Fetal acidosis and hypotension during repeated umbilical cord occlusions are associated with enhanced chemoreflex responses in near-term fetal sheep

Laura Bennet,1,2 Jenny A. Westgate,1,2 Yung-Chi (“Jack”) Liu,2 Guido Wassink,1 and Alistair J. Gunn1,2

1Departments of Physiology, and 2Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand

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VARIABLE FETAL HEART RATE (FHR) decelerations are the most common periodic change in FHR seen during labor (9, 28). The potential clinical significance of changes in the shape of these decelerations remains highly controversial. The initial rapid fall in heart rate is known to be a vagal reflex initiated by hypoxic activation of peripheral arterial chemoreceptors (5, 12), such that it can be delayed by chemodenervation (8, 22, 23). Several studies in near-term fetal sheep have suggested that, during repeated variable decelerations, there is a progressive slowing of this initial fall in FHR (1, 14, 15). However, whereas Akagi and colleagues (1) found that reduced slope during complete occlusions corresponded closely with the development of fetal acidosis and hypotension, others have reported a similar attenuation with repeated partial or complete cord occlusions without significant metabolic deterioration, suggesting that this phenomenon reflects reflex attenuation (14, 15).

This finding, that repeated episodes of hypoxia blunt the chemoreflex response, is in many ways counterintuitive. If, as other evidence suggests (2, 4, 5, 8), the chemoreflex is central to fetal adaptation to severe hypoxia, then we might anticipate that its attenuation would compromise adaptation to labor (14). Alternatively, these findings might suggest that other adaptations to hypoxia become more important with time. However, we must consider whether the response to relatively mild hypoxia, as seen during partial occlusion, accurately reflects the response to more severe hypoxia, which includes deeper, sustained bradycardia (2, 21). Consistent with this speculation, previous studies of acute hypoxia in chronically hypoxic sheep have shown greater or enhanced chemoreflex responses, including cardiovascular centralization of combined ventricular output, compared with normoxic fetuses (7, 11). These data suggest the hypothesis that repeated episodes of brief but severe hypoxia would not attenuate chemoreflex responses and, further, that evidence of greater hypoxic stress as shown by developing arterial hypotension and metabolic acidemia would be associated with enhanced chemoreflex changes.

We have previously reported in near-term fetal sheep that 1-min occlusions of the umbilical cord repeated every 5 min lead to variable FHR decelerations without significant changes in interocclusion fetal arterial blood pressure and very mild changes in acid-base status even after many hours (29, 30). When the frequency of occlusion is increased to 1 min every 2.5 min, however, fetuses consistently develop progressive metabolic acidemia and severe hypotension (29, 30). The aim of the present study was to evaluate in this paradigm whether repeated complete umbilical cord occlusions alone or developing fetal compromise, as shown by hypotension and acidosis, lead to attenuation of the initial slope of fetal variable decelerations.

MATERIALS AND METHODS

Surgical preparation and postoperative care. Sixteen Romney/Suffolk sheep were operated on between 119 and 126 days of gestation (term = 147 days) under halothane anesthesia (2%). All procedures were approved by the Animal Ethics Committee of the University of Auckland. Food, but not water, was withdrawn 18 h before surgery. Ewes were given 5 ml of Streptopen [procaine penicillin (250,000 IU/ml) and dihydrostreptomycin (250 mg/ml), Pitman-Moore, Wellington, New Zealand] intramuscularly for prophylaxis 30 min before the start of surgery. Anesthesia was induced by intravenous (iv) injection of Alphaxalone and Alphadalone (3 mg/kg, Schering-Plough Animal Health, Wellington, New Zealand), and general anesthesia was maintained using 2–3% halothane in oxygen. The depth of anesthesia and maternal respiration were con-
stantly monitored by trained anesthetic staff. Under anesthesia, a 20-gauge iv catheter was placed in a maternal front leg vein to provide a constant-infusion isotonic saline drip to maintain maternal fluid balance for the duration of the surgery.

Using sterile techniques, fetal catheters were placed in the right femoral artery and vein, left and right brachial artery, right brachial vein, and the amniotic sac. Electrocardiogram (ECG) electrodes (AS633-3SSF, Cooner Wire, Chatsworth, CA) were placed subcutaneously over the right shoulder and over the apex of the heart to record the fetal ECG. An inflatable silicone occluder was placed around the umbilical cord of all fetuses (In Vivo Metric, Healdsburg, CA). All leads were exteriorized through the maternal flank, and a maternal long saphenous vein was catheterized to provide access for postoperative care and euthanasia. Gentamicin (80 mg; Rousell, Auckland, New Zealand) was administered into the amniotic sac before closure of the uterus. Amniotic fluid lost during surgery was replaced using normal saline warmed to 37°C.

After surgery, ewes were each housed in an individual metabolic cage with free access to food and water. The housing facility was temperature (16°C) and humidity (50%) controlled, with a 12:12-h cycle. All animals were euthanized by overdose of iv pentobarbital (9 g iv to the ewe: Pentobarb 300, Chemstok International, Christchurch, New Zealand).

Data acquisition. Measurements started at least 12 h before the experiment. Fetal mean arterial blood pressure (MAP), corrected for amniotic pressure (Novatrans II, MX860; Medex, Hilliard, OH), ECG, and FHR were recorded continuously. The MAP signal was collected at 64 Hz and low-pass filtered at 30 Hz. The raw ECG was analog recorded, while the MAP signal was collected by 10.220.33.5 on October 14, 2017 http://jap.physiology.org/ Downloaded from

Table 1. Fetal blood-gas parameters, glucose, and lactate levels in the 1:5 and 1:2.5 groups

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>P02, Torr</th>
<th>Pco2, Torr</th>
<th>BD, mM</th>
<th>Lactate, mM</th>
<th>Glucose, mM</th>
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<tbody>
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<td>Baseline</td>
<td></td>
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<tr>
<td>First 30 min</td>
<td>7.41±0.00</td>
<td>19.9±0.5</td>
<td>45.7±1.1</td>
<td>54.3±2.4</td>
<td>1.0±1.0</td>
<td>0.9±1.0</td>
</tr>
<tr>
<td>1st Occlusion</td>
<td>7.38±0.02</td>
<td>19.9±0.5</td>
<td>46.5±1.4</td>
<td>54.3±2.4</td>
<td>1.0±1.0</td>
<td>0.9±1.0</td>
</tr>
<tr>
<td>6th Occlusion</td>
<td>7.30±0.02</td>
<td>19.9±0.5</td>
<td>47.7±2.5</td>
<td>54.3±2.4</td>
<td>1.0±1.0</td>
<td>0.9±1.0</td>
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<tr>
<td>18th Occlusion</td>
<td>7.34±0.02</td>
<td>19.9±0.5</td>
<td>47.9±1.5</td>
<td>54.3±2.4</td>
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<td>0.9±1.0</td>
</tr>
<tr>
<td>First 30 min</td>
<td>7.40±0.01</td>
<td>21.3±0.6</td>
<td>45.7±1.1</td>
<td>54.3±2.4</td>
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<td>0.9±1.0</td>
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<tr>
<td>1st Occlusion</td>
<td>7.34±0.02</td>
<td>21.3±0.6</td>
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<tr>
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<td>7.27±0.03</td>
<td>21.3±0.6</td>
<td>47.7±2.5</td>
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<td>0.9±1.0</td>
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<tr>
<td>18th Occlusion</td>
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<td>21.3±0.6</td>
<td>47.9±1.5</td>
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<td>0.9±1.0</td>
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<tr>
<td>First 30 min</td>
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<td>45.7±1.1</td>
<td>54.3±2.4</td>
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<td>0.9±1.0</td>
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<tr>
<td>1st Occlusion</td>
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<td>21.3±0.6</td>
<td>46.5±1.4</td>
<td>54.3±2.4</td>
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<td>0.9±1.0</td>
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<tr>
<td>6th Occlusion</td>
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<td>21.3±0.6</td>
<td>47.7±2.5</td>
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<td>0.9±1.0</td>
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<tr>
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<td>54.3±2.4</td>
<td>1.0±1.0</td>
<td>0.9±1.0</td>
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</table>

Values are means ± SE. BD, base deficit; 1:5, repeated total umbilical cord occlusion for 1 min out of every 5 min (n = 8); 1:2.5, repeated total umbilical cord occlusion for 1 min out of every 2.5 min (n = 6) Between-group comparisons by Mann-Whitney U-test. *P<0.05. †P<0.01.

RESULTS

There were no significant differences in gestational age, preocclusion MAP, or baseline acid-base data (Table 1) between the two groups. Each umbilical cord occlusion was accompanied by a variable FHR deceleration. Examination of the 1-s averages of the decelerations showed a distinctive pattern of an initial rapid fall in FHR followed by a more
gradual fall to the nadir of the deceleration (Fig. 1, top). We therefore calculated two slopes for each variable deceleration; the slope of the initial fall in FHR and the slope to the nadir of the FHR deceleration (Fig. 1).

1:5 Occlusion group. All fetuses tolerated 4 h of occlusions without clinically significant acidosis, and the minimum MAP at the end of each occlusion never fell below preocclusion levels. A small fall in pH and rise in base deficit and lactate occurred in the first 30 min of occlusions ($P < 0.001$; Table 1), but thereafter the metabolic status of each fetus remained stable and the pH (7.34 ± 0.03), base deficit (1.1 ± 1.4 mM), and lactate (4.2 ± 1.5 mM) at the end of the experiments were not significantly different from the first 30-min values. There was no significant change over time in either the slope of the initial FHR deceleration (Figs. 1, middle, and 2A) or in the slope to the nadir of the FHR deceleration (Figs. 1, bottom, and 2A). There was no significant within-subject correlation between initial slope and the nadir of MAP at the end of the occlusion ($n = 6$; Fig. 3A).

1:2.5 Occlusion group. All fetuses became progressively hypotensive and acidic. Occlusions were terminated after 76.5 ± 21.2 occlusions. The minimum MAP, at the end of each occlusion, fell progressively from the fourth occlusion, stabilized in the middle 30-min interval, and fell again in the last 30 min of occlusions to 15.5 ± 3.0 mmHg after the last occlusion. By the end of the occlusions, pH was 6.92 ± 0.04, base deficit was 19.7 ± 1.8 mM, and lactate was 14.8 ± 1.2 mM ($P < 0.001$, compared with the 1:5 group; Table 1).

In contrast with the 1:5 group, the 1:2.5 group showed an increase in the initial fall in FHR (i.e., greater slope) over the course of the occlusion series (Figs. 1, middle, and 2B). ANOVA showed both a significant interaction between group and time ($P < 0.05$) and a significant effect of group on the slope of the initial FHR deceleration ($P = 0.001$). The initial slope in the final 30-min period was significantly greater compared with both the first 30-min interval and the 1:5 group in the final 30-min interval ($P < 0.001$; Fig. 1, middle). This increase in slope was associated with a greater fall in FHR during the initial deceleration in the final 30 min of the occlusion series compared with the 1:5 group ($P < 0.05$; Table 2), with a significant interaction between group and time ($P = 0.002$). There was a significant within-subject correlation between initial slope and the nadir of MAP at the end of the occlusion ($r^2 = 0.28$, $n = 6$, $P = 0.001$; Fig. 3B).

There was no significant effect of either group or time on the pattern of changes in the slope to the nadir of the deceleration (Fig. 1, bottom). There was an apparent marked reduction in slope between the first and second occlusion in both groups, which recovered over the first 30 min. Subsequently, there was a modest trend for slope to nadir to fall in the 1:5 group but not in the 1:2.5 group. The depth of deceleration increased over the duration of the experiment in the 1:2.5 but not the 1:5 group, with no overall effect of group or time but a significant interaction between group and time ($P = 0.001$), such that the groups were significantly different in the final 30 min ($P = 0.003$; Table 2).

**DISCUSSION**

The present study has examined an important apparent anomaly in our understanding of fetal cardiovascular adaptation to laborlike insults, the apparent attenuation of the chemoreflex-mediated initial fall in FHR by successive, repeated episodes of hypoxia (14, 15). In contrast, the present study demonstrates that the chemoreflex is not attenuated by repetition of brief but severe episodes of hypoxia continued for up to 4 h. Indeed, more frequent occlusions that were associated with hypotension and metabolic acidosis led to an increase in the rate and depth of the initial fall in FHR in the final 30 min of the occlusion series. Furthermore, we found a significant correlation in this group between the rate of initial fall of the FHR and the severity of evolving hypotension during the episodes of umbilical cord occlusion. These findings are consistent with data indicating that preexisting hypoxemia increases peripheral arterial chemoreceptor activity in several settings (7, 11, 17).
and strongly suggest that attenuation of the chemoreflex only occurs in response to episodes of relatively mild hypoxia (14, 15).

The fall in FHR during hypoxia is a key fetal cardiovascular adaptation that may reduce myocardial work and oxygen requirements. The initial fall is a vagally mediated reflex (4, 23) whose magnitude is closely correlated with the degree of reduction in fetal arterial oxygen saturation (2, 21). If occlusions are continued for 2–3 min, then a non-reflex-mediated hypoxic bradycardia develops (3). The precise afferent inputs that control the fall in FHR during the intermittent hypoxia of labor are not fully understood. There is extensive evidence that during mild to moderate hypoxia the major afferent input is the carotid chemoreflex (2, 4, 8, 12). However, during severe hypoxia, chemodenervation slows but does not abolish the initial fall in FHR (23). Other peripheral chemoreceptors such as those on the aortic arch do not contribute to the initial fall in FHR during moderate inhalational hypoxia (4); however, we may speculate that they might be more important during severe hypoxia as in the present study. For example, it is striking that, in the adult dog, aortic chemoreceptors rather than the carotid body chemoreceptors are the major mediators of the vasomotor responses to severe hypoxia (10), and there is some evidence that the aortic receptors may be more sensitive to different types of hypoxic stress (24). Another candidate might be a baroreflex due to the increase in blood pressure at the start of occlusions, mediated by the well-described peripheral vasoconstriction (12). However, in the present study, the second-by-second time course data in Fig. 2 show that the initial fall in FHR precedes and is more rapid than the modest rise in MAP.

Fig. 2. Time sequence of changes in 1-s average FHR (bpm, ○) and mean arterial blood pressure (MAP; mmHg, □) recordings for the second, middle, and penultimate occlusions for the 1:5 (A) and 1:2.5 (B) occlusion groups. Onset of occlusion was characterized by a rapid initial fall in FHR followed by a transient increase in MAP throughout the occlusion series. Note the development of progressive baseline tachycardia (i.e., between occlusions) in both groups and persistent hypertension during occlusions in the 1:5 group compared with the progressive development of hypotension in the 1:2.5 group. Data are means ± SE.
MAP is not until well after the initial fall in FHR. This is consistent with the finding that hypoxic chemoreceptor activity inhibits the baroreflex fall in heart rate in the adult rat and human (25, 27). Thus it is improbable that the baroreflex is a substantive contributor.

In the present study, we identified a second slower fall to the nadir of the fetal bradycardia following the initial rapid FHR deceleration. The mechanism of this secondary fall is unclear. Speculatively, in part it may be related to further recruitment of other peripheral chemoreceptor input (4) and possibly partly to hypercarbia, which is reported to augment chemoreflex responses (5). Alternatively, although experimental data suggest that primary hypoxic suppression of heart rate in healthy fetuses requires >1 min of occlusion (18, 21), it is possible that early myocardial hypoxia also augments this secondary fall in FHR. The marked delay in recovery of both FHR and MAP after release of occlusion at the time of the penultimate occlusion in the 1:2.5 group (Fig. 2B, bottom) supports this suggestion, at least for this group. This is also consistent with our laboratory’s previous finding of delayed, postocclusion hypotension and reversible cardiac injury in this paradigm (16).

If, as previously suggested (1, 14, 15), there was a progressive decrease in the initial chemoreflex fall in heart rate, this would raise the possibility that episodes of hypoxia earlier in pregnancy might compromise the ability of the fetus to respond fully to acute severe hypoxia in labor. The present study strongly suggests that this is not the case. Two factors may explain our different findings. First, previous studies of repeated hypoxia typically examined the fall to the nadir rather than the initial component of the fall (i.e., comparable to Fig. 1, bottom) (1). Furthermore, in the study of Akagi et al. (1), there was no comparison group that remained hemodynamically and metabolically stable, and the frequency of occlusions was changed over time. Thus the effects of frequency, repetition, and developing fetal compromise could not be distinguished. This is a significant issue, as shown in the present study by the observation that the 1:2.5 group only showed a significantly faster initial fall in FHR than the 1:5 group in the final 30-min interval of occlusions, when severe acidosis and hypotension were present.

The second and more important potential factor is the severity of hypoxia. Previous studies of repeated hypoxia in late gestation used a moderate degree of hypoxemia that did not induce significant fetal acidosis or hypotension. In the study from Giussani et al. (14) the umbilical cord was only partially occluded with a 50% reduction in cord blood flow for 5 min, repeated 12 times, and there was a 15-min interval for recovery between occlusions. Similarly, in the study from Green et al. (15), although complete occlusions were induced for slightly longer than in the present study (90 s vs. 1 min), a much longer, 30-min interval was provided for recovery between the occlusions, and the short series of occlusions was repeated over many days. Under these conditions, we may speculate that the relatively mild fetal stress may allow the chemoreflex to become attenuated. The development of myocardial dysfunction and hypotension during fetal hypoxia is directly related to the extent of depletion of fetal cardiac glycogen reserves, which is only restored in the interval after hypoxia (19). Thus, for example, in the present study, the reduced period of recovery between occlusions in the 1:2.5 group was associated with much greater hemodynamic and metabolic compromise than in the 1:5 group. Furthermore, the relatively long period of recovery in the study from Green and colleagues will have allowed essentially complete restoration of glycogen reserves, which is only restored in the interval after hypoxia (19).

Table 2. Magnitude of the initial and maximal falls in fetal heart rate during umbilical cord occlusions

<table>
<thead>
<tr>
<th></th>
<th>1:5 Group</th>
<th>1:2.5 Group</th>
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<tbody>
<tr>
<td><strong>Initial fall</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First 30 min</td>
<td>64.8±9.7</td>
<td>51.4±5.2</td>
</tr>
<tr>
<td>Mid 30 min</td>
<td>55.2±9.9</td>
<td>63.3±7.1</td>
</tr>
<tr>
<td>Last 30 min</td>
<td>47.0±8.7</td>
<td>71.9±6.5*</td>
</tr>
<tr>
<td><strong>Maximal fall</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First 30 min</td>
<td>100.7±11.1</td>
<td>85.0±6.0</td>
</tr>
<tr>
<td>Mid 30 min</td>
<td>96.8±11.2</td>
<td>110.0±9.8</td>
</tr>
<tr>
<td>Last 30 min</td>
<td>88.3±7.4</td>
<td>132.7±9.8†</td>
</tr>
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</table>

Data are means ± SE. Significant difference vs. 1:5 group: *P < 0.05; †P < 0.01.
of glycogen levels between insults and, therefore, will have prevented hemodynamic compromise (15). In contrast, our findings that the chemoreflex FHR responses to repeated episodes of complete occlusion of the umbilical cord were augmented as hypotension and metabolic acidosis developed are consistent with studies reporting that preexisting, mild hypoxemia increases subsequent peripheral arterial chemoreceptor responses to acute hypoxia in fetal sheep (7, 11). Similarly, mild hypoxemia also increases the contribution of peripheral arterial chemoreceptors to resting ventilation in premature infants (17).

In conclusion, the results of the present study are consistent with clinical observations of progressively deeper decelerations with developing fetal compromise (20, 26). The data demonstrated that, contrary to previous suggestions, the slope of the initial chemoreflex-mediated fall in heart rate was not attenuated by repeated complete occlusions of the umbilical cord. Thus fetal metabolic acidosis and hypotension during repetitive hypoxic insults do not appear to be due to failure of the fetal reflex responses to repeated severe hypoxia. Indeed, the development of severe fetal compromise was associated with augmentation of this response, as demonstrated by the increase in both the slope of initial chemoreflex-mediated fall in heart rate and the absolute magnitude of the fall in FHR. This finding reinforces the concept of the chemoreflex as a central component of fetal adaptation to severe hypoxia, which is robustly maintained during repeated challenges, without attenuation.

GRANTS
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REFERENCES