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Validation of 24-h systemic blood pressure and heart rate monitoring and influence of anaesthesia
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Implantation of telemetric blood pressure transmitters in Göttingen Minipigs: Validation of 24-h systemic blood pressure and heart rate monitoring and influence of anaesthesia

Michelle Fischer Carlsen a, b, Berit Østergaard Christoffersen b, Rikke Lindgaard a, 1, Henrik Duelund Pedersen b, 2, Lisbeth Høier Olsen a, 1

a Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Ridebanevej 9, 1870 Frederiksberg C, Denmark
b Obesity Pharmacology, Global Drug Discovery, Novo Nordisk A/S, Novo Nordisk Park 1, 2760 Måløv, Denmark

1 Corresponding author.
E-mail address: lisbeth.hoier@sund.ku.dk (L.H. Olsen).

Abstract

Introduction: Porcine animal models are used in biomedical research due to anatomical and physiological similarities with human patients. The study aimed to validate telemetric systemic blood pressure (BP) and heart rate (HR) monitoring in Göttingen Minipigs, and in addition to study the effects of three different anaesthesia protocols on telemetric BP and HR measurements.

Methods: Eight female Göttingen Minipigs had telemetry transmitters implanted in the right carotid artery. Over ten weeks, systemic 24-h BP and HR monitoring were repeated four times, each ending with an angiotensin II stimulation test. In addition, systemic BP and HR evaluated by telemetry, intra-arterial catheterisation (IAC) and oscillometric tail-cuff were compared before and after the 10-weeks period. Furthermore, changes in telemetric systemic BP and HR were monitored during anaesthesia in a cross-over design using three different protocols of general anaesthesia: Midazolam/ketamine (MK), propofol, and a combination of tiletamine, zolazepam, xylazine, ketamine and butorphanol (Zoletil-mix).

Results: One minipig was excluded and some data were missing due to central-venous catheter issues. The coefficient of variation was below 10% for the 24-h BP and HR measurements, but higher during angiotensin II stimulation. There was a disagreement between the tail-cuff measurement and telemetry/IAC, however the differences were independent of the BP and HR level. All anaesthesia protocols numerically influenced BP and HR, but only propofol statistically significantly decreased the BP.

Conclusion: The study showed acceptable reproducibility of telemetric measurement of BP and HR over ten weeks in freely moving Göttingen Minipigs. There was a disagreement between direct and indirect BP measurement, and BP and HR were influenced by all anaesthesia protocols.

Keywords: Blood pressure, Heart rate, Hemodynamic Methods, Minipigs, Telemetry, Anaesthesia, Invasive, Non-invasive

1. Introduction

The Göttingen Minipig is a commonly used laboratory animal in biomedical research, due to anatomical and physiological similarities to humans, e.g. in the cardiovascular system (Bode et al., 2010). Compared to domestic swine, the small size of the minipig makes it easier to handle and house, which is ideal in a research setting and especially in long-term studies (Swindle, Makin, Herron, Clubb, & Frazier, 2012). Systemic blood pressure (BP) and heart rate (HR) are essential readouts from many animal experiments, e.g. safety pharmacology or animal models of cardiovascular diseases, and reliable methods for their measurements are therefore needed. Systemic BP can be monitored indirectly (non-invasively) or directly (invasively), with direct monitoring being the gold standard (Gladczak, Shires, Stevens, & Clymer, 2013;...
has been described in other animals such as cynomolgus monkeys, dogs, and mice (Andersen et al., 2017; Brooks et al., 1996; Butz et al., 2018; Tuohy, Raisis, & Drynan, 2017; Ypsilantis, Didilis, & Simopoulos, 2013). A major advantage of the indirect methods including tail or leg cuff measurements using oscilometry or Doppler registration is that they are non-invasive and less expensive compared to the invasive methods. However, they require handling and restraining of conscious animals, which typically cause stress affecting the cardiovascular parameters (Kurtz et al., 2005), and may not allow optimal long-term time-resolved profiles. The direct methods involve implantation of a fluid-filled intra-arterial catheter (IAC) or telemetric transmitter often through either the carotid artery with the tip ending in the aortic arch or through the femoral artery with the tip ending in the abdominal aorta. With surgery, there is a risk of complications such as infections, yet the risk can be minimised with proper surgical aseptic technique and post-operative care. The ICH guideline for safety pharmacology studies for human pharmaceuticals recommends data for evaluation of the cardiovascular system to be obtained from conscious unrestrained animals (ICH, 2000). Telemetric equipment makes it possible to perform continuous measurements in a stress-free way in conscious unrestrained animals (Authier, Pugsley, Troncy, & Curtis, 2010; Kurtz et al., 2005). Twenty-four-hour telemetric measurement in Göttingen Minipigs and Chinese Miniature Experiments Pigs has been described earlier (Stubban et al., 2008; Yuan et al., 2014), but there is no peer-reviewed studies with multiple measurements for several weeks. It has been described in other animals such as cynomolgus monkeys, dogs, and mice (Andersen et al., 2017; Brooks et al., 1996; Butz & Davisson, 2001).

Anaesthesia of laboratory animals can be necessary during biomedical research, and it is well known that anaesthesia can affect the cardiovascular system, why it is crucial to know the effect of different anaesthesia protocols on the cardiovascular readouts. The following three protocols are commonly used for anaesthesia of minipigs: Midazolam and ketamine combination, propofol and a combination of zolazepam, tiletamine, xylazine, ketamine and butorphanol. The aim of this study in Göttingen Minipigs was to validate systemic BP and HR measurements with the DSI PhysioTel PA-C10 telemetry implant by testing repeatability, ability of the method to identify the effect of a known pharmacological agent as positive control (angiotensin II) and agreement with other direct and indirect BP measurements. In addition, practical application of the method for evaluation of the effect of different anaesthesia protocols was performed.

2. Materials and methods

2.1. Animals

The study was approved by the Animal Experiments Inspectorate (license number 2012-15-2934-00715), Ministry of Environment and Food in Denmark, complied with the ARRIVE guidelines and was carried out in accordance with EU Directive 2010/63/EU for animal experiments. Eight healthy naïve female Göttingen Minipigs (Ellegaard Göttingen Minipigs A/S, Denmark) aged approx. 13 months (9–16 months) at study start and weighing approx. 25 kg were included in the study. The minipigs were group-housed for a minimum of two weeks of acclimatisation and single-housed after ear vein catheter and BP transmitter implantation. They were housed in a 12:12 h light-dark cycle and had wood shavings and straw as bedding. They were fed 400 g standard minipig diet once daily on the floor around noon (SDS minipig expanded, Scanbur, Denmark) and had ad libitum access to water. The diet was typically eaten in less than 1 h (individual times were not noted). The animals were fasted before anaesthesia.

Table 1

<table>
<thead>
<tr>
<th>Study week</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Surgical implantation of ear vein catheter + BP transmitter. Simultaneous telemetric and intra-arterial catheter BP and HR measurement.</td>
</tr>
<tr>
<td>1</td>
<td>24-h BP and HR measurement incl. Angiotensin II stimulation (Test round 1)</td>
</tr>
<tr>
<td>2</td>
<td>Anaesthesia with BP and HR measurement</td>
</tr>
<tr>
<td>3</td>
<td>24-h BP and HR measurement incl. Angiotensin II stimulation (Test round 2)</td>
</tr>
<tr>
<td>4</td>
<td>Anaesthesia with BP and HR measurement</td>
</tr>
<tr>
<td>5</td>
<td>24-h BP and HR measurement incl. Angiotensin II stimulation (Test round 3)</td>
</tr>
<tr>
<td>6–9</td>
<td>No study activity</td>
</tr>
<tr>
<td>10</td>
<td>24-h BP and HR measurement incl. Angiotensin II stimulation (Test round 4)</td>
</tr>
<tr>
<td>11</td>
<td>Anaesthesia with BP and HR measurement</td>
</tr>
<tr>
<td>12</td>
<td>Simultaneous telemetric, intra-arterial and non-invasive BP and HR measurement incl. Angiotensin II stimulation. Euthanasia.</td>
</tr>
</tbody>
</table>

2.2. Telemetry system

The telemetric system used to measure BP and HR was the DSI PhysioTel PA-C10 Transmitters and telemetry equipment produced by Data Sciences International (DSI, Saint Paul, MN, USA). The equipment consisted of six components: A transmitter, a magnet and radio for turning the transmitter on and off, an antenna for receiving the signal from the transmitter, a receiver for antenna input, a jacketed external telemetry (JET) device receiver, and a Bluetooth receiver over the pens (one Bluetooth receiver covered four animal pens). Continuous corrections of the blood pressure according to atmospheric pressure were performed in Ponemah using a barometer (Ambient Pressure Reference (APR)). Computer equipment was placed in an adjacent lab. A special vest with pockets for the JET receiver and antenna was custom-made for the minipigs.

2.3. Implantation of telemetry transmitter

The transmitters were implanted one week prior to the first 24-h recording. The animals were fasted from the night before implantation. Preoperative antibiotic prophylaxis (benzylpenicillinprocin; Noropen prolongatun Vet, 0.07 mL/kg IM, ScanVet, Denmark) and analgesia with buprenorphine (Vetgesic® 0.01 mg/kg IM, Orion Pharma Animal Health) and meloxicam was administrated (Metacam®, 0.02 mL/kg IM, Boehringer Ingelheim Animal Health Nordics A/S). General anaesthesia was induced with a combination of anaesthetic and sedative agents (Zoletil mix): 125 mg zolazepam and 125 mg tiletamine (Zoletil® 50 Vet, ChemVet, Denmark) dissolved in ketamine (1.25 mL Ketaminol® Vet 100 mg/mL, MSD Animal Health, Denmark), xylazine (6.5 mL Rompun Vet, 20 mg/mL, Bayer A/S, Denmark) and butorphanol (2.5 mL Torbugesic®, 10 mg/mL, Scanvet, Denmark) (0.1 mL/kg intramuscularly (IM)). Atropine (0.02 mg/kg) was administered IM to stabilise HR and reduce bronchial secretion. The general anaesthesia was maintained with isoflurane. First, permanent central-venous catheters for intravenous (IV) access were introduced through the ear veins with the tip ending in the jugular vein. The catheters were fastened to the pinna using sutures and tape. An incision was made in the jugular sulus and using blunt dissection the left carotid artery was localised and isolated. The BP transmitter was introduced through a small incision in the carotid artery and placed with the tip ending in the aortic arch. At this time during the surgery, the BP was measured concomitantly with the transmitter and an intra-arterial pressure catheter (IAC) as described below. After the IAC had been removed again, a suture was placed around the carotid artery and the transmitter to secure it in place and the
incision site was closed in 3 layers. At the end of the surgery, buprenorfin (Vetgesic® Vet, 0.3 mg/mL, Orion Pharma Animal Health A/S, Denmark) was given IM in a dose of 0.01 mg/kg. Further postoperative analgesia with meloxicam (Metacam®, 0.02 ml/kg IM, Boehringer Ingelheim Animal Health Nordics A/S) was administrated on the following three days.

2.4. Study overview

An overview of the study measurements is shown in Table 1.

2.4.1. 24-h telemetry measurements

Over a total study period of 10 weeks, the systolic (SAP), diastolic (DAP) and mean (MAP) arterial BP and HR were recorded continuously with the DSI PA-C10 transmitter during four 24-h periods, defined as test round 1–4, with each period ending with an angiotensin II stimulation test (see section 2.4.2). HR was calculated from the telemetric pulse wave registrations by dividing 60 with the length of the cardiac cycle in seconds. There was at least two weeks between each 24-h period (Table 1) and the recordings were obtained from 10 a.m. to 10 a.m. the next day. The minipigs were equipped with a special vest for the JET receiver and antenna the day before the 24-h recording, to reduce stress and ensure acclimation towards the vest. Access to the pens was restricted to prevent disturbance, and thus only feeding was allowed around noon.

2.4.2. Angiotensin II stimulation test

In the end of each 24-h period, an angiotensin II stimulation test was performed. The angiotensin II solution (see supplementary) was administrated IV by infusion pump (B. Braun Perfusor® Space, E-Vet) via the ear vein catheter over a period of 10 min in a dose of 2.5 μg/kg/h (2 mL/kg/h) using extension tubes and a swivel system leaving the animals completely freely moving and undisturbed during the infusion. The BP and HR were recorded from 10 min before infusion and during the infusion. After the angiotensin II infusion, ampicillin (1 mL/10 kg Pentrexyl inj., Corden Pharma Latina S.p.A., Latina, Italy, 1 g/10 mL physiological saline) was administered IV, and the catheters were closed with 10 mL sterile saline and 0.8 mL of TauroLock Hep 500 (NorDiaTech A/S, Hedehusene, Denmark).

2.4.3. Comparison of blood pressure measurement methods

During the implantation of the telemetric transmitter and at the end-study evaluations 12 weeks later, systemic BP and HR was measured simultaneously with the telemetry transmitter, IAC and a non-invasive oscillometric tail-cuff system (only at study end) in anaesthetised minipigs. The IAC (Careflow™ Femoral Artery Catheter Kit Seldinger Technique, 4 F, 200 mm (REF 681648, Argon Medical devices)) was placed 10 cm into the left carotid artery together with the telemetry transmitter with the tip placed in the aortic arch during the measurements. The transducer and pressure line were filled with saline, air bubbles removed, and the pressure bag pumped to 300 mmHg. The arterial catheter was connected to the Datex-Ohmeda S/S (GE Healthcare, Brondby, Denmark) through the pressure line, and the transducer was placed in the same height as the heart. The signal was allowed to stabilise for 5 min before starting the recording of BP and HR. At the implantation, BP and HR were noted every 20 s for two minutes for both the IAC and the telemetry transmitter. At end-study, systemic BP and HR were recorded every 2 min for a 6-min baseline period followed by a 10-min angiotensin II stimulation test (IV, 1.25 μg/kg/h (2 mL/kg/h)) simultaneously with telemetry, IAC and oscillometric monitoring using a cuff around the tail of the pig (Cardell™ Veterinary Blood Pressure Monitor 9301 V, CAS Medical Systems Inc., Branford, CT, USA)). The width of the cuff was approximately 40% of the circumference of the tail.

2.4.4. Anaesthesia study

The study was designed as a cross-over study, but all pigs did not complete all protocols due to issues with the function of the central-venous catheters. Anaesthetic protocols were tested with at least two weeks apart. One pig died of unknown reasons after seventeen days in the study. The pig was excluded from the 24-h study and the study comparing different BP methods, but contributed with data to one anaesthetic protocol (Supplemental table S). The three anaesthesia protocols were:

(i) MK protocol: Midazolam in combination with ketamine (induction dose 0.33 mg/kg midazolam (Midazolam B. Braun, 5 mg/mL) IV and 13 mg/kg ketamine (Ketaminol Vet., 100 mg/mL) IV and maintenance dose 1.5 mg/kg/h midazolam and 33 mg/kg/h ketamine

(ii) Propofol protocol: Propofol (Propolipid, 10 mg/mL) (induction dose 4 mg/kg propofol IV and maintenance 8 mg/kg/h propofol)

(iii) Zoletil-mix protocol: A combination of tiletamine, xylazeline, ketamine and butorphanol (1 vial of zoletil (125 mg tiletamine, 125 mg xylazeline), 6.5 mL xylazine (Rompun Vet., 20 mg/mL), 1.25 ketamine (Ketaminol Vet., 100 mg/mL), 2.5 mL butorphanol (Torbugesic Vet., 10 mg/mL) (induction dose 1 mL/10 kg IM and maintenance 0.3 mL/10 kg every 30 min IM)

In the afternoon before the anaesthesia day, all transmitters were turned on, the minipigs were equipped with the vest, and the receivers and antenna were placed in the pocket of the vest. The animals were left with as little disturbance as possible until the next day. The animals were anaesthetised for two hours under observation. Palpebral and interdigital reflexes were monitored every 15 min. The animals were kept warm using blankets. After two hours of anaesthesia, the infusion pump was disconnected, antibiotics (1 mL/10 kg Pentrexyl inj., Corden Pharma Latina S.p.A., Latina, Italy, 1 g/10 mL physiological saline) was administered IV, and the catheters closed with 10 mL sterile saline +0.8 mL of TauroLock Hep 500 (NorDiaTech A/S, Hedehusene, Denmark). SAP, DAP, MAP, and HR recordings were obtained from one hour before anaesthesia and during the two hours of anaesthesia.

2.4.5. Euthanasia

The minipigs were euthanised at study end during anaesthesia with an IV overdose of pentobarbital (400 mg/mL, 6 mL/pig, IV).

2.5. Data analysis and quality control

The software package Ponnemah 6.41 (DSI) was used for BP and HR recordings and analysis. Qualitative control was done using the software noise detection system and by visual assessment. The visual assessment included adjustment and validation of analysis attributes and bad data mark settings to fit the minipig data. See supplementary table 1 and 2 for the adjusted settings and a description of the setting validation.

2.6. Statistical analysis

SAS (Version 9.4, SAS Institute Inc.) was used for statistical analysis and GraphPad Prism (Version 9.1.0, GraphPad Software) for graphic illustrations. Data are presented as means ± standard deviation (SD). P-values <0.05 were considered statistically significant for all tests.

2.6.1. Reproducibility of 24-h BP and HR

The reproducibility of 24-h BP and HR measurements was evaluated by calculating the coefficient of variation (CV%) of all test rounds. The CV% was calculated for each minipig and summarised for all pigs. Four protocols were:

(i) MK protocol: Midazolam in combination with ketamine (induction dose 0.33 mg/kg midazolam (Midazolam B. Braun, 5 mg/mL) IV and 13 mg/kg ketamine (Ketaminol Vet., 100 mg/mL) IV and maintenance dose 1.5 mg/kg/h midazolam and 33 mg/kg/h ketamine

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2.6.1. Reproducibility of 24-h BP and HR

The reproducibility of 24-h BP and HR measurements was evaluated by calculating the coefficient of variation (CV%) of all test rounds. The CV% was calculated for each minipig and summarised for all pigs. Four protocols were defined to investigate diurnal and post-prandial effects: morning (6–10 o’clock), (10–14 o’clock), day (14–18 o’clock) and night (0–4 o’clock). Wilcoxon signed rank test was used to compare the four periods for all the test rounds.
2.6.2. Angiotensin II stimulation test

Two recording periods were defined: baseline (the 5 min before angiotensin II infusion) and infusion (the 10-min period with infusion). The CV% of the percentage difference was calculated per pig for the four study rounds and summarised for all pigs. Wilcoxon signed ranked test was used to compare BP and HR in the baseline and infusion periods in each study round.

2.6.3. Comparison of BP measurements methods

A comparison of the BP and HR measured by the different methods at each time of measurement was made using the Wilcoxon signed rank test. Bland-Altman analysis and plot were used to compare the three BP measurement methods and evaluate the level of agreement.

2.6.4. Anaesthesia study

Wilcoxon signed rank test was used to compare changes in BP and HR during three predefined time periods of anaesthesia: baseline (one hour before anaesthesia induction), the first hour of anaesthesia and the second hour of anaesthesia.

3. Results

The transmitters were successfully implanted in all eight minipigs. One minipig was found dead with no prior clinical observations seventeen days after implantation of the transmitter, leading to the inclusion of only seven minipigs in the study in the 24 h study and the study comparing BP measurements. Furthermore, the pig only contributed with data to one anaesthetic protocol (Supplemental Table 5). No changes explaining the cause of death were observed at necropsy and the transmitter was found in the correct position. In addition, issues with the function of the central-venous catheters resulted in inclusion of fewer minipigs for the angiotensin II stimulation test, the comparison of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test round 1</th>
<th>Test round 2</th>
<th>Test round 3</th>
<th>Test round 4</th>
<th>Mean</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP mmHg</td>
<td>126.3 ± 7.9</td>
<td>136.0 ± 8.9</td>
<td>131.3 ± 4.0</td>
<td>136.4 ± 8.4</td>
<td>132.5 ± 4.7</td>
<td>3.1</td>
</tr>
<tr>
<td>DAP mmHg</td>
<td>92.9 ± 3.3</td>
<td>103.4 ± 6.8</td>
<td>100.9 ± 2.4</td>
<td>103.6 ± 10.7</td>
<td>100.2 ± 5.1</td>
<td>3.4</td>
</tr>
<tr>
<td>MAP mmHg</td>
<td>111.2 ± 3.3</td>
<td>121.1 ± 2.4</td>
<td>117.6 ± 10.7</td>
<td>121.2 ± 117.8</td>
<td>110.7 ± 5.1</td>
<td>3.4</td>
</tr>
<tr>
<td>HR bpm</td>
<td>82.9 ± 7.0</td>
<td>79.3 ± 7.8</td>
<td>85.6 ± 2.8</td>
<td>82.3 ± 9.2</td>
<td>82.5 ± 4.7</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Fig. 1. A. Systolic arterial blood pressure, B. Diastolic arterial blood pressure, C. Mean arterial blood pressure (BP) and D. Heart rate measured for 24 h four times with at least two weeks apart with implanted telemetry transmitters in seven freely moving Göttingen Minipigs. Recording was performed from 10 a.m. to 10 a.m. Data is presented as mean ± SEM.
Table 3
Systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure and heart rate (HR) of seven freely moving Göttingen Minipigs during 24-h telemetry (DSI) measurement, performed four times at least two weeks apart. Morning (6–10 o’clock), feeding (10–14 o’clock), day (14–18 o’clock) and night (00–04 o’clock) were compared. a statistically significantly different from morning (p < 0.05). b statistically significantly different from feeding (p < 0.05). c statistically significantly different from day (p < 0.05). Data are presented as mean ± SD.

<table>
<thead>
<tr>
<th>Test round</th>
<th>SAP (mmHg)</th>
<th>DAP (mmHg)</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Morning</td>
<td>119.8 ± 6.9</td>
<td>86.4 ± 3.3</td>
<td>104.6 ± 5.3</td>
<td>75.6 ± 9.4</td>
</tr>
<tr>
<td>1 Feeding</td>
<td>133.2 ± 9.0a</td>
<td>98.6 ± 4.4a</td>
<td>117.5 ± 7.0a</td>
<td>90.0 ± 13.1a</td>
</tr>
<tr>
<td>1 Day</td>
<td>128.7 ± 6.7</td>
<td>97.4 ± 5.3</td>
<td>114.7 ± 7.3</td>
<td>86.5 ± 7.7ab</td>
</tr>
<tr>
<td>1 Night</td>
<td>125.7 ± 4.0</td>
<td>91.3 ± 2.5abc</td>
<td>110.1 ± 5.4abc</td>
<td>78.6 ± 8.4abc</td>
</tr>
<tr>
<td>2 Morning</td>
<td>135.7 ± 6.2</td>
<td>102.4 ± 5.6</td>
<td>120.2 ± 5.7</td>
<td>77.3 ± 8.5</td>
</tr>
<tr>
<td>2 Feeding</td>
<td>139.0 ± 7.3</td>
<td>105.7 ± 6.0</td>
<td>123.7 ± 6.5a</td>
<td>86.2 ± 6.5a</td>
</tr>
<tr>
<td>2 Day</td>
<td>134.4 ± 8.0</td>
<td>103.2 ± 5.7</td>
<td>120.5 ± 6.8</td>
<td>85.0 ± 5.3</td>
</tr>
<tr>
<td>2 Night</td>
<td>135.4 ± 11.4</td>
<td>102.7 ± 8.7</td>
<td>120.4 ± 10.0</td>
<td>73.7 ± 7.0abc</td>
</tr>
<tr>
<td>3 Morning</td>
<td>130.5 ± 6.1</td>
<td>99.4 ± 3.8</td>
<td>116.5 ± 5.0</td>
<td>78.8 ± 11.3</td>
</tr>
<tr>
<td>3 Feeding</td>
<td>137.0 ± 5.4</td>
<td>105.5 ± 4.3</td>
<td>122.6 ± 4.4</td>
<td>93.5 ± 11.5a</td>
</tr>
<tr>
<td>3 Day</td>
<td>129.5 ± 4.1b</td>
<td>99.7 ± 2.8b</td>
<td>116.2 ± 2.9b</td>
<td>93.4 ± 10.2a</td>
</tr>
<tr>
<td>3 Night</td>
<td>129.8 ± 3.6b</td>
<td>98.2 ± 2.8b</td>
<td>115.6 ± 2.5b</td>
<td>78.7 ± 10.6bc</td>
</tr>
<tr>
<td>4 Morning</td>
<td>134.9 ± 9.9</td>
<td>101.0 ± 11.0</td>
<td>118.8 ± 9.9</td>
<td>73.7 ± 11.1</td>
</tr>
<tr>
<td>4 Feeding</td>
<td>143.0 ± 12.2</td>
<td>112.2 ± 11.3</td>
<td>128.8 ± 14.4</td>
<td>97.3 ± 12.4a</td>
</tr>
<tr>
<td>4 Day</td>
<td>132.6 ± 7.9b</td>
<td>102.4 ± 10.8b</td>
<td>119.1 ± 8.8b</td>
<td>90.0 ± 13.1ab</td>
</tr>
<tr>
<td>4 Night</td>
<td>136.0 ± 5.6</td>
<td>101.1 ± 7.5p</td>
<td>119.6 ± 5.8b</td>
<td>74.8 ± 9.7abc</td>
</tr>
</tbody>
</table>

3.1. 24-h study

3.1.1. Blood pressure and heart rate
The signal from the implanted transmitters was stable with few signal dropouts. Fig. 1 shows the average course of the MAP and HR during the 24-h measurement for each test round. SAP and DAP follows the course of the MAP. The average BP and HR over the full 24-h periods together with the average CV% are summarised in Table 2.

Table 3 summarises the average BP and HR during the different time periods. The BP and HR increased during feeding compared to the morning period, with the increase in HR being statistically significant for all four rounds, while the increase in BP only was statistically significant for test round 1 and 4. While the BP decreased to pre-feeding levels with no difference between morning and day, except for test round 1, the HR remained elevated during the day period compared to morning (not statistically significant for test round 2).

3.1.2. Angiotensin II stimulation test
Fig. 2 show the course of the MAP and HR during the 20-min measurement period. SAP and DAP follows the course of the MAP (Supplementary Fig. 1). Table 4 shows the percentage difference in the BP and HR between the baseline and the angiotensin II infusion, as well as the CV%. The BP increased instantly, as expected, during the angiotensin II stimulation test, and reached a steady state a few minutes after infusion start. The increase was statistically significant for round 1, 2 and 4, but not for round 3, despite numerically similar changes. The average HR rate decreased numerically in all four rounds, although only statistically significantly in test round 1 and 4. The percentage difference and CV% for each individual minipig is listed in the supplementary information (supplemental table 4).

Table 4
The average percentage difference and coefficient of variation (CV%) for the systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure and heart rate (HR) between the baseline and the 10-min angiotensin II stimulation test in seven freely moving Göttingen Minipigs. The angiotensin stimulation test was performed four times, with at least two weeks apart. Data was recorded by telemetry (DSI). Data are presented as mean ± SD. a Statistically significant difference between baseline and infusion periods (p < 0.05).

<table>
<thead>
<tr>
<th>Pig#</th>
<th>Round 1</th>
<th>Round 2</th>
<th>Round 3</th>
<th>Round 4</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>SAP</td>
<td>32.7 ± 4.3*</td>
<td>26.7 ± 4.6*</td>
<td>28.8 ± 4.6*</td>
<td>29.1 ± 4.6*</td>
<td>18.9 ± 9.4*</td>
</tr>
<tr>
<td>(mmHg)</td>
<td>4.1 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>DAP</td>
<td>41.4 ± 7.0 *</td>
<td>32.7 ± 4.6*</td>
<td>34.2 ± 4.6*</td>
<td>31.8 ± 4.6*</td>
<td>18.5 ± 9.4*</td>
</tr>
<tr>
<td>(mmHg)</td>
<td>6.8 ± 1.2</td>
<td>7.0 ± 1.2</td>
<td>7.0 ± 1.2</td>
<td>7.0 ± 1.2</td>
<td>6.8 ± 1.2</td>
</tr>
<tr>
<td>MAP</td>
<td>31.9 ± 5.2*</td>
<td>21.6 ± 5.2*</td>
<td>28.0 ± 5.2*</td>
<td>26.3 ± 5.2*</td>
<td>18.0 ± 9.4*</td>
</tr>
<tr>
<td>(mmHg)</td>
<td>4.2 ± 1.0</td>
<td>4.2 ± 1.0</td>
<td>4.2 ± 1.0</td>
<td>4.2 ± 1.0</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>-17.6 ± 8.4*</td>
<td>-16.5 ± 8.4*</td>
<td>-14.4 ± 8.4*</td>
<td>-15.6 ± 8.4*</td>
<td>-63.0 ± 10.3</td>
</tr>
<tr>
<td>8.4*</td>
<td>10.6</td>
<td>7.8</td>
<td>7.8*</td>
<td>52.1</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. A. Mean arterial blood pressure (BP) and B. Heart rate measured during angiotensin II stimulation test, with implanted telemetry transmitters in seven freely moving Göttingen Minipigs. The dotted line indicates start of infusion. Data is presented as mean ± SEM.
3.2. Comparison of different BP measurement methods

There was no difference in the BP and HR obtained by IAC and telemetry at implantation (Fig. 3). A difference in the BP obtained by the different measurement methods was, however, observed 12 weeks after the telemetry transmitter implantation (Fig. 4). All BP parameters obtained by IAC was statistically significantly higher than the ones obtained by telemetry. A difference plot (Bland-Altman) showed that the underestimation by telemetry was $23.4 \pm 16.4$ mmHg (SAP), $19.8 \pm 14.1$ mmHg (DAP) and $21.5 \pm 15.3$ mmHg (MAP) (Fig. 5). The cuff overestimated the SAP (only statistically significant compared to telemetry) but statistically significantly underestimated the DAP compared to both telemetry and IAC (Fig. 4). The difference plot showed that the average overestimation of the SAP by cuff was $37.4 \pm 11.6$ mmHg compared to telemetry and $14.0 \pm 16.2$ mmHg compared to IAC, and the corresponding underestimation of the DAP was $9.2 \pm 11.2$ mmHg and $29.0 \pm 9.7$ mmHg, respectively. The difference plot (Fig. 5) illustrates that the disagreement between the methods was independent of the BP level.

3.3. Anaesthesia study

The course of the BP and HR during anaesthesia with the three different protocols is shown in Fig. 6. The means ± SD for the BP and HR of the baseline and the two anaesthesia periods for each protocol can be found in the supplementary information (supplemental table 6).

3.3.1. Midazolam/ketamine

The BP slightly decreased non-significantly during the first hour of anaesthesia with MK but started to increase towards baseline levels during the second hour of anaesthesia. The average SAP, DAP and MAP decreased 13.7 mmHg, 8.1 mmHg and 12.5 mmHg respectively from the baseline to the first hour of anaesthesia, and 11.8 mmHg, 4.8 mmHg and 9.5 mmHg respectively from the baseline to the second hour of anaesthesia. The average HR increased non-significantly 4.7 bpm from the baseline to the first hour of anaesthesia and 5.4 bpm from the baseline to the second hour of anaesthesia.

3.3.2. Propofol

The BP and HR decreased during the propofol anaesthesia, with the BP reaching a steady state approximately after half an hour. The average SAP, DAP and MAP decreased 29.3 mmHg, 22.4 mmHg and 12.5 mmHg respectively from the baseline to the first hour of anaesthesia, and 30.9 mmHg, 22.4 mmHg and 26.5 mmHg respectively from the baseline to the second hour of anaesthesia. The decrease for the BP was only statistically significant for the first hour of anaesthesia despite identical values for both first and second hour of anaesthesia, except for SAP where the decrease was statistically significant for both the first and second hour of anaesthesia. The average HR decreased non-significantly 5.2 bpm from baseline to the first hour of anaesthesia and 15.4 bpm from baseline to the second hour of anaesthesia. One minipig had high baseline values of BP before propofol anaesthesia compared to the rest of the minipigs. The statistical analyses were repeated without that minipig, which resulted in the same overall trends, but no statistically significant differences. Data without the minipig concerned are summarised in Supplementary Table 7.
3.3.3. Zoletil mixture for minipigs

The average SAP, DAP and MAP decreased 5.5 mmHg, 10.1 mmHg and 7.5 mmHg from the baseline to the first hour of anaesthesia respectively, and 11.4 mmHg, 14.4 mmHg and 12.3 mmHg from the baseline to the second hour of anaesthesia respectively. The average HR decreased 8.6 bpm from the baseline to the first hour of anaesthesia and 12.5 bpm from the baseline to the second hour of anaesthesia. The decreases in BP and HR from baseline were not statistically significant. One minipig had an unexplainable increase in BP and HR for the last 10 min of the anaesthesia, why the data was tested without the last 10 min, resulting in a statistically significant decrease in SAP and MAP from baseline and first hour of anaesthesia to the second hour of anaesthesia.

The revised data are summarised in Supplementary Table 8.

4. Discussion

This study demonstrated an overall good reproducibility of long-term telemetric BP and HR measurement in Göttingen Minipigs using the DSI PhysioTel PA-C10 transmitter, which make it suitable for long-term telemetric BP and HR measurements in Göttingen Minipigs. The average CV% was below 10, based on four test rounds of 24-h measurements distributed over a period of 10 weeks. However, test round 1 had lower BP values than test round 2–4, the reason for this is unknown, but could potentially indicate that a longer post-operative recovery period is needed. During an angiotensin II stimulation test, the DSI PhysioTel PA-C10 Transmitters and telemetry equipment registered the expected BP increase. The average BP increase ranged from 21.5 to 41.4% compared to the baseline, but with a somewhat higher average CV% of 18.5%. To some extent, this likely reflects that the increase is a calculated read-out based on two measures (average BP before and during the test) – each contributing with variation. Furthermore, it was found that the BP values obtained non-invasively by cuff differed numerically from the BP values measured by a catheter or transmitter implanted in the artery independent of the BP level. Lastly, all the tested anaesthesia protocols numerically affected BP and HR but with large interindividual variations with only propofol anaesthesia giving rise to a statistically significant reduction in BP.

The BP and HR levels found in the present study were numerically higher than the values found in a previous study in freely moving telemetered Göttingen Minipigs (Stubhan et al., 2008), but in the same range as previously reported BP and HR measured by IAC or telemetry in freely moving Göttingen Minipigs or other minipig breeds (Beglinger, Becker, Eggenberger, & Lombard, 1975; Glodek & Oldigs, 1981; Kano et al., 2005; Kuwahara et al., 1999; Yuan et al., 2014). BP and HR obtained from sling restrained animals are reported higher (Beglinger et al., 1975; Glodek & Oldigs, 1981), which could be due to stress associated with the handling of the animals. In the present study, activity (primary related to the feeding period) was associated with the first part of the telemetric BP and HR recording. All pigs followed the same recording protocol, thereby it should not have influenced the findings of the present study. Due to different measuring procedures and equipment, housing conditions and breeds of minipigs, a direct comparison should be made with caution.

In this study, the HR was statistically significantly higher during

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Fig. 4. A. Systolic blood pressure (BP), B. Diastolic BP, C. Mean (BP) and D. Heart rate measured simultaneously by telemetry, intra-arterial catheter (IAC) and cuff at study end in six Göttingen Minipigs. After 6 min an angiotensin II stimulation test was performed, with IV infusion of 2.5 μg/kg/min for 10 min. * = statistically significantly different from telemetry (p < 0.05). # = statistically significantly different from intra-arterial catheter. Data is presented as mean ± SEM, n = 6.
feeding and day, when compared to night and morning, indicating that there might be a diurnal and/or postprandial effect. For a diurnal effect, one would expect the HR to be higher in the morning than the night, but this was not the case. The increase in the HR was postprandial and lasted at least 6 h, which indicates a postprandial effect on the HR rather than a diurnal effect. Several studies in Göttingen Minipigs and other miniature swine breeds have evaluated the diurnal and postprandial effects on the BP and HR and report the same effect of feeding on the HR (Openshaw, Purbrick, Peake, & Meecham, 2008; Stubhan et al., 2008; Yuan et al., 2014). Two studies reported a difference in HR between the light and dark phase, but no feeding time was given, which makes it difficult to evaluate if it was a true diurnal difference (Kuwahara et al., 1999, 2004). In addition, the postprandial HR increase may be affected differently depending on whether the food is given in a bowl or is scattered on the floor encouraging rooting behaviour. In any case, caution should be paid to the time of feeding in relation to experiments with cardiovascular readouts to prevent bias due to postprandial increases in the HR.

The increase in BP after the angiotensin II infusion was expected, due to the vasoconstriction and increased sympathetic output induced by angiotensin II (Lavoie & Sigmund, 2003), and a similar increase in BP after Angiotensin II infusion has been reported in several species (Broome et al., 2001, 2004; Cholewa, Meister, & Mattson, 2005; Marano, Formigari, & Vergari, 1997). In the present study, the HR decreased during angiotensin II infusion, likely reflecting a reflex bradycardia. Studies in anesthetised pigs and rabbits have, however, shown unchanged or dose dependently increased HR after angiotensin II infusion (Broome et al., 2001, 2004; Marano et al., 1997). The difference in measurement methods, time of measurement, species or breed, and use of anaesthesia may have contributed to the difference in results regarding HR. In the present study, the CV% for the angiotensin II stimulation test was high, especially for the HR. Several issues might have contributed to the high CV%; e.g. movement of the pig. Unfortunately, activity was not registered in this study, but many of the telemetry transmitters available can measure activity (DSI, 2019).

When validating non-invasive BP measurement methods in dogs and cats, the difference of paired BP measurements to gold standard invasive methods must not exceed 10 mmHg with a SD of 15 mmHg, according to the guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats by the American College of Veterinary Internal Medicine (ACVIM) (Brown et al., 2007). These criteria were only met for the DAP measured by cuff compared to telemetry and the MAP for cuff compared to IAC. The overestimation of the SAP and underestimation of the DAP by cuff found in this study is supported by another study in minipigs (Lee & Park, 2018). In domestic swine, mixed results are found. Some studies observed the same pattern as this study (Gladczak et al., 2013), while others found that the cuff underestimated all BP parameters compared to IAC (Reed et al., 2018; Tuohy et al., 2017). A better agreement between telemetry and IAC was expected, like at implantation, since they are both invasive. An
explanation for the lack of agreement between telemetry and IAC at end-study could be reduced transmitter sensitivity due to clotting and fibrin accumulation during the 12 weeks between measurements. However, if the sensitivity had been reduced, a decrease in the average blood pressure and a larger variability over the four test rounds would be expected, but this was not the case, why that explanation is disregarded.

All three anaesthesia protocols numerically influenced BP and HR, although with large inter-individual variations, and only propofol statistically significantly decreased the BP. The depth of anaesthesia was not assessed in detail in the current study but could have affected the cardiovascular response to the different anaesthetic protocols. The cardiovascular effect of midazolam administered alone in pigs has been investigated previously with mixed results. Two studies in different pig breeds, showed decreased BP and HR during sedation with midazolam (Bustamante & Valverde, 1997; Goodrich, Lackland, Del Signore, & Swindle, 2001), whereas two other pig studies showed either decreased HR together with increased BP (Smith, Zellner, Spinaie, & Swindle, 1991) or increased HR (Linkenhoker et al., 2010). The inclusion of ketamine in the present study may have minimised the BP lowering, because of its influence on the sympathetic nervous system (Liebe et al., 2017; Peltoniemi, Hagelberg, Olkkola, & Saari, 2016; Tweed, Minuck, & Mymin, 1972). The decrease in BP and HR during the propofol anaesthesia in the present study, is not consistent with earlier reported effects of propofol anaesthesia in pigs, where no changes in BP (Martín-Cancho et al., 2004) or slight increase of SAP (Ido et al., 2019) are reported. In humans and dogs, however, propofol is associated with vasodilation causing hypotension. While for humans there is no change in HR (Ebert, 2005; Sahinovic, Struys, & Absalom, 2018; Shafer, 1993; Trapani, Altomare, Sanna, Biggio, & Liso, 2000), there is a disagreement regarding the effect on the HR for dogs, with reports of HR being unchanged (Musk, Pang, Beths, & Flaherty, 2005), decreased (Brussel et al., 1989; Mannarino, Luna, Monteiro, Beier, & Castro, 2012; Whitwam, Galletly, Ma, & Chakrabarti, 2000) or increased (Cattai, Rabozzi, Ferasin, Isola, & Franci, 2018; Wouters, Van de Velde, Marcus, Deruyter, & Van Aken, 1995). The results of the present study regarding the BP are thus in agreement with data from humans and dogs.

The zoletil-mix for minipigs is a balanced anaesthesia, where the effect on the cardiovascular system is expected to be minimal, which was also the case in the present study. Various protocols with different combinations of the drugs in zoletil-mix have been tested in pigs, but to the authors knowledge, no study has been made with the same protocol as used in this study. Three studies in different pig breeds, using xylazine, tiletamine and zolazepam showed initial increased BP and HR (Lu et al., 2010) or decreased HR (Jee et al., 2010; Selm et al., 2003). In pigs, administration of tiletamine, zolazepam and butorphanol showed decreased HR (Selm et al., 2003), while xylazine, ketamine butorphanol showed initial increased BP followed by decreased BP and unchanged HR (Nishimura et al., 1992). In general, it is difficult to compare the cardiovascular effects directly between studies, since they may depend on breed, dose and combinations of anaesthetics, anaesthesia depth and the methods used for evaluation of the BP and HR.

The low number of animals included in this study is a limitation. A small group size reduces the statistical power of the study, thereby increasing the risk of type II errors (Button et al., 2013). This may be especially true for the anaesthesia study, where the numerical changes in the BP and HR values were biologically relevant for all the protocols.

### Fig. 6

A. Systolic arterial blood pressure (BP), B. Diastolic arterial BP, C. Mean arterial BP and D. Heart rate during midazolam/ketamine, propofol and zoletil-mix anaesthesia in Gottingen Minipigs (n = 6). The solid line indicates induction of anaesthesia. Midazolam/ketamine and propofol were administered IV. Zoletil mix was administered IM every 30 min (dotted lines). Data is presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>MK</th>
<th>Propofol</th>
<th>Zoletil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hour)</td>
<td>0.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>175</td>
<td>150</td>
<td>125</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>120</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>120</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>150</td>
<td>120</td>
<td>100</td>
</tr>
</tbody>
</table>
but not statistically significantly different from baseline in most cases. It cannot be excluded that some of the differences between the reference method (IAC) and the other BP measurements are due to variation in IAC measurements although all methods were standardised as well as possible. Rectal temperature and data related to the recovery period were not recorded in the present study. It could have contributed with further information regarding effects of the anaesthetic protocols. Finally, the study only included female pigs. It cannot be excluded that sex of the pigs may have influenced the findings especially with respect to the 24 h measurements (Poletto et al., 2011).

5. Conclusion

In conclusion, the DSI PhysioTel PA-C10 transmitter is suitable for long-term telemetry of BP and HR measurement in Göttingen Minipigs with a CV% of less than 10% over ten weeks and an ability to register cardiovascular changes, especially if a control group is included. Furthermore, there was a long-lasting postprandial increase in HR, which needs to be considered when doing cardiovascular experiments. The cuff overestimated the systolic BP and underestimated the diastolic BP compared to the invasive methods, but despite this disagreement between non-invasive and invasive BP measurement, the non-invasive measurements by cuff can be used to detect trends in the BP. Lastly, all anaesthesia protocols numerically influenced BP and HR, but due to large inter-individual variations only propofol statistically significantly decreased the BP.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vascn.2022.107168.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References


