Surgical placement of catheters for long-term cardiovascular exercise testing in swine

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J Vis Exp. 2016 Feb 9;(108):e53772.

ABSTRACT

This protocol describes the surgical procedure to chronically instrument swine and the procedure to exercise swine on a motor-driven treadmill. Early cardiopulmonary dysfunction is difficult to diagnose, particularly in animal models, as cardiopulmonary function is often measured invasively, requiring anesthesia. As many anesthetic agents are cardiodepressive, subtle changes in cardiovascular function may be masked. In contrast, chronic instrumentation allows for measurement of cardiopulmonary function in the awake state, so that measurements can be obtained under quiet resting conditions, without the effects of anesthesia and acute surgical trauma. Furthermore, when animals are properly trained, measurements can also be obtained during graded treadmill exercise. Flow probes are placed around the aorta or pulmonary artery for measurement of cardiac output and around the left anterior descending coronary artery for measurement of coronary blood flow. Fluid-filled catheters are implanted in the aorta, pulmonary artery, left atrium, left ventricle and right ventricle for pressure measurement and blood sampling. In addition, a 20 G catheter is positioned in the anterior interventricular vein to allow coronary venous blood sampling. After a week of recovery, swine are placed on a motor-driven treadmill, the catheters are connected to pressure and flow meters, and swine are subjected to a five-stage progressive exercise protocol, with each stage lasting 3 min. Hemodynamic signals are continuously recorded and blood samples are taken during the last 30 sec of each exercise stage. The major advantage of studying chronically instrumented animals is that it allows serial assessment of cardiopulmonary function, not only at rest but also during physical stress such as exercise. Moreover, cardiopulmonary function can be assessed repeatedly during disease development and during chronic treatment, thereby increasing statistical power and hence limiting the number of animals required for a study.

INTRODUCTION

Adequate cardiopulmonary function is essential to supply the body with oxygen and nutrients, particularly during conditions of increased metabolic demand such as during exercise.[1] The cardiopulmonary response to exercise is characterized by a number of adaptations in cardiac function, i.e. an increase in heart rate, contractility and stroke volume, and microvascular function, i.e. vasodilation in the vascular beds supplying exercising muscles as well as in the pulmonary vasculature, and vasoconstriction in the vascular beds supplying the gastrointestinal system as well as inactive muscles.[1] Impaired exercise capacity is an early hallmark of cardiopulmonary dysfunction, and cardiopulmonary exercise testing is used as an effective method to delineate between cardiac dysfunction, vascular dysfunction and/ or pulmonary dysfunction in patients with impaired exercise capacity.[2] Early cardiopulmonary dysfunction is difficult to diagnose, particularly in animal models, as cardiopulmonary function is often measured invasively, requiring anesthesia, with many anesthetic agents possessing cardiodepressive properties.[3]

Chronic instrumentation allows for measurement of cardiopulmonary function in the awake state, and when the animals are fully adjusted to the laboratory conditions measurements can be obtained under quiet resting conditions without the effects of anesthesia and acute surgical trauma. Furthermore, when the animals are appropriately trained, measurements can also be obtained during graded treadmill exercise.[4,5] More specifically, left and right ventricular function can be assessed and related to myocardial perfusion, while regulation of vasomotor tone in the coronary, systemic and pulmonary microcirculation can be determined. The use of fluid-filled catheters allows measurement of pressure as well as taking blood samples without imposing additional stress on the animals. Another advantage of studying chronically instrumented animals is that cardiopulmonary exercise testing can be repeated allowing the use of an animal as its own control, either during disease development or during chronic treatment, thereby increasing statistical power and hence limiting the number of animals required for a study.

Cardiopulmonary anatomy of swine closely resembles that of humans and it is possible to induce various forms of cardiopulmonary disease, such as diabetes[6], myocardial infarction[7], pulmonary hypertension[8,9] and pacing-induced heart failure.[10,11] Moreover, the size of swine allows chronic instrumentation, and repeated blood sampling of sufficient quantity to analyze not only blood gases, but also to perform neurohumoral measurements and/or to search for biomarkers of disease.

This protocol describes the surgery used to chronically instrument swine as well as the protocol for exercising the swine on a motor-driven treadmill.

PROTOCOL

Procedures involving animal subjects have been approved by the Animal Care Committee at Erasmus Medical Center Rotterdam (NL). Swine with weights between 6 and 80 kg have been successfully instrumented using this protocol.

1. Adaptation of the animals to human handling

1.1) After arrival in the facility, house the animals solitarily but enable them to interact with each other.

1.2) Accustomize swine to human handling and transportation from the animal facility to the experimental laboratory, by handling the animal at least once a day for one week.

1.3) Train the animals appropriately for exercise experiments on a motor-driven treadmill by exercising them on the treadmill for a minimum of three times before surgery.

1.4) Animals should be fasted overnight before surgery to prevent nausea, vomiting and thereby potential aspiration of stomach fluids.

2. Preparation for surgery

2.1) Sedation

2.1.1) Prepare medication for sedation in a 10 mL syringe. Premedication consists of tiletamine/zolazepam (5 mg/kg), xylazine, (2.25 mg/kg) and atropine (1 mg).

2.1.2) Inject the medication intramuscularly in the trapezius muscle with a 19G 1.5" needle to sedate the pig.

2.1.3) Wait for approximately 10 minutes and check for muscle relaxation and unconsciousness to confirm appropriate and stable level of sedation.

2.1.4) Place a 20G peripheral safety catheter in an ear vein for subsequent intravenous administration of anesthesia and/or fluids.

2.2) Intubation and ventilation

2.2.1) Place the animal on a table and/or trolley in supine position.

2.2.2) Open the mouth of the animal with an oral spreader.

2.2.3) In case of insufficient relaxation of the jaws or presence of swallowing reflexes, which hinder intubation, administer thiopental (10 mg/kg) intravenously via the ear vein catheter. Alternatively, the pig could be masked with isoflurane to induce sedation

2.2.4) Use a conventional laryngoscope with a light and a Miller blade to allow the laryngoscopist to directly view the larynx. If there is laryngospasm, apply 2% lidocaine to the cords and larynx to reduce the spasm and allow intubation.

2.2.5) Insert an intubating stylet into the endotracheal tube to make the tube conform better to the upper airway anatomy and pass the tube through the mouth and between the vocal cords into the trachea.

2.2.6) Inflate the balloon cuff with a 10 mL syringe to help secure it in place, to prevent leakage of respiratory gases, and to protect the airways from possible aspiration of stomach fluid.

2.2.7) Connect the tube to a breathing filter (heat and moisture exchanger) and to the mechanical ventilator.

2.2.8) Place the animal on its right side on the surgical table.

2.2.9) To achieve pO_2 levels of 100-120 mmHg, ventilate the animal with a mixture of oxygen and nitrogen (1:2 v/v), using the following ventilator settings: Pressure control mode: positive end-expiratory pressure (PEEP) 4 cmH_2O; peak inspiratory pressure 16-18 cmH_2O; breathing frequency depending on the size of the animal, (20 bpm for a 20 kg animal, decrease frequency with increasing body weight) this should result in a tidal volume of ~ 10 ml/kg, monitor ventilation with capnography.

2.2.10) Monitor temperature using a rectal thermometer and maintain temperature between 37-39°C using a heat lamp or heat mat. Moreover, monitor heart rate with electrocardiography.

2.3) Anesthesia

2.3.1) Induce and maintain anesthesia preferably by adding 2.0% of isoflurane (v/v) to the ventilation gas-mixture or alternatively by intravenous administration of fentanyl (10 µg/kg/h) via the ear vein catheter.

2.3.2) Check adequate depth of anesthesia by testing pain reflexes with a hind leg toe pinch before starting surgery. When necessary, add additional anesthesia or wait for a few minutes. Check pain reflexes regularly throughout the surgery.

2.4) Fluids and antibiotics

2.4.1) Administer the first dose of amoxicilline (25 mg/kg) intravenously via the ear vein catheter.

2.4.2) Connect a transfusion system to the ear vein catheter to enable slow infusion of glucose 10% (500 mL) during surgery.

2.5) Sterilization of surgical site

2.5.1) Shave and clean the skin of the animal over an area of approximately 25 cm width from the vertebral column all the way to the left axilla.

2.5.2) Scrub the moisturized skin with povidone-iodine scrub (75 mg/mL) for approximately 5 minutes.

2.5.3) Remove the povidone-iodine soap from the skin with sterile gauzes, before sterilizing the skin with povidone-iodine lotion (100 mg/mL).

2.5.4) Cover the animal with sterile surgical drapes to reduce bacterial transfer and subsequent contamination of the surgical site.

3. Surgery

3.1) Opening the thorax (thoracotomy)

3.1.1) Make an incision in the skin, starting 1 cm caudal to the left inferior angle of the scapula down to the left axilla (Figure 1). Use diathermy to cauterize blood vessels in the skin to prevent excessive bleeding.

Figure 1. Overview of the surgery. Top left panel: The sterile area of the animal, which should be shaved and sterilized lies between the bleu lines. The incision site is depicted as the red dotted line. Bottom left panel: Picture of catheters and flow probes: fluid-filled catheter (A), aorta/ pulmonary flow probe including rubber band (B), coronary venous catheter including 20G needle (C) and the coronary flow probe (D). Top right panel: Schematic overview of placement of the catheters and flow probes. MAP, mean arterial pressure; Cor venous, coronary venous catheter; LAP, left atrial pressure; LVP left ventricular pressure; RVP, right ventricular pressure; PAP, pulmonary artery pressure; CO, cardiac output; CBF, coronary blood flow. Bottom right panel: Tunneled catheters exiting the back secured with a stitch and a knot at approximately 1 cm distance along the suture.

3.1.2) Cut through the serratus muscle and pectoralis major muscle, using the cutting modality of the diathermy. Also use diathermy to cauterize blood vessels in the muscle layer to prevent excessive bleeding.

3.1.3) Use blunt dissection to carefully divide the intercostal muscle of the fourth left intercostal space with a mosquito clamp. Now the costal surface of the left lung covered with visceral and parietal pleura should be exposed.

3.1.4) To enter the pleural cavity, carefully pierce both layers of the pleura and tear them open.

3.1.5) Use a thoracic retractor to separate the edges of the wound and the ribs and to forcefully drive tissues apart to obtain good exposure of the pleural cavity.

3.1.6) Push away the left lung in the caudal direction and keep it in place with a wet gauze. Now the heart and great vessels should be clearly exposed.

3.2) Placement of catheters and flow probes (Figure 1)

3.2.1) Use blunt dissection to remove ~2 cm^2 of the surrounding connective tissue of the descending thoracic aorta.

3.2.2) Perform a purse-string suture, consisting of three stitches, in the aortic wall with a non-absorbable USP3-0 braided silk suture (∅0.2mm).

3.2.3) Penetrate the aortic vessel wall with a stainless steel 16G needle in the middle of the purse-string suture.

3.2.4) Insert the tip of the fluid-filled catheter (until the ring) into the aorta, pull the purse-string suture firmly together and tie the two strings of the suture.

3.2.5) To keep the catheter in place, wind the suture 3 times around the catheter above the ring and again tie the two strings of the suture. Further secure the catheter with a new stitch approximately 1 cm cranial from the insertion place.

3.2.6) Connect the fluid-filled catheter to the calibrated pressure transducer, which is connected to the computer, to monitor the mean arterial pressure during the surgery. Obtain an arterial blood gas to verify or adjust for correct ventilation settings.

3.2.7) Open the pericardium with a crossed cut. Be aware to keep the phrenic nerve that runs over the pericardium intact.

3.2.8) Identify the pulmonary artery and pull it slightly in the caudal direction with a Farabeuf retractor. Now the ascending aorta and aortic arch should be exposed. Monitor mean arterial pressure while retracting the pulmonary artery.

3.2.9) Make a small cut (~1 cm) in the connective tissue between the ascending aorta and the pulmonary artery using Metzenbaum scissors, to be able to dissect either the ascending aorta or the pulmonary artery with a large curved mosquito clamp to place the flow probe.

3.2.10) Place the rubber band of the flow probe around the vessel. To make this easier, place a suture through one end of the rubber band, place this suture around the vessel and pull it until the rubber band surrounds the vessel.

3.2.11) Fix the flow probe measurement device on the rubber band. Connect the flow probe to the computer and check the cardiac output signal on the computer to confirm a correct placement of the flow probe.

3.2.12) Place fluid-filled catheters in the pulmonary artery, right ventricle, left ventricle and left atrium at the same manner as described for the aortic fluid-filled catheter (3.2.2 – 3.2.5). Note that it is not necessary to remove connective tissue before performing a purse-string suture in these structures.

3.2.13) Expose and dissect the proximal part of the left anterior descending coronary artery by first lifting the tissue with a forceps and making a small (2-3 mm) cut with Metzenbaum scissors, followed by carefully teasing the tissue away from the artery with a cotton swab. Ensure complete dissection of the coronary artery by passing a small straight angled mosquito clamp underneath.

3.2.14) Make a stitch parallel to the anterior interventricular coronary vein with a suture, which is connected to the coronary venous catheter.

3.2.15) Puncture the coronary vein with the 20G needle of the coronary venous catheter and insert the cannula of the catheter intravenously.

3.2.16) Remove the needle and secure the catheter with the already performed stitch (3.2.14). Further secure the catheter with a new stitch approximately 1cm from the place of initial puncture.

3.2.17) Place the coronary flow probe around the previously dissected left anterior descending coronary artery. When the artery is constricted and is hardly visible, use lidocaine 10% spray to relax the vessel to get a better exposure of the vessel. Check the signal of the coronary flow on the computer to confirm a correct placement of the flow probe (Figure 2).

3.3) Tunneling

3.3.1) Tunnel the flow probes individually through the third left intercostal space beneath the muscle and above the rib by using a large curved mosquito clamp.

3.3.2) Tunnel the fluid-filled catheters through either the third or the fifth left intercostal space by piercing the intercostal muscle. Clamp off the fluid-filled catheters and remove the three-way stopcock to minimize the piercing area and prevent leakage of the fluid-filled catheters during the tunneling.

3.3.3) Fix the flow probes and the fluid-filled catheters with non-absorbable USP2-0 braided silk (∅0.3mm) by means of a purse string suture on the intercostal muscle. This suture also serves to prevent air leakage after re-instating negative intrathoracic pressure.

Figure 2. Treadmill Experiment. Left panels: Instrumented swine on the treadmill. Fluid-filled catheters are connected to the pressure transducers, placed on the back of the swine. Top right panel: Overview of the total experimental set-up, including treadmill, amplifier and recording computer. Bottom right panel: Typical example of recorded hemodynamic data. From top to bottom; aortic pressure (AoP, blue) and left ventricular pressure (LVP, red); left atrial pressure (LAP,blue) and left ventricular pressure (red); pulmonary artery pressure (PAP, blue) and right ventricular pressure (RVP, red); aortic flow/cardiac output (AoF, blue); coronary blood flow (CBF, red).

3.3.4) Make three incisions in the skin approximately 2 cm sinister and parallel to the vertebral column, approximately 3 cm in length 3 cm apart of each other.

3.3.5) Pierce a trochar beneath the left latissimus dorsi muscle from rostral incision site to the incisions on the back. Tunnel the flow probes and fluid catheters to the back within this trochar.

3.3.6) Place the stopcocks on the fluid-filled catheters and remove the clamp. Withdraw blood to remove clots and air bubbles and fill the fluid-filled catheters with 1000 IU/mL heparin. Coronary venous catheters should be filled with 5000IU/mL heparin.

3.4) Closing the thorax

3.4.1) Make an incision with a length of approximately 1.5 cm, 8 cm caudal and parallel to the first incision.

3.4.2) Lead the drain from the pleural cavity through the sixth intercostal muscles subcutaneously to this incision with a large curved mosquito clamp. Connect the drain to the suction device to remove any remaining fluid and reinstate negative pressure in the pleural cavity during the closing of the thorax.

3.4.3) Relieve and inflate the lung with an end-inspiratory hold. Ensure adequate filling of the lung by visual monitoring.

3.4.4) Close the thorax by pulling the ribs of the fourth intercostal space together at two separate sites with non-absorbable USP6 braided polyester (∅0.8mm).

3.4.5) Close the serratus muscle and pectoralis major muscle with a running stitch and the skin with a running subcuticular suture using non-absorbable USP2-0 braided silk (∅0.3mm).

3.4.6) Suture the incisions on the dorsal side with non-absorbable USP2-0 braided polyester (∅0.3mm) between the catheters. First tie a knot directly onto the skin to close the incision, then fixate the catheters to the suture with a knot 1 cm from the skin. For the flow probes, use an absorbable USP2-0 braided polyglactin (∅0.3mm) suture to prevent cutting of the suture in the flow probe wire (Figure 1).

3.4.7) Carefully remove the drain while applying pressure on the cranial side of the incision to maintain negative pressure in the pleural cavity. Close the incision with a purse string suture using non-absorbable USP2-0 braided polyester (∅0.3mm) and seal the wound with petroleum jelly.

3.5) Termination of anesthesia and recovery from surgery

3.5.1) Stop anesthesia when all incision sites are closed.

3.5.2) Provide analgesia by administering buprenorphine (0.015 mg/kg) i.m. in the gracilis muscle.

3.5.3) Stop the ventilation when the animal is breathing independently and disconnect the tracheal tube from the ventilator. Check regularly if the animal is breathing sufficiently.

3.5.4) Place gauze pads between exteriorization sites of the catheters to absorb wound fluid.

3.5.5) To protect the external segments of the catheters, give the animal an elastic vest and package the catheters between two pieces of artificial sheepskin.

3.5.6) Deflate the balloon of the tracheal tube and extubate when the animal regains its swallowing reflex.

3.5.7) Provide long-term analgesia by means of a Fentanyl slow-release patch (12 µg/h for a 20 kg pig; adjust strength according to bodyweight). Place the patch on a

thin part of the skin (such as the lower abdomen) to ensure adequate delivery of analgesia.

3.5.8) House the animal separately for the entire post-operative period. Provide a heating lamp for the first week after surgery to keep the animal warm.

3.5.9) Supply enough fluid i.v. if the animal is not drinking independently.

3.5.10) Flush the fluid-filled catheters daily, by first withdrawing blood to remove clots, then refilling with saline and finally with heparinized saline (1000-5000 IU/mL) to prevent blood clot formation. Take care not to infuse any air bubbles while flushing the catheters.

3.5.11) Administer amoxicillin (25 mg/kg) i.v. daily for 6 days after surgery to prevent post-surgical infections.

3.5.12) Allow the animal to recover for one week before starting the treadmill experiments.

4. Treadmill experiment (Figure 2)

4.1) Flush the fluid-filled catheters as described (3.5.10) and attach the flushed catheters to the pressure transducers. Measure the rectal temperature to be able to obtain temperature corrected blood gas values.

4.2) Flush the pressure transducers with saline to prevent damping of the signals due to air bubbles. Attach the pressure transducers to the elastic vest on the dorsal side.

4.3) Connect the pressure transducers and flow probes to the amplifier. Start measuring in the computer program and calibrate the pressure transducers and flow probes with 0 mmHg being open to the air (and closed to animal) and 100 mmHg using a manometer.

4.4) Switch the three-way stopcock in a way that the fluid catheters have an open connection with the pressure transducers. Note that the blood pressures can now be obtained. Check signals for shape and amplitude (Figure 2).

4.5) If required, connect an extension line to either of the fluid catheters for sampling of mixed venous and arterial blood.

4.6) Measure hemodynamics when the animal is lying as well as standing quietly on the treadmill. Average blood pressures are measured over a timeframe of 10 sec.

4.7) Obtain arterial and mixed venous blood samples by first withdrawing 5 mL of blood using a 10 mL syringe so that 1 ml of pure blood can be obtained using a heparinized 1 mL syringe. For the coronary venous blood samples, a 2 mL syringe is used instead of the 10 mL syringe and withdrawal of 1 mL is sufficient to obtain pure blood.

4.8) Keep the sealed 1 mL syringes on ice before processing the blood samples with a blood gas analyzer to determine the metabolic and ventilatory condition of the animal.

4.9) Subject the swine to a five-stage exercise protocol on the treadmill, 3 minutes per speed, 1-5 km/h (~85% of maximal heart rate). Obtain hemodynamics and blood gases after 1.5-2 min per speed on each speed as in the resting position.

4.10) After the exercise protocol close the stopcocks and check if drift has occurred in the 0 mmHg calibration, make a note of this calibration. Remove the pressure transducers of the fluid-filled catheters and disconnect the flow probes.

4.11) Flush the fluid-filled catheters with saline and heparin (1000-5000 IU/mL). Protect the catheters and flow probes by putting them beneath the elastic vest between two pieces of artificial sheepskin. The animal can now be returned to its cage.

REPRESENTATIVE RESULTS

Exercise up to 5 km/h resulted in a doubling of cardiac output from 4.3±0.3 to 8.5±0.7 L/min which was principally accomplished by an increase in heart rate from 137±7 to 256±8 beats per minute in combination with a small increase in stroke volume from 32±2 to 36±3 mL (Figure 3). The increase in stroke volume was facilitated by an increase in left ventricular contractility, as evidenced by an increase in the maximum of the first derivative of left ventricular pressure dP/dtmax together with an increased rate of relaxation of the left ventricle and an increase in left atrial pressure, being the filling pressure of the left ventricle (Figure 3). The increase in cardiac output together with an increase in hemoglobin concentration (from 8.5±0.4 to 9.2±0.4 g/dl) and an increase in body oxygen extraction from 45±1 to 71±1% allowed a tripling of body oxygen consumption (Figure 3). Systemic vasodilation occurred as evidenced by an increase in systemic vascular conductance and a decrease in systemic vascular resistance, which accommodated the increase in cardiac output almost completely, so that mean aortic pressure increased only slightly (Figure 3). Exercise also resulted in modest vasodilation in the pulmonary circulation, as evidenced by a 33±8% increase in pulmonary vascular conductance. However, the 101±8% increase in cardiac output, together with the increase in left atrial pressure (from 3±1 to 10 ±1 mmHg), resulted in an increase in pulmonary artery pressure and thereby in an increase in right ventricular afterload (Figure 3).

The increase in heart rate, together with the slight increase in arterial pressure resulted in an increase in left ventricular myocardial oxygen consumption, which was principally met by an increase in coronary blood flow which, in combination with the increase in hemoglobin concentration resulted in an increase in myocardial oxygen delivery (from 310±37 to 738±68 μmol/min). The increase in myocardial oxygen demand was commensurate with the increase in myocardial oxygen supply, as myocardial oxygen extraction (79.8±1.9% at rest 81.6±1.9% during maximal exercise) was essentially maintained constant, resulting in an unchanged coronary venous oxygen saturation and coronary venous oxygen tension (Figure 3).

Figure 3. Typical hemodynamic response to exercise. Body oxygen consumption (BVO2) was used as an index for exercise intensity (x-axes of panel A-L). Shown are the responses of heart rate (HR, panel A), stroke volume (SV, panel B), maximum and minimum of the first derivative of left ventricular pressure (dP/dtmax, panel C and dP/dtmin, panel D resp) as indices of contractility and rate of relaxation, cardiac output (CO, panel E), mean arterial pressure (MAP, panel F), systemic vascular conductance (SVC, panel G), systemic vascular resistance (SVR, panel H), Pulmonary artery pressure (PAP, panel J), left atrial pressure (LAP, panel I), pulmonary vascular conductance (PVC, panel K). Total pulmonary resistance (TPR index for right ventricular afterload increased during exercise, Panel L).
The increase in heart rate, together with the slight increase in arterial pressure resulted in an increase in left ventricular myocardial oxygen consumption (x-axes of panels M-P), which was principally met by an increase in coronary blood flow (CBF, panel M), as myocardial oxygen extraction (MEO2, panel N), coronary venous oxygen saturation (CVSO2, panel O) and coronary venous oxygen tension (cvPO2, panel P) were minimally affected. All data are presented as mean with standard error of the mean (SEM).

DISCUSSION

The present study describes the surgery for chronical instrumentation of swine as well as the protocol for exercising the instrumented swine on a motor-driven treadmill while measuring hemodynamics and taking blood samples for measurement of oxygen content in arterial, mixed venous and coronary venous blood.

Critical steps within the protocol

There are several critical steps within the protocol that start already during the intubation procedure. Thiopental (2.1.5) is a respiratory depressive agent, therefore requiring swift intubation upon administration. Also, it is important to carefully monitor ventilator settings during the procedure. Thus, when the thoracic cavity is opened (step 3.1.4), this results in a loss of the negative intrathoracic pressure. To compensate for this loss and to prevent alveolar collapse, ventilation requires positive end expiratory pressure (PEEP). Moreover, ventilator settings (peak inspiratory pressure) should be adjusted to maintain a tidal volume of ~10 mL/kg. Also note that when the left lung is pushed away (3.1.6.) tidal volume is likely to be decreased because only part of the left lung is ventilated. Ventilator settings should be adjusted based on blood gasses.

Another important note with respect to hemodynamic measurements with fluid filled catheters is that there is a hydrostatic pressure difference between the pressure transducer and the insertion site of the fluid-filled catheter into the cardiovascular system. The height difference between the level of the pressure transducer pressure on the elastic vest (4.2), and the insertion point of the catheter should be estimated during surgery and at sacrifice of the animal and corrected for by interpolation either pre- or post- processing of the data.

Another important point to consider when using this technique is that blood loss, either during surgery or during repeated blood sampling should be minimized, despite the fact that swine are relatively large and consequently have a large blood volume (65 mL/kg). During surgery, blood loss during insertion of the catheters can be minimized by simply applying compression on the puncture wounds. According to animal experimentation guidelines, up to 10% of the circulating blood volume can be taken on a single occasion from normal, healthy animals with minimal adverse effects, but it will take an animal about 14 days to replenish this amount of blood.[15] This means that the recovery from surgery is prolonged when a significant amount of blood is lost.

During the repeated blood sampling during the exercise experiments, a maximum of 1.0% of an animal's circulating blood volume, or 0.6 mL/kg can be removed every 24 hours.[15] This also means that the amount of blood that is sampled during treadmill exercise, should be well-planned and that, after removal of the initial clots that are invariably present in the lumen of the catheter near the tip at the interface with the blood, the remaining blood withdrawn to flush the lines should be given back to the animals.

Modifications and troubleshooting

Implanted fluid-filled catheters should be flushed daily to prevent malfunctioning because of blood clot formation. Depending on the amount of blood clots in the fluid filled catheters, the amount of heparin in each line can be varied from 1000IU/mL to 5000IU/mL. The amount of heparin should be kept to a minimum in the first week after surgery to prevent bleeding from surgical incision wounds due to the presence of the anti-coagulant heparin.

However, even when flushed daily, some fluid-filled catheters will get clogged. When this happens, try withdrawing blood with a smaller 2 mL syringe by applying minimal and/or pulsatile suction. It can take several minutes before the catheter will be unclogged. When this does not work, carefully flush a small amount of saline into the catheter and immediately try to withdraw blood. Be aware that infusion can result in a release of thrombus into the circulation and embolism of distal organs, depending on the site of the catheter. When careful flushing does not work, connect the clogged line to a pressure-transducer to check if there is still a hemodynamic signal. If there is no signal, the fluid filled line should be sealed by several knots and cut off.

Interpretation and limitations

When all points as mentioned above are taken into account, the combination of hemodynamic measurements and blood samples allows for interpretation of the exercise response in terms of whole body and myocardial oxygen consumption, which are better measures for exercise intensity than treadmill speed alone.[7,12-14]

In order to meet the increased metabolic requirements of the body, exercise requires changes in cardiac function as well as changes in local perfusion. Tissue perfusion is regulated by changes in diameter of the small arteries and arterioles of the vascular bed supplying the tissue. Myriad vasoactive factors, derived from neurohumoral systems, the endothelium and local metabolites interact to determine vascular tone and ensure adequate tissue perfusion.[1,5,12,16] Changes in systemic and pulmonary vascular resistance or the inverse, vascular conductance, can be calculated from the blood pressure and flow signals and interpreted in terms of changes in vasomotor tone in the systemic and pulmonary vasculature. Intuitively, vascular resistance is often used to assess changes in vascular tone. However, in our research group, we advocate the use of conductance although conductance and resistance are mathematically related, with conductance being flow normalized for pressure, and resistance equaling pressure divided by flow. Although conductance and resistance are interchangeable if one investigates the effect of only a single stimulus (i.e. exercise)[7,17], interpretation of the two parameters can differ when combining exercise with pharmacological interventions, to investigate the contributions of various vasoactive systems to regulation of vascular tone.[4,5,7,14,18]

During exercise, the systemic circulation transforms from a system at rest that is characterized by a low flow and a high resistance (i.e. low conductance) into a system with high flow

and low resistance, (high conductance). As such, pharmacological vasodilation has different consequences for conductance and resistance during rest versus exercise. The decrease in resistance that is produced by a pharmacological vasodilator at rest is large while the increase in conductance is only small. In contrast, during exercise the same degree of vasodilation translates into a large increase in conductance, but only a small decrease in resistance. Thus, when conductance is used, a greater vasodilation seems to occur during exercise, while when looking at resistance vasodilation appears to be larger at rest. Interpretation of the data thus differs when using resistance or conductance. Although the choice between resistance and conductance may seem rather arbitrary, in physics the variable that undergoes the primary change is designated as the numerator of the index for a response.[7,17,18] Since during exercise aortic blood pressure remains fairly constant whereas cardiac output increases markedly, the most appropriate parameter to describe the systemic vascular response to exercise would appear to be systemic vascular conductance (cardiac output / aortic blood pressure), rather than resistance. Moreover, the systemic circulation consists of a multitude of vascular beds from a variety of organs that are principally perfused in a parallel manner. Since parallel resistors add up reciprocally, while parallel conductors add up in a linear manner, any change in conductance of a particular regional vascular bed translates into an identical (absolute) change of the total systemic vascular conductance. This consideration lends further support to the use of vascular conductance to describe the systemic vascular responses to exercise and pharmacological interventions.

The choice for either resistance or conductance to describe the vascular responses to exercise in the pulmonary bed appears to be less obvious, because exercise produced increases in cardiac output as well as pulmonary artery pressure.[7,17] A choice for either resistance or conductance is also less critical, in view of the relatively minor exercise-induced changes in PVR and PVC as compared to the degree of vasodilation produced by, for example, ET-receptor blockade.[7] As a result, the use of either resistance or conductance to characterize the vascular effects of a pharmacological vasodilator in the pulmonary circulation will yield similar conclusions.

In the coronary circulation, interpretation of the data is even more complex as systemic administration of pharmacological antagonists of endogenous vasoactive substances results not only in alterations in coronary resistance vessel tone, but often also produce pronounced changes in systemic hemodynamic variables.[7,14,17,19] These altered hemodynamics influence cardiac work, and thereby cause changes in coronary blood flow resulting from changes in metabolic requirements of the heart or from autoregulation, rather than as a direct effect of the intervention on coronary vascular tone. For example, blockade of an endogenous vasoconstrictor system decreases mean aortic pressure, as a consequence of systemic vasodilation, and elicits autoregulatory adjustments in coronary microvasclar tone. Moreover, baroreceptor reflex activation acts to increase heart rate and myocardial contractility. Such changes in heart rate and/or blood pressure subsequently will result in alterations in myocardial

metabolism, requiring an adjustment in myocardial oxygen supply and hence in coronary blood flow.

To take into account the effects of such drug-induced alterations in myocardial oxygen consumption, investigators examine the relation between coronary venous oxygen levels and myocardial oxygen consumption (MVO_2)[4,5], as this approach allows assessment of regulation of coronary resistance vessel tone independently of changes in myocardial oxygen demand. Administration of a vasodilator will increase myocardial oxygen delivery at a given level of MVO_2. As this increase in oxygen delivery occurs without a change in oxygen consumption, myocardial oxygen extraction will decrease, thereby leading to increases in coronary venous oxygen content and hence in an upward shift of the relation between MVO_2 and coronary venous oxygen levels. It is therefore imperative to measure both myocardial oxygen demand as well as myocardial oxygen supply in order to correctly study the regulation coronary resistance vessel tone.[4,5]

Notwithstanding its elegance and usefulness, some investigators have pointed out the limitations of this approach.[20] Thus, plotting MVO_2 versus coronary venous PO_2 or coronary venous SO_2 could be considered to be inappropriate because these variables are actually part of the equation to compute MVO_2. Consequently, MVO_2 is not a variable that is independent of coronary venous PO_2 or SO_2. Alternatively, investigators should consider using another index of myocardial work, the rate-pressure product (RPP), which is the product of heart rate and left ventricular systolic pressure. However, as RPP and MVO_2 are almost linearly related, substituting RPP for MVO_2 yields virtually identical results [14], and the relation between MVO_2 and coronary venous oxygen levels is considered a sensitive way of studying alterations in coronary vasomotor tone.

Significance with respect to existing methods

Another method commonly used to assess changes in regulation of vascular tone is the use of isolated coronary and pulmonary small arteries or arterioles in a pressure or wire myograph.[6,14,21] The advantage of myograph studies is that vessels can be studied independent of surrounding tissue and without potentially confounding effect from circulating factors. These in vitro techniques are therefore complementary to the in vivo measurements. However, in vivo and in vitro techniques sometimes give opposing results. For example, the response to the potent vasoconstrictor endothelin was reduced in the intact coronary circulation after myocardial infarction, but was augmented in isolated coronary small arteries from swine with myocardial infarction as compared to healthy control swine.[21] This difference between the in vivo and in vitro data was due to an increased suppression of the vasoconstrictor influence of endothelin by prostanoids in vivo.[21]

Future applications

Given the proposed role of changes in coronary microvascular function in both left and right ventricular dysfunction, assessment of these changes in relevant models of cardiovascular disease is required. The use of chronically instrumented animals allows correlations of the severity of the disease with microvascular (dys)function. Moreover, both coronary and pulmonary microvascular function may appear normal under basal resting conditions, while microvascular dysfunction may be revealed under cardiovascular stress, such as during exercise.

Several swine models of cardiopulmonary disease, such as diabetes[6], myocardial infarction[22], pulmonary hypertension[8,9] and pacing induced heart failure[10] are available and could be combined with chronic instrumentation. A potential drawback is that, when commercially available swine breeds such as Yorkshire, Landrace, Large White etc., are used, adult swine are very large and may therefore be difficult to handle. Therefore, juvenile swine are often used. However, as juvenile swine grow rapidly, positioning and function of flow probes and pressure catheters and patency of fluid-filled catheters may become compromised, limiting the duration of serial measurements within individual animals to approximately 10 weeks. An alternative is the use of adult miniature swine, such as Yucatan or Gottingen swine, of which the adult weight is 40-60 kg.[23]

In conclusion, the use of chronically instrumented animals allows serial assessment of cardiopulmonary function either during development of disease or evaluation of treatment, thereby increasing statistical power and limiting the number of animals required for a study.

ACKNOWLEDGEMENTS

This study was supported by Netherlands Heart Foundation grant 2000T038 (to D.J. Duncker) grant 2000T042 (to D. Merkus), European Commission FP7-Health-2010 grant MEDIA-261409 (to D.J. Duncker and D. Merkus), Netherlands CardioVascular Research Initiative: the Dutch Heart Foundation, the Dutch Federation for University Medical Centers, the Netherlands Organisation for Health Research and Development and the Royal Netherlands Academy of Sciences CVON- ARENA CVON 2011-11 (to D.J. Duncker) and CVON-PHAEDRA CVON 2012-08 (to D. Merkus) and CVON-RECONNECT CVON 2014-11 (to D.J. Duncker and D. Merkus), Sophia Foundation (to D. de Wijs-Meijler, D. Merkus and I.K.M. Reiss).

REFERENCES

1. Laughlin, M. H. et al. Peripheral circulation. Compr Physiol **2**, 321-447, doi:10.1002/cphy.c100048 (2012).
2. Datta, D., Normandin, E. & ZuWallack, R. Cardiopulmonary exercise testing in the assessment of exertional dyspnea. Ann Thorac Med **10**, 77-86, doi:10.4103/1817-1737.151438 (2015).
3. Vatner, S. F. & Braunwald, E. Cardiovascular control mechanisms in the conscious state. N Engl J Med **293**, 970-976, doi:10.1056/NEJM197511062931906 (1975).
4. Duncker, D. J. & Bache, R. J. Regulation of coronary blood flow during exercise. Physiol Rev **88**, 1009-1086, doi:10.1152/physrev.00045.2006 (2008).
5. Tune, J. D., Gorman, M. W. & Feigl, E. O. Matching coronary blood flow to myocardial oxygen consumption. J Appl Physiol (1985) **97**, 404-415, doi:10.1152/japplphysiol.01345.2003 (2004).
6. van den Heuvel, M. et al. Coronary microvascular dysfunction in a porcine model of early atherosclerosis and diabetes. Am J Physiol Heart Circ Physiol **302**, H85-94, doi:10.1152/ajpheart.00311.2011 (2012).
7. Zhou, Z. et al. Pulmonary vasoconstrictor influence of endothelin in exercising swine depends critically on phosphodiesterase 5 activity. Am J Physiol Lung Cell Mol Physiol **306**, L442-452, doi: 10.1152/ajplung.00057.2013 (2014).
8. Pereda, D. et al. Swine model of chronic postcapillary pulmonary hypertension with right ventricular remodeling: long-term characterization by cardiac catheterization, magnetic resonance, and pathology. J Cardiovasc Transl Res **7**, 494-506, doi:10.1007/s12265-014-9564-6 (2014).
9. Mercier, O. et al. Endothelin A receptor blockade improves regression of flow-induced pulmonary vasculopathy in piglets. J Thorac Cardiovasc Surg **140**, 677-683, doi:10.1016/j.jtcvs.2010.01.004 (2010).
10. Spinale, F. G. et al. Chronic supraventricular tachycardia causes ventricular dysfunction and subendocardial injury in swine. Am J Physiol **259**, H218-229 (1990).
11. Yarbrough, W. M. & Spinale, F. G. Large animal models of congestive heart failure: a critical step in translating basic observations into clinical applications. J Nucl Cardiol **10**, 77-86, doi:10.1067/mnc.2003.16 (2003).
12. Duncker, D. J., Stubenitsky, R. & Verdouw, P. D. Autonomic control of vasomotion in the porcine coronary circulation during treadmill exercise: evidence for feed-forward beta-adrenergic control. Circ Res **82**, 1312-1322, doi:10.1161/01.RES.82.12.1312 (1998).
13. Stubenitsky, R., Verdouw, P. D. & Duncker, D. J. Autonomic control of cardiovascular performance and whole body O2 delivery and utilization in swine during treadmill exercise. Cardiovasc Res **39**, 459-474, doi:10.1016/S0008-6363(98)00102-3 (1998).
14. Zhou, Z. et al. Phosphodiesterase-5 activity exerts a coronary vasoconstrictor influence in awake swine that is mediated in part via an increase in endothelin production. Am J Physiol Heart Circ Physiol **306**, H918-927, doi:10.1152/ajpheart.00331.2013 (2014).
15. Gross, D. R. Animal Models in Cardiovascular Research. 3 edn, Springer, doi: 10.1007/978-0-387-95962-7 (2009).
16. Merkus, D. & Duncker, D. J. Perspectives: Coronary microvascular dysfunction in post-infarct remodelled myocardium. Eur Heart J Suppl **16**, A74-A79, doi:10.1093/eurheartj/sut016 (2014).
17. de Beer, V. J., de Graaff, H. J., Hoekstra, M., Duncker, D. J. & Merkus, D. Integrated control of pulmonary vascular tone by endothelin and angiotensin II in exercising swine depends on gender. Am J Physiol Heart Circ Physiol **298**, H1976-1985, doi:10.1152/ajpheart.00459.2009 (2010).

18. Lautt, W. W. Resistance or conductance for expression of arterial vascular tone. Microvasc Res **37**, 230-236, doi:10.1016/S0008-6363(98)00102-3 (1989).
19. Merkus, D. et al. Phosphodiesterase 5 inhibition-induced coronary vasodilation is reduced after myocardial infarction. Am J Physiol Heart Circ Physiol **304**, H1370-1381, doi:10.1152/ajpheart.00410.2012 (2013).
20. Heusch, G. The paradox of alpha-adrenergic coronary vasoconstriction revisited. J Mol Cell Card **51**, 16-23, doi:10.1016/j.yjmcc.2011.03.007 (2011).
21. Merkus, D., Houweling, B., van den Meiracker, A. H., Boomsma, F. & Duncker, D. J. Contribution of endothelin to coronary vasomotor tone is abolished after myocardial infarction. Am J Physiol Heart Circ Physiol **288**, H871-880, doi:10.1152/ajpheart.00429.2004 (2005).
22. Haitsma, D. B. et al. Minimal impairment of myocardial blood flow responses to exercise in the remodeled left ventricle early after myocardial infarction, despite significant hemodynamic and neurohumoral alterations. Cardiovasc Res **52**, 417-428, doi:10.1016/S0008-6363(01)00426-6 (2001).
23. Bender, S. B., van Houwelingen, M. J., Merkus, D., Duncker, D. J. & Laughlin, M. H. Quantitative analysis of exercise-induced enhancement of early- and late-systolic retrograde coronary blood flow. J Appl Physiol (1985) **108**, 507-514, doi:10.1152/japplphysiol.01096.2009 (2010).