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Male disadvantage? Fetal sex and cardiovascular responses to asphyxia in preterm fetal sheep

Laura Bennet, Lindsea C. Booth, Noha Ahmed-Nasef, Justin M. Dean, Joanne Davidson, Josine S. Quaedackers, and Alistair J. Gunn

Department of Physiology, The University of Auckland, Auckland, New Zealand

Bennet L, Booth LC, Ahmed-Nasef N, Dean JM, Davidson J, Quaedackers JS, Gunn AJ. Male disadvantage? Fetal sex and cardiovascular responses to asphyxia in preterm fetal sheep. *Am J Physiol Regul Integr Comp Physiol* 293: R1280–R1286, 2007. First published June 27, 2007; doi:10.1152/ajpregu.00342.2007.—Clinically and experimentally male fetuses are at significantly greater risk of dying or suffering injury at birth, particularly after premature delivery. We undertook a retrospective cohort analysis of 60 female and 65 male singleton preterm fetal sheep (103–104 days, 0.7 gestation) with mean arterial blood pressure (MAP), heart rate, and carotid and femoral blood flow recordings during 25 min of umbilical cord occlusion in utero. Occlusions were stopped early if fetal MAP fell below 8 mmHg or if there was asystole for >20 s. Fetuses that were able to complete the full 25-min period of occlusion showed no differences between sexes for any cardiovascular responses. Similar numbers of occlusions were stopped early in males (mean: 21 min, $n = 16$) and females (mean: 23 min, $n = 16$); however, they showed different responses. Short-occlusion males ($n = 16$) showed a slower initial fall in femoral vascular conductance, followed by greater bradycardia, hypotension, and associated organ hypoperfusion compared with full-occlusion fetuses. In contrast, short-occlusion females ($n = 16$) showed a significantly more rapid early increase in femoral vascular conductance than the full-occlusion fetuses, followed by worsening of bradycardia and hypotension that was intermediate to the full-occlusion fetuses and short-occlusion males. Among all fetuses, MAP at 15 min of occlusion, corresponding with the time of the maximal rate of fall, was correlated with postmortem weight in males ($R^2 = 0.07$) but not females. In conclusion, male and female fetuses showed remarkably similar chemoreflex and hemodynamic responses to severe asphyxia, but some males did show impaired hemodynamic adaptation within the normal weight range.

cardiovascular chemoreflex

IN 1971, Richard Naeye et al. (38) described the increased risk of perinatal mortality and morbidity in boys compared with girls as the "male disadvantage." Numerous studies have since confirmed that sex is an independent risk factor at all stages of gestation (32). In particular, prematurely born boys have up to 22% higher mortality than girls and more frequent adverse neurodevelopmental outcomes, even after adjustment for severity of illness at birth (26, 37, 50).

The mechanisms mediating the influence of sex on perinatal death and disability are poorly understood and likely to be multifactorial. However, higher rates of abnormal fetal heart rate recordings, metabolic acidosis, and need for operative intervention or resuscitation in labor among pregnancies with male fetuses (3, 13, 27, 48, 52) raise the possibility that boys may be less able to adapt to hypoxic stress per se. Male fetuses are on average bigger, grow faster, and have a higher metabolic rate than females (10, 36), suggesting that when oxygen is limited, they might deplete available resources more rapidly. Furthermore, there is evidence that males have relatively delayed maturation of some aspects of autonomic nervous system function, for example, adrenal medullary and lung β-receptor maturation in fetal rabbits (40). Clinically, after exposure to asphyxia at birth, preterm boys are reported to have lower plasma catecholamine levels than girls (23).

The fetal cardiovascular defense responses to hypoxia include redistribution of combined ventricular output and bradycardia (30). These changes help maintain perfusion to key organs such as the heart and brain and are believed to reduce myocardial work and oxygen requirements (14). The chemoreflex responses are mediated through α-adrenergic and vagal afferents, respectively (2, 20, 28), and are well established in preterm fetal sheep (19, 33, 34). During continued asphyxia, the cardiovascular defenses fail, leading to progressive severe hypotension and associated organ hypoperfusion and injury (7, 19).

Surprisingly, we do not know whether there are gender differences in these crucial cardiovascular responses that might contribute to greater male perinatal morbidity and mortality at any fetal age. The data described above suggest the hypothesis that males might be less efficient at maintaining perfusion of central organs during hypoxia due to either reduced peripheral vasoconstriction or impaired maintenance of cardiac output. Thus the aim of this study was to contrast the cardiovascular and cerebrovascular responses of male and female fetuses to an acute asphyxial insult induced by complete umbilical cord occlusion. The study was undertaken in preterm fetal sheep at 0.7 gestation, which in terms of cerebral maturity is comparable to the human brain at 28–32 wk of gestation, before the onset of cortical myelination (35).

METHODS

In this study we analyzed an historical database of experiments conducted in our laboratory over 9 yr (5–9, 42, 43, 45, 46). Fetuses were selected for the study based on the following criteria: they were singletons (confirmed at postmortem); they had a normal partial pressure of oxygen in the baseline period (>20 mmHg) and were not acidotic; they underwent no experimental treatment before umbilical cord occlusion; they were the same age at surgery, experiment, and postmortem ± 1 day; they underwent similar surgical procedures; and they were assigned to the same umbilical cord occlusion protocol.

Experimental preparation. All procedures were approved by the Animal Ethics Committee of the University of Auckland, New Zea-

land. From our database we identified 125 Romney/Suffolk fetal sheep (65 males, 60 females) that fitted our criteria. Details of anesthetic and fluid management, the sterile surgical preparation, and intraoperative and postoperative antibiotic prophylaxis are described in the publications listed above. Fetuses were instrumented using sterile techniques at 97–98 days of gestation (term = 147 days) under general anesthesia maintained with 2–3% halothane.

Catheters were placed in the left fetal femoral artery and vein, right brachial artery, and the amniotic sac. A 3S ultrasound blood flow probe (Transonic Systems, Ithaca, NY) was placed around the left carotid artery to measure carotid artery blood flow (CaBF) as an index of cerebral blood flow (7, 53). A 2R probe was placed around the right femoral artery to measure femoral blood flow (FBF) as a representative peripheral resistance bed. Electrocardiogram (ECG) electrodes (AS633-3SSF; Cooner Wire, Chatsworth, CA) were sewn across the fetal chest to record fetal heart rate (FHR). An inflatable silicone occluder was placed around the umbilical cord (In Vivo Metric, Healdsburg, CA). All fetal leads were exteriorized through the maternal flank, and a maternal long saphenous vein was catheterized to provide access for postoperative care and euthanasia.

A period of 4–5 days of postoperative recovery was allowed before experiments commenced. Fetal catheters were maintained patent by continuous infusion of heparinized saline (20 U/ml at 0.15 ml/h), and the maternal catheter was maintained by daily flushing.

Experimental design. Experiments were conducted at 103–104 days of gestation. Mean arterial pressure (MAP) measured from the femoral artery, mean venous pressure measured from the femoral vein (both corrected for maternal movement by subtraction of amniotic fluid pressure), FHR, FBF, and CaBF were recorded continuously, and the data were stored to disk by custom software for off-line analysis (LabVIEW for Windows; National Instruments, Austin, TX).

Asphyxia was induced by rapid inflation of the umbilical cord occluder for 25 min with sterile saline of a defined volume known to completely inflate the occluder. Arterial blood was taken from the brachial artery 15 min before asphyxia and at 5 and 17 min during occlusion for preductal pH, blood gas (Ciba-Corning Diagnostics 845 blood gas analyzer and co-oximeter; Walpole MA), glucose, and lactate measurements (YSI model 2300; Yellow Springs, OH). Occlusions were stopped before 25 min if either the fetal blood pressure fell to <8 mmHg or asystole occurred for >20 s on continuous ECG monitoring. On completion of the experiment at 72 h after occlusion, ewes and fetuses were killed with an overdose of pentobarbitone sodium (9 g iv to the ewe; Pentobarb 300; Chemstock International, Christchurch, New Zealand). Fetal body weights were recorded.

Data analysis and statistics. In this study cardiovascular data are presented for the baseline, occlusion, and first 15 min of recovery. Further data are not available because many fetuses were subsequently assigned to treatment protocols that may affect cardiovascular function. Carotid (CVC) and femoral artery vascular conductance (FVC) were calculated as blood flow (ml/min)/MAP − mean venous pressure (mmHg). Conductance was calculated instead of the reciprocal, vascular resistance, because during umbilical cord occlusion, the denominator, peripheral blood flow, transiently falls to near zero, and thus resistance increases nonlinearly (43, 46). We defined four groups in this study: fetuses that completed the 25-min occlusion period, full-occlusion males and full-occlusion females, and those whose occlusions were stopped early, short-occlusion males and short-occlusion females. Because there were no differences for any parameter between full-occlusion males and full-occlusion females, for subsequent analyses we refer to this group as the full-occlusion combined group, and we statistically compared the responses of the full-occlusion combined group to those of the short-occlusion male and short-occlusion female groups. Data are displayed and analyzed in the short-occlusion groups only for times when the number of fetuses is six or more: 24 min for most variables, and 23 min for blood flow and conductance because of very low signals.

Time-series data were analyzed by ANOVA with time as a repeated measure (SPSS version 12; SPSS, Chicago, IL) followed by Fisher's protected least significant difference (LSD) post hoc test when a significant overall effect was found. Incidences were compared using Fishers exact test. The relationship between fetal weight at post mortem and fetal MAP at 15 min of occlusion was assessed using linear regression analysis. Fetal MAP at 15 min was examined because this corresponded with the time of maximal rate of fall. Statistical significance was accepted when $P < 0.05$. Data are means ± SE.

RESULTS

Duration of occlusion. The majority of fetuses in both sex groups tolerated the full 25 min of occlusion. Sixteen fetuses of each sex had their occlusion stopped early (short-occlusion groups). There was no significant difference between groups in the rate of assignment to postocclusion intervention. For the short-occlusion females, the mean duration of occlusion was 23 min (range: 20–24 min). For the short-occlusion males, the mean duration of occlusion was 21 min (range: 15–24 min, $P = 0.06$, males vs. females). The major reason recorded for short occlusions in females was asystole (12 of 16 fetuses), whereas the major reason in males was hypotension (14 of 16 fetuses, $P = 0.001$). The incidence of profound hypotension <8 mmHg before 25 min was significantly greater in males (14/65 vs. 4/60, $P = 0.02$).

Fetal weights. The full-occlusion females weighed 1,716 ± 46 g at postmortem, and the short-occlusion females weighed 1,644 ± 59 g [4.2% less, not significant (NS)]. The full-occlusion males weighed 1,831 ± 43 g, and the short-occlusion males weighed 1,719 ± 66 g (6.1% less, NS).

Blood chemistry. The arterial pH, blood gas, glucose, and lactate values for the full- and short-occlusion groups, before and during asphyxia, are presented in Table 1. There were no differences in any baseline values. In addition, there was no difference in the baseline hematocrit or hemoglobin concentrations among groups (data not shown). All fetuses became significantly acidotic, hypercapnic, and hypoxic during occlusion compared with baseline values ($P < 0.001$). Overall, the short-occlusion groups showed a significantly reduced rise in arterial partial pressure of CO_2 (Pa_{CO_2}) during occlusion; this was significant in short-occlusion males compared with full-occlusion males ($P < 0.005$), with a borderline effect in short-occlusion females compared with full-occlusion females ($P = 0.05$ at 5 and 17 min). Short-occlusion males also had lower blood lactate values compared with full-occlusion males at 17 min ($P < 0.005$).

Overall cardiovascular responses. There were no significant differences between the full-occlusion and short-occlusion groups for any cardiovascular parameter during the baseline period. There was no difference between full-occlusion males and full-occlusion females during occlusion or the first 15 min after occlusion. Differences between the full-occlusion combined and short-occlusion groups during and after occlusion are discussed below (Fig. 1).

Fetal heart rate. All groups showed a rapid initial fall in FHR after occlusion, followed by a slower progressive fall throughout the remainder of occlusion. Similar to our previous report (19), when episodes of asystole occurred as noted above, they were closely associated with intermittent tachycardia, and thus increased FHR variability, but with little effect on mean

Table 1. *Fetal arterial pH, blood gases, glucose, and lactate values before and during asphyxia induced by complete umbilical cord occlusion*

Time	Group	pHa	Pa_{CO_2}, mmHg	Pa_{O_2}, mmHg	BE, mM	Lactate, mM	Glucose, mM
Control	Male full	7.37±0.0	47.8±1.0	24.3±0.7	1.6±0.4	0.7±0.0	1.0±0.0
	Female full	7.37±0.0	48.4±1.5	22.8±1.1	1.1±0.9	0.6±0.1	1.0±0.0
	Male short	7.37±0.0	44.9±1.5	25.0±0.7	0.6±1.1	0.7±0.1	1.1±0.0
	Female short	7.37±0.0	47.8±1.1	24.0±0.7	1.8±0.8	0.7±0.0	1.0±0.0
5 min	Male full	7.06±0.0§	97.0±2.4§	6.6±0.4§	−4.7±0.8§	3.8±0.1§	0.3±0.1§
	Female full	7.03±0.0§	95.5±3.2§	7.3±0.7§	−6.5±0.9§	3.9±0.2§	0.3±0.0§
	Male short	7.07±0.0§	85.7±3.1§#	7.5±0.6§	−6.7±0.7§*	3.3±0.2§	0.4±0.0§
	Female short	7.05±0.0§	88.9±2.1§	7.8±0.4§	−6.8±0.5§	3.6±0.1§	0.3±0.0§
17 min	Male full	6.86±0.0§	136.1±3.3§	8.9±0.4§	−10.1±0.4§	6.3±0.1§	0.6±0.1§
	Female full	6.83±0.0§	140.0±4.1§	9.2±0.8§	−12.2±0.9§	6.6±0.2§	0.7±0.1§
	Male short	6.88±0.0§	117.7±5.1§#	8.3±0.7§	−12.1±0.8§*	5.5±0.2§#	0.6±0.1§
	Female short	6.87±0.0§	129.3±3.9§	9.7±0.8§	−11.1±0.5§	6.1±0.2§	0.6±0.1§

Values are means ± SE of measurements taken 15 min before (control) and during (5 and 17 min) asphyxia induced by complete umbilical cord occlusion. pHa, arterial pH; Pa_{CO_2}, fetal arterial partial pressure of CO_2; Pa_{O_2}, fetal arterial partial pressure of O_2. BE, base excess. §$P < 0.001$ vs. control values for each group. *$P < 0.05$; #$P < 0.005$, full-occlusion males vs. short-occlusion males.

FHR. Short-occlusion males showed significantly lower FHR compared with the full-occlusion combined group between 12 and 21 min of occlusion ($P < 0.05$, Fig. 1). In contrast, short-occlusion females were not significantly different from either the full-occlusion combined or short-occlusion male groups. During recovery, there were no differences among any of the groups.

Mean arterial blood pressure. MAP initially rose in all groups, peaking at 3 min after the start of occlusion, followed by a progressive fall with continued occlusion. In short-occlusion males, MAP was significantly lower than in the full-occlusion combined group in the first minute ($P < 0.05$) and then from 8 min until the end of occlusion ($P < 0.05$, Fig. 1). In short-occlusion females, MAP was significantly lower than in full-occlusion combined fetuses from 10 to 23 min of occlusion ($P < 0.05$, Fig. 1). MAP was significantly lower in short-occlusion males than in short-occlusion females from 12 to 18 min ($P < 0.05$). After release of occlusion, MAP was significantly lower in short-occlusion males than in the full-occlusion combined group from 5 to 10 min ($P < 0.05$).

Carotid and femoral blood flows. CaBF was maintained around baseline values in all groups for the first 5–7 min after the start of occlusion and then progressively fell throughout occlusion. In short-occlusion males, CaBF was significantly lower than in the full-occlusion combined group from 9 min until the end of occlusion ($P < 0.05$, Fig. 1). In short-occlusion females, CaBF was significantly lower than in the full-occlusion combined group at 19 and 20 min ($P < 0.05$) and significantly higher than in short-occlusion males at 10 and 11 min ($P < 0.05$). There were no significant differences among groups during the recovery period.

FBF showed a triphasic pattern of changes during occlusion in all groups: there was a rapid initial fall to near zero flow, followed by partial reperfusion and then a secondary slower fall in the latter phase of occlusion (Fig. 1). The short-occlusion females showed a faster initial fall in FBF such that FBF was significantly lower than in both the full-occlusion combined and short-occlusion male groups during the first 2 min and significantly lower than in the short-occlusion male group at the third minute ($P < 0.05$). From 3 to 5 min after the start of occlusion, FBF rose in all groups and was significantly higher in short-occlusion females compared with the other groups at 6 to 8 min and higher than in short-occlusion males between 20 and 22 min ($P < 0.05$). Short-occlusion males had lower FBF than the combined group from 14 min until the end of occlusion ($P < 0.05$). During recovery, short-occlusion
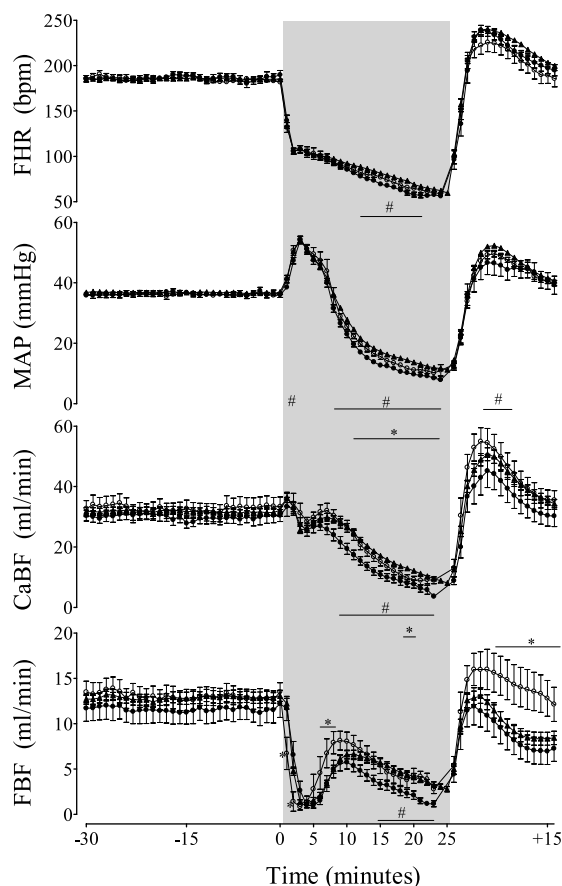

Fig. 1. Time sequence of changes in fetal heart rate [FHR; beats/min (bpm)], mean arterial blood pressure (MAP), carotid blood flow (CaBF), and femoral blood flow (FBF) for short-occlusion females (○), short-occlusion males (●), and full-occlusion-combined fetuses (▲). Data are 1-min averages ± SE from 30 min before umbilical cord occlusion (shaded region) until 15 min after occlusion. Horizontal bars denote the periods of significance. *$P < 0.05$, short-occlusion females vs. full-occlusion combined group. #$P < 0.05$, short-occlusion males vs. full-occlusion combined group.
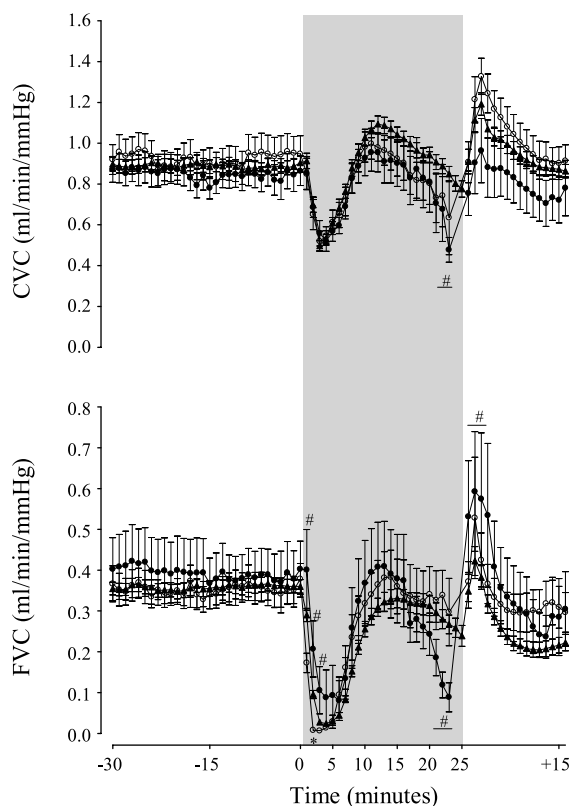
Fig. 2. Time sequence of changes in carotid artery vascular conductance (CVC) and femoral artery vascular conductance (FVC) for short-occlusion females (○), short-occlusion males (●), and full-occlusion combined fetuses (▲). Data are 1-min averages ± SE from 30 min before umbilical cord occlusion (shaded region) until 15 min after occlusion. Horizontal bars denote the periods of significance. *$P < 0.05$, short-occlusion females vs. full-occlusion combined group. #$P < 0.05$, short-occlusion males vs. full-occlusion combined group.

females had higher FBF compared with the combined group from 8 min postocclusion ($P < 0.05$) and from 10 min compared with short-occlusion males ($P < 0.05$).

Vascular conductance. CVC fell after the start of occlusion, reaching a nadir at 3 to 4 min in all groups, followed by a secondary increase to baseline values or a little higher at ~12 min and then a late fall (Fig. 2). There were no differences in carotid conductance between groups until late in the occlusion phase, when CVC was significantly lower in the short-occlusion male group compared with the full-occlusion combined group from 22 min ($P < 0.05$, Fig. 2). After release of occlusion, CVC was transiently higher in short-occlusion females compared with short-occlusion males at 3 to 4 min.

FVC showed a similar triphasic pattern of changes during occlusion, with an initial profound reduction in FVC reaching a nadir at 2 to 5 min, recovery to approximately baseline values, and a gradual late fall. In short-occlusion females, FVC was significantly lower than in short-occlusion males in the first 3 min and than in the full-occlusion combined group in the second minute ($P < 0.05$). FVC was briefly greater in short-occlusion females than in full-occlusion combined fetuses at 7 min and short-occlusion males from 22 min ($P < 0.05$, Fig. 2). In short-occlusion males, FVC was significantly higher than in both the other groups in the first 3 min ($P < 0.05$) and lower than in the full-occlusion combined group at 22 and 23 min

($P < 0.05$). During recovery, the short-occlusion male group had transiently higher FVC compared with the full-occlusion combined group at 3 to 4 min postocclusion. There were no other statistical differences among groups.

Fetal weight vs. MAP. There was a borderline relationship between fetal weight and MAP at 15 min of occlusion for all fetuses ($P = 0.05$). Subgroup analysis suggested that there was a small, significant relationship between weight and MAP in male fetuses ($P < 0.05$, $R^2 = 0.07$, Fig. 3, *bottom*) but not in female fetuses ($P = 0.6$, Fig. 3, *top*). There was no significant relationship between baseline blood gases, pH, glucose, or lactate and MAP at 15 min.

DISCUSSION

This study has demonstrated that, contrary to our initial hypothesis, sex per se did not significantly alter the cardiovascular responses of healthy singleton preterm fetal sheep to an acute, profound asphyxial insult. Neither the average responses nor the incidence or timing of failure to complete the full period of umbilical cord occlusion were significantly different between the sexes. However, overall, significantly more males developed profound hypotension (<8 mmHg) before the end
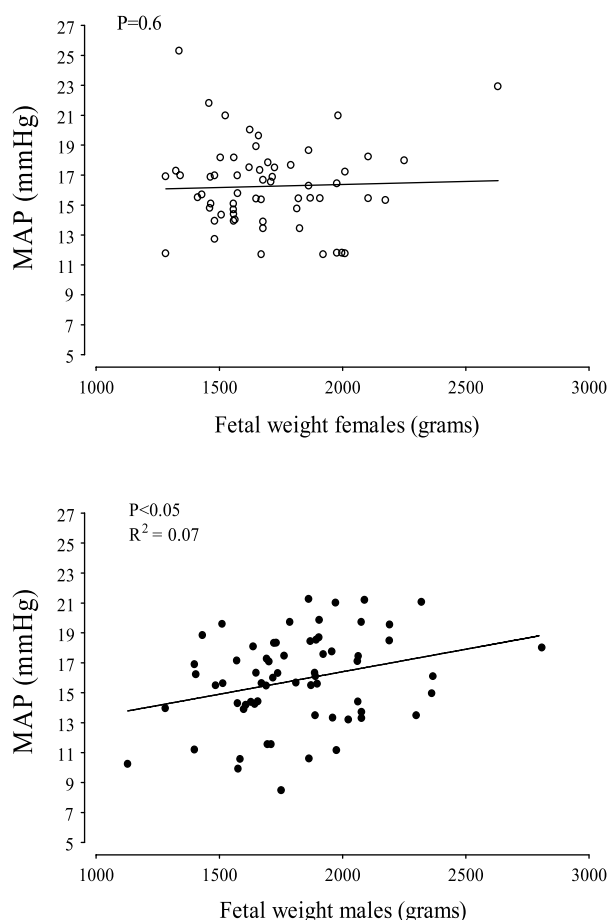
Fig. 3. Relationship between fetal weight for all fetuses, measured at postmortem, and fetal mean arterial blood pressure (MAP) at 15 min of occlusion, a time that corresponded with the time of maximal rate of fall in MAP. There was no significant relationship between fetal weight and MAP for the female fetuses (*top*), but there was a statistical association between weight and MAP for the male fetuses (*bottom*, linear regression, $P < 0.05$).

of the occlusion period and intriguingly, male but not female fetuses showed a significant correlation between postmortem weight and severity of the fall in arterial blood pressure after 15 min of occlusion. Furthermore, the causes of failure to complete the occlusions differed markedly. These differences were associated in turn with changes that suggest altered chemoreflex and cardiac responses between the sexes in this subgroup. The short-occlusion males demonstrated slower and reduced initial peripheral vasoconstriction compared with the full-occlusion fetuses. This was followed by earlier and significantly greater hypotension, associated with greater falls in heart rate and carotid and femoral blood flow. In contrast, short-occlusion females showed a markedly more rapid onset of initial vasoconstriction of the femoral bed and subsequent falls in blood pressure and heart rate that were intermediate between those of the full-occlusion fetuses and short-occlusion males. It is improbable that these differences relate to placental function, since fetal body weight (measured 3 days after occlusion) and pH, blood gas, glucose, and lactate values before occlusion were not different among the groups.

Conceptually, the cardiovascular responses of the fetus to acute severe hypoxia can be considered in two phases, the initial, rapid chemoreflex-mediated adaptations (1, 20) and the subsequent longer period of progressive hypoxic decompensation (1). The present study demonstrated that all groups, regardless of the ultimate duration of occlusion, mounted broadly similar initial chemoreflex responses and, in particular, identical rates and magnitudes of the fall in heart rate. However, the short-occlusion females showed a significantly more rapid fall in femoral blood flow than full-occlusion fetuses, mediated by a more rapid reduction in femoral vascular conductance. In contrast, the short-occlusion males showed a slower fall in femoral conductance than full-occlusion fetuses. The mechanism of this apparent sensitization of the α-adrenergic receptor-mediated peripheral arc of the chemoreflex (20) in a subset of female fetuses and blunting in the corresponding males is unknown. There is some evidence for higher fetal production or release of epinephrine and norepinephrine in females in the rabbit (40) and premature infants after asphyxia (23). However, the present study strongly indicates that there does not seem to be a general sex difference, since in the majority of fetuses the initial peripheral chemoreflex-mediated vasoconstriction was not different between sexes.

Alternatively, we might speculate that the short-occlusion groups had been exposed to previous stimuli that altered fetal development. For example, experimental placental insufficiency is associated with increased peripheral sympathetic nervous system tone in the sheep that predates the development of significant growth restriction (12). Similarly, in the unhatched chick, chronic moderate hypoxia leads to sympathetic hyperinnervation of the arterial system (47). Although the effect of sex in fetal life is unknown, fetal growth restriction is associated with enhanced hypothalamic adrenal responses in boys but enhanced autonomic responses in girls (41). These examples relate to pathological stressors; however, in the current study, baseline blood gases and body weights were not significantly different.

There is now evidence that less severe or transient adverse stimuli in utero can lead to persistent changes, including resetting of chemoreflex and baroreflex responses within the normal weight range (11, 16, 17, 25). Of particular relevance to the present findings, moderate maternal undernutrition in pregnant rats that had only a transient effect on growth of the pups was associated with increased sympathetic nervous system activity in female but not male rats (29). Similarly, maternal undernutrition during the first 30 days of gestation in sheep led to enhanced adrenal steroidogenic potential in female but not male offspring (18).

In the present study, the initial peripheral vasoconstriction was only sustained for the first 5 to 6 min of occlusion in all fetuses and was followed not by overt vasodilatation but rather a return to control values, similar to previous reports (6, 31, 43, 54). This is unlikely to be due to local accumulation of metabolites, since a similar biphasic pattern occurs even during a more moderate insult such as partial umbilical cord occlusion (21). Furthermore, in the present study, fetuses, particularly the short-occlusion males, were able to increase vascular tone later during occlusion, when severe respiratory and metabolic acidosis was present. Preliminary data from this paradigm in preterm fetal sheep suggest that loss of renal vasoconstriction is closely associated in time with attenuation of the renal sympathetic response to asphyxia (4), suggesting a primarily central mechanism.

Loss of initial vasoconstriction was associated with a further, progressive fall in heart rate and, after ~10 min in all groups, with overt hypotension. There is no evidence for continuing reflex mechanisms at this time (1). Likely contributors to impaired cardiac function include hypoxia, acidosis, depletion of myocardial glycogen, and cardiomyocyte injury (24). It is notable that after resolution of the initial hypertension, the fall in blood pressure in the second half of occlusion slowed to a steady pace of around 1 mmHg per minute, in parallel with the continued fall in heart rate. Given the approximately baseline levels of peripheral vascular tone at this time, fetal MAP must have been primarily determined by cardiac output. In turn, since stroke volume is relatively constrained in the fetus (22), cardiac output will have been a function primarily of the fall in heart rate. This analysis is highly consistent with the finding of parallel worsening of hypotension and bradycardia in short-occlusion-males relative to full-occlusion fetuses in the present study.

The ability of the fetus to survive prolonged asphyxia is closely linked to levels of cardiac glycogen (49). Thus we speculate that the short-occlusion males, and to a lesser extent females, may have had lower cardiac glycogen stores. Interestingly, in the present study, the short-occlusion males were significantly less hypercapnic during hypoxia than either the short-occlusion females or full-occlusion fetuses, consistent with at least one report at term (31), and circulating lactate was significantly lower in the short-occlusion males after 17 min of occlusion. This curious observation supports the hypothesis that the short-occlusion males had reduced glycogen stores, and thus reduced anaerobic metabolism, leading to reduced CO_2 and lactate production.

Although cardiac glycogen is reduced during clinical growth restriction (51), and after a limited period of maternal fasting in rats (39), baseline plasma glucose values in the current study were normal. However, it is important to appreciate that glucose uptake in fetal sheep can be altered after maternal nutritional restriction during early to midgestation in the absence of any change in birth weight in males but not females (15). Furthermore, clinically, it is notable that relatively small

reductions in birth weight are associated with a significantly greater mortality in boys compared with girls (32). These observations are highly consistent with the present observation of a significant overall relationship between body weight and blood pressure during occlusion in male but not female fetuses.

Alternatively, there is some evidence that females may have greater resistance to hypoxic-ischemic injury of cardiomyocytes (44). In the present study, although female fetuses had a similar rate of early termination of occlusion to males and a greater fall in heart rate and associated worsening of hypotension and organ hypoperfusion relative to the full-occlusion fetuses, the deterioration was of later onset and milder than in the short-occlusion males. The cause of the greater incidence of periodic asystole that led to occlusions being stopped is unclear but suggests the possibility of compromise of the fetal cardiac pacemaker.

In conclusion, the present study has demonstrated for the first time that the hemodynamic responses of male and female preterm sheep fetuses to a prolonged episode of asphyxia are remarkably similar. In part, this likely reflects the healthy, normoxic, well-grown singletons in this cohort; possibly greater divergence would be seen among twins or overtly growth-restricted fetuses. However, there was evidence in the complete cohort that males showed both an increased rate of terminal hypotension and a significant positive correlation between body weight at postmortem and MAP at the time of the most rapid fall, which was not seen in female fetuses. We speculate that these differences indicate a differential effect of sex within the normal spectrum. Potentially, the present findings could represent a part of the poorly understood and complex differences between boys and girls that lead to higher perinatal morbidly and mortality in boys (50).

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