

# Heart rate-mediated blood pressure control in preterm fetal sheep under normal and hypoxic-ischemic conditions

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**BACKGROUND:** The understanding of hypoxemia-induced changes in baroreflex function is limited and may be studied in a fetal sheep experiment before, during, and after standardized hypoxic conditions.

**METHODS:** Preterm fetal lambs were instrumented at 102 d gestation (term: 146 d). At 106 d, intrauterine hypoxia-ischemia was induced by 25 min of umbilical cord occlusion (UCO). Baroreflex-related fluctuations were calculated at 30-min intervals during the first week after UCO by transfer function (cross-spectral) analysis between systolic blood pressure (SBP) and R-R interval fluctuations, estimated in the low-frequency (LF, 0.04–0.15 Hz) band. LF transfer gain (baroreflex sensitivity) and delay (s) reflect the baroreflex function.

**RESULTS:** Baseline did not differ in LF transfer gain and delay between controls and the UCO group. In controls, LF gain showed postnatal increase. By contrast, LF gain gradually decreased in the UCO group, resulting in significantly lower values 4–7 d after UCO. In the UCO group, LF delay increased and differed significantly from controls.

**CONCLUSION:** Our results show that intrauterine hypoxia-ischemia results in reduced baroreflex sensitivity over a period of 7 d, indicating limited efficacy to buffer BP changes by adapting heart rate. Cardiovascular dysregulation may augment already present cerebral damage after systemic hypoxia-ischemia in the reperfusion period.

Preterm infants have a higher incidence of neurological morbidity and mortality as compared with term infants (1). Neurological sequelae in preterm infants may be the consequence of hypoxia-ischemia during fetal and early postnatal life (2). Hemodynamic factors during hypoxia-ischemia and the posthypoxia-ischemia reperfusion phase contribute considerably to these neurological disorders (3). To ensure adequate blood flow to organs throughout the body, including the brain, blood pressure (BP) is controlled by baroreflex and chemoreflex mechanisms. Under normoxemia, the

baroreflex stabilizes perfusion pressure in the face of disturbances of circulatory homeostasis by adapting heart rate, myocardial contraction, and vascular resistance. A poorly developed baroreflex function could contribute to BP instabilities, which may lead to impaired cerebral perfusion or hemorrhage (4). A better understanding of the dynamics underlying the control mechanisms regulating BP may be useful to improve diagnosis of these disorders.

Low-frequency (LF) fluctuations in arterial BP with a wavelength of approximately 10 s (also called Mayer waves) have historically been attributed to baroreflex activity (5). The oscillations are assumed to be caused by a feedback control and mediated through the sympathetic and parasympathetic innervations of the baroreflex (6). Assessment of the baroreflex function (i.e., heart rate-mediated BP control) can be performed through quantification of baroreceptor sensitivity. Baroreceptor sensitivity (ms/mm Hg) may simply be defined as the change in heart rate (or R-R interval) in response to changes in arterial BP and may be estimated from LF cross-spectral analysis of spontaneous fluctuations in R-R intervals and BP (7–10).

Our understanding of the cardiovascular response of the human preterm infant is limited (11,12), and knowledge of fetal baroreflex is mostly based on animal (sheep) models (13,14). There is, however, conflicting evidence on the fetal and postnatal development of baroreceptor reflex function. Although some studies show increasing baroreflex sensitivity with age, other studies show no significant maturational change (15–18). The cardiovascular system responds to hypoxia-ischemia by changing heart rate and redistributing cardiac output to preserve the limited energy resources and maintain adequate supply to the brain. Current knowledge about how the preterm fetus, hampered by an immature BP regulation, responds to hypoxia is limited. Experiments have shown that the immature sheep fetus can survive prolonged periods of hypoxia-ischemia, but resulting hypotension and hypoperfusion may contribute to cerebral injury as seen in human preterm fetuses (13,19,20). These studies mainly focus

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on cerebrovascular and fetal heart rate response following hypoxia–ischemia for 72 h. Heart rate–mediated BP control has not been examined for longer than several days after the initial period of hypoxia–ischemia.

The aim of this study was to (a) quantify baroreflex-mediated heart rate response (baroreflex sensitivity) by transfer function analysis between LF R–R and systolic BP (SBP) fluctuations in an instrumented preterm fetal sheep model and (b) evaluate longitudinal changes of baroreflex-mediated heart rate response during the first week following umbilical cord occlusion (UCO)–induced hypoxia–ischemia.

## RESULTS

### Cardiovascular and Transfer Function Parameters at Baseline

Baseline values for both the UCO and the control group were derived in the 24 h before the UCO (Table 1). Time domain estimates of variability were comparable in both groups. A slight but significant difference in coherence values was noted between both groups. The baseline values for LF transfer gain, delay, and frequency were comparable in both groups.

### Cardiovascular and Transfer Function Parameters at 144–168 h

At the end of the experiment, mean R–R interval, mean SBP, and SBP SD (SD-SBP) did not differ between the intervention

group and controls (Table 1). By contrast, the R–R interval SD (SD-RR) was significantly reduced after UCO at a comparable endpoint. Spectral power analysis showed significant differences in LF and total power for the R–R interval and SBP series between the UCO group and controls. In the UCO group, LF and total power for the R–R interval showed a 15–20 times reduction as compared with controls. For the SBP series, LF and total power were reduced by a factor of 2. Transfer function analysis showed no difference in coherence or LF frequency. A slight increase in LF delay after UCO was observed in comparison with the controls. Following UCO, both time domain estimate for baroreflex sensitivity (SD-RR/SD-SBP) and transfer gain were reduced by approximately 40%.

### Maturation of Cardiovascular and Transfer Function Parameters

Figure 1 shows the changes in short-term variability of R–R interval time series (SD-RR) and SBP (SD-SBP) time series. After UCO, the decrease in SD-RR is reflected by a decrease from 72 to 168 h in SD-RR/SD-SBP, the time domain estimate for baroreflex sensitivity. Figure 2 shows the changes in coherence, LF transfer gain, and delay. From 96 h, LF transfer gain is decreased as compared with that of controls.

In addition, the maturational effects on time and frequency domain spectral measures were evaluated by multilevel

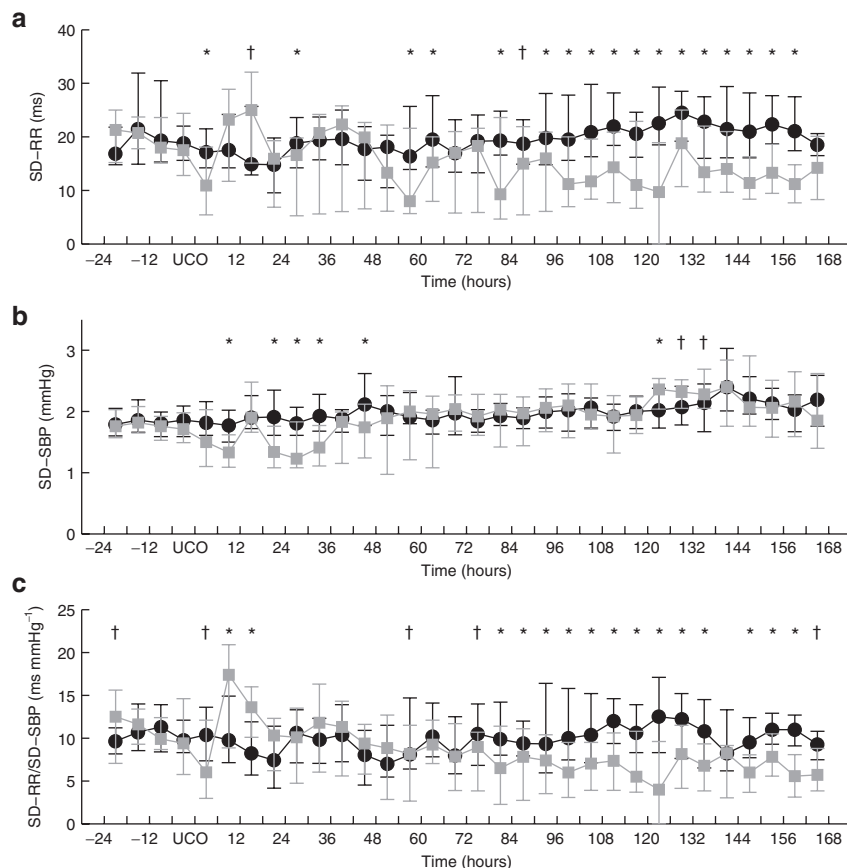
**Table 1.** Baseline values and long-term monitoring of blood gas, cardiovascular, and transfer function parameters

	Control group		UCO	
	Baseline	T = 144–168 h	Baseline	T = 144–168 h
<b>Blood gas analysis</b>				
pH	7.41 ± 0.04	7.38 ± 0.05	7.41 ± 0.03	7.40 ± 0.07
Arterial pCO <sub>2</sub> (mm Hg)	42 ± 6	31 ± 10	41 ± 9	41 ± 10
Arterial pO <sub>2</sub> (mm Hg)	17 ± 2	17 ± 3	15 ± 4	19 ± 6
<b>R–R interval series</b>				
R–R interval (ms)	328 (315–341)	345 (327–363)	332 (322–341)	347 (332–357)
SD-RR (ms)	21 (15–25)	21 (18–27)	21 (17–24)	11 (9–16)*
Total power (ms <sup>2</sup> )	184 (54–451)	271 (149–463)	258 (46–406)	13 (2–71)*
LF power (ms <sup>2</sup> )	138 (44–144)	214 (144–335)	116 (37–359)	13 (1–62)*
<b>SBP series</b>				
SBP (mm Hg)	46 (43–48)	49 (45–50)	47 (39–55)	52 (45–57)
SD-SBP (mm Hg)	1.8 (1.7–2.1)	2.2 (2.0–2.3)	1.8 (1.7–1.9)	1.9 (1.7–2.4)
Total power (10 <sup>-2</sup> mm Hg <sup>2</sup> )	1.7 (1.2–2.7)	3.2 (2.1–4.1)	1.7 (1.0–2.0)	1.3 (0.7–2.2)†
LF power (10 <sup>-2</sup> mm Hg <sup>2</sup> )	1.5 (1.1–2.5)	2.4 (1.9–3.5)	1.6 (0.9–1.8)	1.3 (0.6–2.2)†
SD-RR/SD-SBP (ms/mm Hg)	10.4 (8.6–11.9)	10.5 (8.7–12.0)	12.2 (8.6–13.2)	6.2 (3.4–8.0)*
<b>Transfer function</b>				
Frequency (Hz)	0.09 (0.09–0.10)	0.09 (0.09–0.11)	0.09 (0.09–0.11)	0.09 (0.08–0.09)
Coherence	0.62 (0.61–0.65)	0.65 (0.63–0.68)	0.64 (0.63–0.67)†	0.63 (0.61–0.65)
Gain (ms/mm Hg)	6.2 (5.6–8.4)	7.7 (6.7–10.6)	7.8 (5.6–11.4)	4.8 (3.1–6.7)*
Delay (s)	5.5 (3.3–6.8)	4.3 (3.2–5.9)	4.0 (3.3–5.6)	6.6 (3.7–7.6)†

Blood gas analyses, cardiovascular parameters, and transfer function parameters during the baseline period and on the seventh day (T = 144–168 h) after UCO. Time domain and transfer function measures were determined from 30-min segments during 24 h before and 144–168 h after occlusion, respectively. Values are expressed as means ± SEM or medians (IQR). Assessment of change between baseline and the following days within a group was performed by multilevel analysis (Table 2).

IQR, interquartile range; LF, low frequency; SBP, systolic blood pressure; UCO, umbilical cord occlusion.

\*P < 0.01; †P < 0.05 (comparison between the UCO and control groups at corresponding times).



**Figure 1.** Longitudinal changes in SD-RR, SD-SBP, and SD-RR/SD-SBP. Plots of the longitudinal development of (a) heart rate variability (SD-RR), (b) blood pressure variability (SD-SBP), and (c) time domain measure of baroreflex sensitivity (SD-RR/SD-SBP). The x-axis represents time in hours. The closed circles and closed squares indicate the control and UCO groups, respectively. In the first 24 h after UCO, significant differences are observed between the UCO and control groups, inherent to the experimental occlusion. After 72 h, a consistent decrease in SD-RR and SD-RR/SD-SBP is noted. Data are shown as medians and IQRs for each 6-h period. \* $P < 0.01$ ; † $P < 0.05$ . IQR, interquartile range; SBP, systolic blood pressure; UCO, umbilical cord occlusion.

regression analysis for both the control and the UCO groups using pooled 6-h values during the first week after UCO. This model corrects for the repeated measurements within and between subjects. The results of the multilevel analysis are shown in **Table 2**. The control group showed a significant increase in R-R interval, SD-RR, SBP, and SD-SBP. The UCO group showed a significant increase in SD-SBP, approximately 2.5 times the magnitude of the SD-SBP increase in the control group. The multilevel analysis supported the finding that baroreflex sensitivity increases in controls, but decreases gradually after UCO-induced asphyxia. Both the time domain estimate for baroreflex sensitivity (SD-RR/SD-SBP) and the LF transfer gain decreased by the same amount following UCO, 0.7 ms/mm Hg/day.

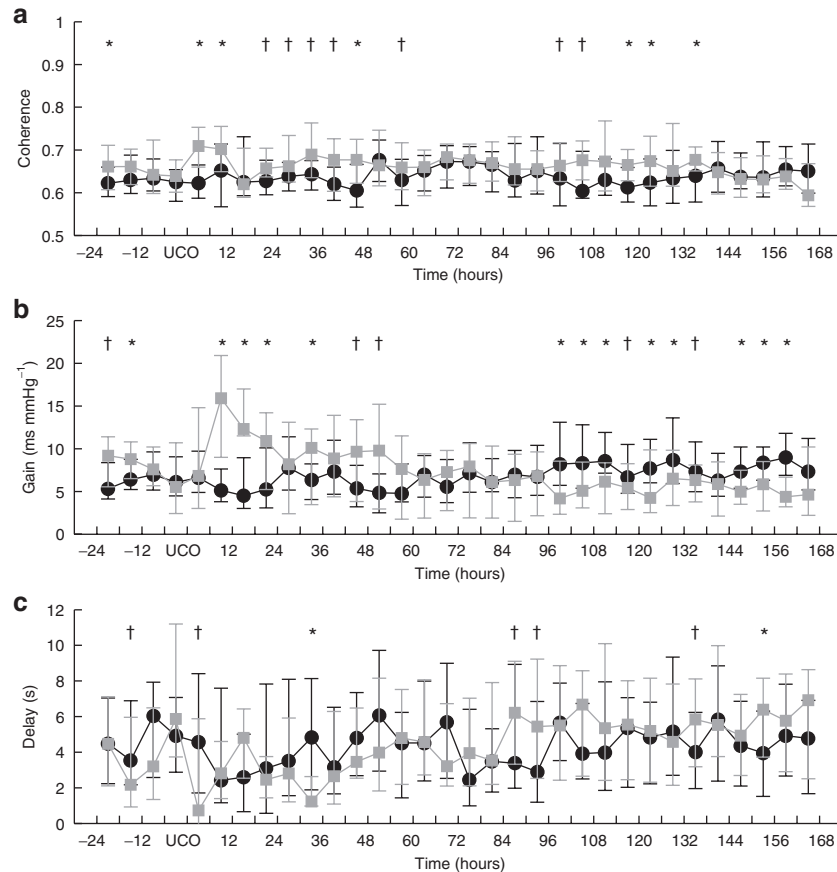
## DISCUSSION

The present study demonstrates that intrauterine-induced hypoxia-ischemia in preterm fetal sheep has long-term effects on baroreflex function. During the first week following prolonged UCO, heart rate variability decreases dramatically and baroreflex sensitivity decreases gradually to show lower values than those of controls at 4–7 d after UCO. These results suggest that intrauterine hypoxia-ischemia limits the baroreflex

activity to buffer changes in SBP by adapting fetal R-R interval. The dysregulation of the baroreflex may augment already present cerebral damage after systemic hypoxia-ischemia in the reperfusion period. As a consequence of this cardiovascular instability, the preterm brain may be prone to BP fluctuations, leading to disruption of the immature vascular architecture and neonatal encephalopathy (21).

## Transfer Function Analysis and Baroreflex Function

In the present study, we focused on the baroreflex-mediated interactions between LF fluctuations of SBP and R-R intervals during the normoxemic recovery state after the immediate cardiovascular effects of acute hypoxia and hypoxia-ischemia. Under normoxemic conditions, baroreflex rather than chemoreflex is involved in the interactions between arterial BP and fetal heart rate (22). We were interested in the long-term effect of induced fetal hypoxia-ischemia on baroreflex function (i.e., baroreflex sensitivity and delay of the reflex arc). The evaluation of baroreflex function is useful for the assessment of fetal autonomic cardiovascular control (23). Evaluation of baroreflex function by spectral methods is based on the concept that each spontaneous oscillation in BP elicits an oscillation at the same frequency in R-R interval by the effect of arterial



**Figure 2.** Longitudinal changes in coherence, LF transfer gain, and delay. Plots of the longitudinal development of (a) coherence, (b) transfer gain, and (c) delay. The x-axis represents time in hours. The closed circles and closed squares indicate the control and UCO groups, respectively. In the first 24 h after UCO, significant differences are observed between the UCO and control groups, inherent to the experimental occlusion. After 96 h, a consistent decrease in LF transfer gain is noted. Data are shown as medians and IQRs for each 6-h period. \* $P < 0.01$ ; † $P < 0.05$ . IQR, interquartile range; LF, low frequency; UCO, umbilical cord occlusion.

baroreflex activity (7). As found in other fetal sheep experiments, we observed an oscillation frequency associated with fetal baroreflex activity centered around 0.1 Hz (24). According to the transfer function method, baroreflex sensitivity was calculated as the value of the transfer function modulus and phase (i.e., gain and delay) between SBP and R-R interval in the LF, considering a coherence  $\geq 0.5$  (25). The transfer function methodology has been used in several studies of human preterm infants (11,26). Because the values of SBP and R-R intervals obtained from the fetal sheep are comparable with the values found in preterm infants, the same spectral analysis methodology was followed. Transfer function analysis in this study showed relatively high coherence values in the LF band, suggesting reliable estimates of transfer gain and delay.

#### Long-Term Effects of Hypoxia–Ischemia

During longitudinal monitoring of the UCO group, the LF transfer gain gradually decreased over time, leading to a significantly lower LF transfer gain post-UCO than that in the control group. Consistent with this finding is the time domain observation of a decreased SD-RR, whereas SD-SBP remains unchanged. By contrast, the LF delay between BP variations and subsequent heart rate variations increased as compared

with that of the control group. The gradual decrease of baroreflex sensitivity and increase in delay between variations in heart rate and SBP suggest that hypoxia–ischemia results in a deregulation of the baroreceptor reflex arc. A decreased LF transfer gain and increased delay may result from changes in the dynamics of the sympathetic and parasympathetic pathways and/or from changes in the balance between these pathways (6,17,18,23). Because the parasympathetic effect on heart rate changes is fast as compared with the sympathetic effect, increased delay may indicate a greater relative contribution of sympathetic regulation. This result is supported by the finding that normalized LF power (LF/total power) increased to nearly 100% following UCO, whereas it remained stable in controls (approximately 75%).

The afferent signals from the baroreceptor output are projected on the nucleus of the solitary tract, which is located in the medulla region, to be processed and transferred to the BP regulation effectors (27). Previous findings in 0.6 gestation fetal sheep showed that suppressed fetal heart rate variability following 30-min UCO was associated with severe neuronal loss in the brain stem 3 d after the insult (20). This neuronal loss may affect the processing of afferent nerve signals and therefore the baroreflex function, thereby decreasing regulation

**Table 2.** Maturation of cardiovascular and transfer function parameters

	Control; <i>b</i> (change/d)	UCO; <i>b</i> (change/d)
R–R interval series		
R–R interval (ms)	2.8 ± 0.6*	NS
SD-RR (ms)	0.8 ± 0.2*	NS
SBP series		
SBP (mm Hg)	0.4 ± 0.1*	NS
SD-SBP (mm Hg)	0.04 ± 0.02 <sup>†</sup>	0.10 ± 0.02*
SD-RR/SD-SBP (ms/mm Hg)	NS	–0.7 ± 0.1*
Transfer function		
Frequency (Hz)	NS	NS
Coherence	NS	NS
LF gain (ms/mm Hg)	0.4 ± 0.1*	–0.7 ± 0.1*
LF delay (s)	NS	0.5 ± 0.1*

Results of multilevel regression analysis using a generalized linear model to correct for repeated measurements. Regression analysis is performed according to:  $f(t) = a + bx$ , where  $a$  is the offset;  $b$ , the slope; and  $t$ , time (in days), with  $t = 0$  corresponding to the time of occlusion for the UCO group. Values are expressed as means ± SD.

LF, low frequency; NS, not significant; SBP, systolic blood pressure; UCO, umbilical cord occlusion.

\* $P < 0.01$ ; <sup>†</sup> $P < 0.05$ .

efficacy. Although we did not study brain stem histology, our findings showed that deregulation of baroreflex function gradually increases over the course of 7 d, suggesting evolving neuronal loss in the brain stem. This finding also highlights the importance of the duration of the study. At 3 d after UCO, the endpoint for most other longitudinal studies (19,20), baroreflex transfer function parameters of our UCO group did not significantly differ from that of the control group, potentially obscuring long-term functional loss induced by hypoxia–ischemia. We speculate that gradual functional loss may be related to the evolution of cytotoxic edema associated with hypoxia–ischemia during the first week, as seen by magnetic resonance imaging studies in human infants (28). Fetal heart rate variability (SD-RR) was already significantly decreased at 3 d post-UCO and gradually decreased further during the remainder of the study. This observation is in agreement with that of Ikeda *et al.*, who showed that decreased long-term fetal heart rate variability at 24–72 h of recovery after UCO is an indicator of the severity of histological damage in term fetal brain (29). To our knowledge, our study is the first to describe this change in fetal heart rate variability in preterm asphyxiated fetal lambs.

#### Maturation Effects of Baroreflex Activity

The results of our study indicate that the baroreflex function matures during fetal life, as shown by the increase in LF transfer gain for the control group between 0.75 and 0.80 gestational age (GA). These results confirm findings from other experiments, which indicated that the baroreflex function was active from approximately 85 d of gestation (0.6 GA) and that the magnitude of fetal heart rate response to BP changes increased toward term (15). Other studies found a decrease in baroreflex

sensitivity during development (30–32). Several studies showed no significant maturation of the baroreflex sensitivity (16,17). There may be several explanations for these conflicting findings. First, the different pathways of the baroreflex feedback system mature at different rates. In preterm sheep of 0.7 GA, there is probably no significant control by the renal sympathetic nerve, which regulates vascular resistance (18,33), whereas in term sheep, vascular resistance control is present (31). In our study, fetal sheep were instrumented at 0.7 GA and maturational changes were studied between 0.75 and 0.80 GA. Other studies have little to no variation in GA, or their results lack the temporal resolution required to compare results with our findings directly (16,17,30,31). Second, the discrepancies may be the result of methodological differences. Quantification of spontaneous baroreflex sensitivity by spectral methods and by injection of drugs or aortic compression does not necessarily produce comparable results (34). Spontaneous variations in BP are normally in the order of several mm Hg, whereas experiments using invasive quantification methods generally study heart rate responses to larger BP changes. Third, heart rate response to BP changes is nonlinear and can be characterized by a sigmoidal input–output relation with maximum responses around the (normal) operating point and decreased regulation capacity at the extremes (8).

#### Pathophysiological Implications

Reduction in fetal heart rate variability is associated with fetal hypoxia and acidemia in human preterm (35) and term fetuses (36). However, despite the highly adverse outcomes of moderate to severe encephalopathy around birth, the predictive value of abnormal fetal heart rate patterns for cerebral palsy is consistently weak (37). This study demonstrates an association between fetal hypoxia–ischemia and reduced baroreflex sensitivity. For obvious reasons, direct measurement of fetal BP in humans is not possible. Future studies may explore the use of noninvasive (Doppler) estimation of fetal BP and spectral-derived variability in assessing hypoxemia-induced changes in brain stem mechanisms for cardiovascular control (38).

In conclusion, this is the first study to quantify baroreflex-mediated heart rate response in a preterm fetal sheep model by transfer function analysis of R–R interval and SBP fluctuations and to show long-term effects on baroreflex function after intrauterine UCO-induced hypoxia–ischemia. Our results show that intrauterine hypoxia–ischemia limits baroreflex efficacy to buffer changes in SBP by adapting fetal R–R interval following UCO. As a consequence, the preterm brain may be prone to BP fluctuations, possibly leading to disruption of the immature vascular architecture and augmenting already present cerebral damage after systemic hypoxia–ischemia in the reperfusion period.

#### METHODS

##### Animals

Texel ewes were date-mated and the fetuses were randomized to the UCO group or the control group. The study group consisted of 14 fetal sheep (9 in the control group and 5 in the UCO group). Fetal instrumentation was performed at 0.7 GA (102 d, term 146 d).



The study was conducted at the Maastricht University Medical Center, Maastricht, the Netherlands, and was approved by the local Animal Ethics Research Committee.

### Experimental Preparations

The pregnant ewes were deprived of food, but not water, 24 h before surgery. They were anesthetized using 1–2% isoflurane, guided by depth of sedation. After a lower midline incision, the fetus was carefully extracted through a small incision in the uterus. The fetus was instrumented with a 3.5 French Argyle umbilical vessel catheter (Tyco Health Care Group, Mansfield, MA) in the femoral artery. The arterial catheter was utilized for continuous monitoring of fetal arterial pressure. A vascular occluder (OC16HD, *in vivo* Metric, Healdsburg, CA) was placed around the umbilical cord. Three custom-made silver electrodes with fixed leads (Cooner, Chatsworth, CA) were inserted in the subdermis and fixed with sutures. These electrodes were used for electrocardiography (ECG) and formed an Einthoven triangle. The fetus was then repositioned in the uterus. A catheter was inserted into the amniotic sac to monitor the amniotic fluid pressure. Before closure, the amniotic cavity was filled with warm sterile saline (NaCl 0.9%). All fetal catheters and electrode leads were exteriorized through the flank of the ewe. The lower midline incision was securely sutured. After surgery, the ewe was allowed to recover and housed in a confined space to allow continuous perfusion of catheters with heparinized saline. Postsurgery ewes received 5 d of intravenous amoxicillin (1000 mg)/clavulanic acid (200 mg). During the complete course of the experiment (11 d), the animals had *ad libitum* access to water and food.

### Experimental Design

Experiments were conducted at 102 (operation) to 113 (killed) d of gestation. After a recovery period of 4 d (GA, 106 d), the umbilical cord of the fetuses in the UCO group was occluded by rapidly inflating the occluder with sterile saline of a defined volume known to completely inflate the occluder. Verification of the occlusion was obtained by observation of a sudden drop in heart rate. The occlusion was discontinued after 25 min or sooner when mean arterial BP dropped below 8 mm Hg or when asystole occurred. At the end of the occlusion, the lambs always suffered from severe bradycardia (heart rate < 90 bpm; normal is approximately 180 bpm) or even complete cardiac arrest. If there was no increase in heart rate within 20 s after the end of occlusion, resuscitation was started using an injection of adrenalin (0.1 mg/kg). Arterial blood gas analysis ( $pO_2$ ,  $pCO_2$ , pH) was performed before, during, and after UCO to monitor the metabolic state during hypoxia–ischemia and recovery. Fetuses were studied for 7 d postocclusion. At the end of the experiment, both ewe and fetus were euthanized by administration of pentobarbital (200 mg/kg).

### Data Acquisition

BP, amniotic pressure, and ECG data were acquired and digitized by an MPAQ unit (Maastricht-Programmable Acquisition system, Maastricht Instruments BV, Maastricht, The Netherlands). Analog filtering was applied to the ECG data with a 1- to 200-Hz bandpass filter. Arterial BP and amniotic pressure data were not filtered. All data were sampled at 1000 Hz. Finally, data were stored on a hard drive for off-line analysis.

### Data Analysis

Data were processed off-line using MATLAB R2009a (The MathWorks, Inc., Natick, MA). Recordings were divided into 30-min segments for analysis. ECG data were filtered using a 50-Hz infinite impulse response notch filter and 20–80 Hz fourth-order Butterworth bandpass filter, empirically found to be optimal for the R-peak detection algorithm used. Fetal BP signals were filtered using a 50-Hz infinite impulse response notch filter and 2- to 16-Hz fourth-order Butterworth bandpass filter to facilitate detection by the waveform analysis algorithm. Fetal BP was corrected for offset by subtraction of the low pass–filtered amniotic fluid pressure.

In each 30-min segment, R-peaks of the QRS-complex and SBP were detected by adaptive filtering of the ECG and BP waveforms, respectively. Segments containing artifacts were excluded (<5% of the data).

The unevenly time-spaced R–R interval and SBP series were convolved with a boxcar function and subsequently resampled at 4 Hz using nearest-neighbor interpolation to obtain equidistant sample points (26).

### Spectral Analysis and Transfer Function Analysis

Spectral analysis offers the opportunity to decompose spontaneously occurring fluctuations in SBP and R–R interval time series into a power spectrum and to relate the character of the fluctuations to physiological processes. Transfer function analysis (cross-spectral analysis) between SBP and R–R interval series in the LF band (0.04–0.15 Hz) is a useful estimate of baroreflex sensitivity (7,25,39). This methodology was adapted for preterm infants and we refer to previous papers for further details (11,26). Because R–R interval and BP values obtained from fetal sheep are comparable with values found in preterm infants, the same spectral analysis methodology was followed. Each 30-min segment of R–R interval and SBP series was divided into nine 192-s segments. In the 192-s segments, total spectral power (0.04–2 Hz) and LF spectral power (0.04–0.15 Hz) were calculated from the autospectra of R–R interval and SBP series. From the cross-spectra of R–R interval and SBP series, the coherence, transfer gain, and transfer phase were calculated for the LF band. Because the reliability of transfer function depends on coherence between the signals, transfer function parameters were only assessed if coherence was > 0.50. In 41% of all 192-s segments coherence was > 0.50, and these segments were included for transfer function analysis. Baroreflex sensitivity was calculated as the LF transfer gain and assessed at the frequency with the highest coherence within the LF band. Baroreflex delay was calculated by dividing the transfer phase at the corresponding frequency bin by  $2\pi$  times this frequency. Time domain cross correlation of the R–R interval and SBP series was used to correct for  $n-2\pi$  phase shifts.

### Time Domain Estimates of Variability

Heart rate variability was assessed for each 192-s segment by calculating the mean R–R interval and SD (SD-RR) (39). Likewise, BP variability was assessed by calculating the mean SBP and SD (SD-SBP). To verify results obtained by spectral analysis, baroreceptor sensitivity was also analyzed in the time domain. To obtain a time domain estimate for baroreceptor sensitivity, SD-RR was divided by SD-SBP (26).

### Data Processing

Median values per 30-min segment were calculated from the underlying 192-s segments. For further analysis, 30-min segments were pooled as 6- or 24-h values (median; interquartile range), depending on the mode of presentation in a graph or table. The median was used because normality was not *a priori* assumed and to reduce the effect of outliers. Baseline values were assessed in the 24 h before UCO and median values for the long-term follow-up were assessed at 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 h after UCO.

### Statistics

Blood gas data were expressed as means  $\pm$  SEM. Group comparisons for blood gas analysis were performed using the two-side unpaired Student *t* test. Time and frequency domain estimates were expressed as median and interquartile range and group comparisons were analyzed using the Mann–Whitney *U* test. Because the 6-h values were normally distributed, the maturational effects on time domain and frequency domain spectral measures were evaluated using a multi-level analysis. In this analysis, linear regression is estimated using the random effects model (SPSS 19.0.0, Inc., Chicago, IL), which extends the generalized linear model to allow for analysis of repeated 6-h values between and within subjects. Regression analysis results are shown with the coefficients *a* and *b* (*a*, intercept; *b*, slope of regression line). A *p* value < 0.05 was considered significant.

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