Pre-existing hypoxia is associated with a delayed but more sustained rise in T/QRS ratio during prolonged umbilical cord occlusion in near-term fetal sheep

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Running Title: Pre-existing hypoxia and acute umbilical cord occlusion
Abstract.

There is limited information on whether pre-existing fetal hypoxia alters hemodynamic responses and changes in T/QRS ratio and ST waveform shape during subsequent severe asphyxia. Chronically instrumented near-term sheep fetuses (124±1 days) were identified as either normoxic PaO₂ > 17 mmHg (n=9) or hypoxic PaO₂ ≤ 17 mmHg (n=5), and then received complete occlusion of the umbilical cord for 15-minutes. Umbilical cord occlusion led to sustained bradycardia, severe acidosis and transient hypertension followed by profound hypotension in both groups. Pre-existing hypoxia did not affect changes in mean arterial blood pressure, but was associated with a more rapid initial fall in femoral blood flow and vascular conductance and with transiently higher fetal heart rate at 2 minutes and from 9 to 11 minutes of occlusion compared with previously normoxic fetuses. Occlusion was associated with a significant but transient rise in T/QRS ratio; pre-existing hypoxia was associated with a significant delay in this rise (maxima 3.7 ± 0.4 vs. 6.2 ± 0.5 minutes), but a slower rate of fall. There was a similar elevation in troponin-T levels 6 hours after occlusion in the two groups (median (range) 0.43 (0.08, 1.32) vs. 0.55 (0.16, 2.32) µg/L, N.S.). In conclusion, mild pre-existing hypoxia in normally grown singleton fetal sheep is associated with more rapid centralization of circulation after umbilical cord occlusion and delayed elevation of the ST waveform and slower fall, suggesting that chronic hypoxia alters myocardial dynamics during asphyxia.

*Key words: Asphyxia; fetal ECG monitoring; T/QRS ratio; ST segment; sheep*
Introduction

Antenatal hypoxia is associated with an increased incidence of stillbirth and metabolic acidosis (30, 38) and, in the long-term, with abnormal neurodevelopment (38). It is thus important to identify compromised infants as early as possible in labor. The presence of elevation of the ST segment of the fetal electrocardiogram (ECG), typically measured relative to the QRS complex (the T/QRS ratio), is a marker for the development of fetal metabolic acidosis in clinical and experimental studies (1, 2, 19, 35, 43). The relationship between ST segment changes and fetal compromise is complex (40), however, there is some evidence that pre-existing hypoxia can enhance the rise in T/QRS ratio and affect ST waveform shape during subsequent insults (39, 45).

Further, chronic fetal compromise can either improve or impair some fetal responses to subsequent acute insults. For example, chronically hypoxic fetal sheep exposed to acute hypoxia exhibited more pronounced centralization of circulation (6), with enhanced femoral vasoconstriction associated with greater increases in plasma noradrenaline and vasopressin (12). In contrast, when hypoxic twin or triplet fetuses were exposed to repeated umbilical cord occlusion, they developed severe hypotension and metabolic acidosis much more rapidly (44). It is highly likely that these differences reflect the observation that fetal compromise is not a single entity; it may be relatively pure fetal hypoxia, or varying degrees of substrate limitation or acidosis (12). The effect of pre-existing hypoxia on responses to prolonged intervals of severe hypoxia is unknown.

We have previously reported that prolonged asphyxia induced by umbilical cord occlusion in near-term fetal sheep is associated with a transient rise in the T/QRS ratio in parallel with initial hypertension, followed by a fall in T/QRS and profound hypotension (44). In the present study we examined the effect of pre-existing, spontaneous fetal hypoxemia on...
hemodynamic and T/QRS responses to this insult in singleton fetal sheep (29). Troponin T
levels were measured to evaluate whether pre-existing hypoxia increased fetal myocardial
injury after occlusion.
Material and Methods

Surgery

Romney/Suffolk fetal sheep were instrumented between 119 and 126 days gestation (term = 147 days) under general anesthesia (2% halothane in O2) using sterile techniques as previously described in detail (13, 21, 40). All procedures were approved by the Animal Ethics Committee of the University of Auckland. Catheters were placed in the right femoral artery and vein, left and right brachial artery and the amniotic sac. Ultrasound blood flow probes (size 3S, Transonic Systems Inc., Ithaca, NY, USA) were placed around the left femoral artery and the right carotid artery for measurement of femoral blood flow (FBF) and carotid artery blood flow (CaBF). Electrocardiogram (ECG) electrodes were placed subcutaneously over the right shoulder and chest at apex level to record the fetal ECG. An inflatable silicone occluder was placed around the umbilical cord of all fetuses (In Vivo Metric, Healdsburg, CA, USA). All leads were exteriorized through the maternal flank and a maternal long saphenous vein was catheterized to provide access for post-operative care and euthanasia. The maternal skin incision was infiltrated with a local analgesic, 10 ml 0.5% bupivacaine plus adrenaline (AstraZeneca Ltd., Auckland, NZ). Post-operatively, fetal catheters were maintained patent by continuous infusion of heparinized saline (20 U/ml at 0.2 ml/h) and the maternal catheter maintained by daily flushing.

Recordings

Fetal mean arterial blood pressure (MAP, Novatrans II, MX860; Medex Inc., Hilliard, OH, USA), corrected by subtraction of amniotic pressure, FBF, CaBF, and ECG were recorded continuously. The blood pressure signal was collected at 64 Hz and low pass filtered at 30 Hz. The fetal ECG was analog filtered between 0.05 and 80 Hz and digitized at 512 Hz. All
data were stored to disk using custom software (Labview for Windows, National Instruments Ltd., Austin, Texas, USA) for off-line analysis.

**Experimental procedures**

Experiments were conducted 4 to 5 days after surgery, and recordings were begun 24 hours before experiments. Fetuses were assigned to either the normoxia group (N, n=9) if their baseline PaO\textsubscript{2} was > 17 mmHg or the pre-existing hypoxia group (H, n=5) if PaO\textsubscript{2} \leq 17 mmHg (10, 25), on the day of study. Fetal asphyxia was induced by rapid inflation of the umbilical occluder with sterile saline of a defined volume known to completely inflate the occluder. Occlusion was confirmed by observation of an immediate sharp rise in MAP and a fall in FHR. On completion of the occlusion period, the occluder was deflated over 10 to 15 seconds to prevent excessively rapid changes in the circulating blood volume. When bradycardia persisted for more than 30 seconds or fetal blood pressure did not increase to over 25 mmHg in the first 60 seconds after release of occlusion then a dose of 0.3 ml of epinephrine (0.1 ml/kg estimated weight) of 1:10000 epinephrine was given by slow i.v. push.

Fetal arterial blood samples (0.3 ml) were taken 60 minutes prior to occlusion, after 2 and 12 minutes of occlusion, then 30 minutes, 1, 2, 3, 4 and 6 hours after release of the occluder for pH, blood gas, base excess (BE), hematocrit (Hct) and hemoglobin (Hb) determination (Ciba-Corning Diagnostics 845 blood gas analyzer and co-oximeter, MA, USA) and for glucose and lactate measurements (YSI model 2300, Yellow Springs, Ohio, USA). Plasma samples (0.5 ml) were frozen at –80°C for later measurement of cardiac troponin T values using the Elecsys 2010 immunoassay system (Roche-Boehringer, Mannheim, Germany). The adult normal values for our laboratory are < 0.03 µg/L, and there is a 10% coefficient of variation above this value (26). On completion of the experiment the ewes and fetuses were
killed by an overdose of sodium pentobarbitone (9 g i.v. to the ewe: Pentobarb 300, Chemstock International, Christchurch, New Zealand).

Data analysis and statistics

Off-line physiological data analysis was performed using Labview-based customized programmes (National Instruments, Austin, Texas, USA). One-minute and 5-second averages of FHR, MAP, FBF and CaBF were calculated for each fetus. Femoral and carotid vascular conductance (FVC and CVC respectively) were calculated by dividing mean blood flow by MAP. Conductance was calculated instead of the reciprocal, vascular resistance, because during umbilical cord occlusion the denominator of resistance, blood flow, approaches zero leading to highly nonlinear, non-parametric changes. In contrast, conductance changes much more linearly, allowing parametric statistics to be used (23). The 5-second average data were used to assess changes in hemodynamic variables in the first 3 minutes between groups; one minute average data were used to compare all other changes. The ECG waveform was averaged with respect to the S wave over 5 second intervals. For each averaged waveform the ratio between the T height, measured from the level of the PQ interval, and the QRS amplitude was calculated (T/QRS ratio) (44). The raw ECG data for each averaged waveform were visually assessed (B.W.), after coding of the files by a staff member not involved in the experimental study, in order to identify ST waveform shape changes and to verify software identification of the T-wave.

The effects of occlusion on time sequence data were evaluated by two-way ANOVA with time treated as a repeated measure (SPSS v10, SPSS Inc., Chicago, IL, USA). The baseline period was taken as the mean of the hour before occlusion. Where an effect of group or an interaction between group and time were observed, inter-group comparisons for selected data were performed by one way ANOVA. Baseline, survival data and troponin T data were
compared by Mann-Whitney U test. Statistical significance was accepted when P<0.05. Data are presented as mean ± SEM or median (range).
Results

We have previously reported FHR, MAP, FBF, T/QRS ratio and blood gas and acid base changes in the normoxic group (44). There were no differences between groups in mean gestational age at surgery or fetal weight at postmortem (Table 1). The pre-existing hypoxia group showed significantly lower baseline PaO₂ and higher Hb, but no significant difference in baseline oxygen content, or pH, PaCO₂, BE, lactate and glucose (Table 2). PaO₂ values were also significantly higher in the normoxia group 48 and 24 hours before occlusion (22.3±1.1, 21.5±0.9 mmHg respectively) than in the pre-existing hypoxia group (14.7±1.8, 14.0±1.4 mmHg, P<0.05).

Changes in fetal blood gases, glucose and lactate

Umbilical cord occlusion was associated with profound metabolic acidemia, hypoxemia and hypercarbia in both groups (Table 2). 7/9 normoxic and 2/5 hypoxic animals received epinephrine i.v. for fetal resuscitation after release of occlusion. Six normoxic fetuses and three hypoxic fetuses survived to 6 hours after occlusion.

There was no significant difference between groups for PaO₂ or for oxygen content during occlusion. Fetal Hb concentrations were significantly higher in the pre-existing hypoxia group at 12 minutes of occlusion. Similarly, hematocrit tended to be higher overall in the pre-existing hypoxic group, and was significantly higher than in the normoxic group at 12 minutes of occlusion (P<0.05, data not shown). Shortly after occlusion, both groups showed a continuing, similar metabolic acidosis, with a mild but persistent hypercarbia (Table 2); the pre-existing hypoxia group had significantly lower values for PaO₂ and higher hemoglobin concentrations and hematocrit.

Fetal heart rate and mean arterial blood pressure
Occlusion elicited a rapid fall in FHR in both groups, with no significant difference between groups in the rate or depth of this initial fall (Figure 1). This was followed by a brief relative increase in FHR in both groups, maximal approximately 90 to 120 seconds after occlusion, that was significantly greater in the pre-existing hypoxia group (N: 115.4±5.5 vs. H: 163±19.8 bpm, P<0.05, ANOVA). From approximately 5 to 7 minutes onwards all fetuses showed a steady fall in FHR to a nadir at the end of occlusion (N.S. between groups, Figure 1). The hypoxic group showed a transiently higher FHR from 9 to 11 minutes of occlusion than the normoxic group (P<0.05, ANOVA).

There was no significant difference in baseline MAP or changes during umbilical cord occlusion between groups. In both groups there was an initial rise in MAP after the start of occlusion to a maxima after 1.9±0.1 vs. 2.1±0.2 minutes (N vs. H, N.S.). MAP then progressively fell reaching baseline values after 6.8±0.2 vs. 6.6±0.5 minutes (N.S.), with no significant difference in the nadir of MAP at the end of occlusion (9.3±1.0 vs. 11.1±1.1 mmHg, N.S.). After release of occlusion, the pre-existing hypoxia group showed a significantly greater transient increase in MAP compared with the normoxic group from 5 to 12 minutes after release of occlusion.

Femoral blood flow and vascular conductance

Occlusion was associated with a rapid fall in FBF and FVC to a nadir over 1 to 2 minutes in both groups (Figure 2). This fall was significantly more rapid in the pre-existing hypoxia group, but of similar ultimate magnitude. From the fourth minute of occlusion onwards, fetal FVC increased over the remainder of occlusion although it remained significantly lower than baseline values, with no significant difference between groups. This was associated with a brief proportionate increase in FBF that was maximal at 7 minutes, followed by a progressive fall in association with hypotension. There was no significant difference in
changes in FVC or FBF after release of occlusion.

*Carotid blood flow and vascular conductance*

CaBF and CVC were not significantly different between groups during occlusion (Figure 2). CaBF was maintained around baseline values in all groups for the first 4 minutes after the start of occlusion and then progressively fell to a nadir at the end of occlusion. CVC fell after the start of occlusion, reaching a nadir at 2 minutes in both groups, followed by a secondary increase to baseline values at approximately 9 minutes, with no further change. CaBF but not CVC was transiently greater in the hypoxia group, from 3 to 6 minutes after release of occlusion (P<0.05).

*T/QRS and ST segment changes*

The initial T wave orientation was variable but predominantly negative before occlusion. Only one hypoxic animal had a positive baseline T-wave configuration. All fetuses showed a rapid change to a positive T-wave and ST elevation at the onset of occlusion (Figure 3). The T/QRS ratio increased markedly, and peak values of T/QRS were reached at 3.7±0.4 minutes in normoxic fetuses. In contrast the pre-existing hypoxia group showed a significant delay of 3 minutes before T/QRS began to rise, and the peak was reached after 6.2±0.5 minutes (Figure 1, P<0.01). With continued occlusion, the T/QRS ratio fell more slowly in the pre-existing hypoxia group, such that T/QRS values were higher from 8 to 10 minutes of occlusion, but the groups were not significantly different thereafter. Although a few fetuses in both groups showed brief periods of negative T-waves the average T/QRS ratio remained significantly higher than baseline levels.

The ST waveform shape changes showed a typical sequence of events during baseline, occlusion and recovery (Figure 3). With the onset of occlusion, six normoxic animals
developed ST elevation (and positive T-waves) (44). Three animals showed a negative ST-segment at the onset of occlusion. All five hypoxic animals showed a positive ST-segment configuration. During continued occlusion no fetuses developed biphasic waveforms; however, two fetuses in both groups showed ST depression towards the end of occlusion. Two animals in the normoxic group developed heart block; these fetuses did not respond to epinephrine after release of occlusion and died soon after release of occlusion. In the phase of reperfusion 7 normoxic and 3 hypoxic animals showed biphasic waveforms for 3.5 ± 0.9 and 2.4 ± 0.5 minutes, respectively (N.S.).

_Troponin T_

Both groups showed a similar increase in troponin T after the end of occlusion. The normoxic and pre-existing hypoxic groups showed an increase from 0.01 (0.01, 0.03) vs. 0.04 (0.02, 0.53) µg/L at baseline, to 0.32 (0.1, 0.82) vs. 0.36 (0.09, 0.82) at 3 hours and 0.43 (0.08, 1.32) vs. 0.55 (0.16, 2.32) µg/L at 6 hours after release of occlusion (N.S. between groups, P<0.001 vs. baseline).
Discussion

The present study has demonstrated that spontaneous, compensated fetal hypoxia in otherwise well grown singleton fetal sheep was associated with a significantly delayed but ultimately more sustained rise in the fetal ST waveform during prolonged complete umbilical cord occlusion. This change was associated with more rapid initial centralization of blood flow and a slower late fall in fetal heart rate during occlusion. In contrast, pre-existing hypoxia did not affect the time course of hypotension or metabolic acidosis during occlusion.

Clinically, similar unpredictable acute, catastrophic events, such as abruptio placentae or umbilical cord prolapse remain a significant cause of perinatal morbidity (42). There are conflicting data on how preceding hypoxia affects fetal adaptation to such acute severe, periods of asphyxia (6, 12, 39). The present study examined singleton fetuses with spontaneous hypoxia, but without evidence of other metabolic abnormalities, in order to avoid confounding the effects of hypoxia with those of limited nutrient supply and growth restriction (12). A limitation of this approach is that we cannot be sure of the precise etiology or timing of the onset of hypoxia, although the partial pressures of oxygen were significantly lower than in the normoxic group for 48 hours before the study. Further, there was evidence of compensation by increased hemoglobin levels, allowing similar fetal arterial oxygen contents to the normoxic group despite lower oxygen tensions, that would be consistent with a significant period of hypoxia (11). Given that the fetuses were not significantly different in weight at post-mortem and also had normal glucose values, these data suggest a relatively late onset limitation of placental oxygen exchange.

Dynamic ECG changes have been suggested to reflect anaerobic myocardial metabolism and depletion of myocardial glycogen reserve, augmented by beta-adrenergic stimulation (19,
Our previous report showed that the elevation in ST waveform height during severe asphyxia occurs rapidly at a time when blood pressure is marked elevated (44), and then falls in parallel with the development of progressive hypotension. These data suggest that ST waveform elevation reflects anaerobic cardiac metabolism, and thus the subsequent fall in ST waveform height reflects depletion of the major substrate for anaerobic metabolism, cardiac glycogen (19). The present data demonstrate for the first time that pre-existing hypoxia was associated with a delayed but more sustained rise in ST waveform height. The period of sustained elevation of the ST waveform in the pre-existing hypoxia group was associated with a transiently higher fetal heart rate than in normoxic fetuses, suggesting that pre-existing hypoxia improved the ability to maintain anaerobic cardiac metabolism.

The mechanism of the delay in the initial rise in ST waveform height in fetuses with pre-existing hypoxia is unclear. Limited data, from studies of chronic exposure to high altitude, suggest that hypoxia can be associated with reduced cardiac beta-1 adrenoreceptor responsiveness in fetal sheep, through post-receptor mechanisms (8), which could attenuate fetal ECG changes (19, 20). However, this would not be consistent either with the brief but markedly greater increase in fetal heart rate in the pre-existing hypoxia group during occlusion, just before T/QRS height began to rise in the present study, or the very similar pattern of changes in arterial blood pressure in the two groups during occlusion. Further, there is evidence as discussed below that sympathetic neural activity is augmented by chronic hypoxia (9, 36). We propose that a combination of changes, including more rapid initial peripheral vasoconstriction, and changes in neurotransmitters and intracellular events induced by hypoxia (34) altered cardiomyocyte responses and thus potentially attenuated initial anaerobic stress on the heart (7).

Conceptually, the cardiovascular responses of the fetus to acute severe hypoxia can be
considered in two phases, the initial, rapid chemoreflex mediated adaptations (3, 14), and the
subsequent longer period of progressive hypoxic-decompensation (4, 27, 28) that cannot be
prevented by vagotomy (3). The chemoreflex responses help maintain perfusion to key
organs such as the heart, adrenals and brain, and are believed to reduce myocardial work (10,
27). The present study demonstrated that there was no significant effect of pre-existing
hypoxia on the initial chemoreflex mediated bradycardia. However, consistent with previous
data (6, 12), the spontaneous hypoxia group showed a significantly faster initial fall in
femoral blood flow, mediated by a correspondingly reduction in femoral conductance. These
data suggest that the alpha-adrenergic receptor-mediated peripheral arc of the chemoreflex
was sensitized by previous hypoxia (14). We may speculate that this helps support fetal
adaptation by further reducing myocardial work (10). This effect was specific, since there
was no significant difference in the changes in carotid artery flow or conductance, as an
index central vascular bed. The mechanism is unlikely to be more severe hypoxia during
occlusion as there was no significant difference between groups in oxygen contents after 2
minutes of occlusion. A more likely mechanism is increased peripheral sympathetic nervous
system tone, which has been reported to occur during chronic hypoxia induced by
experimental placental insufficiency before the development of significant growth restriction
in fetal sheep (9). This increase has been related to sympathetic hyperinnervation of the
arterial system in unhatched chicks (36), and to greater sensitivity of the peripheral
vasculature to noradrenaline in lambs (18).

Although bradycardia was sustained throughout the period of occlusion, a brief increase in
FHR was seen in both groups during the early phase of occlusion, that was greater in the pre-
existing hypoxia group. In both groups this transient increase corresponded closely with the
initial increase in arterial blood pressure. It is unlikely to be a baroreflex because blood
pressure had not fallen below baseline values (37). At this developmental age, sympathetic
activity acts to limit the fall in FHR in the first five minutes of umbilical cord occlusion or hypoxia (22, 31). This transient relative increase in FHR provides further evidence of enhanced sympathetic responses with chronic hypoxia (9, 36).

In the present study the initial peripheral vasoconstriction was only sustained for the first four minutes of occlusion in all fetuses, and was followed by partial return to baseline values, consistent with previous data in a range of vascular beds during both complete and partial occlusion of the umbilical cord (5, 15, 24, 33, 44). A similar response is also seen after partial occlusion in near-term fetal sheep (15), suggesting that it is not simply an effect of profound hypoxia or acidosis. Loss of peripheral vasoconstriction was associated with a further, progressive fall in heart rate, and, after approximately 6 minutes, with overt hypotension. There is no evidence for continuing reflex mechanisms at this time (3); likely contributors to impaired cardiac function include hypoxia, acidosis, depletion of myocardial glycogen and cardiomyocyte injury (17).

Given that peripheral vascular tone never rose above baseline levels, hypotension must have been primarily caused by a fall in combined ventricular output. Since stroke volume is relatively constrained in the fetus (16), cardiac output will have been a function primarily of the fall in heart rate. Thus the higher fetal heart rate in the pre-existing hypoxia group in this phase, from 9 to 11 minutes of occlusion, suggests better maintenance of cardiac output. Further, the pre-existing hypoxia group showed better blood pressure and improved carotid blood flow in the immediate recovery period. Although these data are difficult to interpret in view of the confounding effects of cardiac arrest and adrenaline (27), the rate of adrenaline administration in the pre-existing hypoxia group (40%) tended to be less than in normoxic fetuses (78%). These data indicate the possibility of more robust recovery of cardiac function after asphyxia in fetuses with isolated, well compensated pre-existing hypoxia.
In experimental studies, prior tissue hypoxia or ischemia can improve cardiac recovery under specific circumstances (34). Speculatively, this “so called” preconditioning may provide a mechanism by which the hypoxic group may have been able to transiently better tolerate asphyxia (34). This may be partly mediated through enhanced adenosine levels (32), which are seen during moderate reductions in oxygen tension comparable with those seen in the pre-existing hypoxia group (7). The significant rise in troponin T values after occlusion in the present study was similar in the two groups suggesting that any difference was related to improved functional recovery rather than reduced myocardial injury per se. Although we cannot rule out a type II error, the pre-existing hypoxia group showed a small trend to higher troponin values.

In conclusion, the present data reinforce previous studies suggesting that elevation of the T/QRS ratio in isolation is a marker for anaerobic cardiac metabolism due to fetal hypoxia rather than for the development of hypotension (41). Moderate pre-existing hypoxia, in normally grown singleton fetuses was associated with enhanced centralization of circulation after umbilical cord occlusion, a significant delay before elevation of the ST waveform but a slower subsequent fall and a slower late fall in fetal heart rate after the onset of hypotension, suggesting that pre-existing hypoxia can improve myocardial dynamics during subsequent severe asphyxia.

Acknowledgements

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References


15. Giussani DA, Unno N, Jenkins SL, Wentworth RA, Derks JB, Collins JH, and


22. Hyman AI, Haworth G, Bowe ET, Daniel SS, and James LS. Effects of sympathetic


38. Soothill PW, Ajayi RA, Campbell S, Ross EM, and Nicolaides KH. Fetal oxygenation at cordocentesis, maternal smoking and childhood neuro-development. *Eur J*


Figure Legends

Figure 1. The time sequence of changes in fetal heart rate (FHR, bpm, top panel), mean arterial pressure (MAP, mmHg, second panel) and T wave relatively measured to the QRS segment (T/QRS ratio, third panel) during 15-minutes of complete umbilical cord occlusion of Hypoxic (●) and Normoxic (○) animals. Values are presented as 15-second averages for the first 3 minutes of occlusion and 60-second averages for all other data. The period of umbilical cord occlusion is denoted by the grey area. Data are mean ± SE. * P<0.05.

Figure 2. The time sequence of changes in femoral blood flow (FBF, ml/min, first panel), femoral vascular conductance (FVC, min/ml/mmHg, second panel), carotid blood flow (CaBF, ml/min, third panel) and carotid vascular conductance (CaVC, min/ml/mmHg, bottom panel) in the pre-existing hypoxia (●) and normoxia (○) during 15-minutes of complete umbilical cord occlusion. Values are presented as 15-second averages for the first 3 minutes of occlusion, and 60-second averages for all other data. Umbilical cord occlusion is denoted by the grey area. Data are mean ± SE. * P<0.05.

Figure 3. An example of the changes in FHR (top panel, bpm), and T/QRS ratio (bottom panel) and ECG complex changes (below the panels) during 15-minutes of complete umbilical cord occlusion in a fetus with pre-existing hypoxia. Data are 15-second averages. Note the change from a negative T wave before occlusion to a positive orientation after occlusion, and the transient increase in the height of the ST segment and T wave during continued occlusion.
Table 1. Fetal parameters for normoxia and pre-existing hypoxia groups

<table>
<thead>
<tr>
<th></th>
<th>Weight (grams)</th>
<th>Gestation (days)</th>
<th>Survival (hours)</th>
<th>Sex (female : male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia 9</td>
<td>3349.3 ± 184.2</td>
<td>124.4 ± 0.4</td>
<td>12 (0, 72)</td>
<td>6 : 3</td>
</tr>
<tr>
<td>Hypoxia 5</td>
<td>3244.4 ± 184.1</td>
<td>123.0 ± 0.8</td>
<td>16 (1, 72)</td>
<td>2 : 3</td>
</tr>
</tbody>
</table>

Data are mean ± SEM or median (range).
Table 2. Fetal acid-base balance, blood gases and serum glucose and lactate levels before, during and after 15 minutes of umbilical cord occlusion.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>2 min</th>
<th>12 min</th>
<th>30 min post</th>
<th>1h post</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>N</td>
<td>7.36±0.01</td>
<td>7.22±0.017</td>
<td>6.93±0.01</td>
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<tr>
<td></td>
<td>H</td>
<td>7.35±0.02</td>
<td>7.20±0.02</td>
<td>6.97±0.02</td>
<td>7.21±0.02</td>
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<tr>
<td>PaCO₂</td>
<td>N</td>
<td>44.7±1.9</td>
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<td>PaO₂</td>
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<td>8.8±0.6</td>
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<tr>
<td></td>
<td>H</td>
<td>13.6±1.3†</td>
<td>3.8±0.79</td>
<td>8.4±1.2</td>
<td>19.4±2.5*</td>
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<td>BE</td>
<td>N</td>
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<td>-0.8±0.8</td>
<td>-9.8±0.7</td>
<td>-8.8±1.5</td>
</tr>
<tr>
<td>mmol/L</td>
<td>H</td>
<td>2.2±1.9</td>
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<tr>
<td>Lactate</td>
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<td>mmol/L</td>
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<tr>
<td>mmol/L</td>
<td>H</td>
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<td>0.3±0.1</td>
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<td>10.6±0.4</td>
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<tr>
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<td>13.2±2.4</td>
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<td>14.2±1.0†</td>
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<tr>
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<td>0.5±0.0</td>
<td>0.5±0.0</td>
<td>4.0±0.1</td>
</tr>
<tr>
<td>mmol/L</td>
<td>H</td>
<td>3.3±0.9</td>
<td>0.4±0.0</td>
<td>0.6±0.2</td>
<td>3.6±0.5</td>
</tr>
</tbody>
</table>

N= normoxic and H= pre-existing hypoxia. Min: minutes Data are mean±SEM. Blood samples were taken during occlusion at 2 and 12 minutes. * P < 0.05 and † P < 0.01.
Figure 2
Figure 3