Effects of moderate and severe hypocapnia on intracerebral perfusion and brain tissue oxygenation in piglets

Effects of moderate and severe hypocapnia on intracerebral perfusion and brain tissue oxygenation in piglets

Running head (short title): hypocapnia and cerebral tissue oxygenation

Article category: Research Report

Simone K. Ringer1*, Nicola G. Clausen2,3,4, Nelly Spielmann2,3, Markus Weiss2,3

1Section Anaesthesiology Vetsuisse Faculty University of Zurich, Switzerland
2Department of Anaesthesiology, University Children’s Hospital Zurich, Switzerland
3Children’s Research Centre, University Children’s Hospital of Zurich, University of Zurich, Switzerland
4Department of Anaesthesiology and Intensive Care, Odense University Hospital, Denmark

*Corresponding author: Dr. S. K. Ringer, Department of Clinical Diagnostics and Services, Section Anaesthesiology, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 258c, 8057 Zürich (Switzerland). E-mail: sringer@vetclinics.uzh.ch

What is already known:

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/PAN.13736

This article is protected by copyright. All rights reserved
- hypocapnia is common during pediatric anesthesia and might lead to cerebral damage
- hypocapnia during pediatric anesthesia is frequently accompanied by hypotension

**What this article adds:**
- this article investigates the effects of hypocapnia, and hypocapnia combined with hypotension on cerebral perfusion and brain tissue oxygenation in young piglets
- use of multiparameter brain monitoring including invasive tissue partial pressure measurements of oxygen and cerebral blood flow, as well as non-invasive near infrared spectroscopy (NIRS) monitoring

**Abstract**

**Background:** Hypocapnia is a common alteration during anesthesia in neonates.

**Aim:** To investigate the effects of hypocapnia and hypocapnia combined with hypotension (HCT) on cerebral perfusion and tissue oxygenation in anesthetized piglets.

**Method:** Thirty anesthetized piglets were randomly allocated to groups: moderate hypocapnia (mHC), severe hypocapnia (sHC) and HCT. Cerebral monitoring comprised a tissue oxygen partial pressure and a laser Doppler probe inserted into the brain tissue as well as a near-infrared spectroscopy (NIRS) sensor placed on the skin, measuring regional oxygen saturation. Hypocapnia was induced by hyperventilation (target $\text{PaCO}_2$: mHC: 3.7 – 4; sHC: 3.1 – 3.3 kPa) and hypotension by blood withdrawal and nitroprusside infusion (mean blood pressure: 35 – 38 mmHg). Data were analyzed at baseline, during (Tr20, Tr40, Tr60) and after (Post20, Post40, Post60) treatment.

**Results:** Compared to baseline, tissue oxygen partial pressure decreased significantly and equally during all treatments [mean(SD) at baseline: mHC 35.7(32.45); sHC: 28.1(20.24); HCT 25.4(10.3) and at Tr60: mHC: 29.9(27.36); sHC: 22.2(18.37); HCT: 18.4(9.5) mmHg]. Decreased laser Doppler flow was detected with all treatments at Tr20 [mHC: 0.9(0.18); sHC: 0.88(0.15); HCT: 0.97(0.13) proportion from baseline]. Independently of group, regional oxygen saturation varied only after reverting and not during treatment. Blood lactate, pH, $\text{HCO}_3^-$ and $\text{PaO}_2$ increased during treatment with no differences between groups.

**Conclusion:** This animal model revealed reduced cerebral blood flow and brain tissue oxygenation during hypocapnia without detectable changes in regional oxygen saturation as measured by NIRS. Changes occurred as early as during moderate hypocapnia.

**Keywords:** Anesthesia, Inhalation; Cerebral Circulation; Hypocapnia; Monitoring, Intraoperative Neurophysiological; Pigs; Vascular Hypotension

**Introduction**

Hypocapnia is a common event that has been reported to occur in 69% of neonates undergoing general anesthesia.\(^1\) Arterial carbon dioxide partial pressure ($\text{PaCO}_2$) influences cerebral autoregulation with hypocapnia, resulting in vasoconstriction and decreased cerebral blood flow (CBF).\(^2,3\) Consequently, hypocapnia during anesthesia may considerably lower cerebral perfusion, potentially resulting in serious consequences for the developing brain.\(^4-7\) In addition, hypocapnia has negative effects on cardiovascular function leading to hypotension.\(^8\)

The effects of hypocapnia on cerebral oxygenation and perfusion have been investigated by using
invasive tissue partial pressure measurements of oxygen (PtO$_2$) in pigs, cats, dogs and humans$^{9-16}$ and by using invasive CBF monitoring in pigs.$^{9,13}$ Today, near infrared spectroscopy (NIRS) is an increasingly used non-invasive technique used to monitor cerebral oxygenation during pediatric anesthesia.$^{17}$ Furthermore, NIRS has recently been recommended in identifying and preventing PCO$_2$-induced cerebral hypo- and hyperperfusion.$^{18}$ However, so far, there is only limited evidence as to whether NIRS can adequately reflect disturbances in PtO$_2$ and CBF caused by hypocapnia in anesthetized infants. Since in pediatric anesthesia hypocapnia is often associated with hypotension, their combined effect also needs to be investigated.$^1$

The aim of the present study was to investigate the effects of isolated moderate and severe hypocapnia as well as hypocapnia combined with hypotension on cerebral tissue oxygenation and perfusion using PtO$_2$, laser Doppler flow and NIRS measurements. Our hypothesis was that cerebral perfusion and oxygenation would be reduced proportionally to the degree of hypocapnia, and that the effects would be aggravated by adding hypotension. A further hypothesis was that NIRS would prove to be valuable in detecting hypocapnia-associated decreases in cerebral oxygenation.

**Methods**

*Study design*

The study was approved by the local Ethics Committee for Animal Experiments (license number ZH175/16) and relevant aspects of the ARRIVE guidelines were followed. Thirty 4- to 6-week-old female piglets were randomly allocated (by picking slips of paper from an envelope) into 3 treatment groups (10 animals each): moderate hypocapnia (mHC), severe hypocapnia (sHC) and moderate hypocapnia combined with hypotension (HCT). Cerebral PtO$_2$ was set as the primary outcome, while laser Doppler flow, NIRS, end-tidal sevoflurane, heart rate (HR) and blood gases were considered secondary outcome measures. Sample size calculation revealed that a minimum of 5 animals per group were necessary to show a difference in PtO$_2$ of 10 mmHg with a power of 80% and a level of significance of 5%.

*Anesthesia*

Piglets were collected from the breeding farm on the day of the experiment. As soon as they arrived at the research facility, anesthesia was induced using sevoflurane in oxygen applied by facemask. Once sufficiently anesthetized, a peripheral intravenous (IV) cannula was inserted into the middle ear vein and 0.2 mg kg$^{-1}$ midazolam (Dormicum®; Roche Pharma, Reinach, Switzerland) injected. The piglets’ tracheas were orally intubated with a cuffed tracheal tube and mechanical ventilation (volume-controlled ventilation with a tidal volume (VT) of 10 ml kg$^{-1}$ and a positive end-expiratory pressure (PEEP) of 5 cmH$_2$O) started to maintain physiologic arterial partial pressures of carbon dioxide (PaCO$_2$ = 4.7 – 6 kPa). Anesthesia was maintained using sevoflurane in oxygen and air combined...
with intravenous midazolam (1 mg kg\(^{-1}\)h\(^{-1}\)). The inspired oxygen fraction (FiO\(_2\): 0.21 – 0.35) was adjusted to obtain hemoglobin oxygen saturation (SpO\(_2\) ≥ 97%). Anesthetic depth was adjusted according to the clinical judgement of an experienced veterinarian anesthetist (SKR). Initial monitoring consisted in pulsoxymetry, ECG, non-invasive blood pressure, composition of respiratory gases, spirometry and rectal temperature (S/5TM anesthesia monitor, Datex-Ohmeda, Helsinki, Finland). The femoral artery was catheterized using a cut-down technique (BD Insyte-A™ 22G; Becton Dickinson Infusion Therapy, Systems Inc. Sandy, Uhta, USA) for continuous arterial blood pressure monitoring and for blood withdrawal.

Intravenous Ringer's acetate infusion containing 1% glucose (Ringeracetat Glucose 1%; Bichsel, Interlaken, Switzerland) was started (5 ml kg\(^{-1}\)h\(^{-1}\)) immediately after induction and was replaced by a glucose-free solution (Ringer-Acetat Fresenius i.v.; Fresenius Kabi AG, Oberdorf, Switzerland) once blood glucose was > 6 mmol L\(^{-1}\). Hypoglycemia (blood glucose < 3 mmol L\(^{-1}\)) was corrected using boluses (0.5 ml kg\(^{-1}\)) of 50% glucose solution (Glucose-Lösung 50%; AlleMan Pharma GmbH, Pfullingen, Germany). Noradrenaline (Noradrenaline Sintetica, Sintetica S.A., Mendrisio, Switzerland) continuous infusion (CRI) was added (starting rate at 0.03 μg kg\(^{-1}\)min\(^{-1}\) and adapted to effect) in the case of unintended hypotension (mean arterial pressure (MAP) < 50 mmHg).

Cerebral monitoring

Piglets were instrumented with two craniotomy bolts (drill-bit diameter 3.8 mm) (LICOX®IMC Model IM1 Single Lumen Introducer Kit, Integra, Sophia Antipolis Cedex, France), using the same technique as described elsewhere.\(^1\) The incision site had been previously injected with 0.1 ml lidocaine (Lidocain HCL 2%, Bichsel, Interlaken, Switzerland) and bupivacaine 0.1 ml (Carbostesin 0.5%; AstraZeneca AG, Zug, Switzerland), while intravenous Fentanyl (10 mcg kg\(^{-1}\)) (Sintenyl; Sintetica SA, Mendrisio, Switzerland) was administered for additional analgesia. Afterwards, a tissue oxygen partial pressure (PtO\(_2\)) (Licox® Brain Tissue Oxygen Monitoring, Integra, Sophia Antipolis Cedex, France) and a laser Doppler flow (LD-flow) probe (moorVMS™-laser Doppler blood flow monitor, Moor Instruments Devon, UK) were inserted through the craniotomy bolts into the frontal white matter. A neonatal near-infrared spectroscopy (NIRS) sensor (OxyAlert® NIRSensor Neonatal, Covidien Inc, Minneapolis, MN, USA) was placed on the skin caudally to the cerebral probes and cranially to the base of the ears to measure regional cerebral oxygen saturation (rSO\(_2\)) (figure 1). Cerebral tissue blood flow (CBF) measured by LD-flow technique, PtO\(_2\), and rSO\(_2\) (INVOS™ 5100C Cerebral/Somatic Oximeter, Covidien Inc, Minneapolis, MN, USA) were monitored continuously.

A study flow diagram is presented in figure 2. Baseline (B) recordings were made at the earliest 2 hours after PtO\(_2\) probe positioning and once all monitored variables were stable within physiological limits. Afterwards moderate hypocapnia (mHC), severe hypocapnia (sHC) or hypocapnia combined with hypotension (HCT) was started. Hypocapnia was induced by increasing the respiratory rate settings on the ventilator to achieve a PaCO\(_2\) of 3.7 – 4 kPa (mHC) and 3.1 – 3.3 kPa (sHC). Treatment HCT comprised moderate hypocapnia combined with moderate hypotension induced by withdrawing blood (10 ml kg\(^{-1}\) body weight over 5 minutes) via the femoral artery catheter and the use of continuous infusion of nitroprusside (Nitrate® 50 mg, SERB S.A.S., Paris, France) titrated to effect
(starting dose 6 \( \mu g \) kg\(^{-1}\) min\(^{-1} \) and adapted as needed). Target MAP value was 35 – 38 mmHg (HCT). Treatment targets were implemented gradually over 20 minutes. Once treatment goals were achieved (Treatment), they were stably maintained for 60 minutes with recordings repeated every 20 minutes (Tr20, Tr40, Tr60). After the Tr60 recordings, the blood volume that had been removed (HCT group) was replaced by substituting balanced hydroxyethyl starch 130/0.4 i.v. (Voluven® 6% balanced, Fresenius Kabi (Schweiz) AG, Oberdorf, Switzerland) and the ventilator settings turned back to baseline. Recordings were continued for another hour (postTr20, postTr40, postTr60). Vital sign data, respiratory and anesthesia gases, CBF, PtO\(_2\), and rSO\(_2\) were recorded every 5 minutes. Extended blood gas analyses including pH, arterial partial pressure of oxygen (PaO\(_2\)) and PaCO\(_2\), bicarbonate (HCO\(_3^-\)), base excess (BE), co-oximetry, hemoglobin, lactate and glucose measurements were performed at B, Treatment, 30 and 60 minutes after starting treatment (Tr30, Tr60), and 30 and 60 minutes after discontinuing treatment (postTr30, postTr60). At the end of the procedure, all piglets were euthanized while still anesthetized.

Statistical analyses

Data (PtO\(_2\), CBF, rSO\(_2\), end-tidal sevoflurane and HR were analysed before (B), during (Tr20, Tr40, Tr60) and after (PostTr20, PostTr40, PostTr60) treatment using IBM® SPSS® Statistics Version 22 (IBM Corp) and Graph Pad Prism 5 for Mac OS X software. Doppler flow measurements were obtained in non-absolute values and therefore data were analyzed as proportion change compared to baseline (normalized flow) values for each piglet.\(^{20}\) Measurement points B, Treatment, Tr30, Tr60, postTr30 and PostTr60 were included for analysis of extended blood gas measurements. Kolmogorov-Smirnov and Shapiro-Wilk were used to test for normality distribution. One-way-ANOVA was used to compare the three treatments regarding age, weight and total anesthesia duration. Repeated measures mixed ANOVA followed by Sidak post-hoc tests was used to investigate changes over time and between groups (\( p < 0.05 \)). All data are presented as mean ± SD.

A \( p < 0.05 \) was considered statistically significant.

Results

All piglets were in good condition on arrival and successfully completed the treatment as randomized. No significant differences in demographics or anesthesia duration were detected between groups (Table 1). One piglet (HCT) had to be completely excluded from analysis due to missing data, which did not allow mixed ANOVA analysis. Additionally, flow data for one piglet (sHC) were removed from the study because it appeared to be an outlier during initial graphical evaluation of data.

Three piglets (HCT), 3 (mHC), and 6 (sHC) needed noradrenaline CRI at baseline. In 5 (HCT), 7 (mHC), and 7 (sHC) piglets, noradrenaline had to be started during hypocapnia (mHC, sHC) or while returning MAP and ventilation to baseline values (reverting treatment) (HCT). Two piglets in treatment groups HCT and mHC respectively, and one piglet from group sHC needed a bolus dose of glucose to correct blood glucose to > 3 mmol L\(^{-1}\).

This article is protected by copyright. All rights reserved
At baseline, no differences between groups were detected for any measured variable. Mean arterial blood pressure and PaCO\textsubscript{2} values are presented in figure 3. No differences in end tidal sevoflurane concentrations were detected over time (p = 0.701) or between groups (p = 0.63).

Results for PtO\textsubscript{2}, LD-flow, rSO\textsubscript{2} and HR are presented in figure 4. A significant decrease in PtO\textsubscript{2} was observed during Tr20 – Tr60 (mHC: 29.9 ± 27.37, sHC: 22.2 ± 18.37, HCT: 18.4 ± 9.52 mmHg at Tr60) compared to B (mHC: 35.7 ± 32.47, sHC: 28.1 ± 20.25, 25.44 ± 10.31 mmHg) in all groups (p < 0.000). The difference in mean PtO\textsubscript{2} measurements from B to Tr60 was 5.8 (mHC), 5.9 (sHC), and 7 (HCT) mmHg. No significant difference was detected between treatments. Significant changes over time, independently of treatment, were detected for normalized LD-flow (p = 0.001). Decreased LD-flow was detected with all treatments at Tr20 [mHC: 0.9(0.18); sHC: 0.88(0.15); HCT: 0.97(0.13)] proportion from B]. Highly individual flow responses were observed with HCT and mHC. However, with sHC all piglets showed a decrease in normalized LD-flow during treatment and an increase afterwards. A significant interaction time x treatment was observed for rSO\textsubscript{2}. However, no differences were detected between the groups at the individual time points. Also, no differences compared to B were detected during Tr20 – Tr60. A significant increase compared to baseline was observed for all groups after returning to normocapnia. A significant interaction time x treatment was observed for HR. Compared to B, HR increased significantly in HCT from postTr30 – postTr60, and was higher compared to sHC but not mHC. No changes in HR over time were detected with mHC and sHC.

Blood lactate, PaO\textsubscript{2}, pH, HCO\textsubscript{3}, BE and Hb are presented in figure 5. Lactate and PaO\textsubscript{2} increased during treatment and decreased afterwards in all groups, (p < 0.000). A significant interaction time x group was detected for pH (p < 0.000) with a significant increase during treatment and no differences between groups at the individual measurement points. Also, HCO\textsubscript{3} was significantly higher at Tr60 compared to B. No differences compared to B were detected for BE. A significant interaction time x group was observed for Hb (p < 0.000). Hemoglobin decreased with HCT compared to B. However, no differences between groups were detected.

**Discussion**

The main findings of this laboratory trial were that hypocapnia with and without hypotension caused a decrease in brain tissue oxygenation and cerebral blood flow, measured invasively, without detectable changes in cerebral NIRS. No differences were detected between moderate and severe hypocapnia or between hypotension plus hypocapnia and the other two. Blood lactate increased during all treatments. Hyperventilation with subsequent hypocapnia is an often-observed accidental disturbance of physiological homeostasis during anesthesia and is sometimes even deliberately induced.\textsuperscript{1} Furthermore, negative arterial to end-tidal carbon dioxide differences (ETCO\textsubscript{2} > PaCO\textsubscript{2}) in children have been reported to erroneously lead to overestimation of ETCO\textsubscript{2} with subsequent risk of unrecognized hypocapnia.\textsuperscript{21}

In agreement with previous studies, the current animal model demonstrated a decrease in PtO\textsubscript{2} during hypocapnia.\textsuperscript{9-15} The observed effect in the present study is even more convincing when the simultaneous increase in P\textsubscript{a}O\textsubscript{2} caused by hyperventilation is taken into account.\textsuperscript{22} Contrary to a

This article is protected by copyright. All rights reserved
previous report showing linear CO$_2$ reactivity over an ETCO$_2$ range of 2.7 to 8 kPa, no differences between moderate and severe hypocapnia were detected in the present study. This might be due to only small differences in PaCO$_2$ goals (0.7 kPa) between moderate- and severe-HC in this study.

A decrease in CBF was detected during all treatments, by using intracranial LD-flow. This is in line with results of previous studies using thermal diffusion probes placed in the frontal matter of pigs and using different monitoring techniques in brain-injured humans. Individual responses of CBF to hypocapnia have been described in previous studies. Similarly, in the present study all animals showed a decrease in CBF during sHC, while responses were inconsistent during mHC.

No changes in rSO$_2$ were detected, despite a decrease in CBF and PtO$_2$, and an increase in blood lactate during treatments. This is surprising, as NIRS monitoring has recently been recommended in identifying and preventing PCO$_2$-induced cerebral hypo- and hyperperfusion. The present study might have been underpowered to detect alterations in rSO$_2$, especially as NIRS measurements have a high inherent physiological variation. Additionally, readings can vary depending on the chosen NIRS device and the optical target size might be different in this animal model compared to in humans.

Furthermore, near-infrared spectroscopy readings can be affected by contamination from extracranial tissues. Therefore, the NIRS sensors in the present study were positioned on the basis of computer tomography images obtained from a previous experiment to be appropriate for brain tissue sampling. (Supporting information). Near infrared spectroscopy is a non-invasive monitor that measures intravascular hemoglobin (75% venous) oxygen saturation rather than oxygenation at tissue level. Reduced cerebral perfusion is expected to increase cerebral oxygen extraction (ERO$_2$), thereby reducing rSO$_2$. On the other hand, alkalosis increases the oxygen affinity of hemoglobin, shifting the oxyhemoglobin curve to the left and therefore decreasing oxygen release to the tissue. This might explain maintenance of rSO$_2$ despite a decreased CBF and PtO$_2$. The lower oxygen release to the tissue probably further contributed to or could even be the primary reason for the observed decrease in PtO$_2$. Whether more severe hypocapnia would lead to increased ERO$_2$ and, consequently, detectable changes in rSO$_2$ needs further detailed investigation. Especially, as studies investigating the effects of different degrees of hypocapnia on rSO$_2$ while maintaining other physiological parameters within normal ranges are scarce.

A strength of the current study compared to previous publications using multiparameter brain monitoring was the investigation of isolated hypocapnia while maintaining systemic normotension. Interestingly, no differences were detected between the combined hypocapnia-hypotension and the individual hypocapnia groups. The effects of hypotension and hypocapnia on cerebral resistance vessels are opposite: the former dilates while the latter constricts. Various studies investigating the addition of hypocapnia in already hypotensive individuals concluded that cerebrovascular reactivity to hypocapnia is significantly attenuated or eliminated during hemorrhage-, drug-, or anesthesia-induced hypotension. Whether the reduction in PtO$_2$ and CBF in the HCT group of the present study was primarily due to hypocapnia, hypotension or their combination is unknown. However, at least part of the effect could be attributed to hypocapnia, as no decrease in CBF was observed in a previous study using a similar animal model and investigating isolated hypotension. In contrast to the present results, a study using magnetic resonance imaging to detect changes in cerebral perfusion and
metabolism in piglets reported that effects were more severe when hypocapnia and hypotension were combined. However, hypocapnia (2.7 – 3.3 kPa) and hypotension (27 – 33 mmHg) goals were lower than those of the present trial. Also, a study investigating the effects of severe hypocapnia (3.3 kPa) combined with mild hypotension (50 mmHg MAP) on PtO$_2$ and cerebrospinal fluid metabolites in adult dogs showed more severe changes in cerebral oxygenation compared to hypocapnia alone. It is thus possible that the statistical power might have been insufficient to detect small differences between treatments in the present study.

Regional oxygen saturation (rSO$_2$) increased significantly once normotension and/or normocapnia were restored. This has already been described in previous studies and can be explained as an “overshoot” of tissue oxygen after tissue hypoxia. The effect has been attributed to altered cellular oxygen utilization following tissue hypoxia. The exact mechanism leading to an increase in HR after normotension and normocapnia have been restored in the HCT-group is unknown. However, the same was observed in a previous study after the restoration of mild hypotension, also induced by nitroprusside.

Although a major limitation of the study is the use of a piglet instead of an infant model, it would be unethical to subject children to hypocapnia and hypotension for research purposes. Piglets are, however, proposed to be an ideal model to investigate anesthesia-associated brain complications. Regarding brain growth, 4- to 6-week-old piglets correspond to 1- to 2-year-old children. No control group was included in the present experiment; this was decided in order to reduce the number of animals (3R principle), as control animals had shown stable PtO$_2$, rSO$_2$ and HR over time in a previous study with a similar setup. Based on these results, changes observed in the present study are unlikely due to anesthesia duration itself, and can therefore be attributed to the induced treatments. Brain tissue partial pressure of oxygen (PtO$_2$) is not an ischemia monitor, and integration of brain chemistry (microdialysis) would have been helpful to further investigate adequacy of cerebral oxygenation in the present study. However, PtO$_2$ has been shown to be a reliable marker of brain tissue perfusion and is considered to reflect the balance between oxygen delivery and demand at tissue level. Furthermore, low PtO$_2$ has been shown to be associated with poor outcome in brain injured patients.

In conclusion, this animal model revealed reduced, invasively measured cerebral blood flow and brain tissue oxygenation and a systemic increase in blood lactate even during moderate hypocapnia. No changes in rSO$_2$, measured by NIRS, were detected, despite decreased brain tissue oxygenation and reduced cerebral blood flow. Consequently, the use of NIRS to detect cerebral hypoperfusion and tissue hypoxia due to hypocapnia needs further investigation.

Disclosures:
The study was approved by the local ethics committee of the Canton of Zurich (ZH175/16).

This article is protected by copyright. All rights reserved
This work was supported by the Anna Mueller Grocholski-Foundation, Zurich with a Research Grant (2016) (no grant number).

Conflict of interest: No conflicts of interest declared.

Acknowledgements:
The authors thank Siemens Healthcare Diagnostics AG (Zurich, Switzerland) for providing the blood gas analysis kits. We further acknowledge Medtronic Schweiz AG (Münchenbuchsee, Switzerland) and Integra Life Sciences (Lausanne, Switzerland) for providing the monitors used during the trials. The authors thank Matthias Haab, scientific illustrator of the Equine Department, Vetsuisse Faculty Zurich, for the original design of Figure 1.

References


Table 1: Demographic data (mean (SD)) of 29 piglets randomly allocated to three groups: moderate hypocapnia (mHC), severe hypocapnia (mHT), and combined hypocapnia-hypotension (HCT).

<table>
<thead>
<tr>
<th></th>
<th>mHC</th>
<th>sHC</th>
<th>HCT</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Age (days)</td>
<td>32.5 (4.84)</td>
<td>34.0 (6.25)</td>
<td>34.1 (5.80)</td>
<td>p = 0.78</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>6.5 (0.72)</td>
<td>6.3 (0.72)</td>
<td>6.9 (0.74)</td>
<td>p = 0.14</td>
</tr>
<tr>
<td>Anesthesia time (min)</td>
<td>334.7 (21.85)</td>
<td>333.1 (29.46)</td>
<td>337.0 (23.24)</td>
<td>p = 0.94</td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swiss Landrace (SL)</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Large White (LW) x SL</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>(LW x SL) x Duroc</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Figure captions:

Figure 1: Schematic diagram demonstrating instrumentation for brain monitoring in piglets. A tissue oxygen partial pressure (PtO\textsubscript{2}) and a laser Doppler flow (LD-flow) probe are advanced into the frontal white matter. Additionally, a neonatal near-infrared spectroscopy (NIRS) sensor is placed on the skin surface immediately behind the intracranial probes. The photon path of the NIRS sensor is shown in a cross-sectional view.

Figure 2: Study Flow Diagram.

Figure 3: Mean and standard deviation values for arterial partial pressure of carbon dioxide (PaCO\textsubscript{2}) and mean arterial pressure (MAP) of 29 piglets anesthetized with sevoflurane-midazolam and divided into three groups: mHC (mild hypocapnia; PaCO\textsubscript{2} = 3.7 – 4 kPa, n = 10), sHC (severe hypocapnia; PaCO\textsubscript{2} = 3.1 – 3.3 kPa, n = 10) and HCT (combined hypocapnia and hypotension; PaCO\textsubscript{2} = 3.7 – 4 kPa and MAP: 35 – 38 mmHg, n = 9). Treatment time is marked with a gray bar. B: baseline, Tr20 – Tr60: treatment, Post20 - Post60: time after restoring normocapnia and normotension.

Figure 4: Mean and standard deviation values for brain tissue oxygen partial pressure (PtO\textsubscript{2}), regional cerebral oxygen saturation (rSO\textsubscript{2}), laser Doppler flow (normalized flow = divided by the baseline value) and heart rate (HR) of 29 piglets anesthetized with sevoflurane-midazolam and divided into three groups: mHC (mild hypocapnia; PaCO\textsubscript{2} = 3.7 – 4 kPa, n = 10), sHC (severe hypocapnia; PaCO\textsubscript{2} = 3.1 – 3.3 kPa, n = 10) and HCT (combined hypocapnia and hypotension; PaCO\textsubscript{2} = 3.7 – 4 kPa and MAP: 35 – 38 mmHg, n = 9). Treatment time is marked with a gray bar. B: baseline, Tr20 – Tr60: treatment, Post20 - Post60: time after restoring normocapnia and normotension.
Flow data for group sHC includes only 9 piglets.

Figure 5: Mean and standard deviation values for arterial blood lactate, pH, arterial partial pressure of oxygen (PaO₂), base excess (BE), hemoglobin (Hb), and bicarbonate (HCO₃⁻), from 29 piglets anesthetized with sevoflurane-midazolam and divided into three groups: mHC (mild hypocapnia; PaCO₂ = 3.7 – 4 kPa, n = 10), sHC (severe hypocapnia; PaCO₂ = 3.1 – 3.3 kPa, n = 10) and 9 HCT (combined hypocapnia and hypotension; PaCO₂ = 3.7 – 4 kPa and MAP: 35 – 38 mmHg, n = 9). Treatment time is marked with a gray bar. B: baseline, Treatment – Tr60: treatment, Post30-Post60: time after restoring normocapnia and normotension.

° significantly different compared to baseline of the same group
× significant difference to B independent of group. P < 0.05.
Research piglets (n=30)

mHC (n = 10)

sHC (n = 10)

HCT (n = 10)

PaCO₂: 3.7 – 4 kPa
MAP: > 50 mmHg

PaCO₂: 3.1 – 3.3 kPa
MAP: > 50 mmHg

PaCO₂: 3.7 – 4 kPa
MAP: 35 – 38 mmHg

1 piglet excluded

Euthanasia

Instrumentation

Two hours of stabilization

Baseline recordings (6)

Randomisation

Implementation of Treatment Targets over 20 minutes

Treatment Targets achieved (Treatment)

Maintenance of Targets for 60 minutes
Tr20
Tr40
Tr60

Restoration of Normo-capnia/tension
Post20
Post40
Post60

Normocapnia restored
Normocapnia restored
Normocapnia and Normotension restored

pan_13736_f2.tiff