% isoflurane, 2 L/min oxygen). The size of the endotracheal tube depends on the individual anatomic characteristics of each animal and ranges between 6.0- to 7.5-mm.

1.2.4. Cannulate the ear vein with 18 G needle and start administration of 5% glucose in Ringer solution (1 L/hour).

1.3. CMRI

1.3.1. Transfer animal to CMRI facility and administer 0.4-0.5 mg/kg atracurium besylate i.v.. Atracurium besylate is a nondepolarizing, skeletal muscle relaxant that is used to avoid respiratory artefacts during CMRI measurements. Start positive pressure ventilation (16/min frequency, 350 mL volume, 25-30 mmHg positive pressure).

1.3.2. Position the animals in the supine position. Place flexible coils on the chest and 32-channel coils are placed in CMRI bed. Perform non-contrast cardiac MRI with a 1.5T scanner, using a phased array coil and a vector electrocardiogram (ECG) system to assess the cardiac function and morphology (ejection fraction (EF), cardiac output (CO), chamber and wall dimensions). Acquire cine MRI images using a retrospectively ECG-gated, steady-state free precession cine MRI technique in short-axis and long-axis views of the heart using 1.2 ms echo time, 40 ms repetition time, 50-degree flip angle, 300 mm field-of-view, 8 mm slice thickness, and at least 256x256 image matrix.

1.3.3. Quantify left and right ventricular end-diastolic (LVEDV and RVEDV) and end-systolic volumes (LVESV and RVESV), stroke volumes (LVSV and RVSV), EF-s (LVEF and RVEF) and masses by manual planimetry of end-diastolic (LVED mass) and end-systolic (RVED mass) short-axis cine images. Quantify left atrial volume by tracings on the two- and four-chamber cine images. Correct the left atrial volumes to BSA to get left atrial volume indexed to body surface area (LAVi). Assess the presence of pulmonary oedema on the localizer images.

1.3.4. For calculation of cardiac index (CI) use BSA and cardiac output.

1.3.5. Terminate the anesthesia by withdrawal of isoflurane. When spontaneous breathing returns, extubate the animal, remove i.v. cannula and return it to its cage.

2. Premedication, vascular access and coronary artery occlusion

2.1. Premedication

2.1.1. One day prior to surgical procedure administer 500 mg of acetyl salicylic acid and 300 mg of clopidogrel orally.

2.1.2. Apply analgesia (meloxicam 0.4 mg/kg body weight) and antibiotic cocktail (benzylpenicillin-procain (24.8 mg/mL), benzylpenicillin-benzatine (83.6 mg/mL), Ldihidrostreptomycine-sulfate (156.3 mg/mL), 3 mL/50 kg body weight) by intramuscular injections on the day of coronary artery occlusion.

2.1.3. Repeat the steps described in 1.2.1-1.2.4 sections.

2.1.4. Use the ear vein cannula for fluid replacement and drug administration. Administer 1g

magnesium sulfate throughout the procedure via ear vein every 30 min to prevent ventricular tachycardia (VT) and ventricular fibrillation (VF).

2.2. Vascular access

2.2.1. Place the animal on the operating table, fix the limbs, and apply wedges to immobilize the animal in the supine position.

2.2.2. Disinfect the surgical site with povidone-iodine. The surgical site is around the skin fold between the gracilis and sartorius muscle.

2.2.3. Remove the hair at the surgical site with a razor.

2.2.4. Place surface ECG electrodes in Einthoven's triangle. This triangle is formed by the two anterior limbs and left hind limb and the electrodes are placed on limbs.

2.2.5. Start positive pressure ventilation (16/min frequency, 350 mL volume, 25-30 mmHg positive pressure).

2.2.6. Isolate the disinfected surgical area with a surgical drape.

2.2.7. Approach the femoral region as described in detail by K. S. Ettrup et al. [19]. In brief, make a longitudinal incision to the skin between the gracilis and sartorius muscles. Separate the subcutaneous tissue and fascia. Isolate the femoral artery and put two surgical sutures below it to control hemorrhage.

2.2.8. Puncture and cannulate the femoral artery with a 6F-ACT introducer using the Seldinger technique [20,21].

2.2.9. Fix the sheath to the skin.

2.2.10. Use the artery for blood sampling for further biochemical analyses.

2.2.11. Administer 5000 IU heparin via the femoral sheath to secure adequate anticoagulation and prevent thrombosis during the surgical intervention. Readminister 2500 IU heparin every 60 min throughout the procedure. The animals received approximately 370-440 IU/kg heparin during the whole intervention.

2.2.12. Attach a pressure sensor to the femoral vessel to monitor the arterial blood pressure throughout the surgical intervention.

2.2.13. For calibration of pressure place the pressure recording system on the level of the heart of each animal. After removing the air bubbles, the zero pressure calibration is performed when the three-way stopcock is opened to the direction of the free air.

2.3. Coronary artery occlusion, reperfusion and intracoronary drug administration

2.3.1. Note that this intervention should be performed only by trained interventional cardiologist. Through the femoral sheath, introduce and advance the guidewire to the aortic arch and introduce the 5F guiding catheter over the guidewire. First, advance the guidewire to approach aortic root atraumatically. Perform deep intubation by a thin, 5F guiding catheter to avoid significant obstruction of the blood flow.

2.3.2. Position the fluoroscope in antero-posterior position.

2.3.3. Ensure that there is no thrombus or air bubble within the catheter with the aspiration of at least 5 mL of blood, the volume of the catheter, with the syringe connected to the catheter.

2.3.4. Connect the outer portion of the catheter to a syringe filled with radiocontrast agent (iobitridol 1.1 mL/50 kg body weight).

2.3.5. Take care that the syringe is held elevated to prevent infusion of air bubbles into the coronary artery.

2.3.6. To perform baseline angiography, intubate separately and fill with contrast agent selectively the ostia of right coronary artery and left main coronary artery. For more technical details, refer to catheterization textbooks [20,21].

2.3.7. Perform BARI (Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index) scoring after the baseline angiography. A score to all terminal arteries (terminal portion of the left anterior descending, left circumflex, and right coronary artery, as well as the ramus, diagonals, obtuse marginals, posterior descending and posterolateral branches) is assigned based on their length and caliber according to specific criteria [22,23]. A value of 0 represents an almost insignificant vessel size. In contrast, a value of 3 defines a large-sized artery with a length of two thirds the distance between the base and cardiac apex. Do not take right ventricular marginals and posterior descending artery septal branches into account.

2.3.7.1. Calculate the final BARI score (% of the left ventricle at risk) by dividing the total value from the infarct-related artery by the total values of all arteries (**Figure 2A-D**) supplying the LV. Choose the occlusion site on the left anterior descendent (LAD) coronary artery to achieve approximately 25-30% myocardium at risk as assessed by BARI scoring.

2.3.8. Insert the percutaneous transluminal coronary angioplasty (PTCA) guidewire through the guiding catheter. Position it distally to the planned site of the occlusion under fluoroscopic guidance, and check angiography for potential complications (e.g., coronary dissection, perforation).

2.3.9. Determine by visual estimation the optimal balloon size based on coronary artery

diameter.

2.3.10. Place the balloon catheter (balloon diameter 2.5 mm and balloon length 12 mm) over the PTCA guidewire and advance it to the planned position.

2.3.11. Fill the balloon with contrast agent and check the position of the balloon catheter by angiography.

2.3.12. Inflate the balloon below the nominal pressure (7-9 atmospheres) of the balloon to develop the soft touch between the balloon side-wall and the surface of the vessel. Soft-touch is defined as interaction of balloon side-wall that is enough to occlude the vessel without causing injury to the vessel wall.

2.3.13. Confirm the occlusion (TIMI 0) with angiography by visualizing the stop of the contrast flow. Keep in place the guidewire and the balloon and pull back the guiding catheter from the ostium of the coronary artery to avoid diffuse cardiac ischemia.

2.3.14. Tape instruments to the surgical drape to avoid dislocation of the intracoronary balloon.

2.3.15. Record and document the ECG sign of occlusion by ST elevation.

2.3.16. During the whole procedure, carefully monitor the vital signs, heart rate (HR), blood pressure, core temperature using rectal probe, and pulse oximetry.

2.3.17. Cover the animal with a heating device to maintain the core temperature.

2.3.18. Administer 1 g of magnesium sulfate as an intravenous bolus if pulseless VT or VF occurs and start chest compressions with a frequency of 100/min immediately. Apply 300J DC shock and lidocaine 2-4 mg/kg as an intravenous bolus. Treat asystole with 1 mg of epinephrine as an intravenous bolus.

2.3.19. Check balloon pressure every 30 min during the coronary occlusion. If there is a decrease of more than 0.5 bar in balloon pressure, set it back to initial values.

2.3.20. Perform angiography just before the end of coronary occlusion to verify the maintained balloon placement and absence of flow distally to the occlusion site.

2.3.21. Administer 2500 IU of heparin and 1 g of magnesium sulfate intracoronarily as a slow bolus to prevent thrombosis and arrhythmias.

2.3.22. Initiate the reperfusion with balloon deflation after 120 min cardiac ischemia in Göttingen minipigs and after 90 min in Landrace pigs.

2.3.23. Remove the deflated balloon.

2.3.24. Confirm the success of reperfusion with coronary angiography to demonstrate the blood flow at the distal part of the coronary vessel (TIMI 3).

3. Intracoronary drug administration

3.1. To prevent coronary artery embolization, fill the therapeutic perfusion microcatheter with saline.

3.2. Place the microcatheter over the PTCA guidewire.

3.3. Advance and confirm the position of the microcatheter. The tip of the microcatheter should be placed at the level of occlusion.

3.4. Remove the PTCA guidewire.

3.5. Connect the microcatheter with the perfusion pump and initiate intracoronary administration 5 minutes after initiation of reperfusion.

3.6. After drug administration remove the microcatheter.

3.7. Make control angiography to check the TIMI 3-grade flow of the contrast and to exclude that intervention led to air emboli or coronary dissection.

4. Wound closure and post-operative care

4.1. Remove the arterial sheath and tie down the femoral artery proximal to the puncture site. Occlusion of femoral artery following the angiographic intervention has no effect on the function of legs in pigs as assessed by daily veterinarian observations.

4.2. Close the wound using continuous sutures and apply antiseptic coating.

4.3. Terminate the anesthesia by withdrawal of isoflurane.

4.4. Closely monitor the animals in the recovery period and inspect them every 12 hours until postoperation day 3, then every 24 hours until the end of the study. Particular attention should be given to eating and drinking behavior, lethargy, signs of infection, painful condition, weight change, mobility, and general health status. Following the procedure, the animals were transported with a van in small groups in cages to avoid unnecessary stress in early postoperative period.

5. Post-MI CMRI and its evaluation

5.1. Anesthesia

5.1.1. Use the anesthetic protocol described in sections 1.2.1-1.2.4.

5.2. CMRI

5.2.1. Administer an intravenous bolus of contrast agent, 0.2 mmol/kg gadobutrol at a rate of 4 mL/sec, using a manual injector.

5.2.2. Take delayed enhancement images using an inversion recovery-prepared, gradient-echo sequence. Obtain short-axis and long-axis images 10 to 15 minutes after the administration of contrast agent.

5.2.3. Evaluation

5.2.3.1. Perform evaluation using MASS 7.6 analysis software in a blinded fashion.

5.2.3.2. Assess end-diastolic segmental wall thickness on short-axis cine images.

5.2.3.3. Measure scar transmurality on short-axis delayed enhancement images.

5.2.3.4. Quantify myocardial necrosis with manual planimetry on the delayed contrast enhancement images by delineating the myocardium with signal intensity 5 SDs above the mean signal obtained in the remote, non-infarcted myocardium.

6. Statistics

6.1. Show continuous data as mean ± standard error.

6.2. Evaluate the difference using repeated measures one-way ANOVA followed by Fisher's LSD test in Göttingen minipigs and paired t-test in Landrace pigs. BARI scores were compared with unpaired t-test and mortality rates with the chi-square test between the two breeds.

6.3. Use GraphPad Prism for data evaluation. The differences were claimed to be statistically significant if $p<0.05$.

REPRESENTATIVE RESULTS:

Mortality

Out of 13 Göttingen minipigs subjected to myocardial infarction, two animals died (15.4% mortality), one during the ischemic period due to irreversible VT and one owing to asystole in reperfusion. In Göttingen minipigs, one animal was successfully resuscitated during cardiac ischemia. The mortality rate was 0% in Landrace pigs, ten out of ten animals survived, two of them required resuscitation due to VF in the ischemic period. Mortality did not differ significantly between the two breeds.

Myocardial scar sizes were comparable between the two breeds

To measure the extent of cardiac scar as a consequence of MI, CMRI was performed. Scar sizes and BARI scores were comparable between the two breeds measured at the 2nd month of follow-up in Landrace pigs, and at the 3rd and 6th month in Göttingen minipigs (**Figure 2E,F**). No differences were observed when scar sizes were related to the BARI scores in Landrace pigs at 2 months (0.55 ± 0.1) and in Göttingen minipigs at 3 months and 6 months respectively (0.75 ± 0.12 and 0.57 ± 0.08). The scars were localized in the anterior, anteroseptal, septal, anteroapical and apical segments of the heart in both breeds. The lateral wall was affected only in Göttingen minipigs. Right ventricular infarction was negligible, affected only one animal out of eleven surviving Göttingen minipigs and one out of ten Landrace pigs (2.11 ± 2.11 vs. 0.97 ± 0.97).

Increase in left ventricular mass was more pronounced in Landrace pigs during follow-up

The cardiac growth rate was measured by CMRI. LVED mass in Göttingen minipigs increased only moderately (8%) at 6 months (**Figure 3A**). In contrast, in Landrace pigs, LVED mass increased by almost 100% at 2 months (**Figure 3B**).

Left ventricular ejection fraction decreased only in Göttingen minipigs

LVEF, as the most widely used parameter of left ventricular systolic function, was measured by CMRI. MI resulted in a significant decrease in LVEF in minipigs at 3 months and 6 months (**Figure 4A**). In Landrace pigs, LVEF did not change after 2 months (**Figure 4B**).

Post-infarction LVESV and LVEDV increased significantly in both breeds (**Table 1**). LVESV increased by 69% and 80% in Göttingen minipigs after 3 and 6 months, respectively, and by 80% in Landrace pigs after 2 months. LVEDV showed a 28% increase after 3 months and a 42% increase after 6 months in Göttingen minipigs and an 82% increase in Landrace pigs after 2 months. LVSV of Landrace pigs increased by 85% in 2 months and LVSV of Göttingen minipigs did not increase significantly even at 6 months.

Left atrial volume indexed to body surface area increased only in Göttingen minipigs, but both the breeds developed pulmonary oedema following myocardial infarction

In order to further examine signs of HF, we performed measurement of the left atrial volume indexed to body surface area (LAVi). LAVi increased by 34% in Göttingen minipigs after 6 months (**Figure 5A**) and did not change significantly in Landrace pigs after 2 months (**Figure 5B**). Representative images show the tracing of the left atria (**Figure 5C-D**). Moreover, the presence or absence of pulmonary oedema was assessed by CMRI on the localizer images (**Figure 5E**). Pulmonary oedema was observed in both breeds as a result of cardiac decompensation. Ten out of eleven Göttingen minipigs and nine out of ten Landrace pigs showed obvious signs of pulmonary oedema.

Increase in body weight was more pronounced in Landrace pigs during follow-up

In Göttingen minipigs body weight gain was only 8% after 3 months and 30% after 6 months (**Figure 6A**), whereas increased heart weight was accompanied by a nearly 100% increase in body weight in Landrace pigs at 2 months (**Figure 6B**).

Trends in cardiac functional parameters differ between Göttingen minipigs and Landrace pigs

Coronary artery occlusion led to an almost significant decrease in mean arterial pressure (MAP) in Göttingen minipigs (57.9 ± 3.98 mmHg vs. 49.89 ± 1.24 mmHg) and decreased significantly in Landrace pigs (65.4 ± 5.97 mmHg vs. 45.47 ± 4.79* mmHg) in the early reperfusion phase as compared to the baseline (pre-infarction) values.

CI is a reliable indicator of cardiac performance, which relates left ventricular CO to BSA. In Göttingen minipigs, CI didn't change at the measured time points (**Figure 7A**), whereas in Landrace pigs a tendency to increase was detected in cardiac index (**Figure 7B**).

HR of Göttingen minipigs increased significantly at 3 (20%) and 6 months (22%) after MI compared to baseline values (**Table 2**).

In contrast, the HR of Landrace pigs didn't change significantly during the follow-up period. In Göttingen minipigs CO showed a significant 32% increase only at 6 months of follow-up, whereas CO was increased by 76% in Landrace pigs after 2 months due to a significant increase in LVSV (**Table 2**). BSA increased significantly in both breeds at the measured time points (**Table 2**). BSA increased by 4% and 19% in Göttingen minipigs after 3 and 6 months, respectively, and by 54% in Landrace pigs after 2 months.

Increase in right ventricular morphofunctional parameters were observed in both Göttingen minipigs and Landrace pigs

MI affected not only left ventricular function, but it also resulted in a significant increase of RVEF in both breeds (**Figure 8**) measured by CMRI, despite the negligible right ventricular scar size. RVED mass increased in Landrace pigs only (**Table 3**).

RVESV did not change during follow up in any of the breeds. RVEDV increased significantly by 37% only in Landrace pigs (**Table 3**). While RVSV in Göttingen minipigs increased significantly by 23% only after 6 months, in Landrace pigs a significant 80% increase in RVSV was observed at 2 months.

FIGURE AND TABLE LEGENDS:

Figure 1. Experimental protocol for post-myocardial infarction-induced heart failure in Landrace pigs and Göttingen minipigs. CMRI – cardiac magnetic resonance imaging.

Figure 2. Estimation of the myocardium at risk based on the BARI (Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index) score (A-D). The total value of the infarct-related artery is divided by the sum of the 3 total values of each coronary artery, the right coronary artery (RCA), the left circumflex coronary artery (LCX), and the left anterior descending coronary artery (LAD). Left ventricular scar sizes in Göttingen minipigs and Landrace pigs measured by cardiac magnetic resonance imaging (**E**). Scar size is shown as a ratio of mass of infarction to the mass of left ventricle at end of diastole (LVED). BARI scores in Göttingen minipigs and Landrace pigs measured before coronary occlusion (**F**).

Figure 3. Left ventricular end-diastolic (LVED) mass (g) of Göttingen minipigs (A) and Landrace pigs (B) measured by cardiac magnetic resonance imaging. *p<0.05 vs. corresponding baseline (repeated measures one-way ANOVA followed by Fisher's LSD test in Göttingen minipigs; paired t-test in Landrace pigs).

Figure 4. Left ventricular (LV) ejection fraction (%) of Göttingen minipigs (A) and Landrace pigs (B) measured by cardiac magnetic resonance imaging. *p<0.05 vs. corresponding baseline (repeated measures one-way ANOVA followed by Fisher's LSD test in Göttingen minipigs; paired t-test in Landrace pigs).

Table 1. Left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), and left ventricular stroke volume (LVSV) at the measured time points in Landrace pigs and Göttingen minipigs. *p<0.05 vs. corresponding baseline (repeated measures one-way ANOVA followed by Fisher's LSD test in Göttingen minipigs; paired t-test in Landrace pigs).

Figure 5. Left atrial volume indexed to body surface area (LAVi) in mL/m^2 in Göttingen minipigs (A) and Landrace pigs (B) measured by cardiac magnetic resonance imaging. Representative images of left atrial volumes, tracings were made on the two- (C) and four chamber (D) cine images. The white arrows show the presence of pulmonary oedema on the representative localizer image (E). *p<0.05 vs. corresponding baseline (paired t-test in Göttingen minipigs and Landrace pigs).

Figure 6. Bodyweights (kg) of Göttingen minipigs (A) and Landrace pigs (B). *p<0.05 vs. corresponding baseline (repeated measures one-way ANOVA followed by Fisher's LSD test in Göttingen minipigs; paired t-test in Landrace pigs).

Figure 7. Left ventricular (LV) cardiac indices (L/min/m^2) of Göttingen minipigs (A) and Landrace pigs (B).

Table 2. Heart rate (HR), cardiac output (CO), and body surface area (BSA) of Göttingen minipigs (A) and Landrace pigs (B). *p<0.05 vs. corresponding baseline (repeated measures one-way ANOVA followed by Fisher's LSD test in Göttingen minipigs; paired t-test in Landrace pigs).

Figure 8. Right ventricular (RV) ejection fractions (%) of Göttingen minipigs (A), and Landrace pigs (B). *p<0.05 vs. corresponding baseline (repeated measures one-way ANOVA followed by Fisher's LSD test in Göttingen minipigs; paired t-test in Landrace pigs).

Table 3. **Right ventricular end-diastolic (RVED) mass, right ventricular end-systolic volume (RVESV), right ventricular end-diastolic volume (RVEDV), and right ventricular stroke volume (RVSV) in Göttingen minipigs and Landrace pigs.** *p<0.05 vs. corresponding baseline (repeated measures one-way ANOVA followed by Fisher's LSD test in Göttingen minipigs; paired t-test in Landrace pigs).

DISCUSSION:

Here we described a detailed protocol highlighting the critical steps of a technique of induction of acute MI and the evaluation of post-MI HF in a closed-chest model of adult Göttingen minipigs. We also described the method of intracoronary drug administration, BARI scoring, and reported left and right ventricular cardiac morpho-functional changes in a translational post-MI HF model. This is the first characterization of post-MI HF in Göttingen minipigs in comparison to Landrace pigs, showing that the Göttingen minipig model reflects post-MI HF parameters comparable to humans. We conclude that the Göttingen minipig model is superior to the Landrace pig to follow-up the development of post-MI HF. Clinically relevant pig models of post-MI HF are prerequisites for final proof-of-concept studies before entering into clinical trials in most of the cardiovascular drug and medical device development projects [6,7,12]. Indeed, pig models resemble humans in anatomy, physiology, and biochemical properties in particular in the field of MI research as they develop trans-mural infarctions due to the lack of collateral perfusion [14]. Therefore, pig models can serve as models for the analysis of cardioprotective therapies and their mechanisms [24-29].

Here we have found that despite the equal scar sizes, mortality rates, and BARI scores in the two breeds, left ventricular dysfunction characterized by decreased LVEF was observed only in Göttingen minipigs. Here we observed a 15.4% acute mortality in Göttingen minipigs and no mortality in the follow-up period, the letter is comparable to that in clinical studies. Indeed, a patient-level meta-analysis of 10 randomized clinical trials found that the Kaplan-Meier estimated 1-year rate of all-cause mortality was as low as 2.2% following myocardial infarction [30]. Scar sizes reported here are comparable to those in clinical trials. In clinical trials performed by Lonborg et al and Stone et al in patients surviving ST-elevation myocardial infarction the median scar sizes, measured as % of left ventricular myocardial mass were 9.5% and 17.9 % respectively [30,31]. Moreover, scar sizes in the present study accord with those reported in previous publications in Göttingen minipigs (12-25%) [32-37] and in Landrace pigs (14-18%) [38-40]. The present finding on baseline LVEF in Landrace swine is according to data reported by others in large swine [13,41,42]. These values in large swine are smaller as compared to healthy human LVEF reference ranges (58-61%)[43] and baseline (pre-infarction) values in Göttingen minipigs (55-73%) [33,44,45]. Nevertheless, it is worth noting that only the post-infarction data or delta changes of LVEF are reported in most publications [46-50]. In accordance with the present results, previous studies of either post-MI HF induced by 45 to 90 min LAD occlusion followed by reperfusion or by permanent LAD occlusion have demonstrated either no reduction or modest reduction of LVEF in Landrace or Yorkshire swine after 4-6 weeks follow up as compared to baseline (pre-infarction) LVEF [51-53]. However, Schuleri et al. compared morphofunctional parameters between Göttingen minipigs and Yorkshire swine and found that both breeds showed a decrease of LVEF 8 weeks after induction of MI by 120 to 150 min LAD occlusion-reperfusion; however, baseline LVEF values in Yorkshire swine were not reported [54]. In other experiments in female Dalland Landrace pigs post-MI adverse remodeling was induced by 90 min LAD occlusion, however, LVEF was not reported after 4 weeks of follow-up [55]. In contrast to our findings, in a study by de Jong et al., LVEF markedly decreased in Landrace pigs subjected to open chest LAD occlusion and followed by a 12-week follow-up [56]. This difference can be attributed to substantially longer ischemic period (150 min), which resulted in larger infarct size (23.4 ± 2.1% of LV). Elsewhere, 120-min closed-chest occlusion of left circumflex (LCX) coronary artery in German Landrace pigs led to a significant reduction in LVEF after eight weeks of reperfusion, suggesting that the different

location of MI may also affect global left ventricular function [57]. Our present findings are consistent with others showing significant reduction in LVEF in post-MI HF in Göttingen minipigs after long-term follow up [33,44,45].

The reduction of LVEF in Göttingen minipigs following MI is consistent with clinical data showing cardiac dysfunction as a consequence of ventricular remodeling in patients after AMI [58]. In conclusion, Göttingen minipigs better mimic the human conditions, since pre-infarction LVEF, scar size, post-infarction LVEF, and mortality all are comparable to these parameters found in humans.

Here we observed a (8%) increase in LVED mass after six months in Göttingen minipigs and a markedly higher (97%) increase in LVED masses in Landrace pigs after two months. Similar data were reported by Schuleri et al. in Yorkshire pigs, where a 40% increase in heart weight was observed after two months. In contrast, in other experiments of closed-chest post-MI HF in Göttingen minipigs no significant changes in left ventricular masses were observed [33,44]. Therefore, differences between the two breeds regarding LVEF can be attributed to an intensive cardiac growth rate in Landrace pigs and thus altered cardiac remodeling.

In clinical settings, besides the LVEF, left ventricular volume provides valuable insight into long-term prognosis and mortality rate in post-MI patients [59]. LVESV is the primary determinant of both early and late mortality in patients after AMI [60,61]. Here we have shown that ventricular volume assessed by CMRI increased significantly in both breeds. Post-MI remodeling induced a more pronounced increase in LVESV than in LVEDV in Göttingen minipigs, while both LVESV and LVEDV were increased by a similar rate in Landrace pigs. Consequently, left ventricular ejection fraction (LVEF) was significantly decreased at 3 and 6 months only in Göttingen minipigs but not in Landrace pigs after 2 months. These results must be interpreted with caution in Landrace pigs, where increased LVESV, LVEDV, and LVSV (calculated as the difference between the LVESV and LVEDV) are more likely related to an intensive increase in cardiac mass. Increased LVESV and LVEDV are consistent with clinical data of patients with post-MI HF [62-64]. Moreover, adverse left ventricular remodeling was defined as an increase of 15% or more in the LVEDV in clinical studies[65,66] and we found here a 28% increase after 3 months and a 42% increase after 6 months in LVEDV in Göttingen minipigs showing a clinically relevant adverse remodeling. In addition, here we have shown that LAVi increased only in Göttingen minipigs, but not in Landrace pigs. Increase of left atrial volume is an additional key structural alteration in the context of HF and is an independent predictor of death and HF hospitalization in patients surviving MI [67].

Right ventricular function is rarely studied in post-MI HF models. Here we have found that right ventricular ejection fraction increased in both breeds. Although RV was practically not involved in myocardial necrosis, RVEF increased significantly in both breeds indicating RV volume overload and hence left ventricular dysfunction. Similarly, a clinical study enrolling 2008 patients with chronic systolic HF showed that 733 patients (37%) belonged to normal right ventricular function category with RVEF≥40% [68].

In conclusion, we have shown here that the adult Göttingen minipig model with long-term follow-

up mimics functional and morphological parameters of post-MI HF comparable to humans. Our present data also show that Landrace pigs are not suitable for the evaluation of post-MI HF mainly due to consequences of the rapid increase in body and heart weight that does not allow long-term follow-up and interferes with post-MI HF pathology. Landrace pigs might be suitable to assess the consequences of acute myocardial infarction. The present comprehensive characterization of the closed-chest infarction models in Landrace and Göttingen minipigs will be useful for choosing the optimal large animal models to study post-MI HF and developing novel therapies against this pathology.

The current experiment was performed only in female pigs, therefore, the potential effect of the different sexes on post-MI HF remains unknown in these models [69]. Signs of HF were assessed by CMRI, according to recommendations of a recent guideline on the relevance of rigor and reproducibility in preclinical studies on cardioprotection [12]. However, the use of more targeted angulation of CMRI imaging planes and more targeted sequence may result in better estimation of left atrial volumes, and pulmonary oedema. Although we haven't measured biomarkers and histological signs of post-MI HF in this study, these models are suitable for analysis of any biomarkers since the availability of plasma and tissue samples. Due to the different susceptibility of the 2 breeds to ischemia/reperfusion injury, different durations of coronary occlusions were selected here that may although limit the comparison of the 2 models, however, by this approach we achieved similar infarct size. The follow-up time in the 2 breeds was different as in the Landrace pigs only 2 months follow up time can be achieved due to technical reasons, i.e. rapid increase in body weight that shows a major limitation of the Landrace model. A further limitation is the lack of different risk factors and comorbidities and thus the present large animal models do not completely mimic the clinical situation in terms of the presence of multiple risk factors including co-morbidities and their medications. However, currently, there are no established large animal models with multiple comorbidities for routine use. These large animal models cannot be powered for mortality analysis due to animal ethical reasons and the high cost of these studies.

ACKNOWLEDGMENTS:

This study was funded by Quark Pharmaceuticals Inc where S.A. and E.F. are employees. This study was also supported by the National Research, Development and Innovation Office of Hungary (NKFIA; NVKP-16-1-2016-0017 National Heart Program), and by the Higher Education Institutional Excellence Program of the Ministry of Human Capacities in Hungary, within the framework of the Therapeutic Development thematic program of the Semmelweis University. GB.B. was supported by EFOP-3.6.3-VEKOP-16-2017-00009 and Gedeon Richter Plc. Scholarship. Z.G. was supported by a János Bolyai Research Scholarships of the Hungarian Academy of Sciences and by the ÚNKP-19-4 New National Excellence Program of the Ministry of Human Capacities.

DISCLOSURES:

PF is the founder and CEO of Pharmahungary Group, a Group of R&D companies www.pharmahungary.com.

REFERENCES:

1 Gerber, Y. et al. A contemporary appraisal of the heart failure epidemic in Olmsted County, Minnesota, 2000 to 2010. *JAMA Internal Medicine.* **175** (6), 996-1004, doi:10.1001/jamainternmed.2015.0924 (2015).

2 Gerber, Y. et al. Mortality Associated With Heart Failure After Myocardial Infarction: A Contemporary Community Perspective. *Circulation: Heart Failure.* **9** (1), e002460, doi:10.1161/circheartfailure.115.002460 (2016).

3 Paradies, V., Chan, M. H. H., Hausenloy, D. J. in *Primary Angioplasty: A Practical Guide* eds T. J. Watson, P. J. L. Ong, & J. E. Tcheng) 307-322 (Springer Copyright 2018, The Author(s). 2018).

4 Ponikowski, P. et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *European Heart Journal.* **37** (27), 2129-2200, doi:10.1093/eurheartj/ehw128 (2016).

5 Windecker, S. et al. 2014 ESC/EACTS Guidelines on myocardial revascularization: The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS)Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). *European Heart Journal.* **35** (37), 2541-2619, doi:10.1093/eurheartj/ehu278 (2014).

6 Hausenloy, D. J. et al. Novel targets and future strategies for acute cardioprotection: Position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart. *Cardiovascular Research.* **113** (6), 564-585, doi:10.1093/cvr/cvx049 (2017).

7 Lecour, S. et al. ESC working group cellular biology of the heart: position paper: improving the preclinical assessment of novel cardioprotective therapies. *Cardiovascular Research.* **104** (3), 399-411, doi:10.1093/cvr/cvu225 (2014).

8 Ferdinandy, P., Hausenloy, D. J., Heusch, G., Baxter, G. F., Schulz, R. Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. *Pharmacological Reviews.* **66** (4), 1142-1174, doi:10.1124/pr.113.008300 (2014).

9 Gaspar, A. et al. Randomized controlled trial of remote ischaemic conditioning in ST-elevation myocardial infarction as adjuvant to primary angioplasty (RIC-STEMI). *Basic Research in Cardiology.* **113** (3), 14, doi:10.1007/s00395-018-0672-3 (2018).

10 Hausenloy, D. J. et al. Effect of remote ischaemic conditioning on clinical outcomes in patients with acute myocardial infarction (CONDI-2/ERIC-PPCI): a single-blind randomised controlled trial. *Lancet.* **394** (10207), 1415-1424, doi:10.1016/s0140-6736(19)32039-2 (2019).

11 Heusch, G. Cardioprotection research must leave its comfort zone. *European Heart Journal.* **39** (36), 3393-3395, doi:10.1093/eurheartj/ehy253 (2018).

12 Bøtker, H. E. et al. Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. *Basic Research in Cardiology.* **113** (5), 39, doi:10.1007/s00395-018-0696-8 (2018).

13 McCall, F. C. et al. Myocardial infarction and intramyocardial injection models in swine. *Nature Protocols.* **7** (8), 1479-1496, doi:10.1038/nprot.2012.075 (2012).

14 Cesarovic, N., Lipiski, M., Falk, V., Emmert, M. Y. Animals in cardiovascular research. *European Heart Journal.* **41** (2), 200-203, doi:10.1093/eurheartj/ehz933 (2020).

15 Gutierrez, K., Dicks, N., Glanzner, W. G., Agellon, L. B., Bordignon, V. Efficacy of the porcine species in biomedical research. *Frontiers in Genetics.* **6** 293, doi:10.3389/fgene.2015.00293 (2015).
16 Lelovas, P. P., Kostomitsopoulos, N. G., Xanthos, T. T. A comparative anatomic and physiologic overview of the porcine heart. *Journal of the American Association for Laboratory Animal Science.* **53** (5), 432-438 (2014).
17 Itoh, T. et al. Body surface area measurement in laboratory miniature pigs using a computed tomography scanner. *Journal of Toxicological Sciences.* **41** (5), 637-644, doi:10.2131/jts.41.637 (2016).
18 Swindle, M. M., Makin, A., Herron, A. J., Clubb, F. J., Jr., Frazier, K. S. Swine as models in biomedical research and toxicology testing. *Veterinary Pathology.* **49** (2), 344-356, doi:10.1177/0300985811402846 (2012).
19 Ettrup, K. S. et al. Basic surgical techniques in the Gottingen minipig: intubation, bladder catheterization, femoral vessel catheterization, and transcardial perfusion. *Journal of Visualized Experiments.* (52), doi:10.3791/2652 (2011).
20 Pepine, C. J., Hill, J. A., Labert, C. R. *Diagnostic and therapeutic cardiac catheterization*. (Williams & Wilkins, 1998).
21 Thompson, C. A. *Textbook Of Cardiovascular Intervention*. (Springer London LTD, 2016).
22 Moral, S. et al. Quantification of myocardial area at risk: validation of coronary angiographic scores with cardiovascular magnetic resonance methods. *Revista Española de Cardiología (English Edition).* **65** (11), 1010-1017, doi:10.1016/j.recesp.2012.04.020 (2012).
23 Candell-Riera, J. et al. Culprit lesion and jeopardized myocardium: correlation between coronary angiography and single-photon emission computed tomography. *Clinical Cardiology.* **20** (4), 345-350, doi:10.1002/clc.4960200409 (1997).
24 Baranyai, T. et al. In vivo MRI and ex vivo histological assessment of the cardioprotection induced by ischemic preconditioning, postconditioning and remote conditioning in a closed-chest porcine model of reperfused acute myocardial infarction: importance of microvasculature. *Journal of Translational Medicine.* **15** (1), 67, doi:10.1186/s12967-017-1166-z (2017).
25 Giricz, Z. et al. Swiprosin-1/EFhD-2 Expression in Cardiac Remodeling and Post-Infarct Repair: Effect of Ischemic Conditioning. *International Journal of Molecular Sciences.* **21** (9), doi:10.3390/ijms21093359 (2020).
26 Gyöngyösi, M. et al. Inhibition of interleukin-1beta convertase is associated with decrease of neointimal hyperplasia after coronary artery stenting in pigs. *Molecular and Cellular Biochemistry.* **249** (1-2), 39-43 (2003).
27 Gyöngyösi, M. et al. Platelet activation and high tissue factor level predict acute stent thrombosis in pig coronary arteries: prothrombogenic response of drug-eluting or bare stent implantation within the first 24 hours. *Thrombosis and Haemostasis.* **96** (2), 202-209, doi:10.1160/th06-03-0178 (2006).
28 Lukovic, D. et al. Transcriptional Alterations by Ischaemic Postconditioning in a Pig Infarction Model: Impact on Microvascular Protection. *International Journal of Molecular Sciences.* **20** (2), doi:10.3390/ijms20020344 (2019).
29 Pavo, N. et al. On-line visualization of ischemic burden during repetitive ischemia/reperfusion. *JACC Cardiovascular Imaging.* **7** (9), 956-958, doi:10.1016/j.jcmg.2014.03.019 (2014).

30 Stone, G. W. et al. Relationship Between Infarct Size and Outcomes Following Primary PCI: Patient-Level Analysis From 10 Randomized Trials. *Journal of the American College of Cardiology.* **67** (14), 1674-1683, doi:10.1016/j.jacc.2016.01.069 (2016).
31 Lønborg, J. et al. Final infarct size measured by cardiovascular magnetic resonance in patients with ST elevation myocardial infarction predicts long-term clinical outcome: an observational study. *European Heart Journal: Cardiovascular Imaging.* **14** (4), 387-395, doi:10.1093/ehjci/jes271 (2013).
32 Karantalis, V. et al. Synergistic Effects of Combined Cell Therapy for Chronic Ischemic Cardiomyopathy. *Journal of the American College of Cardiology.* **66** (18), 1990-1999, doi:10.1016/j.jacc.2015.08.879 (2015).
33 Natsumeda, M. et al. A Combination of Allogeneic Stem Cells Promotes Cardiac Regeneration. *Journal of the American College of Cardiology.* **70** (20), 2504-2515, doi:10.1016/j.jacc.2017.09.036 (2017).
34 Quevedo, H. C. et al. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. *Proceedings of the National Academy of Sciences of the United States of America.* **106** (33), 14022-14027, doi:10.1073/pnas.0903201106 (2009).
35 Schuleri, K. H. et al. CT for evaluation of myocardial cell therapy in heart failure: a comparison with CMR imaging. *JACC: Cardiovascular Imaging.* **4** (12), 1284-1293, doi:10.1016/j.jcmg.2011.09.013 (2011).
36 Schuleri, K. H. et al. Cardiovascular magnetic resonance characterization of peri-infarct zone remodeling following myocardial infarction. *Journal of Cardiovascular Magnetic Resonance.* **14** (1), 24, doi:10.1186/1532-429x-14-24 (2012).
37 Schuleri, K. H. et al. Autologous mesenchymal stem cells produce reverse remodelling in chronic ischaemic cardiomyopathy. *European Heart Journal.* **30** (22), 2722-2732, doi:10.1093/eurheartj/ehp265 (2009).
38 Jansen of Lorkeers, S. J. et al. Xenotransplantation of Human Cardiomyocyte Progenitor Cells Does Not Improve Cardiac Function in a Porcine Model of Chronic Ischemic Heart Failure. Results from a Randomized, Blinded, Placebo Controlled Trial. *PLoS One.* **10** (12), e0143953, doi:10.1371/journal.pone.0143953 (2015).
39 van Hout, G. P. et al. Admittance-based pressure-volume loops versus gold standard cardiac magnetic resonance imaging in a porcine model of myocardial infarction. *Physiological Report.* **2** (4), e00287, doi:10.14814/phy2.287 (2014).
40 Thavapalachandran, S. et al. Platelet-derived growth factor-AB improves scar mechanics and vascularity after myocardial infarction. *Science Translational Medicine.* **12** (524), doi:10.1126/scitranslmed.aay2140 (2020).
41 Pahlm, U. S. et al. Regional wall function before and after acute myocardial infarction; an experimental study in pigs. *BMC Cardiovascular Disorders.* **14** 118, doi:10.1186/1471-2261-14-118 (2014).
42 Baranyai, T. et al. In vivo MRI and ex vivo histological assessment of the cardioprotection induced by ischemic preconditioning, postconditioning and remote conditioning in a closed-chest porcine model of reperfused acute myocardial infarction: importance of microvasculature. *Journal of Translational Medicine.* **15** (1), 67, doi:10.1186/s12967-017-1166-z (2017).
43 Petersen, S. E. et al. Reference ranges for cardiac structure and function using

837 cardiovascular magnetic resonance (CMR) in Caucasians from the UK Biobank population cohort.
838 *Journal of Cardiovascular Magnetic Resonance.* **19** (1), 18, doi:10.1186/s12968-017-0327-9
839 (2017).
840 44 Bellera, N. et al. Single intracoronary injection of encapsulated antagomir-92a promotes
841 angiogenesis and prevents adverse infarct remodeling. *Journal of the American Heart*
842 *Association.* **3** (5), e000946, doi:10.1161/jaha.114.000946 (2014).
843 45 Sharp, T. E., 3rd et al. Cortical Bone Stem Cell Therapy Preserves Cardiac Structure and
844 Function After Myocardial Infarction. *Circulation Research.* **121** (11), 1263-1278,
845 doi:10.1161/circresaha.117.311174 (2017).
846 46 Crisostomo, V. et al. Delayed administration of allogeneic cardiac stem cell therapy for
847 acute myocardial infarction could ameliorate adverse remodeling: experimental study in swine.
848 *Journal of Translational Medicine.* **13** 156, doi:10.1186/s12967-015-0512-2 (2015).
849 47 Uitterdijk, A. et al. VEGF165A microsphere therapy for myocardial infarction suppresses
850 acute cytokine release and increases microvascular density but does not improve cardiac
851 function. *American Journal of Physiology-Heart and Circulatory Physiology.* **309** (3), H396-406,
852 doi:10.1152/ajpheart.00698.2014 (2015).
853 48 Vilahur, G. et al. HMG-CoA reductase inhibition prior reperfusion improves reparative
854 fibrosis post-myocardial infarction in a preclinical experimental model. *International Journal of*
855 *Cardiology.* **175** (3), 528-538, doi:10.1016/j.ijcard.2014.06.040 (2014).
856 49 Vilahur, G. et al. Reperfusion-triggered stress protein response in the myocardium is
857 blocked by post-conditioning. Systems biology pathway analysis highlights the key role of the
858 canonical aryl-hydrocarbon receptor pathway. *European Heart Journal.* **34** (27), 2082-2093,
859 doi:10.1093/eurheartj/ehs211 (2013).
860 50 Zalewski, J. et al. Cyclosporine A reduces microvascular obstruction and preserves left
861 ventricular function deterioration following myocardial ischemia and reperfusion. *Basic Research*
862 *in Cardiology.* **110** (2), 18, doi:10.1007/s00395-015-0475-8 (2015).
863 51 Galvez-Monton, C. et al. Comparison of two preclinical myocardial infarct models:
864 coronary coil deployment versus surgical ligation. *Journal of Translational Medicine.* **12** 137,
865 doi:10.1186/1479-5876-12-137 (2014).
866 52 Ghugre, N. R., Pop, M., Barry, J., Connelly, K. A., Wright, G. A. Quantitative magnetic
867 resonance imaging can distinguish remodeling mechanisms after acute myocardial infarction
868 based on the severity of ischemic insult. *Magnetic Resonance in Medicine.* **70** (4), 1095-1105,
869 doi:10.1002/mrm.24531 (2013).
870 53 Sim, D. S. et al. Cardioprotective effect of fimasartan, a new angiotensin receptor blocker,
871 in a porcine model of acute myocardial infarction. *Journal of Korean Medical Science.* **30** (1), 34-
872 43, doi:10.3346/jkms.2015.30.1.34 (2015).
873 54 Schuleri, K. H. et al. The adult Gottingen minipig as a model for chronic heart failure after
874 myocardial infarction: focus on cardiovascular imaging and regenerative therapies. *Comparative*
875 *Medicine.* **58** (6), 568-579 (2008).
876 55 Koudstaal, S. et al. Myocardial infarction and functional outcome assessment in pigs.
877 *Journal of Visualized Experiments.* (86), doi:10.3791/51269 (2014).
878 56 de Jong, R. et al. Cardiac function in a long-term follow-up study of moderate and severe
879 porcine model of chronic myocardial infarction. *BioMed Research International.* **2015** 209315,
880 doi:10.1155/2015/209315 (2015).

57 Raake, P. W. J. et al. Comprehensive cardiac phenotyping in large animals: comparison of pressure-volume analysis and cardiac magnetic resonance imaging in pig post-myocardial infarction systolic heart failure.International Journal of Cardiovascular Imaging. **35** (9), 1691-1699, doi:10.1007/s10554-019-01610-z (2019).
58 Burns, R. J. et al. The relationships of left ventricular ejection fraction, end-systolic volume index and infarct size to six-month mortality after hospital discharge following myocardial infarction treated by thrombolysis. *Journal of the American College of Cardiology.* **39** (1), 30-36, doi:10.1016/s0735-1097(01)01711-9 (2002).
59 Cohn, J. N., Ferrari, R., Sharpe, N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *Journal of the American College of Cardiology.* **35** (3), 569-582, doi:10.1016/s0735-1097(99)00630-0 (2000).
60 Migrino, R. Q. et al. End-systolic volume index at 90 to 180 minutes into reperfusion therapy for acute myocardial infarction is a strong predictor of early and late mortality. The Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO)-I Angiographic Investigators. *Circulation.* **96** (1), 116-121, doi:10.1161/01.cir.96.1.116 (1997).
61 White, H. D. et al. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation.* **76** (1), 44-51, doi:10.1161/01.cir.76.1.44 (1987).
62 Asgeirsson, D. et al. Longitudinal shortening remains the principal component of left ventricular pumping in patients with chronic myocardial infarction even when the absolute atrioventricular plane displacement is decreased. *BMC Cardiovascular Disorders.* **17** (1), 208, doi:10.1186/s12872-017-0641-z (2017).
63 Pfeffer, M. A., Lamas, G. A., Vaughan, D. E., Parisi, A. F., Braunwald, E. Effect of captopril on progressive ventricular dilatation after anterior myocardial infarction. *New England Journal of Medicine.* **319** (2), 80-86, doi:10.1056/nejm198807143190204 (1988).
64 McKay, R. G. et al. Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. *Circulation.* **74** (4), 693-702, doi:10.1161/01.cir.74.4.693 (1986).
65 Cung, T. T. et al. Cyclosporine before PCI in Patients with Acute Myocardial Infarction. *New England Journal of Medicine.* **373** (11), 1021-1031, doi:10.1056/NEJMoa1505489 (2015).
66 Savoye, C. et al. Left ventricular remodeling after anterior wall acute myocardial infarction in modern clinical practice (from the REmodelage VEntriculaire [REVE] study group). *American Journal of Cardiology.* **98** (9), 1144-1149, doi:10.1016/j.amjcard.2006.06.011 (2006).
67 Meris, A. et al. Left atrial remodelling in patients with myocardial infarction complicated by heart failure, left ventricular dysfunction, or both: the VALIANT Echo study. *European Heart Journal.* **30** (1), 56-65, doi:10.1093/eurheartj/ehn499 (2009).
68 Meyer, P. et al. Effects of right ventricular ejection fraction on outcomes in chronic systolic heart failure. *Circulation.* **121** (2), 252-258, doi:10.1161/circulationaha.109.887570 (2010).
69 Perrino, C. et al. Improving Translational Research in Sex-specific Effects of Comorbidities and Risk Factors in Ischemic Heart Disease and Cardioprotection: Position Paper and Recommendations of the ESC Working Group on Cellular Biology of the Heart. *Cardiovascular Research.* doi:10.1093/cvr/cvaa155 (2020).

Figure 1

Click here to access/download;Figure;Figure1_R1.pdf

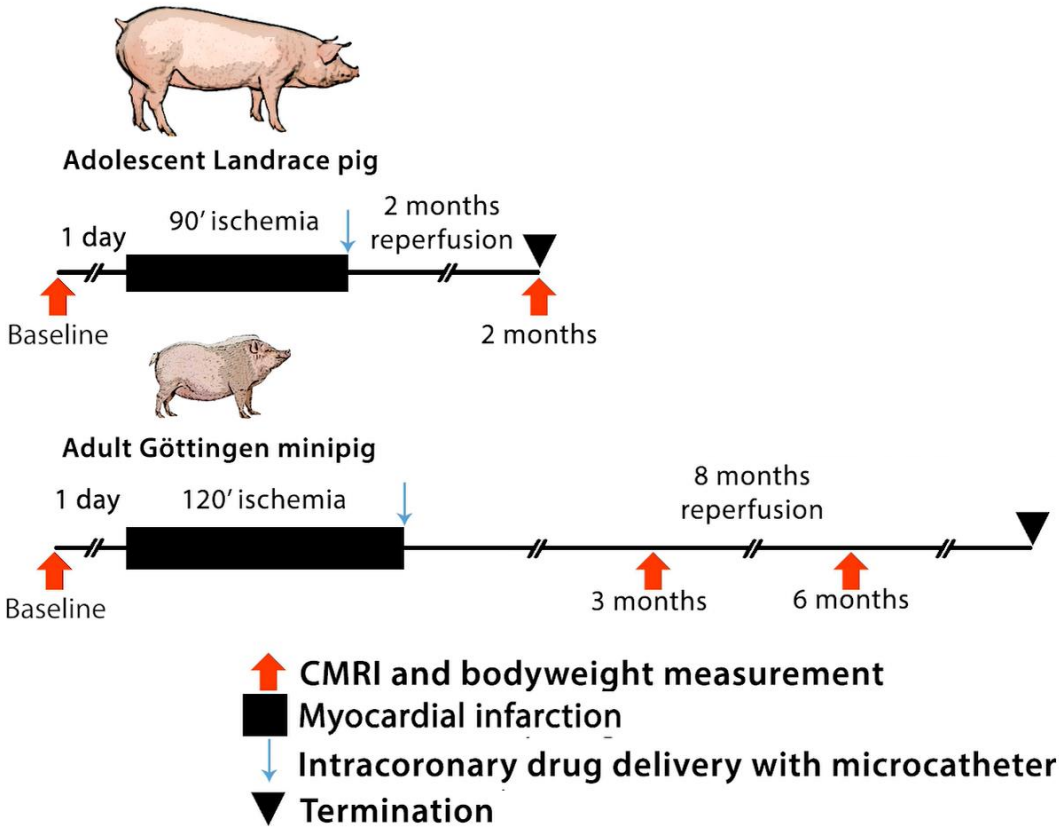
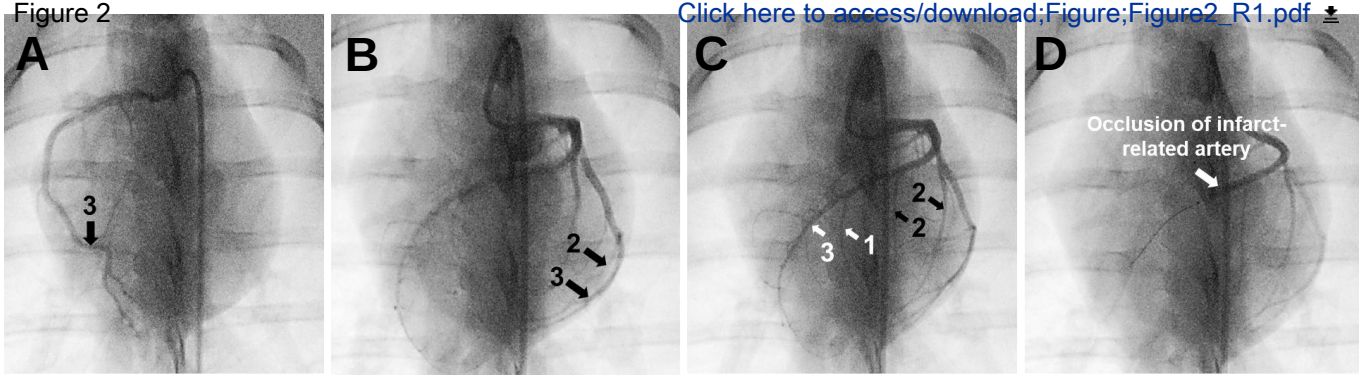


Figure 2



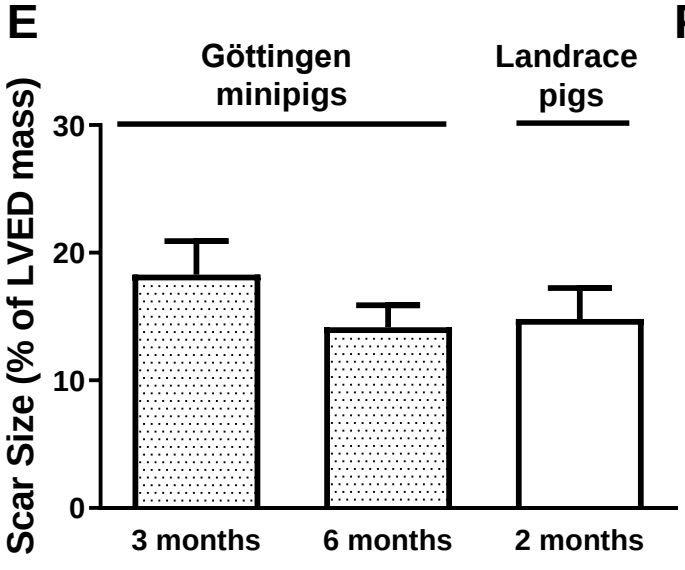
RCA
Total value:
3

LCX
Total value:
2+3 = 5

LAD
Total value:
2+2+1+3 = 8

Total value of the infarct related artery:
3+1 = 4

BARI score (% of left ventricle at risk) = total value of the infarct-related artery divided by the sum of the 3 total values of each coronary artery = 4/16 = 25%



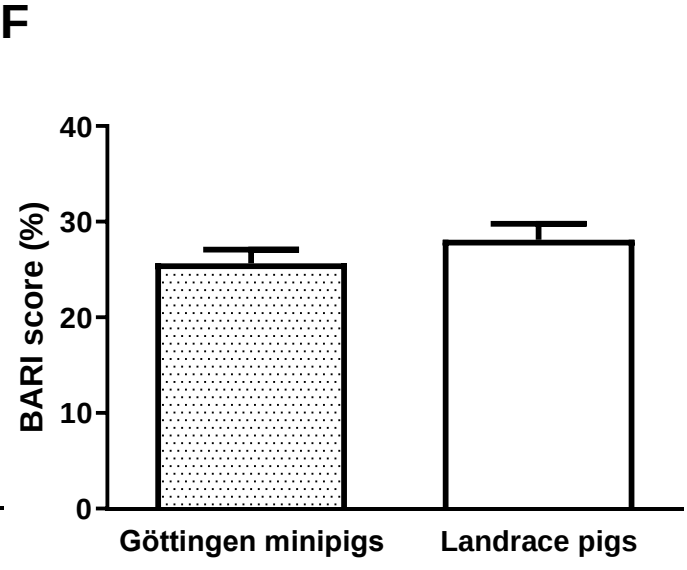


Figure 3

Click here to access/download;Figure;Figure3_R1.pdf

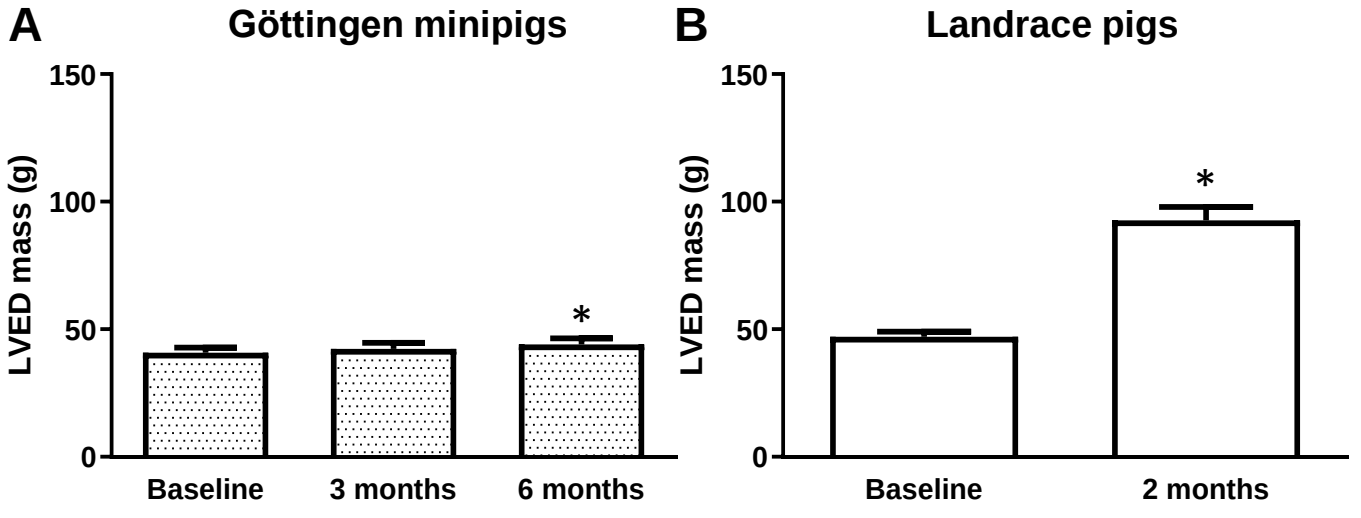


Figure 4

Click here to access/download;Figure;Figure4_R1.pdf

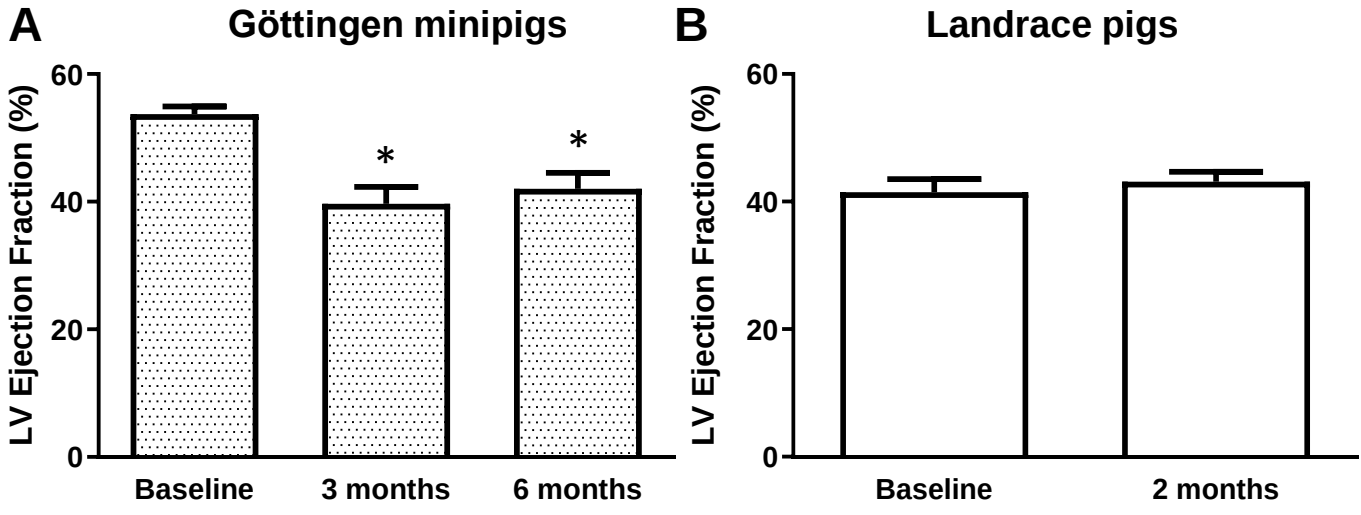


Figure 5

Click here to access/download;Figure;Figure5_R1.pdf

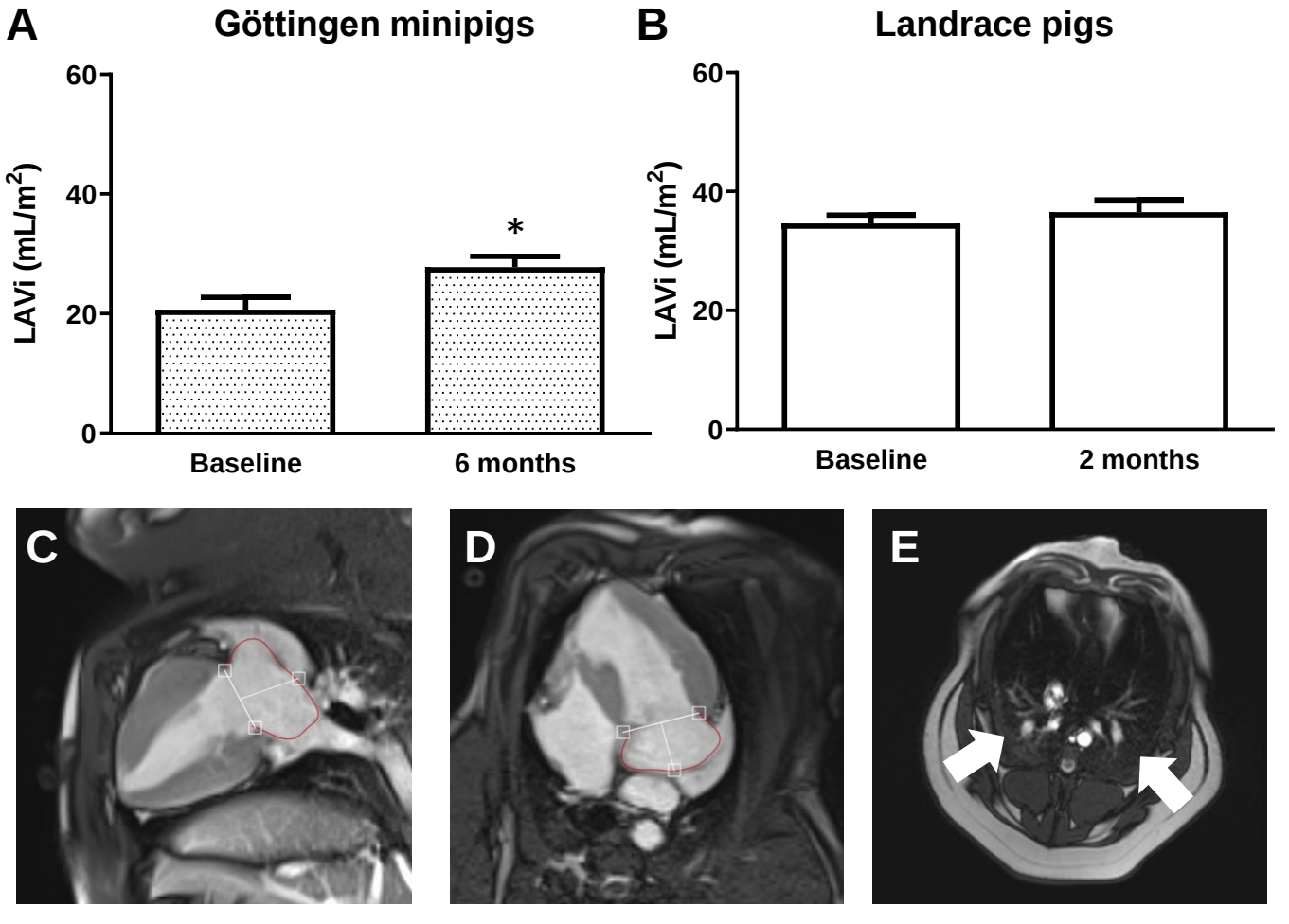


Figure 6

Click here to access/download;Figure;Figure6_R1.pdf

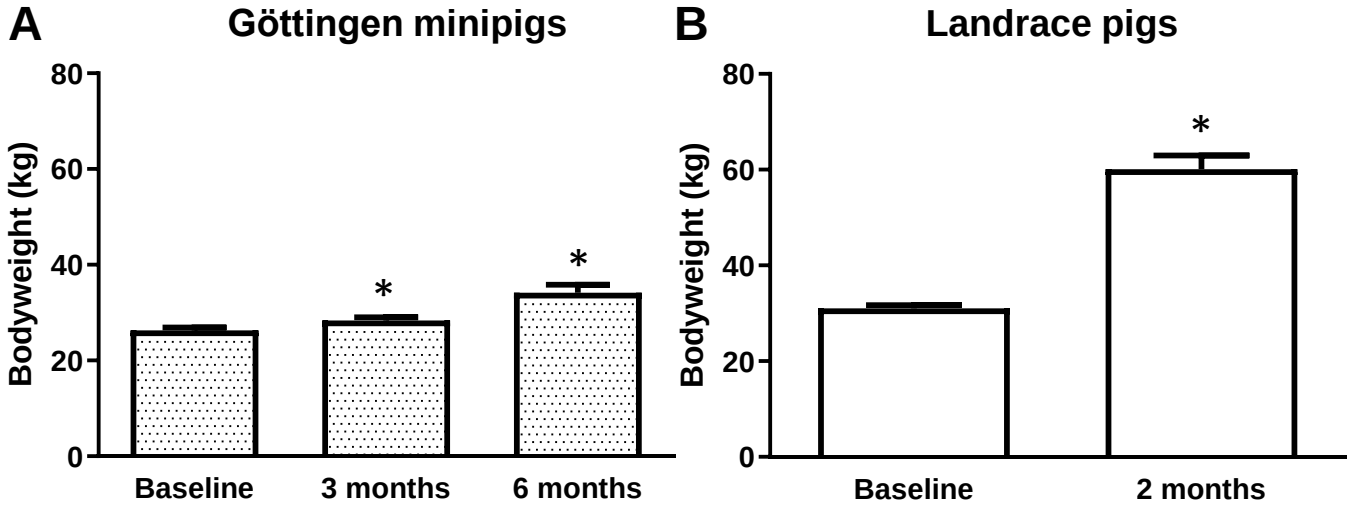


Figure 7

Click here to access/download;Figure;Figure7_R1.pdf

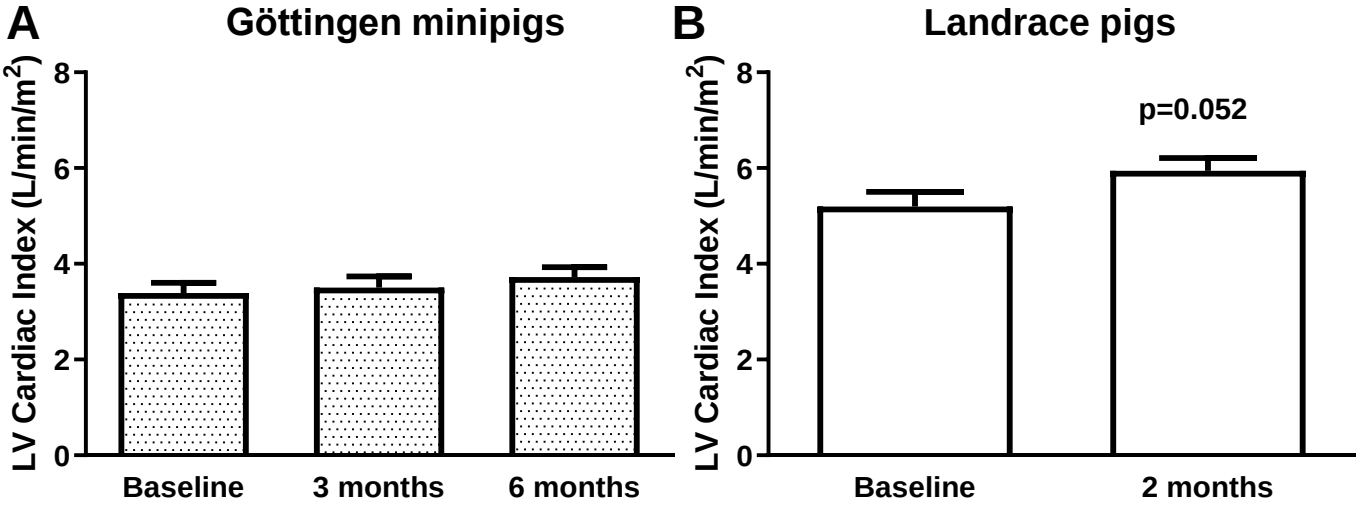


Figure 8

Click here to access/download;Figure;Figure8_R1.pdf

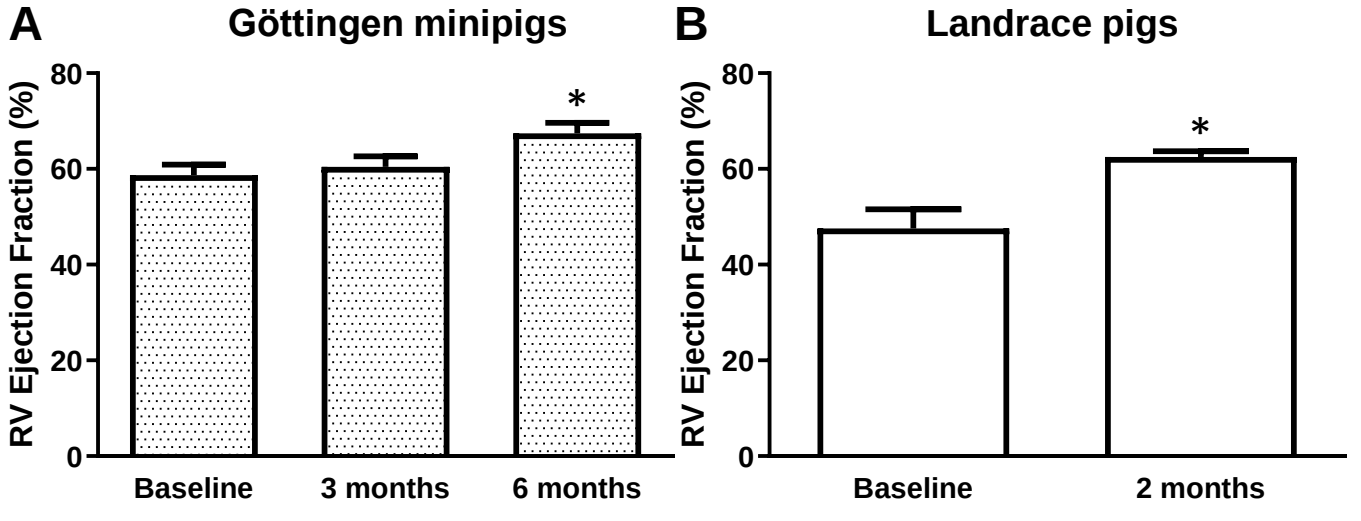


Table 1

Measured parameter	Göttingen minipigs			Landrace pigs	
	Baseline	3 months	6 months	Baseline	2 months
LVESV [ml]	25.77 ± 1.73	43.65 ± 4.53*	46.28 ± 4.35*	54.59 ± 2.00	98.26 ± 8.60*
LVEDV [ml]	55.49 ± 3.14	71.08 ± 5.25*	78.81 ± 5.46*	93.99 ± 3.85	171.20 ± 11.50*
LVSV [ml]	29.71 ± 1.65	27.44 ± 1.97	32.52 ± 2.37	39.40 ± 3.05	72.94 ± 3.99*

Table 2

Measured parameter	Göttingen minipigs			Landrace pigs	
	Baseline	3 months	6 months	Baseline	2 months
HR [1/min]	79.64 ± 4.03	95.55 ± 5.34*	97.00 ± 4.46*	93.44 ± 2.73	88.00 ± 2.52
CO [L/min]	2.37 ± 0.16	2.58 ± 0.20	3.12 ± 0.24*	3.65 ± 0.25	6.41 ± 0.39*
BSA [m^2]	0.70 ± 0.01	0.73 ± 0.01*	0.83 ± 0.03*	0.70 ± 0.01	1.08 ± 0.03*

Table 3

Measured parameter	Göttingen minipigs			Landrace pigs	
	Baseline	3 months	6 months	Baseline	2 months
RVED mass [g]	8.64 ± 0.68	8.98 ± 0.76	7.94 ± 0.77	16.49 ± 0.90	23.61 ± 1.40*
RVESV [ml]	18.27 ± 1.47	16.91 ± 1.80	14.57 ± 1.02	43.59 ± 3.68	42.65 ± 2.37
RVEDV [ml]	44.16 ± 2.61	42.14 ± 2.83	46.27 ± 3.45	83.03 ± 3.42	113.72 ± 5.12*
RVSV [ml]	25.82 ± 1.72	25.25 ± 1.67	31.71 ± 2.99*	39.44 ± 3.52	71.06 ± 3.38*

Name of Material/Equipment	Company
Special Diet Services pig chow	SDS, Witham, England, Hungarian distributor: Akronom Kft.
maintenance minipig diet	no. 9023, Altromin
pregnant sow chow	Bonafarm-Bábolna Takarmány Plc
ketamine hydrochloride	Richter Pharma AG
xylazine	Medicus Partner
atropine	Egis
endotracheal tube	Portex
isoflurane	Abbot
anesthetic machine	Dräger Julian
18 G needle	Anhul Kangda Medical Products Co. Ltd.
5% glucose in Ringer solution	B Braun
atracurium besylate	GSK
cardiac magnetic resonance machine	Siemens Healthineers Medical GmbH
acetyl salicylic acid	Bayer
clopidogrel	Zentiva
meloxicam (meloxidyl)	Ceva
antibiotic coctail (tardomyocel) comp III.	Norbrook
ear vein cannula	B Braun Melsungen AG
magnesium sulfate	Wörwag Pharma GmbH
povidone-iodine	Egis
ECG electrodes	Leonhard Lang GmbH
6F-ACT introducer	St Jude Medical
heparin	TEVA
arterial pressure sensor and monitoring system	GE Healthcare
guidewire	PT^2MS Boston Scientific

5F guiding catheter	Medtronic Launcher, 5F
fluoroscope, C-bow	Siemens Medical GmbH
Iobitridol (Xenetix)	Guerbet
balloon catheter	Boston Scientific, EMERGE, 2.5mm x 12mm
heating device	3M
rectal probe	Vatner Kft
pulse oxymeter	Comen medical
epinephrine	Richter Gedeon Rt.
lidocaine	EGIS
microcatheter	Caravel ASAHI
defibrillator	GE Marquette Responder 1100
perfusion pump	TSE system
antiseptic coating	Friedrich Huber aeronova GmbH&Co
gadobutrol	Bayer
MASS 7.6 analysis software	Medis Medical Imaging Software, Leiden

250 years of EXCELLENCE in medical education, research & innovation and healthcare

SEMMELWEIS UNIVERSITY

Faculty of Medicine

Department of Pharmacology and Pharmacotherapy

Director

Péter Ferdinandy, MD, PhD, DSc, MBA

18th January, 2021.

RE: revised manuscript, ID: JoVE61901, by Brenner GB, Giricz Z *et al.*

Dear Editors,

We have revised the manuscript, figures and the video according to the valuable comments of the editors and the reviewers. Please find attached our revised manuscript and here the detailed response to the comments of the editors and production.

We hope that our manuscript and video will meet the high standards of JoVe and it will be considered for publishing.

Yours sincerely,

Péter Ferdinandy, MD, PhD, DSc, MBA

Address: H-1089 Budapest, Orczy út 2-4. HUNGARY
Postal address: P.O. Box 2, Budapest, H-1428;
Üllői út 26., Budapest, H-1085;

Phone: (+36-1) 210-4416, Fax: (+36-1) 210-4412
E-mail: ferdinandy.peter@med.semmelweis-univ.hu
E-mail office: pharmacology@med.semmelweis-univ.hu
web: semmelweis.hu/pharmacology

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

According to the request of the Editorial team, the proofreading was done.

2. Please revise the title for conciseness.

We have shortened the title as follows:

"Post-myocardial infarction heart failure in closed-chest coronary occlusion/reperfusion model in Göttingen minipigs and Landrace pigs"

3. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s). Please remove the brackets as well.

Answer: The style of the references was changed to as required.

4. Please specify the age/gender of the animal used.

According to the request of the Editorial team we amended the protocol section accordingly as follows on page 4.

"13 healthy and sexually mature female Göttingen minipigs (age between 12 and 14 months) and 10 healthy and sexually immature female Landrace pigs (age between 2 and 3 months) were housed in pig stalls conforming to the size recommendations of the most recent Guide for the Care and Use of Laboratory Animals DHEW and EU Guidelines 63/2010. Animals were not spayed. The temperature of the animal rooms was controlled, and animals were kept at a 12-hour light/dark cycle and vermin-free."

5. Please discuss limitations of the protocol in the discussion.

Answer: According to the request of the Editorial we amended the text with a limitation section as follows on page 14 and 15.

"The current experiment was performed only in female pigs, therefore, the potential effect of the different sexes on post-MI HF remains unknown in these models. Signs of HF were assessed by CMRI, according to a recent guideline on the relevance of rigor and reproducibility in preclinical studies on cardioprotection recommends. However, the use of more targeted angulation of CMRI imaging planes and more targeted sequence may result in better estimation of left atrial volumes, and pulmonary oedema. Although we haven't measured biomarkers and histological signs of post-MI HF in this study, these models are suitable for analysis of any biomarkers since the availability of plasma and tissue samples. Due to the different susceptibility of the 2 breeds to ischemia/reperfusion injury, different durations of coronary occlusions were selected here that may although limit the comparison of the 2 models, however, by this approach we achieved similar infarct size. The follow-up time in the 2 breeds was different as in the Landrace pigs only 2 months follow up time can be achieved due to technical reasons, i.e. rapid increase in body weight that shows a major limitation of the Landrace model. A further limitation is the lack of different risk factors and comorbidities and thus the present large animal models do not completely mimic the clinical situation in terms of the presence of multiple risk factors including co-morbidities and their medications. However, currently, there are no established large

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

animal models with multiple comorbidities for routine use. These large animal models cannot be powered for mortality analysis due to animal ethical reasons and the high cost of these studies."

In addition, please note that all the suggestions of the production team were involved in the video. These suggestions were the next:

1. Please reduce the video length to be 15 min or less. The video is currently 15:02 long.
2. Please increase the homogeneity between the video and the written manuscript. Ideally, all figures in the video would appear in the written manuscript and vice versa. The video and the written manuscript should be reflections of each other.
3. Title Cards & Text:

• 01:35 - 01:42. The name caption shows a bit more info than what we normally show. You can simplify the text by using their name and the university they're affiliated with.
• 02:55 - 02:57 Placement of lower 3rd should be on the right
• 03:17 - 03:17 The ethics card appears 3 minutes into this video. The intro usually is a minute, minute and a half tops, I would shorten the intro to reduce redundancy.
• 03:19 - 03:19 The titles should fade out then fade in.
• 03:22 - 03:22 Titles should fade out, video or graphics should fade in. No straight cuts.
• 03:44 - 03:44 Fade out Fade in text
• 03:53 - 03:53 Lower text fades out, while top text stays a couple frames before the cut
• 04:00 - 04:00 Fade out video, fade in Text
• 04:05 - 04:05 Fade out text, fade in video
• 04:31 - 04:31 Cross Dissolve, fade up text
• 04:36 - 04:36 Text fades out, but doesn't fade in
• 06:07 - 06:13 Text fades out, but cuts in wierdly
• 06:23 - 06:30 Text doesn't fade in or out
• 06:31 - 06:31 Fade out video fade in text
• 06:36 - 06:36 Fade out text, fade in graphics
• 07:39 - 07:39 Text doesn't have a drop shadow like the previous text. Not consistent. Also it cuts in and fades out.
• 07:59 - 07:59 Text cuts in
• 08:54 - 08:54 Text cuts in and out
• 09:40 - 09:40 Text randomly drifts upwards
• 12:13 - 12:13 Fade out, fade in
• 12:18 - 12:18 Fade out fade in
• 14:04 - 14:04 Fade out Fade in
• 14:08 - 14:08 Fade Out Fade In
• 14:09 - 14:09 Show the name caption again

4. Video Editing & Pacing:

• 00:01 - 0:01 Let the video fade up first before the audio starts.
• 01:32 - 01:32 This dip to black is jarring and starts right as the person talks on screen.
• 02:05 - 02:05 Abrupt jump cut between the same talent, maybe cover it with some b-roll footage.
• 02:33 - 02:33 Cuts away too soon from previous talent
• 02:53 - 02:53 I would cut out the breath in before talking
• 03:39 - 03:39 Fade out video, fade in text, add a second more space between title and VO.
• 05:02 - 05:12 Too much space here without VO
• 05:18 - 05:22 Too much space without VO

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

• 05:28 - 05:38 There's a lot of space without VO. I would cut out anything extra, unnecessary, or redundant; in order to keep the video concise
• 07:56 - 07:56 The cut here seems to happen a second too late into the VO
• 09:51 - 09:51 Fade out video, Fade in text
• 09:57 - 09:57 Fade out text, Fade in graphics
• 10:17 - 10:22 Long pause without VO, can be cut up with cross dissolves
• 10:26 - 10:30 Long pause wehre nothing happens on camera, and no VO
• 10:34 - 10:37 Too much space with no VO
• 11:12 - 11:12 Fade out video, fade in text
• 11:18 - 11:18 Fade out video, fade in text
• 11:51 - 11:51 Fade out video, fade in text
• 11:56 - 11:56 Fade out text, fade in video
• 14:18 - 14:18 Cut out sooner, the cut in is a bit too late, it cuts off a bit of the beginning of his words.
• 14:50 - 14:50 Fade Out, pause a second, then fade up text
• 14:50 - 15:02 15 seconds might be too long for credits, 5 seconds should be enough.

5. Cross Dissolves
• 04:10 - Cross dissolve between these two shots of the pig being placed on the table and then being tied to the table
• 04:14, 04:20, 04:23, 04:25 - Other points where cross dissolves need to happen.
• 05:40 - 05:55 Alot of this could cut up and shortened using cross dissolves.

6. Results:
• 12:31 - 12:31 Give more space around the frame margins by shrinking or moving the figures a bit
• 12:50 - 12:50 Scale the figures down a bit so they don't look claustrophobic

7. Narration Audio:
• 00:30 - 0:30 Raise the overall volume a little bit.
• 07:31 - 07:31 Consider re-editing this section of narration, as it is somewhat cut-up, the statements should be more united.

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

250 years of EXCELLENCE in medical education, research & innovation and healthcare

SEMMELWEIS UNIVERSITY

Faculty of Medicine

Department of Pharmacology and Pharmacotherapy

Director

Péter Ferdinandy, MD, PhD, DSc, MBA

18th January, 2021.

RE: revised manuscript, ID: JoVE61901, by Brenner GB, Giricz Z *et al.*

Dear Reviewer 1,

We have revised the manuscript, figures and the video according to Your valuable comments. Please find attached our revised manuscript and a detailed response to Your comments here in this letter, see below.

We hope that our manuscript and video will meet the high standards of JoVe and it will be considered for publishing.

Yours sincerely,

Péter Ferdinandy, MD, PhD, DSc, MBA

Address: H-1089 Budapest, Orczy út 2-4. HUNGARY
Postal address: P.O. Box 2, Budapest, H-1428;
Üllői út 26., Budapest, H-1085;

Phone: (+36-1) 210-4416, Fax: (+36-1) 210-4412
E-mail: ferdinandy.peter@med.semmelweis-univ.hu
E-mail office: pharmacology@med.semmelweis-univ.hu
web: semmelweis.hu/pharmacology

Major Concerns:

1. Missing details of animal conditions, such as age of initial experiment, sex, puberty or not, castration or not. Is there any sex effect in minipigs during post-MI period?

According to the request of Reviewer #1 as per the missing details of animal conditions, sex, age, puberty, castration we amended the manuscript on page 4:

"13 healthy and sexually mature female Göttingen minipigs (age between 12 and 14 months) and 10 healthy and sexually immature female Landrace pigs (age between 2 and 3 months) were housed in pig stalls conforming to the size recommendations of the most recent Guide for the Care and Use of Laboratory Animals DHEW and EU Guidelines 63/2010. Animals were not spayed. The temperature of the animal rooms was controlled, and animals were kept at a 12-hour light/dark cycle and vermin-free."

As per the question related to the "sex effect in minipigs during post-MI period". The sex effect on post-MI HF parameters is possible, but the current experiment was performed only in female pigs, therefore, the potential effect of the different sexes on post-MI HF remains unknown in these models. Sex effects on outcome of cardiovascular diseases were described in details in a recent position paper in which one of our senior authors was an author (Perrino C et al, Cardiovasc Res, 2020).

According to this comment of Reviewer #1 we amended the limitation section as follows on page 14:

"The current experiment was performed only in female pigs, therefore, the potential effect of the different sexes on post-MI HF remains unknown in these models."

2. Dietary intake and diet composition should be addressed in the study (ad libitum or ?% of body weight/day, type of diet? commerical diet or high fat diet?). Is dietary intake amount for Landrace pig the reason for increasing body weight twice in two months? Does Landrace pig recive a higher caloric diet than Göttingen minipig?

Answer: Ad libitum feeding would lead to overt weight gain in both Landrace pigs and Göttingen minipigs. The two strains got different chow, but both were restricted feedings. The Göttingen minipigs received chow according to the breeder's recommendations and the Landrace pigs received pregnant sow chow, 1.5% of body weight given two times a day according to PIC Wean to Finish Manual 2008 and 2013.

According to the request of Reviewer #1 we amended the text of the protocol as follows on page 3:

"Ad libitum feeding leads to overt weight gain in both Göttingen minipigs and Landrace pigs, therefore, pigs from both the breeds were fed with a restricted diet regimen. Göttingen minipigs were put on restricted diet as early as they arrived to animal facility and for the whole study duration. Special Diet Services pig chow 180-220g/meal/animal was given twice daily according to "Taking good care of Ellegaard Göttingen Minipigs®" guideline (revision date: 13 March, 2013) in the first 2 days. Between day 3 and 12 animals were fed 50% Special Diet Services pig chow and 50% maintenance minipig diet. From day 14 until the end of the study animals were fed a maintenance minipig diet. Landrace pigs received pregnant sow chow, 1.5% of body weight given two times a day according to PIC Wean to Finish Manual 2008 and 2013. All the animals received food individually dispensed and food intake was

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

monitored to avoid competition for chow. Animals with feeding difficulties were fed individually aided by tending personnel. All animals received tap water ad libitum."

3. How about the blood pressure for these pigs post-MI period?

Answer: here we show baseline (before coronary artery occlusion) mean arterial blood pressure values and values in early reperfusion. We did not want to perform invasive measurements in the follow-up period. We found that MAP decreased almost significantly in Göttingen minipigs and decreased significantly in Landrace pigs in early reperfusion phase.

According to the request of Reviewer #1 we amended the text of result section as follows on page 10:

"Coronary artery occlusion led to an almost significant decrease in mean arterial pressure (MAP) in Göttingen minipigs (57.9 ± 3.98 mmHg vs. 49.89 ± 1.24 mmHg) and decreased significantly in Landrace pigs (65.4 ± 5.97 mmHg vs. 45.47 ± 4.79 mmHg) in the early reperfusion phase as compared to the baseline (pre-infarction) values."*

Minor Concerns:

《 There are some typos

Corrected according to the request.

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

250 years of EXCELLENCE in medical education, research & innovation and healthcare

SEMMELWEIS UNIVERSITY

Faculty of Medicine

Department of Pharmacology and Pharmacotherapy

Director

Péter Ferdinandy, MD, PhD, DSc, MBA

18th January, 2021.

RE: revised manuscript, ID: JoVE61901, by Brenner GB, Giricz Z *et al.*

Dear Reviewer 2,

We have revised the manuscript, figures and the video according to Your valuable comments. Please find attached our revised manuscript and a detailed response to Your comments here in this letter, see below.

We hope that our manuscript and video will meet the high standards of JoVe and it will be considered for publishing.

Yours sincerely,

Péter Ferdinandy, MD, PhD, DSc, MBA

Address: H-1089 Budapest, Orczy út 2-4. HUNGARY
Postal address: P.O. Box 2, Budapest, H-1428;
Üllői út 26., Budapest, H-1085;

Phone: (+36-1) 210-4416, Fax: (+36-1) 210-4412
E-mail: ferdinandy.peter@med.semmelweis-univ.hu
E-mail office: pharmacology@med.semmelweis-univ.hu
web: semmelweis.hu/pharmacology

1. Clinical studies on cardioprotection for which a translational model is sought use mortality and hospitalization for heart failure as clinical outcome endpoints. This model had zero mortality after reperfusion (when a patient would enter a study) and no reduction in cardiac index. Clinical signs of heart failure are not reported. Therefore, there is no evidence for heart failure, just evidence for moderate LV dysfunction which would not show up in a clinical outcome endpoint.

Answer: We agree that these large animal models do not completely mimic the clinical situation in terms of multiple risk factors including co-morbidities and their medications, but currently there are no more suitable large animal models for routine use before entering into a clinical trial. Here we have shown 15.4% acute mortality rate in young adult Göttingen minipigs with no comorbidities, and no mortality was observed in follow-up period. Indeed, risk factors affects the outcome of myocardial infarction and cardioprotection as reviewed by us repeatedly (Ferdinandy et al, Pharmacol Rev, 2014, 2007; Trend Pharmacol Sci, 1998). It should be noted that low mortality rate is comparable to clinical studies (Stone GW et al, J Am Coll Cardiol, 2016). Experimental studies with larger group sizes in large animals with different comorbidities powered for mortality analysis or for other cardiovascular events as endpoints with even longer follow-up are definitely needed, however, this would serious raise concerns in animal-ethics and finances.
Thank you for Your valuable feedback regarding cardiac parameters and cardiac index. Here we have focused on signs of heart failure that can be measured by cardiac magnetic resonance imaging and found reduction in left ventricular ejection fraction in Göttingen minipigs but not in Landrace pigs.
Moreover, adverse left ventricular remodeling was defined as an increase of 15% or more in the LVEDV in clinical settings and we found 28% increase after 3 months and a 42% increase after 6 months in LVEDV in Göttingen minipigs (Cung TT et al, N Engl J Med, 2015, Savoye C et al, Am J Cardiol., 2006). In order to further examine signs of heart failure, we performed measurement of the the left atrial volume indexed to body surface area (LAVi). LAVi increased by 34% in Göttingen minipigs after 6 months (Figure 5A, see below) and did not change significantly in Landrace pigs after 2 months (Figure 5B, see below). Moreover, the presence or absence of pulmonary oedema was assessed by cardiac MRI on the localizer images. Pulmonary oedema was observed in both breeds as a result of cardiac decompensation. Ten out of eleven Göttingen minipigs (see also below representative figure 5E) and nine out of ten Landrace pigs showed obvious signs of pulmonary oedema.

According to the request of Reviewer #2 we amended the discussion section as follows on page 13:
"Here we observed a 15.4% acute mortality in Göttingen minipigs and no mortality in the follow-up period, the let-ter is comparable to that in clinical studies. Indeed, a patient-level meta-analysis of 10 randomized clinical trials found that the Kaplan-Meier estimated 1-year rate of all-cause mortality was as low as 2.2% following myocardial infarction".

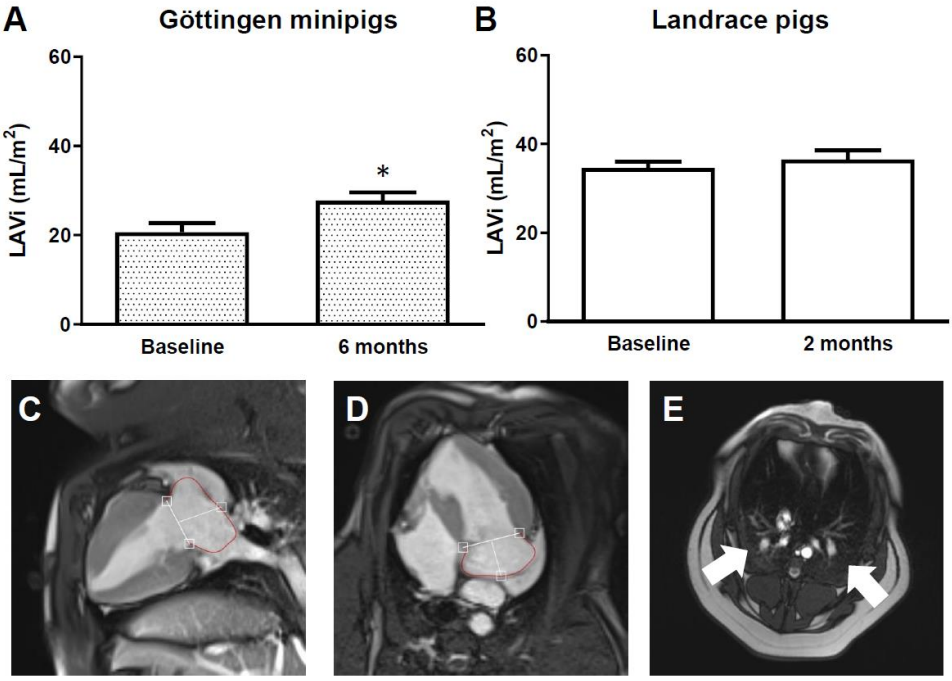
According to the suggestion of Reviewer #2 we amended the discussion section as follows on pages 14:
"Moreover, adverse left ventricular remodeling was defined as an increase of 15% or more in the LVEDV in clinical studies and we found here a 28% increase after 3 months and a 42% increase after 6 months in LVEDV in Göttingen minipigs showing a clinically relevant adverse remodeling."

According to the suggestion of Reviewer #2 we amended the limitations section as follows on pages 14 and 15:
"Signs of HF were assessed by CMRI, according to a recent guideline on the relevance of rigor and reproducibility in preclinical studies on cardioprotection recommends… A further limitation is the lack of different risk factors and comorbidities and thus the present large animal models do not completely mimic the clinical situation in terms of the presence of multiple risk factors including co-morbidities and their medications. However, currently, there are no established large animal models with multiple

comorbidities for routine use. These large animal models cannot be powered for mortality analysis due to animal ethical reasons and the high cost of these studies."

Also, according to the request of Reviewer #2 we amended the results section with a new figure and text as follows on page 10 and 11:

"In order to further examine signs of HF, we performed measurement of the left atrial volume indexed to body surface area (LAVi). LAVi increased by 34% in Göttingen minipigs after 6 months (Figure 5A) and did not change significantly in Landrace pigs after 2 months (Figure 5B). Representative images show the tracing of the left atria (Figure 5 C-D). Moreover, the presence or absence of pulmonary oedema was assessed by CMRI on the localizer images (Figure E). Pulmonary oedema was observed in both breeds as a result of cardiac decompensation. Ten out of eleven Göttingen minipigs and nine out of ten Landrace pigs showed obvious signs of pulmonary oedema."



Also, we amended the figure and table legends section as follows on page 12:

*"Figure 5. Left atrial volume indexed to body surface area (LAVi) in mL/m2 in Göttingen minipigs (A) and Landrace pigs (B) measured by cardiac magnetic resonance imaging. Representative images of left atrial volumes, tracings were made on the two- (C) and four chamber (D) cine images. The white arrows show the presence of pulmonary oedema on the representative localizer image (E). *p<0.05 vs. corresponding baseline (paired t-test in Göttingen minipigs and Landrace pigs)."*

Also the protocol section is amended as follows on page 4:

"Quantify left atrial volume by tracings on the two- and four-chamber cine images. Correct the left atrial volumes to BSA to get left atrial volume indexed to body surface area (LAVi). Assess the presence of pulmonary oedema on the localizer images."

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

The discussion section is amended as follows on page 14:

"In addition, here we have shown that LAVi increased only in Göttingen minipigs, but not in Landrace pigs. Increase of left atrial volume is an additional key structural alteration in the context of HF and is an independent predictor of death and HF hospitalization in patients surviving MI."

In addition, we amended the limitation section as follows on page 15:

"… the use of more targeted angulation of CMRI imaging planes and more targeted sequence may result in better estimation of left atrial volumes, and pulmonary oedema."

2. I am surprised that the authors used 120 min LAD occlusion and still found only moderate scar size during follow-up. My concern is that they occluded the cannulated limb and induced remote pre-, per- and postconditioning. This cardioprotection would limit the potential for further cardioprotection. In fact, the authors did not provide any evidence that they could induce cardioprotection in their model.

Answer: We kindly disagree with this statement because these scar sizes are comparable to those in clinical trials. In clinical trials performed by Lonborg *et al* and Stone *et al* in patients surviving ST elevation myocardial infarction (the 2 studies involved 2941 patients), the median scar sizes (% left ventricular myocardial mass) were 9.5% and 17.9 % respectively (see ref 30 and 31 in revised manuscript). These scar sizes accord with our observations. Moreover, what can be seen in the literature is that the scar sizes/LVmass reported in publications for Göttingen minipigs ranges between 12-25% (see ref 32 to 37 in revised manuscript) and in Landrace pigs ranges between 14-18% (see ref 38 to 40 in revised manuscript).

Nevertheless, to further clarify this issue, we amended the discussion section as follows on page 13:

"Scar sizes reported here are comparable to those in clinical trials. In clinical trials performed by Lonborg et al and Stone et al in patients surviving ST-elevation myocardial infarction the median scar sizes, measured as % of left ventricular myocardial mass were 9.5% and 17.9 % respectively. Moreover, scar sizes in our present study accord with those reported in previous publications in Göttingen minipigs (12-25%) and in Landrace pigs (14-18%)".

As to the reviewers concern on that the occluded and cannulated limb induced remote pre-, per- and postconditioning we believe that remote conditioning was not performed in these settings, since the definition of remote conditioning includes brief and reversible episodes of ischemia with reperfusion in one vascular bed, tissue or organ Heusch G et al, J Am Coll Cardiol, 2015). In addition, remote ischemic conditioning failed to affect myocardial infarction sizes in Landrace pigs (Baranyai T et al, J Transl Med, 2017) and failed to reduce infarct size and to improve clinical outcomes in a recent single-blind, randomized controlled trial (CONDI-2/ERIC-PPCI) performed at 33 centers across Europe in STEMI patients (Hausenloy DJ et al, Lancet, 2019). In addition, occlusion of femoral artery following the angiographic intervention had no effect on the function of legs in pigs. Moreover, this is an animal model, control and treated groups would undergo the same treatment.

Along with the comments of Reviewer #2 we amended the methods section as follows on page 8:

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

"Occlusion of femoral artery following the angiographic intervention has no effect on the function of legs in pigs as assessed by daily veterinarian observations."

Minor issues:

Please report scar size/BARI area at risk, too.

Answer: According to the request, we report here the IS%/BARI%. No differences were observed between the groups, when IS%/BARI% was measured.
We amended the results section accordingly as follows on page 9:

"No differences were observed when scar sizes were related to the BARI scores in Landrace pigs at 2 months (0.55 ± 0.1) and in Göttingen minipigs at 3 months and 6 months respectively (0.75 ± 0.12 and 0.57 ± 0.08)."

Was temperature and/or blood flow in the cannulated limb measured? Could the pig walk after femoral ligation and recovery?

Answer: Temperature and blood flow of the cannulated hind limb was not measured, however, no signs of critical limb ischemia were observed in pigs by the daily observation of the vet.

According to the request of Reviewer #2 we amended the methods section as follows on page 8:

"Occlusion of femoral artery following the angiographic intervention has no effect on the function of legs in pigs as assessed by daily veterinarian observations."

Antibiotics and/or analgesics after the occlusion/reperfusion?

Answer: Please note that the details on admission of antibiotics and analgesics were described under point 2.1.2. Monitoring of the animals for general health status, discomfort, mortality was reported under point 4.4, and now we have amended with some more details. As per the antiinfective therapy, antibiotic cocktail (benzylpenicillin-procain, benzylpenicillin-benzatine, dihidrostreptomycine-sulfate) was administered parenterally. This single injection maintains therapeutic plasma concentrations for long periods, thus readministration is not needed. No signs of wound infections were observed on daily veterinarian checks.

According to the request of Reviewer #2 we amended the methods section as follows on page 8:

"4.4. Closely monitor the animals in the recovery period and inspect them every 12 hours until postoperation day 3, then every 24 hours until the end of the study. Particular attention should be given to eating and drinking behavior, lethargy, signs of infection, painful condition, weight change, mobility, and general health status."

What was the time interval between the onset of reperfusion and the start of the intracoronary infusion of saline? I am concerned that the crucial early reperfusion phase could be missed.

Answer: The time interval between the onset of reperfusion and start of intracoronary infusion of saline was 5 min due to technical reasons as this time is required to perform control angiography to confirm the success of reperfusion and to introduce the microcatheter for therapy administration. We believe that the

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

crucial early reperfusion time is longer than 5 minutes. One of the most obvious and serious deficiency in the field of cardioprotection is the lack of the studies of optimal dosing and timing to estimate efficacy and safety of cardioprotective therapies (Heusch G, Circ Res, 2017). In addition, preclinical data on efficacy of delayed ischemic postconditioning (IPost) showed that IPost applied 30 or 45 min after the start of reperfusion reduced infarct sizes in rats, suggesting that the time window for cardioprotection can be much longer than 5 min (Barsukevich V et al, Basic Res Cardiol, 2015).

According to the suggestion of Reviewer #2 we amended the methods section as follows on page 8:

"3.1.5. Connect the microcatheter with the perfusion pump and initiate intracoronary administration 5 minutes after initiation of reperfusion."

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

250 years of EXCELLENCE in medical education, research & innovation and healthcare

SEMMELWEIS UNIVERSITY

Faculty of Medicine

Department of Pharmacology and Pharmacotherapy

Director

Péter Ferdinandy, MD, PhD, DSc, MBA

18th January, 2021.

RE: revised manuscript, ID: JoVE61901, by Brenner GB, Giricz Z *et al.*

Dear Reviewer 3,

We have revised the manuscript, figures and the video according to Your valuable comments. Please find attached our revised manuscript and a detailed response to Your comments here in this letter, see below.

We hope that our manuscript and video will meet the high standards of JoVe and it will be considered for publishing.

Yours sincerely,

Péter Ferdinandy, MD, PhD, DSc, MBA

Address: H-1089 Budapest, Orczy út 2-4. HUNGARY
Postal address: P.O. Box 2, Budapest, H-1428;
Üllői út 26., Budapest, H-1085;

Phone: (+36-1) 210-4416, Fax: (+36-1) 210-4412
E-mail: ferdinandy.peter@med.semmelweis-univ.hu
E-mail office: pharmacology@med.semmelweis-univ.hu
web: semmelweis.hu/pharmacology

Major Concerns:

1. Please add information about the age and body weight of the animals used. One of the most important findings in the manuscript is that the animal's growth is critical in measurements of cardiac parameters. However, without the information of the age and/or the bodyweight of the animal used, this finding might be challenging for the readers to reproduce.

Answer: The text has been amended accordingly. Please note that the bodyweights are reported in Figure 6:

We also amended the methods section as follows on page 4:

"13 healthy and sexually mature female Göttingen minipigs (age between 12 and 14 months) and 10 healthy and sexually immature female Landrace pigs (age between 2 and 3 months) were housed in pig stalls conforming to the size recommendations of the most recent Guide for the Care and Use of Laboratory Animals DHEW and EU Guidelines 63/2010. Animals were not spayed. The temperature of the animal rooms was controlled, and animals were kept at a 12-hour light/dark cycle and vermin-free."

2. PROTOCOL section 2.3.12: Please add more specific information about the balloon size and the inflation pressure. For example, "choose the same-sized balloon of the vessel diameter and inflate at 3/4 nominal pressure". "Soft-touch between the balloon side-wall and the surface of the vessel" does make sense to experienced PCI operators, but for those starting to learn the procedure would have a hard time figuring out.

Answer: The balloon catheter was purchased from Boston Scientific, EMERGE, with balloon diameter 2.5 mm and balloon length 12 mm. The inflation pressure ranged between 7-9 atmospheres. The nominal pressure of the balloon is the pressure at which the balloon reaches its labeled diameter. The balloon is filled with contrast agent first and the soft touch is defined as interaction of balloon side-wall that is enough to occlude the vessel without causing injury to vessel wall. This intervention should be performed only by trained interventional cardiologist. Specific skill labs are available to gain skills for this intervention using different means of models. It is unethical to learn in animals during practicing the intervention and to cause even fatal injury.

According to the request of the Reviewer #3 we amended the methods section as follows on page 7:

"2.3.10. Place the balloon catheter (balloon diameter 2.5 mm and balloon length 12mm) over the PTCA guidewire and advance it to the planned position.

2.3.11. Fill the balloon with contrast agent and check the position of the balloon catheter by angiography.

2.3.12. Inflate the balloon below the nominal pressure (7-9 atmospheres) of the balloon to develop the soft touch between the balloon side-wall and the surface of the vessel. Soft-touch is defined as interaction of balloon side-wall that is enough to occlude the vessel without causing injury to the vessel wall."

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

3. VIDEO, 12:45-13:30: Did the LVEF in Landrace pigs moderately increased? In the manuscript it is depicted as not changed. Also, I would suggest mentioning in the video that the reason of unchanged LVEF in Landrace pigs is possibly related to the significant increase in heart weight to avoid confusion.

Answer: According to the request of Reviewer #3 we have changed the narration and cleared the "moderate increase" and added that this is probably due to increase in cardiac size in the video (12 min 01 sec).

Minor Concerns:

1. PROTOCOL section 2.2.8: Please add a reference for the Seldinger technique (again for beginners).

Answer: references are added to Seldinger technique (see ref 20 and 21 in revised manuscript).

2. PROTOCOL section 2.2.13: Can you add detailed suggestions for calibrating zero pressure at the right atrium level in both breeds?

According to the request of Reviewer #3 we changed the text on page 6:

"2.2.13. For calibration of pressure place the pressure recording system on the level of the heart of each animal. After removing the air bubbles, the zero pressure calibration is performed when the three-way stopcock is opened to the direction of the free air."

3. REPRESENTATIVE RESULTS section, Line 342-353: I suggest that the statement from Line 351-353 (LVEF, as the most~) should be the first statement in this paragraph since this is the most critical finding in this paragraph. Furthermore, since the reason for unchanged LVEF in Landrace pigs were most likely by the significant increase in heart weight, I would suggest the next paragraph (line 355-362) to be the 3rd paragraph in this REPRESENTATIVE RESULTS section. Also, this causality of increased heart weight and unchanged LVEF in Landrace pigs should be mention in the video as well.

According to the request of Reviewer #3 we changed the text of the result and figure and table legends section on page 9-12 and the EF and cardiac size results are rearranged as follows and this causality is mentioned in the video as well. In addition, new section was added here with LAVi and pulmonary oedema results:

"Increase in left ventricular mass was more pronounced in Landrace pigs during follow-up

The cardiac growth rate was measured by CMRI. LVED mass in Göttingen minipigs increased only moderately (8%) at 6 months (Figure 3A). In contrast, in Landrace pigs, LVED mass increased by almost 100% at 2 months (Figure 3B).

Left ventricular ejection fraction decreased only in Göttingen minipigs

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

LVEF, as the most widely used parameter of left ventricular systolic function, was measured by CMRI. MI resulted in a significant decrease in LVEF in minipigs at 3 months and 6 months (Figure 4A). In Landrace pigs, LVEF did not change after 2 months (Figure 4B).

Post-infarction LVESV and LVEDV increased significantly in both breeds (Table 1). LVESV increased by 69% and 80% in Göttingen minipigs after 3 and 6 months, respectively, and by 80% in Landrace pigs after 2 months.

LVEDV showed a 28% increase after 3 months and a 42% increase after 6 months in Göttingen minipigs and an 82% increase in Landrace pigs after 2 months.

LVSV of Landrace pigs increased by 85% in 2 months and LVSV of Göttingen minipigs did not increase significantly even at 6 months.

Left atrial volume indexed to body surface area increased only in Göttingen minipigs, but both the breeds developed pulmonary oedema following myocardial infarction

In order to further examine signs of HF, we performed measurement of the left atrial volume indexed to body surface area (LAVi). LAVi increased by 34% in Göttingen minipigs after 6 months (Figure 5A) and did not change significantly in Landrace pigs after 2 months (Figure 5B). Representative images show the tracing of the left atria (Figure 5 C-D). Moreover, the presence or absence of pulmonary oedema was assessed by CMRI on the localizer images (Figure E). Pulmonary oedema was observed in both breeds as a result of cardiac decompensation. Ten out of eleven Göttingen minipigs and nine out of ten Landrace pigs showed obvious signs of pulmonary oedema.

Increase in body weight was more pronounced in Landrace pigs during follow-up

In Göttingen minipigs body weight gain was only 8% after 3 months and 30% after 6 months (Figure 6A), whereas increased heart weight was accompanied by a nearly 100% increase in body weight in Landrace pigs at 2 months (Figure 6B).”

4. REPRESENTATIVE RESULTS section, Line 385: Table 1 should be Table 3.

According to the request of the Reviewer #3 we changed the number of the tables on page 11.

5. DISCUSSION section, Line 465: Was the mortality rate in both breeds the same? Wasn't that 15.4% vs. 0%?

Answer: Please note that these differences in mortality rates are statistically nonsignificant, measured by chi-square test.

6. DISCUSSION section, Line 474: What does "relevance of measurement of post-MI adverse remodeling" means?

Answer: thank You, we change the text.

According to the request of Reviewer #3 we changed the text on page 13 as follows:

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

"In other experiments in female Dalland Landrace pigs post-MI adverse remodeling was induced by 90 min LAD occlusion, however, LVEF was not reported after 4 weeks of follow-up."

7. DISCUSSION section, Line 476-477: What does the pressure-overload-induced heart failure model have a role in this discussion?

Answer: According to the suggestion of Reviewer #3 we removed this sentence on page 13.

8. DISCUSSIOn section, Line 486: I would suggest adding a line break before "The reduction of LVEF in ....." since the following few sentences have a significant role in the discussion.

Answer: According to the request of the Reviewer #3 we put a line break before the sentence on page 13.

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

250 years of EXCELLENCE in medical education, research & innovation and healthcare

SEMMELWEIS UNIVERSITY

Faculty of Medicine

Department of Pharmacology and Pharmacotherapy

Director

Péter Ferdinandy, MD, PhD, DSc, MBA

18th January, 2021.

RE: revised manuscript, ID: JoVE61901, by Brenner GB, Giricz Z *et al.*

Dear Reviewer 4,

We have revised the manuscript, figures and the video according to Your valuable comments. Please find attached our revised manuscript and a detailed response to Your comments here in this letter, see below.

We hope that our manuscript and video will meet the high standards of JoVe and it will be considered for publishing.

Yours sincerely,

Péter Ferdinandy, MD, PhD, DSc, MBA

Address: H-1089 Budapest, Orczy út 2-4. HUNGARY
Postal address: P.O. Box 2, Budapest, H-1428;
Üllői út 26., Budapest, H-1085;

Phone: (+36-1) 210-4416, Fax: (+36-1) 210-4412
E-mail: ferdinandy.peter@med.semmelweis-univ.hu
E-mail office: pharmacology@med.semmelweis-univ.hu
web: semmelweis.hu/pharmacology

May main concerns are that the study uses different occlusion times and different intervals to MRI scanning for each group making comparission difficult - this is not discussed in the manuscript. Furthermore, only MRI data is reported, leaving out other measures of heart failure (histological findings, blood samples, histology, electrical remodeling).

Answer: The different durations of cardiac ischemia times (90 min in Landrace pigs and 120 min in Göttingen minipigs) have been selected due to the different susceptibility of the two breeds to ischemic injury. These ischemia times were selected based on literature for Göttingen minipigs (Schuleri, KH et al, Eur Heart J, 2009; Chang HJ et al, JACC Cardiovasc Imaging, 2009; Schuleri KH et al, JACC Cardiovasc Imaging, 2011; Schuleri, KH et al, J Cardiovasc Magn Reson., 2012; Fanton Y et al, Int J Cardiol. 2015), and for large swine (Hoetzenecker K et al, Basic Res Cardiol, 2012; Chen Y et al, J Chin Med Assoc, 2013; Vilahur G et al, Eur Heart J, 2013; Jablonowski R, Acad Radiol, 2014; Lichtenauer M et al, Eur J Clin Invest, 2014; Varga-Szemes A et al, Int J Cardiovasc Imaging 2014; Vilahur G et al, Int J Cardiol. 2014; Crisostomo V et al, J Transl Med, 2015).
These ischemia times can provide predictable and reproducible myocardial infarction sizes and localizations. In our present study the same scar sizes were achieved in the two breeds that justifies the selection of different durations of cardiac ischemia. The different intervals to MRI scanning can be explained by the initial fast growth rate of Landrace pigs that did not allow us to extend the study length to 3 months. Moreover, please note that with exception of the scar sizes and the mortality rate, none of the other measured parameters were directly compared between the two breeds. Signs of HF were assessed only by CMRI, however, a guideline on the relevance of rigor and reproducibility in preclinical studies on cardioprotection recommends cardiac magnetic resonance imaging (CMRI) as a clinically relevant method for measurement of ventricular function in pigs (Bøtker HE et al, *Basic Res Cardiol.* 2018). To further prove that pigs had HF we measured other clinically relevant signs of HF such as left atrial size and pulmonary oedema (please see the answer for Reviewer 2 question 1 and figure 5).

According to the request of Reviewer #4 we included in the text of the limitation section the next on page 14-15:

"Signs of HF were assessed by CMRI, according to recommendations of a recent guideline on the relevance of rigor and reproducibility in preclinical studies on cardioprotection… Although we haven't measured biomarkers and histological signs of post-MI HF in this study, these models are suitable for analysis of any biomarkers since the availability of plasma and tissue samples. Due to the different susceptibility of the 2 breeds to ischemia/reperfusion injury, different durations of coronary occlusions were selected here that may although limit the comparison of the 2 models, however, by this approach we achieved similar infarct size. The follow-up time in the 2 breeds was different as in the Landrace pigs only 2 months follow up time can be achieved due to technical reasons, i.e. rapid increase in body weight that shows a major limitation of the Landrace model."

I have the following concerns and comments that may contribute to improve the manuscript.

Abstract:

74: Please explain abbreviation for BARI.

Answer: Thank You!

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

According to the request of Reviewer #4 we amended the abstract section as follows on page 2:

„In addition, here we used BARI scoring (Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index) for standardization of myocardium at risk, we also describe the procedure of intracoronary administration of the drug and report right ventricular morphofunctional parameters."

Please mention to time of coronary occlusion in the abstract

Answer: Answer: Thank You!

According to the request of Reviewer #4 we amended the abstract section as follows on page 2:

"MI was induced by intraluminal balloon occlusion of the left anterior descending coronary artery for 120 min in Göttingen minipigs and for 90 min in Landrace pigs, followed by reperfusion."

Introduction

Lines 97 to 107 are not relevant to understand the context of the study and could be shorted or removed.

Answer: According to the request of Reviewer #4 we shortened the introduction section by deleting unnecessary data on page 3:

"Numerous cardioprotective therapies effective even in large animal models have been described, but only remote ischemic conditioning (RIC) seemed to improve clinical outcomes of post-MI HF in a small clinical trial. However, this encouraging result on the efficacy of RIC was questioned in a single-blind, randomized controlled trial (CONDI-2/ERIC-PPCI) performed at 33 centers across Europe in STEMI patients, where RIC failed to improve clinical outcomes. Potential reasons for the failed translation of the preclinical data might be the use of suboptimal post-MI HF animal models with low clinical relevance."

The value of the introduction could be improved by adding specific information on how and why the protocol was chosen.

Answer (work on it): Thank You on this suggestion.

According to the request of Reviewer #4 we tried to improve the introduction section as follows on page 3 and 4:

Corrected text: "To analyze the scientific interest on post-MI HF in pigs we performed literature search on PubMed using the following search string: "(pig OR swine OR porcine OR sus-scrofa OR minipig OR mini-pig OR miniature-pig OR miniature-swine) AND (infarct OR ischem* OR ischaem* OR reperfus*) AND (heart OR cardi* OR myocard*) AND (LAD OR left-anterior* OR LCX OR left-circumflex OR RCA) AND (heart-failure OR lvef OR ejection-fraction OR infarct-size OR infarction-size)" and found that pig models of cardiac ischemia/reperfusion are frequently used to study MI and post-MI HF, but only 17% (71 out of 425 articles) of studies involved minipigs and 7% (30 out of 425 articles) used Göttingen minipigs. Only about 1% (5 out of 425) of studies used Göttingen minipigs and clinically relevant protocols with long-term follow-up (1-9 months of reperfusion) and CMRI to analyze cardiac function. The small number of clinically relevant studies highlights the translational gap*

between basic research and clinical trials. Therefore, a comprehensive characterization of the closed-chest post-MI HF models in Göttingen minipigs and Landrace pigs with repeated assessment of left and right ventricular function and anatomy using CMRI during long-term follow up is required. Here we aimed to focus on the technical feasibility and clinical relevance of two post-MI HF models to describe standardized and reproducible experimental protocols for post-MI HF studies that may be used to assess cardioprotective drug and/or medical device therapies."

Methods

The authors provide an easy-to-understand point-by-point approach to describe their methods. I would encourage to either add vendor information on the used equipment (c-bow, anesthesia system, monitoring system etc for better reproducibility)

According to the helpful request of Reviewer #4 we amended the excel of materials with vendor information of materials excel including c-bow, anesthesia system etc.

As this paper focuses on methods, a short section on pitfalls and/or learning experiences the authors had while setting these models up and include information on how pigs were resituated. This could help other groups from making the same mistakes and reduce the number of used animals in the future.

Answer: According to the request of Reviewer #4 we amended the protocol section on page 6 and page 8 as follows:

"2.3.1. Note that this intervention should be performed only by trained interventional cardiologist."

"4.4. Following the procedure, the animals were transported with a van in small groups in cages to avoid unnecessary stress in early postoperative period."

Occlusion period of the two races differ. How does 90 in landrace pigs compare to 120 min in minipigs. Why were different times chosen? Why different reperfusion periods/MRI timepoints?

Answer: this question was raised by Reviewer #4 previously and answered above.

1.2.2: Please provide the formulas here

Answer: According to the request of Reviewer #4 we amended the protocol as follows on page 4:

"1.2.2. Measure the body weight and length of animals. The calculation of the body surface areas (BSA) formulas were described by Itok et al. for Göttingen minipigs (BSA [m^2] = (7.98 × BW [kg]$^{2/3}$)/100) and by Swindle et al. for Landrace pigs (BSA [m^2] = (7.34 × BW [kg]$^{0.656}$)/100)."

1.2.3: Were 6.0 tubes used for both species? Please specify

Answer: the size of the endotracheal tube depends on the individual anatomic characteristics of each animal. Endotracheal intubation was performed by a 6.0- to 7.5-mm endotracheal tubes.

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

According to the request of Reviewer #4 we changed the text on page 4 as follows:

"1.2.3. Intubate animals, maintain anesthesia with isoflurane (2% isoflurane, 2 L/min oxygen). The size of the endotracheal tube depends on the individual anatomic characteristics of each animal and ranges between 6.0- to 7.5-mm."

1.3.1: Please use generic names for drugs, fx. Atracurium instead of tracrium. What is the reason for muscle relaxant? Were 350 ml tidal volumes used for both species? Please specify

Answer: For cardiac MRI measurements it is necessary to avoid respiratory artefact that come from the changes of cardiac position during the breathing. Atracurium, as a muscle relaxant was administered to avoid these respiratory artefacts. In humans this can be achieved by asking the patients to hold the breath.

According to the request of Reviewer #4 we changed the text on page 5 as follows:

"1.3.1. Transfer animal to CMRI facility and administer 0.4-0.5 mg/kg atracurium besylate i.v.. Atracurium besylate is a nondepolarizing, skeletal muscle relaxant that is used to avoid respiratory artefacts during CMRI measurements. Start positive pressure ventilation (16/min frequency, 350ml volume, 25-30 Hgmm positive pressure)."

1.3.2: Please provide information on how the pig was positioned in the scanner and how coils were placed

According to the request of Reviewer #4 we changed the text on page 5 as follows:

"1.3.2. Position the animals in the supine position. Place flexible coils on the chest and 32-channel coils are placed in CMRI bed."

2.1.1: Please specify the concentration of the different antibiotics used in the cocktail

Answer: OK, thank You.
According to the request of Reviewer #4 we changed the text on page 5 as follows:

"2.1.2. Apply analgesia (meloxicam 0.4 mg/kg body weight) and antibiotic cocktail (benzylpenicillin-procain (24.8 mg/mL), benzylpenicillin-benzatine (83.6 mg/mL), dihidrostreptomycine-sulfate (156.3 mg/mL), 3 ml/50 kg body weight) by intramuscular injections on the day of coronary artery occlusion."

2.2.2.: Where is the surgical site?

Answer: the area around the skin fold between the gracilis and Sartorius muscle
According to the request of Reviewer #4 we changed the text on page 5 as follows:

"2.2.2. The surgical site is around the skin fold between the gracilis and sartorius muscle."

2.2.4: Please specify ECG lead positions. Are electrodes placed on the limbs, close to the limbs or on torso?

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

Answer: According to the request of Reviewer #4 we amended the text on page 6 as follows:

"2.2.4. Place surface ECG electrodes in Einthoven's triangle. This triangle is formed by the two anterior limbs and left hind limb and the electrodes are placed on limbs."

2.2.5: Again, specify tidal volume.

Please see the answer above.

2.2.11: Was the same dose used for landrace and minipigs? Did you adjust for weight?

Answer: We have clarified the text as follows on page 5:
"The animals received approximately 370-440 IU/kg heparin during the whole intervention."

2..3.2: Please specify the guiding catheter, vendor, type?

Answer: Medtronic Launcher, 5F guiding catheter was used.
According to the request of Reviewer #4 we amended the excel of materials.

2.3.6: How was the coronary ostium intubated? Was the guidewire used to approach the aortic valve? Was only the guiding advanced? Please add this step as the catheter in the protocol has not moved from the aortic arch…

2.3.6: What is meant by "selective filling" Was angiography performed separately for LAD, LCx, RCA?

In line with the two comments above, we clarified the text on page 6 as follows:

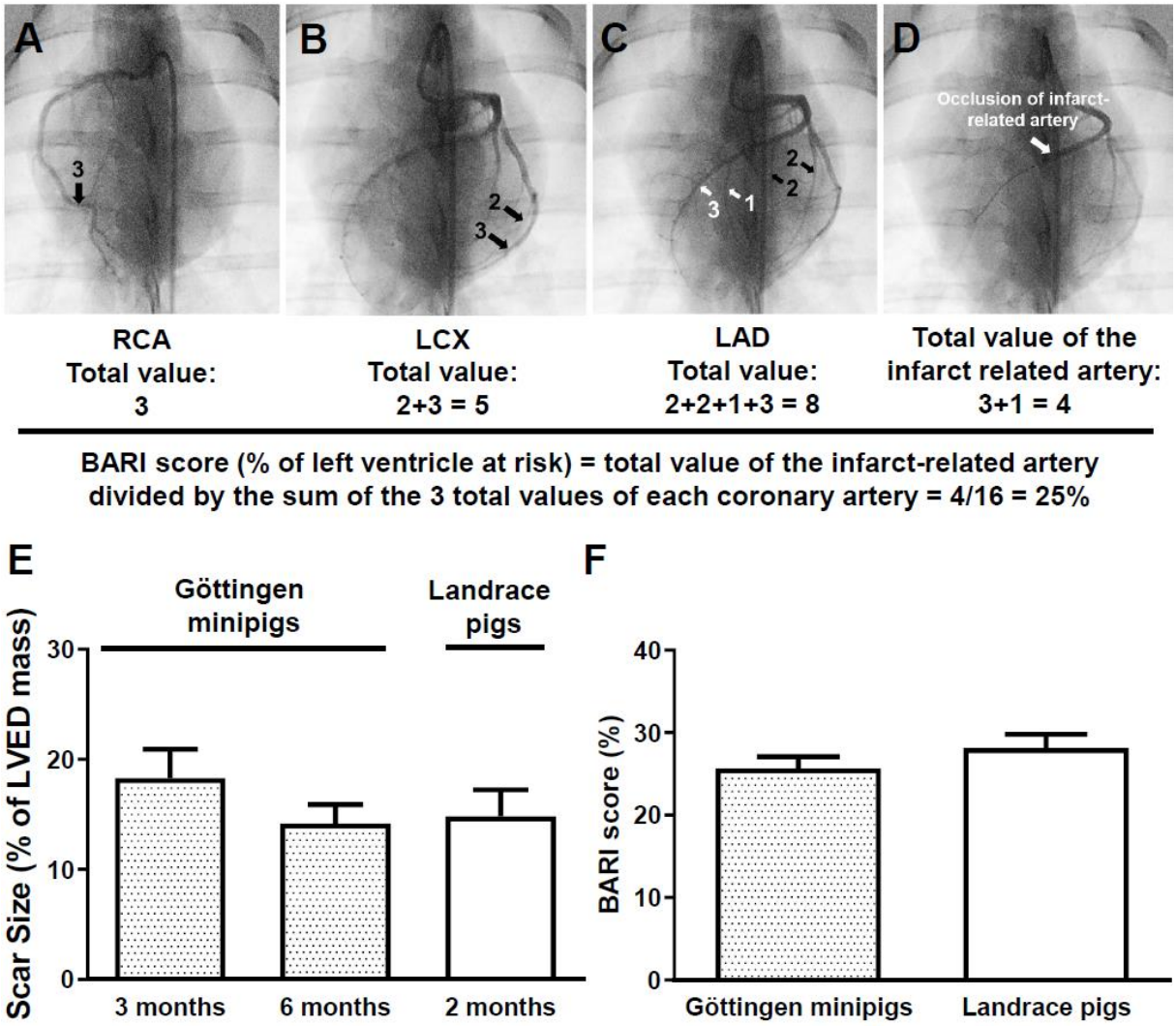
"2.3.1. … First, advance the guidewire to approach aortic root atraumatically. Perform deep intubation by a thin, 5F guiding catheter to avoid significant obstruction of the blood flow.
"2.3.6. 2.3.6. To perform baseline angiography, intubate separately and fill with contrast agent selectively the ostia of right coronary artery and left main coronary artery. For more technical details, refer to catheterization textbooks." – references are also added here, see in the revised manuscript ref 20 and 21.

2.3.7.: This seams an important step in deciding position of occlusion. As this is a methodological paper, the authors could consider showing a concrete example with a figure explaining the steps.

According to the request of Reviewer #4 we amended the protocol and the results section with a new figure and text as follows on page 6 and 7:
"2.3.7. Perform BARI (Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index) scoring after the baseline angiography. A score to all terminal arteries (terminal portion of the left anterior descending, left circumflex, and right coronary artery, as well as the ramus, diagonals, obtuse marginals, posterior descending and posterolateral branches) is assigned based on their length and caliber according to specific criteria. A value of 0 represents an almost insignificant vessel size. In contrast, a value of 3 defines a large-sized artery with a length of two thirds the distance between the base and cardiac apex. Do not take right ventricular marginals and posterior descending artery septal branches into account. Calculate the final BARI score (% of the left ventricle at risk) by dividing the

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

total value from the infarct-related artery by the total values of all arteries (Figure 2 A-D) supplying the LV. Choose the occlusion site on the left anterior descendent (LAD) coronary artery to achieve approximately 25-30% myocardium at risk as assessed by BARI scoring."



Also, we amended the figure and table legends section as follows on page 11:

"Figure 2. Estimation of the myocardium at risk based on the BARI (Bypass Angioplasty Revascularization Investigation Myocardial Jeopardy Index) score (A-D). The total value of the infarct-related artery is divided by the sum of the 3 total values of each coronary artery, the right coronary artery (RCA), the left circumflex coronary artery (LCX), and the left anterior descending coronary artery (LAD). Left ventricular scar sizes in Göttingen minipigs and Landrace pigs measured by cardiac magnetic resonance imaging (E). Scar size is shown as a ratio of mass of infarction to the mass of left ventricle at end of diastole (LVED). BARI scores in Göttingen minipigs and Landrace pigs measured before coronary occlusion (F)."

2.3.16: What was done/could be done if HR/BP or temperature were out of normal ranges?

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

Answer: To maintain the temperature we used heating device as described under the point 2.3.17., to maintain the blood pressure continuous infusion of in Ringer solution (1 L/hour) was given as described under the point 1.2.4. in the protocol. Magnesium sulfate was administered throughout the procedure to prevent ventricular tachycardia (VT) and ventricular fibrillation (VF) as described under the point 2.1.4. in the protocol. The resuscitation protocol was described under the 2.3.18. point.

2.3.22: Should read 120 and 90, respectively depending on the race.

According to the request of the Reviewer #4 we changed the text on page 8 as follows:

"Initiate the reperfusion with balloon deflation after 120 min cardiac ischemia in Göttingen minipigs and after 90 min in Landrace pigs."

2.3.23: Was the guiding kept in place during occlusion? Could this have influence on left side perfusion? Was a guiding with side-holes used?

According to the helpful comment of the Reviewer, we changed the text on page 7 as follows

"Keep in place the guidewire and the balloon and pull back the guiding catheter from the ostium of the coronary artery to avoid diffuse cardiac ischemia."

How exactly was infarct size calculated? Did the authors assess border zone as well?

Answer: the border zone is not assessed separately. The quantification was performed as described in the protocol on page 9.

"5.2.3.4. Quantify myocardial necrosis with manual planimetry on the delayed contrast enhancement images by delineating the myocardium with signal intensity 5 SDs above the mean signal obtained in the remote, non-infarcted myocardium."

Results

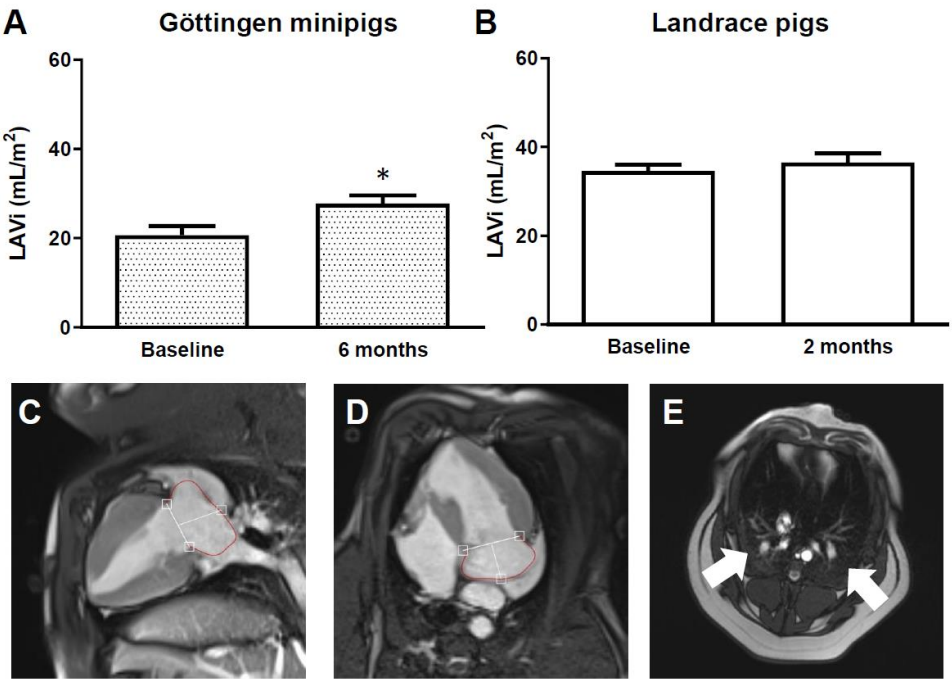
The findings mainly include MRI data. It would be interesting to know if the authors have also looked for clinical signs of heart failure, elevation in proBNP or other cardiac markers during the experiment and, finally, histology.

Answer: We have not looked for biomarkers and histology.
Also, according to the request of Reviewer #4 and Reviewer #2 we amended the results section with a new figure and text as follows on page 10:

"In order to further examine signs of HF, we performed measurement of the left atrial volume indexed to body surface area (LAVi). LAVi increased by 34% in Göttingen minipigs after 6 months (Figure 5A) and did not change significantly in Landrace pigs after 2 months (Figure 5B). Representative images show the tracing of the left atria (Figure 5 C-D). Moreover, the presence or absence of pulmonary oedema was assessed by CMRI on the localizer images (Figure E). Pulmonary oedema was observed in both breeds as a result of cardiac decompensation. Ten out of eleven Göttingen minipigs and nine out of ten Landrace pigs showed obvious signs of pulmonary oedema."

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim



Also, we amended the figure and table legends section as follows on page 12:

*"Figure 5. Left atrial volume indexed to body surface area (LAVi) in mL/m^2 in Göttingen minipigs (A) and Landrace pigs (B) measured by cardiac magnetic resonance imaging. Representative images of left atrial volumes, tracings were made on the two- (C) and four chamber (D) cine images. The white arrows show the presence of pulmonary oedema on the representative localizer image (E). *p<0.05 vs. corresponding baseline (paired t-test in Göttingen minipigs and Landrace pigs)."*

Also the protocol section is amended as follows on page 4:

"Quantify left atrial volume by tracings on the two- and four-chamber cine images. Correct the left atrial volumes to BSA to get left atrial volume indexed to body surface area (LAVi). Assess the presence of pulmonary oedema on the localizer images."

As to the question of other parameters of heart failure we amended the limitations section on page 15 as follows:

"Although we haven't measured biomarkers and histological signs of post-MI HF in this study, these models are suitable for analysis of any biomarkers since the availability of plasma and tissue samples."

Were ECGs performed during/at the end of the follow-up and if yes, were changes in activation or repolarization present?

Answer: we have not performed ECG examinations in the follow-up.

It seems that the cardiac index is increasing during the experiments compared to baseline despite myocardial infarction. Furthermore, baseline LVEF is reduced in Landrace pigs. Do the authors have any explanation?

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu
Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

Answer: We do not know the exact explanation for baseline LVEF in Landrace pigs. Our present finding on baseline LVEF in Landrace swine is according to data reported by others in large swine (see references 13,41,42 in revised manuscript). These values in large swine are smaller as compared to healthy human LVEF reference ranges (58-61%) (see reference 43 in revised manuscript) and baseline (pre-infarction) values in Göttingen minipigs (55-73%) (see references 33,44,45 in revised manuscript). Nevertheless, it is worth noting that only the post-infarction data or delta changes of LVEF are reported in most publications (see references 46-50 in revised manuscript). These data further support that Göttingen minipigs better mimic the human conditions since pre-infarction LVEF, scar size, post-infarction LVEF, and mortality all are comparable to these parameters found in humans (see the discussion section of the revised manuscript on pages 12-14).

According to the request of the Reviewer #4 we amended the text of discussion on page 14 as follows:

"Our present finding on baseline LVEF in Landrace swine is according to data reported by others in large swine. These values in large swine are smaller as compared to healthy human LVEF reference ranges (58-61%) and baseline (pre-infarction) values in Göttingen minipigs (55-73%). Nevertheless, it is worth noting that only the post-infarction data or delta changes of LVEF are reported in most publications."

Line 327: should read "one animal"

Answer: Thank You, we made the changes.

Discussion

Line 442ff: The authors state, that "Göttingen minipig model reflects post-MI HF parameters comparable to humans. We conclude that the Göttingen minipig model is superior to the Landrace pig to follow up the development of post-MI HF". To my opinion this is not supported by the results presented. Cardiac output increases over time, so does CI, LVEF was already decreased at baseline for Landrace pigs.

It is true that LVEF decreased in minipigs after MI, but this is not reflected in cardiac output. Were other parameters assed? Are there signs of diastolic heart failure? Elevated proBNP? Invasive pressure measurements before euthanizing the animals? ECG changes? The statement should therefore be rephrased or supporting data added to the results.

Even mentioned multiple times in the discussion, rapid growth rates in Landrace pigs makes comparison difficult. Increase in cardiac diameters, CO, and CI are therefore hard to interpret. This should be made clear in a limitation section. Further limitations should include that only MRI was used to quantify the effects, occlusion times and MRI times were different for the groups.

Answer: according to Your valuable suggestions we improved the discussion section (please see the new discussion section on pages 12-14 in revised manuscript).

Pros/Cons of the used protocol should be discussed.

According to the request of the Reviewer #4 we amended the text with a limitation section on page 15 as follows:

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

"The current experiment was performed only in female pigs, therefore, the potential effect of the different sexes on post-MI HF remains unknown in these models. Signs of HF were assessed by CMRI, according to recommendations of a recent guideline on the relevance of rigor and reproducibility in preclinical studies on cardioprotection. However, the use of more targeted angulation of CMRI imaging planes and more targeted sequence may result in better estimation of left atrial volumes, and pulmonary oedema. Although we haven't measured biomarkers and histological signs of post-MI HF in this study, these models are suitable for analysis of any biomarkers since the availability of plasma and tissue samples. Due to the different susceptibility of the 2 breeds to ischemia/reperfusion injury, different durations of coronary occlusions were selected here that may although limit the comparison of the 2 models, however, by this approach we achieved similar infarct size. The follow-up time in the 2 breeds was different as in the Landrace pigs only 2 months follow up time can be achieved due to technical reasons, i.e. rapid increase in body weight that shows a major limitation of the Landrace model. A further limitation is the lack of different risk factors and comorbidities and thus the present large animal models do not completely mimic the clinical situation in terms of the presence of multiple risk factors including co-morbidities and their medications. However, currently, there are no established large animal models with multiple comorbidities for routine use. These large animal models cannot be powered for mortality analysis due to animal ethical reasons and the high cost of these studies."

Line 480: "significantly" should be deleted in that context.

Answer: thank You, "significantly" was deleted.

Address: H-1085 Budapest, Üllői út 26.HUNGARY
P.O.Box: 1085 Budapest, Üllői út 26.; 1428 Budapest, Pf. 2.
Email: minta.nev@semmelweis-univ.hu

Phone: (06-1) 459-1234, (06-1) 459-1500/12345
Fax: (06-1) 459-1234, (06-1) 459-1500/12345
Web: http://semmelweis.hu/alhonlapcim

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Translational value of post-myocardial infarction-induced heart failure in closed-chest coronary occlusion/reperfusion model in Göttingen minipigs: comparison with Landrace pigs

Author(s): G. B. Brenner, Z. Gyöngyösi, R. Garamvölgyi, A. Mankovics, Zs. Ondrik, N.V. Sayour, T. Gergely, T. Baranyai, Ö. Petneházy, A. Csiszovszki, Zs. Petrásovits, D. Kárpáti, Gy. P. Szabó, H. Jávor, Zs. Dohy, Cs. Czimbalmos, B. Merkely, I. G. Kiss-Vitán, P. Ferdinandy

Item 1: The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via:

☐ Standard Access ☒ Open Access

Item 2: Please select one of the following items:

☒ The Author is **NOT** a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and(c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video – Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video – Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this **Section 6** is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Protection of the Work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

9. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

10. **Author Warranties.** The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

11. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

12. **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13. **Fees.** To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14. **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

CORRESPONDING AUTHOR

Name:	Peter Ferdinandy, MD, PhD, MBA
Department:	Department of Pharmacology and Pharmacotherapy
Institution:	Semmelweis University, Budapest, Hungary; Pharmahungary Group, Szeged, Hungary
Title:	Professor, Founder and CEO
Signature:	
Date:	26 June, 2020

Please submit a **signed** and **dated** copy of this license by one of the following three methods:

1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140