Tonometry to estimate intestinal perfusion in newborn piglets

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Abstract
Aim—To determine the correlation between gastric intramucosal pH and superior mesenteric artery (SMA) flow in newborn piglets.

Methods—Fourteen newborn piglets were randomly assigned to either a control or to an epinephrine group which received 0, 1, 2, 4, 0 μg/kg/min of epinephrine for 60 minutes, each dose. Gastric tonometry was performed, SMA flow was measured, and intramucosal pH and the ratio of tonometer pCO₂ over arterial pCO₂ (rCO₂) were calculated.

Results—Intramucosal pH decreased over time in both groups, but tended to be lower in the epinephrine group. With increasing dose of epinephrine, SMA flow decreased; in the epinephrine group. With increasing time in both groups, but tended to be lower (p = 0.04) with a tendency to decrease intramucosal pH (p = 0.06). Conclusions—Gastric tonometry may be useful in human neonates to evaluate gut ischaemia.

Keywords: tonometry; ischemia; inotropes; bowel

Tonometry is a minimally invasive method of calculating intracellular pH in a hollow viscus. Intramucosal pH in the gastric mucosa can be estimated indirectly by measuring partial pressure of carbon dioxide (PCO₂) in the lining of the stomach with a silicone balloon catheter, and the arterial bicarbonate concentration. These two values are then substituted in the Henderson–Hasselbalch equation:

\[ \text{pHim} = 6.1 + \log[HCO_3^-]/(PCO_2 \times 0.031). \]

For gastric tonometry, a modified gastric tube is inserted into the stomach with a silastic gas permeable balloon at the end into which 0.9% saline or phosphate buffered saline is instilled. After the buffer has equilibrated with the PCO₂ in the superficial mucosa it is analysed in a blood gas analyser.

Animal studies have shown that intramucosal pH falls in response to haemorrhagic shock 12 and in mechanically induced gut ischaemia.12,13 In clinical practice, the presence of an abnormally low gastric intramucosal pH has been correlated with increased mortality in both critically ill adults14,15 and in children with septic shock.16 A low gastric intramucosal pH is a relatively frequent finding postoperatively in patients who have undergone heart surgery.17

Mesenteric ischaemia is one of the factors implicated in the pathogenesis of necrotising enterocolitis. This has an incidence of 1.3 to 2.4 per 1000 live births with a reported prevalence of up to 10% in very low birthweight infants.18 Epinephrine is frequently used to treat hypotension in neonates; high doses reduce superior mesenteric artery (SMA) blood flow in newborn piglets.19,20 It is not known whether gastric tonometry correlates with mesenteric perfusion in newborn infants receiving epinephrine.

The objectives of this pilot study were to investigate whether increasing amounts of epinephrine reduce SMA flow, inducing a fall in gastric intramucosal pH and a rise in rCO₂, and whether tonometry is a useful tool for estimating intestinal perfusion changes in the newborn piglet.

Methods
Fourteen 1–3 day old piglets (Camborough/Canabreed) who had been fasted for a minimum of 4 hours were randomly assigned to one of two groups. After 45 minutes of stabilisation the control group was studied for 4 hours while kept anaesthetised. The study group similarly stabilised and received a stepwise increasing infusion of 1, 2, and 4 mcg/kg/min of epinephrine for 60 minutes for each dose, followed by 60 minutes of regular maintenance fluid without epinephrine. Both groups received 20 ml/kg/hour of 0.9% saline/5% dextrose as maintenance solution.

Under general anaesthesia with halothane, a double lumen catheter was placed in the external jugular vein through a midline neck incision, while a single lumen catheter was inserted into the carotid artery. After tracheotomy and placement of an endotracheal tube, assisted ventilation was started. Animals were ventilated (Health Dyna 105 Ventilator) with room air at pressures and rates to maintain normal arterial PCO₂ between 35 and 45 mm Hg and arterial pH between 7.30 and 7.45.

Halothane was then discontinued, boluses of ace promazine (0.2 mg/kg) and fentanyl (10 µg/kg) were administered followed by a continuous fentanyl infusion (0.1 mg/kg/hour). Paralysis was maintained using pancuronium bromide (0.2 mg/kg initially followed by 0.1 mg/kg every 60 minutes). To inhibit gastric acid production, a loading dose of ranitidine (1 mg/kg) was administered, followed by a continuous infusion (0.125 mg/kg/hour).

The aorta was exposed retroperitoneally by a left flank incision and the SMA was identified. A 3 mm extraluminal flow probe (Transonic Systems Inc., Ithaca, NY, USA) was gently placed around the SMA. After inserting the probe the incision was closed and good signals
of flow measurement were ensured. SMA flow, mean arterial blood pressure, heart rate and pulse oximetry oxygen saturation were continuously monitored, and the analog outputs were digitised and stored on hard disc. Fifteen minute segments of the digitised data recordings at the end of each 60 minute period were analysed and averaged.

The animals were then left to stabilise for 45 minutes before baseline recordings were made. Stability was defined as variability of 10% or less for blood pressure and heart rate, oxygen saturations > 90%, pH between 7.30 and 7.45, and PaCO₂ between 35 and 45 mm Hg. A tonometer catheter (TRIP 7F Sigmoid, Tonometrics Inc, Worchester, MA) was inserted into the stomach. The balloon was instilled with 2.5 ml of a phosphate buffered saline solution and left to equilibrate for 60 minutes. Sampling was performed according to the manufacturer’s recommendations and PaCO₂ was measured in a standard blood gas analyser. Samples were removed before each change of epinephrine dose; each new sample had been allowed to equilibrate for 60 minutes. Epinephrine was administered using high precision intravenous pumps at 1 µg/kg/min between 0 and 60 minutes, 2 µg/kg/min between 60 and 120 minutes, 4 µg/kg/min between 120 and 180 minutes and was discontinued between 180 and 240 minutes. Blood and tonometry samples were collected at baseline and at the end of every 60 minutes. Blood samples were analysed for arterial blood gases, electrolytes, glucose and lactate. For lactate analysis, blood was collected on ice, plasma was separated, and stored immediately at −80°C. Lactate was measured using an enzymatic-spectrophotometric method (Sigma Diagnostic, St Louis, MO, USA), as described before (Sigma Diagnostics) and our laboratory achieved a linearity of r = 0.99 at 0 to 13.3 mM/l of lactate in calibration and a test–retest stability of 0.99. ²¹

Animals were killed at the end of the study with sodium pentobarbital (30 mg/kg). No animals died during the 4 hour recording.

Approval of the Health Sciences Animal Welfare Committee of the University of Alberta was obtained for all procedures, according to the guidelines of the Canadian Council of Animal Care.

All values are expressed as mean and standard deviation. Using ANOVA, assuming a 50% difference in SMA flow between groups receiving 4 µg/kg/min of epinephrine, ²² and assuming a standard deviation of 15, a minimum sample size of seven for each group would be needed to reach a power of 80% with an α value of 0.05 (Sigmastat 1.0 for Windows, Jandel Scientific, San Rafael, CA, USA). Differences within each group were analysed using one way repeated measures analysis of variance, between groups using two way analysis of variance, and for multiple comparisons the Student Newman–Keuls test was used. When the normality or equal variance test failed, a Kruskal–Wallis one way ANOVA on ranks was used: p < 0.05 was considered significant.

Figure 1 In the epinephrine group gastric intramucosal pH decreased with decreasing SMA flow (p=0.04). SMA flow is expressed as % change from baseline. For the epinephrine group: 0, 60, 120, 180 and 240 minutes represent 0, 1, 2, 4, and 0 µg/kg/min epinephrine, respectively.

Intramucosal pH and the ratios of PaCO₂ in tonometer over arterial blood (rCO₂) were calculated as follows:

\[ \text{pHim} = 6.1 + \log \left( \frac{[\text{HCO}_3^-]}{\text{P}_{\text{CO}_2} x 0.031} \right) \]

\[ r_{\text{CO}_2} = \frac{\text{P}_{\text{CO}_2}}{\text{PaCO}_2} \]

where [HCO₃⁻] is arterial bicarbonate concentration, P₃CO₂ is tonometer partial pressure of carbon dioxide, PaCO₂ is arterial partial pressure of carbon dioxide and 0.031 solubility of carbon dioxide in plasma.

Repeated measures regression analysis was used to investigate the association between the continuous variable SMA% (percentage change from baseline for superior mesenteric artery flow during epinephrine infusion) with each of the continuous variables rCO₂ and intramucosal pH. Repeated measures regression was implemented by using the generalised linear mixed model (GLMM), as outlined in SAS System for Mixed Models 1996 (SAS Institute Inc., Cary, NC, USA). This approach allows for a regression model to be specified while adjusting for the correlated model error terms induced by repeated measurements. To ensure valid inference on model parameters it is important to choose a covariance structure that adequately reflects the
Table 1 Mean (SD) SMA flow, gastric intramucosal pH, rCO2, serum lactate

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% change in SMA flow</th>
<th>Gastric intramucosal pH</th>
<th>rCO2</th>
<th>Lactate (mM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Epinephrine</td>
<td>Controls</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0.04</td>
<td>7.32 (0.06)</td>
<td>7.28 (0.17)</td>
</tr>
<tr>
<td>60</td>
<td>20.3 (18.7)</td>
<td>10.1 (17.6)</td>
<td>7.22 (0.07)</td>
<td>7.13 (0.12)</td>
</tr>
<tr>
<td>120</td>
<td>17.9 (19.1)</td>
<td>21.5 (25.2)</td>
<td>7.10 (0.08)</td>
<td>7.10 (0.11)</td>
</tr>
<tr>
<td>180</td>
<td>15.8 (27.9)</td>
<td>47.7 (16.7)</td>
<td>7.05 (0.13)</td>
<td>7.05 (0.13)</td>
</tr>
<tr>
<td>240</td>
<td>14.7 (38.8)</td>
<td>14.8 (24.2)</td>
<td>7.09 (0.16)</td>
<td>7.09 (0.16)</td>
</tr>
</tbody>
</table>

SMA: superior mesenteric artery; rCO2: ratio of PCO2 in tonometer over PCO2 in arterial blood; *p<0.05 vs controls
**p<0.01 vs controls
Numbers with different superscript are significantly different within the same column.
During haemorrhagic shock,8–11 in bowel
Discussion
Criterion, and Residual Log Likelihood values.

In neonates undergoing corrective cardiovascular surgery for left heart
ischaemia,12–14 and the presence of low intramu-
cosal pH was shown, supporting an
association between intestinal blood flow and
intramucosal pH.23

In this pilot study we investigated the value
of gastric tonometry in a newborn animal
model. This model is known to develop
splanchnic vasoconstriction at high epine-
phrine doses22 which might allow us to
determine whether changes in SMA blood flow
flow can be used to predict gastric mucosal pH,
and vice versa. Our preliminary data confirm
that SMA flow decreases with increasing epinephrine dose,21 22 returning to baseline
values once epinephrine has been discontin-
ued. However, inaccuracies in estimating intra-
mucosal pH from gastric pH can arise through
back diffusion of acid and/or production of
CO₂ from neutralising gastric acid by duodenal
bicarbonate. By administering ranitidine, an
H₂-receptor blocker, we should have minimised
this effect.24 25

After an initial drop within 60 minutes, gas-
tric intramucosal pH was not significantly
altered in either the control or epinephrine
group. The fact that intramucosal pH dropped
from baseline in both groups may have been the
consequence of the surgical manipulation
for line insertion and tracheostomy, or, more
likely, it may have been the result of manipulat-
ing the SMA flow itself during flow probe
placement. The fact that there was no signifi-
cant change in SMA flow from baseline in the
control group supports the fact that the
haemodynamics of these animals remained
stable during the 4 hour study. Moreover, arte-
rial pH, base excess, blood pressure, and
oxygen saturations did not alter in either group
for the duration of the entire study. Although
there was a modest dose dependent decrease in
intramucosal pH with epinephrine infusion, we
could not show a difference between the
groups for the respective epinephrine doses. It
has been shown that intramucosal pH only falls
significantly when SMA flow is reduced to less
than 60% of baseline flow,19 which is about the
level of SMA flow reduction the animals receiv-
ing 4 μg/kg/min of epinephrine experi-
enced. The three animals whose SMA flows
dropped by more than 50% had the largest
drop in intramucosal pH—0.3 or more. rCO₂
increased with decreasing SMA blood flow (p
= 0.04), and intramucosal pH tended to
decrease with decreasing SMA flow (p = 0.06).
However, the large scatter and small sample
size would preclude us from accurately predict-
ing intramucosal pH and/or rCO₂ from SMA
flow measurements. Furthermore, we used
very high doses of epinephrine at 2 and 4
μg/kg/min to obtain circulatory and pH effects
in the gastrointestinal system. Therefore, the
usefulness of this technology remains to be
established in preterm infants who probably
experience much smaller changes in blood flow
distribution.

In adults intramucosal pH monitoring has
been used to influence the timing of weaning
from artificial ventilation and the introduction of
total enteral feeding.26 27 A significant fall in
gastric intramucosal pH in association with
maximal ventilatory weaning, presumably due
to diversion of blood away from the intestine to
the respiratory muscle system, has been
reported.26 Similarly, introduction of enteral
feeding has been associated with a drop in
intramucosal pH,27 probably as a result of the
regional intestinal perfusion not being able to
maintain enough blood flow to satisfy the
metabolic demands of the bowel in response to
feeds.28 Whereas the studies in adults investigat-
ed gastric intramucosal pH, our findings on
individual predictability was of limited value. In
preterm infants changes in mesenteric blood flow
have been associated with the development of
cotcotising enterocolitis.29 While the role of
tonometry in the critically ill neonate remains
to be proved, the application may provide
information supplementing the clinical man-
agement of necrotising enterocolitis or bowel
ischaemia, even in the presence of normal
haemodynamic and biochemical data.

Serum lactate concentrations decreased over
time in the control group. Starting values are
within the range of previously reported values
for piglets,26 and the fact that values further
decreased with time supports that our animals
were not hypoxic or hypotensive. The fact that
lactate increased with increasing epinephrine
doses does not necessarily indicate increased
lactate production due to peripheral tissue
hypoxia and anaerobic respiration, but may
merely reflect β-adrenoceptor stimulation at
low doses of epinephrine infusion.29 Furthermore,
high doses of epinephrine infusion may
result in impaired hepatic use of lactate due to
decreased hepatic perfusion, and/or enzymatic
inhibition due to α-stimulation.29 30

In conclusion, our pilot study shows that in
newborn pigs, using epinephrine as a pharma-
cological means of decreasing the SMA
flow, intramucosal pH decreases and rCO₂
increases with decreasing SMA flow. Although
gastric tonometry may be useful to sequentially
monitor intramucosal pH, rCO₂, and splan-
chnic perfusion within the individual patient,
normal reference group values need to be
established in larger groups of infants, and prefer-
ablely of different gestational and postnatal ages.

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