

# Effects of caffeine on renal and pulmonary function in preterm newborn lambs

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**INTRODUCTION:** Administration of caffeine is associated with a reduction in bronchopulmonary dysplasia, assisted ventilation, patent ductus arteriosus (DA), and cerebral palsy in preterm infants, but the mechanisms are unknown. Our aim was to determine the effects of acute caffeine administration on renal and pulmonary functions in preterm lambs.

**METHODS:** Lambs were delivered by cesarean section at ~126 d of gestation and ventilated with a tidal volume of 5 ml/kg, 60 breaths/min and 5 cmH<sub>2</sub>O positive end-expiratory pressure. After 30 min, lambs received 40 mg/kg caffeine i.v. ( $n = 7$ ) or saline (controls;  $n = 6$ ) over 30 min and were ventilated for 2 h.

**RESULTS:** Arterial concentrations of caffeine reached 35.9 ± 7.8 mg/l. Urine output and mean heart rate were significantly higher after caffeine treatment than in controls ( $5.86 \pm 1.95$  vs.  $0.76 \pm 0.94$  ml/kg, area under the curve,  $P = 0.041$  and  $211 \pm 8$  vs.  $169 \pm 15$  beats per minute,  $P < 0.05$ , respectively). Heart rate remained higher for the experimental period.

**DISCUSSION:** Caffeine did not affect pulmonary artery or DA blood flows or other renal, respiratory, or cardiovascular parameters examined. Neonatal administration of caffeine increased heart rate and urine output but had little effect on pulmonary function in ventilated preterm lambs.

Preterm birth, defined as birth before 37 complete weeks of gestation, occurs in 7–12% of all births in developed countries and accounts for over 70% of neonatal mortality and morbidity (1). Apnea of prematurity is one of the most common problems encountered in the clinical management of preterm infants, occurring in 85% of infants born at <34 wk gestation (2). Apnea of prematurity (usually defined as a cessation of breathing >15 s) is often associated with bradycardia and arterial desaturation (3) and is common in preterm infants because they have immature respiratory control mechanisms. Methylxanthines (theophylline and caffeine) and continuous positive pressure ventilation have been the mainstay for managing apnea in preterm infants for more than 30 y (3). Methylxanthines increase central inspiratory drive and minute ventilation; they also improve CO<sub>2</sub> sensitivity, decrease hypoxic depression of breathing, and relax bronchial smooth muscle (4–6). Caffeine is the methylxanthine that is most commonly

used to treat apnea because it has a longer half-life, a higher therapeutic index, and lower toxicity than theophylline (7). Caffeine treatment improves dynamic respiratory compliance during the first week after birth in preterm infants with respiratory distress syndrome (8) and improves diaphragmatic contractility (9,10), both of which improve ventilation and gas exchange. At therapeutic concentrations, the actions of caffeine appear to be mediated primarily by competitive antagonism of adenosine (A1 and A2a) receptors (11).

At birth, the airways must be cleared of liquid to allow the entry of air and the onset of pulmonary gas exchange (10,12,13). The process of lung aeration initiates major changes in cardiopulmonary physiology that are essential for the transition to air-breathing after birth, including a large increase in pulmonary blood flow (PBF) and left-to-right shunting through the ductus arteriosus (DA) before it closes (14–17). Failure of the lungs to fully aerate after birth is a common problem in very preterm infants that impedes the transition to postnatal life. As a result, these infants usually require some form of assisted ventilation to survive, but this intervention can initiate or exacerbate lung injury, which can lead to bronchopulmonary dysplasia.

A recent study demonstrated that caffeine therapy for very preterm infants is associated with significant reductions in the duration of assisted ventilation, the incidence of patent DA, and the rate of bronchopulmonary dysplasia, by ~10%, and that it reduces the incidence of cerebral palsy as compared with placebo-treated infants (18,19). Although these are very important outcomes, the mechanisms responsible are unknown. In addition to promoting the stability of respiratory drive, it is possible that caffeine has a direct effect on the lungs, through its diuretic action, leading to improved lung liquid clearance. Following its clearance from the airways, the liquid enters the pulmonary tissue to form perivascular cuffs (20), leading to increased pulmonary interstitial tissue pressure (21). This liquid takes hours to clear from lung tissue (20,21), and sustained high interstitial tissue pressures promotes liquid movement back into the airways (22,23); the presence of pulmonary hypertension may compound this problem. We hypothesized that caffeine would increase urine output, thereby enhancing the rate of pulmonary fluid clearance and improving

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pulmonary function. Our aim was to determine the effect of an acute, high-dose caffeine infusion on renal and pulmonary functions in ventilated preterm lambs.

## RESULTS

Gestational age at birth, birth weight, and wet lung weight or wet/dry lung weight ratio were not significantly different between the caffeine and control groups (Table 1).

### Plasma and Urine Caffeine Concentrations

Arterial plasma caffeine concentrations were significantly elevated in the caffeine-treated lambs within 15 min of administration of caffeine ( $P < 0.001$ ; Figure 1a). The concentrations of caffeine increased further in the 30-min period after the infusion and then remained around this concentration for the duration of the experiment ( $P < 0.001$ ; Figure 1a). Concentrations of caffeine in urine were significantly elevated above control levels within 15 min of administration of caffeine and increased further in the 30-min period after the infusion ( $P < 0.001$ ; Figure 1a). The urinary caffeine concentration remained elevated for the remainder of the experiment. The concentrations of caffeine in plasma and urine in the control lambs were negligible (Figure 1a).

### Renal Function

Over the experimental period, area under the curve urine output was significantly greater in caffeine-treated lambs ( $5.86 \pm 1.95$  ml/kg) than in controls ( $0.76 \pm 0.94$  ml/kg;  $P = 0.041$ ) (Figure 1b). At 30 min after the infusion started, mean urine flow rates were  $10.2 \pm 2.5$  ml/kg/h in caffeine-treated lambs and  $5.3 \pm 1.1$  ml/kg/h in controls. For the following hour, urine flow rates remained high in the caffeine-treated lambs as compared with the controls, (Figure 1b). Urine output for the 30-min period immediately after the cessation of caffeine or saline infusion was significantly greater in both groups of lambs than urine output at 60–90 and 90–120 min after the infusion period ( $P = 0.046$ ). The volume of fluid infused into the lambs during the experiment was similar in the two groups (caffeine,  $10.3 \pm 1.2$  ml/kg; controls,  $9.6 \pm 0.33$  ml/kg).

Glomerular filtration rate was similar in the two groups of lambs (Figure 1c) as was fractional sodium clearance (Figure 1d). Concentrations of sodium, potassium, glucose, and urea in urine and plasma were similar for the two groups, as was the urinary excretion of sodium and potassium (data not shown).

**Table 1.** Gestational parameters and weights

|                           | Saline control<br>(n = 6) | 40 mg/kg caffeine<br>(n = 7) |
|---------------------------|---------------------------|------------------------------|
| Gestational age (d)       | 128 ± 1                   | 128 ± 1                      |
| Male:female ratio         | 3:3                       | 3:4                          |
| Body weight (kg)          | 3.08 ± 0.1                | 3.66 ± 0.2                   |
| Wet lung weight (g)       | 88.7 ± 7.1                | 90.2 ± 5.9                   |
| Wet/dry lung weight ratio | 8.5 ± 0.6                 | 8.5 ± 0.5                    |

Data shown as mean ± SEM. Statistical analyses were performed using unpaired t test.

### Cardiopulmonary Function

Heart rates were greater in caffeine-treated lambs as compared with controls ( $P = 0.012$ ; Figure 2a) 10 min after infusion onset; heart rates remained elevated for the duration of the experiment in caffeine-treated lambs (Figure 2a). Heart rate increased over the course of the experimental period in caffeine-treated lambs but decreased in the controls ( $P = 0.007$ ). Mean arterial pressure was not different between groups (Figure 2b).

PBF and DA blood flow, pulmonary arterial pressure, and pulmonary vascular resistance (PVR) were not significantly different between groups (Figure 2c–e). In both groups, after the initial increase in PBF and decrease in DA flow, which occurred during the 30-min stabilization period, PBF ( $P < 0.001$ ) decreased and DA flow ( $P < 0.001$ ) increased with time. Mean carotid arterial pressure ( $P < 0.001$ ), mean pulmonary artery pressure ( $P < 0.001$ ), PaO<sub>2</sub> ( $P = 0.004$ ; Figure 3a), and oxygen saturation ( $P = 0.024$ ; Figure 3b) decreased during the experiment in both groups. Conversely, PVR ( $P < 0.001$ ; Figure 2e) and PaCO<sub>2</sub> ( $P = 0.014$ ; Figure 3d) increased during the experiment in both groups. There were no significant differences in PaO<sub>2</sub>, percentage oxygen saturation of hemoglobin, PaCO<sub>2</sub>, or arterial pH between the treatment groups (Figure 3a–d).

### Ventilation Parameters

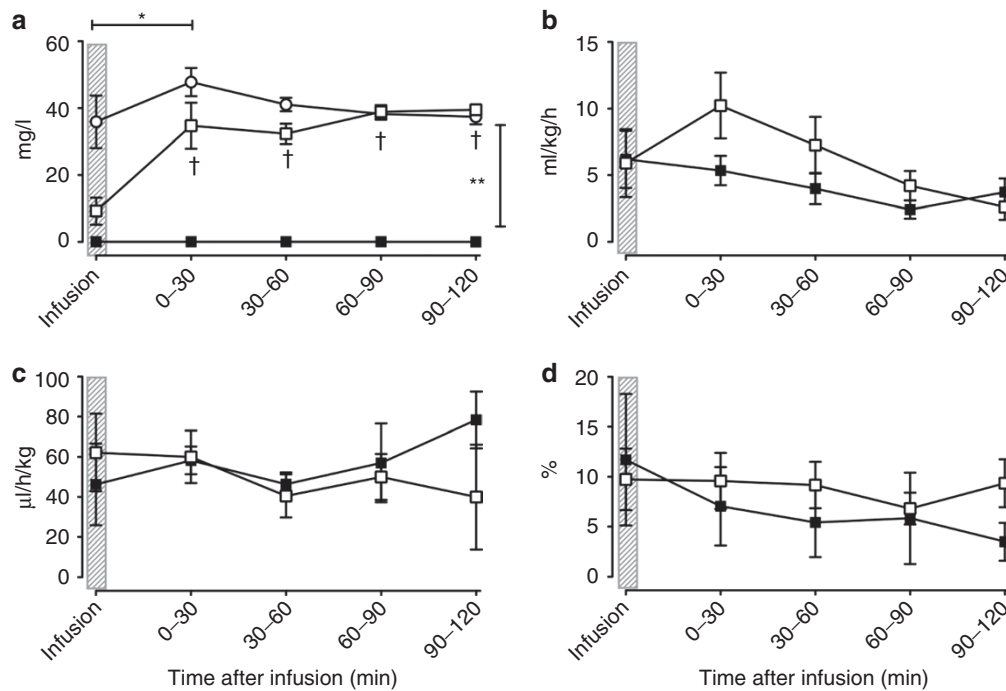
Dynamic respiratory system compliance and mean airway pressure were not significantly different between the groups (Figure 3e,f). Mean airway pressure ( $P = 0.018$ ) decreased during the stabilization period and then tended to increase in both groups. There were no significant differences in ventilator efficiency index or oxygenation index between the groups (data not shown).

## DISCUSSION

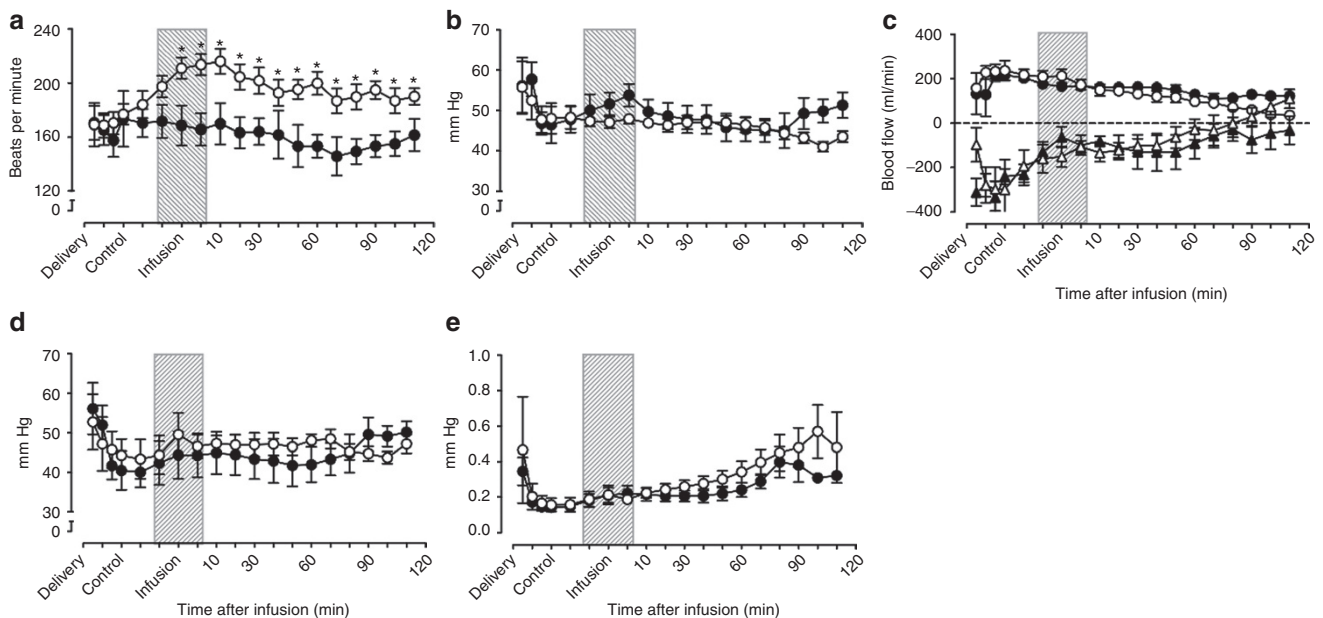
We have demonstrated that intravenous administration of caffeine, sufficient to increase the heart rate and urine output, has a limited effect on renal function and no acute effect on cardiopulmonary function in ventilated preterm lambs. To our knowledge, this is the first study to investigate all of these functional parameters concurrently in ventilated preterm experimental subjects with lung maturation similar to that of infants born around 30–34 wk gestation.

### Caffeine Concentration

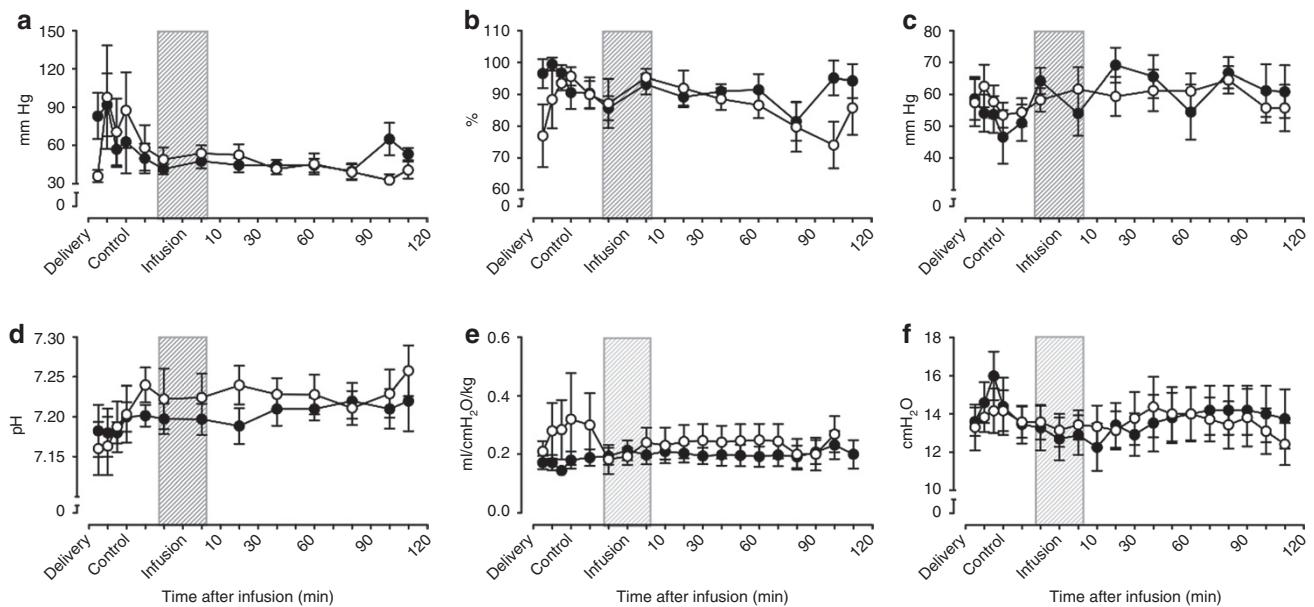
We chose a dose of caffeine within the upper end of the range of doses used clinically. The current commonly prescribed dosing regimen for preterm infants with apnea is a loading dose of 10–20 mg/kg caffeine base intravenously or orally, followed by a daily maintenance dose of 2.5–5 mg/kg caffeine base (24). This regimen has proven effective for more than 30 y and is sufficient to prevent or decrease apnea and increase respiratory drive (7). The higher dose used in our study is one that may be used to reduce apnea and facilitate extubation in preterm infants being weaned from mechanical ventilation (25). We have demonstrated that concentrations of caffeine are rapidly increased in both plasma (40 mg/l) and urine (30 mg/l),



**Figure 1.** Data obtained from caffeine-treated (white symbols) and control (black symbols) lambs over the experimental period. The shaded bars show the period of caffeine infusion. **(a)** Concentrations of caffeine in plasma (circles) and urine (squares); \* indicates significant difference in caffeine concentrations at time points indicated by bar ( $P < 0.001$ ); † indicates significant difference in urinary concentrations of caffeine at those time points compared with the infusion period ( $P < 0.001$ ); \*\* indicates significant difference in plasma and urinary concentrations of caffeine between the treatment groups ( $P < 0.001$ ); **(b)** urine flow rates ( $P = 0.041$ ); **(c)** glomerular filtration rate (not significant); **(d)** urinary fractional sodium clearance (not significant). Urine flow rates over the experimental period were assessed by measuring the area under the curve and differences between the groups were compared using an unpaired *t* test ( $P = 0.041$ ). Values were obtained at 30-min intervals, and data are presented as mean  $\pm$  SEM.



**Figure 2.** Physiological data obtained in caffeine-treated (white symbols) and control (black symbols) lambs. The shaded bars show the period of caffeine infusion. **(a)** Heart rate; \* indicates significant difference between caffeine-treated and control lambs at those time points during the experiment ( $P = 0.012$ ). Heart rate increased over the course of the experiment in the caffeine group but decreased in the saline control group ( $P = 0.007$ ); **(b)** mean arterial pressure decreased ( $P < 0.001$ ) during the experiment in both the groups; **(c)** left pulmonary artery blood flow (circles) decreased and ductus arteriosus blood flow (triangles) increased during the experiment in both groups ( $P < 0.001$ ); **(d)** pulmonary artery pressure decreased ( $P < 0.001$ ) after delivery; and **(e)** pulmonary vascular resistance increased ( $P < 0.001$ ) in both groups. The figure shows values recorded just after delivery, during the 30-min control period, during the 30-min infusion period, and at regular intervals after the infusion. Data are presented as mean  $\pm$  SEM.



**Figure 3.** Comparison between caffeine-treated (white symbols) and control (black symbols) lambs for (a) partial pressure of oxygen in arterial blood, which significantly decreased ( $P = 0.004$ ); (b) arterial blood saturation, which decreased ( $P = 0.024$ ); (c) partial pressure of carbon dioxide in arterial blood, which increased ( $P = 0.01$ ); (d) arterial pH, which was unchanged during the experiment in either group; (e) dynamic respiratory compliance; (f) mean airway pressure, which decreased over the experimental period in both the groups ( $P = 0.018$ ). The shaded bars show the period of caffeine infusion. The figure shows values recorded just after delivery, during the 30-min control period, during the 30-min infusion period, and at regular intervals after the infusion. Data are presented as mean  $\pm$  SEM.

reaching a maximum within 60 min of commencing the infusion; these high concentrations were maintained for the remainder of the experimental period. The plasma concentrations of caffeine detected are comparable with the therapeutic range for the treatment of apnea in preterm infants (5–50 mg/l) (9). We anticipated maintenance of plasma caffeine concentrations after our 30-min infusion because the half-life of caffeine is estimated to be 60–140 h in preterm infants (26).

### Renal Function

The methylxanthine theophylline has been shown to improve renal function during the first 2 d after birth in very preterm infants with respiratory distress syndrome (27) and the renal effects of methylxanthines appear to be dose- and time-related in newborn rabbits (28). We hypothesized that caffeine would reduce liquid accumulation in the lungs through its diuretic action, therefore facilitating improved lung function. Although we found no evidence of a change in pulmonary water content (wet/dry lung weight ratio) at the end of the experiment or pulmonary function throughout the experiment, caffeine did increase urine flow. At 30–60 min after beginning the caffeine infusion, urine flow rates were approximately double those measured in control lambs. As a result, caffeine-treated lambs lost ~5 ml/kg of liquid more than saline-treated lambs over the 2-h experiment.

Apart from an increase in urine flow, there were no changes in glomerular filtration rate, urinary excretion of sodium and potassium, or the fractional clearance of sodium, indicating minimal effects on renal function. Previous studies in preterm infants have shown that administration of caffeine increased

urine flow rate, increased creatinine clearance (as an indicator of glomerular filtration rate), and affected other renal functions (including urinary sodium excretion) (29). However, these effects were observed more than 12 h after commencing caffeine and occurred in response to a much lower dose (7.5 mg/kg) than we used. Presumably, therefore, the effects of caffeine on aspects of renal function other than urine output take some time to manifest.

### Cardiovascular Function

The physiological responses to caffeine infusion observed in our study included an increase in heart rate and urine output. However, these responses did not translate into any other physiological changes, including effects on mean arterial pressure, pulmonary artery pressure, PVR, or blood-gas parameters. These findings are in contrast to other studies that found intravenous caffeine increased cardiac output and blood pressure in preterm infants, around 60 min after administration, even at a lower dose (5 mg/kg caffeine base) than we used (30). In dogs, methylxanthines decreased airway resistance, pulmonary artery pressure, and PVR, and increased lung compliance and PBF (31,32). Again, these previously observed changes presumably take some time to manifest and our 2-h experimental period may not have been long enough to induce these additional physiological responses.

The recently reported clinical trial of infants treated with caffeine found that administration of caffeine resulted in infants being less likely to require pharmacological or surgical closure of a patent DA (18). Therefore, we considered that caffeine might have a direct effect on the pulmonary vasculature and DA.



However, we found no difference in PBF or blood flow through the DA between caffeine and saline-treated lambs. Our findings are consistent with those of a recent study demonstrating that, in isolated DA from fetal sheep, caffeine had no direct effect on vessel contractility (33). The temporal changes in PBF and DA blood flow we observed are consistent with our previous studies, which have shown that immediately after birth retrograde flow (left-to-right shunting) through the DA contributes to the increase in PBF. Subsequently, PBF gradually decreases with time, mostly because of a gradual reduction in the contribution of left-to-right flow through the DA as it closes (17).

### Pulmonary Function

In preterm infants, caffeine has been shown to increase breathing frequency, decrease the number of apneic events, and reduce PaCO<sub>2</sub>, the need for mechanical ventilation, and the duration of ventilation (4,34). Caffeine may also improve ventilation in preterm infants by improving or stabilizing diaphragm muscle function and preventing diaphragm fatigue (9). Our experimental design deliberately obviated our ability to determine effects on breathing frequency or diaphragm function, as the lambs were anesthetized and mechanically ventilated because we wanted to avoid the confounding effects of caffeine on respiratory drive.

Caffeine has been shown to improve respiratory compliance and reduce supplemental oxygen requirements during the first postnatal week in preterm infants with respiratory distress syndrome, 24 h after treatment (35). Improvement in lung mechanics has previously been shown to occur in infants with bronchopulmonary dysplasia within 1 h of caffeine therapy (36). We did not find any changes in dynamic lung compliance and it is unlikely that airway resistance was altered in our lambs treated with caffeine. Further, a study in preterm baboons treated with caffeine demonstrated improvement in lung function and a twofold increase in urine output in the initial 24 h after birth (37). Our study shows that these established effects of neonatal administration of caffeine are not a consequence of the immediate, acute effects of the treatment. Our inability to detect an effect of caffeine on lung water content suggests that the physiological benefit of administration of caffeine observed in previous studies is not a consequence of improved liquid clearance from the neonatal lungs.

### Experimental Model

We chose to investigate functional parameters concurrently in ventilated preterm experimental subjects with lung maturation similar to that of infants born around 30–34 wk gestation. At this gestational age (~126 d gestation) lambs have very immature lungs and are not viable without respiratory support. This study was purposely designed to understand the physiological concepts involved in the relationship of caffeine to renal, and pulmonary functions. We were not trying to exactly replicate the protocols and procedures used in the clinical setting, instead we were trying to answer a specific scientific question that is relevant to and improves our understanding of the clinical use of caffeine in neonates.

### Conclusion

We conclude that intravenous administration of caffeine, which causes an increase in heart rate and urine output, has no effect on cardiopulmonary function during 2 h of ventilation in preterm lambs. Findings from this study demonstrate that acute, high-dose caffeine treatment in ventilated preterm lambs is unlikely to influence cardiopulmonary function or exacerbate poor outcomes in the short term. This suggests that the beneficial effects of caffeine in preterm infants may be central, rather than pulmonary, in origin.

### METHODS

#### Experimental Protocol

The relevant Monash University animal ethics committee approved all experimental procedures. Aseptic surgery was conducted on 13 pregnant ewes at  $\sim 122 \pm 2$  d (mean  $\pm$  SD) of gestation (term is  $\sim 147$  d) using established techniques (16). Briefly, catheters were inserted into a fetal carotid artery and jugular vein, the amniotic sac and left pulmonary artery; ultrasonic flow probes (Transonic Systems, Ithaca, NY) of 4 mm diameter were placed around the left pulmonary artery and DA. After recovery from surgery, ewes were kept in individual cages. Fetal well-being was monitored daily by measuring fetal PaO<sub>2</sub>, PaCO<sub>2</sub>, pH, and percentage oxygen saturation of hemoglobin (ABL30, Radiometer, Denmark). Mean arterial blood pressure, pulmonary arterial pressure, and pulmonary and DA blood flows were recorded digitally (ADInstruments, N.S.W., Australia).

#### Delivery and Ventilation

At  $126 \pm 2$  d of gestation, ewes and fetuses were anesthetized by maternal administration of sodium thiopentone (100 mg i.v.) followed by 2% isoflurane in O<sub>2</sub> and the fetal head and neck exposed via caesarean section. The fetal trachea was orally intubated with a 3.5 mm cuffed tube and lung liquid was drained passively before the umbilical cord was clamped and cut. Lambs were then delivered, dried, weighed, and placed under a radiant heater and ventilated (Babylog 8000+; Dräger, Germany; in volume guarantee mode) with an expired tidal volume of 5 ml/kg, ventilator rate of 60 breaths/min, a positive end-expiratory pressure of 5 cmH<sub>2</sub>O, and an initial fraction of inspired oxygen of 1.0. Expiratory time and initial fraction of inspired oxygen were altered (depending on blood-gas values) to maintain arterial pH between 7.30 and 7.45, PaCO<sub>2</sub> between 40 and 55 mm Hg, and percentage oxygen saturation of hemoglobin between 90 and 95%. Arterial blood gases (pH, PaCO<sub>2</sub>, PaO<sub>2</sub>, and percentage oxygen saturation of hemoglobin) were collected and adjusted for body temperature every 5 min for the first 20 min, then every 10 min for the rest of the experimental period. Lambs were sedated (Alfaxalone, 15 mg/h i.v., Jurox, Australia) in 5% dextrose (6 ml/h i.v.) to prevent spontaneous breathing. All lambs were ventilated for a 30-min stabilization period, during which a bladder catheter was inserted to permit timed collections of urine. An incision was made below the umbilical cord in the abdomen and the bladder was located by blunt dissection. A catheter (size 5.0 French) was secured into the bladder using a purse-string suture.

Following the initial 30-min ventilation period, caffeine base (Auspman, WA, Australia) in a 10 mg/ml solution was administered intravenously at 40 mg/kg ( $n = 7$ ) over a 30-min period. Control lambs ( $n = 6$ ) were infused with saline over the same period. Lambs were then ventilated for a further 120 min.

At 126 d gestation lambs have very immature lungs and are not viable without respiratory support. As this study was purposely designed to understand the physiological concepts involved in the relationship between caffeine and renal and pulmonary functions, we did not administer antenatal glucocorticoids or postnatal surfactant.

#### Analytical Methods

**Renal function.** Serial urine samples were collected after the initial stabilization period over 30-min intervals throughout the experiment.

An arterial blood sample was collected midway between successive urine collections. Blood samples were transferred immediately to lithium heparin tubes and centrifuged at 4 °C and 2,500 rpm. Plasma and urine samples were frozen at -20 °C for subsequent measurement of caffeine, sodium, potassium, creatinine, glucose, and urea (Synchron CX5 Delta clinical analyzer, Beckman Coulter, Brea, CA). The following parameters were calculated:

1. Urine flow rate (ml/h/kg)
2. Glomerular filtration rate ( $\mu\text{l/h/kg}$ ; assessed using creatinine clearance); glomerular filtration rate = urine flow rate  $\times$  urine  $\frac{\text{creatinine}}{\text{plasma creatinine}}$
3. Urinary fractional sodium clearance (%); urinary fractional sodium clearance = (excretion rate  $\frac{\text{sodium}}{\text{filtered sodium}}$ )  $\times$  100;

where: excretion rate  $\frac{\text{sodium}}{\text{filtered sodium}}$  = urine flow rate  $\times$  urine  $\frac{\text{sodium}}{\text{plasma sodium}}$ ; and filtered  $\frac{\text{sodium}}{\text{glomerular filtration rate}} \times \text{plasma sodium}$ .

**Pulmonary function.** Mean arterial pressure, left pulmonary artery pressure and blood flow, and DA blood flow were recorded continuously. PVR was calculated using the formula  $\text{PVR} = \text{Ppa} - \text{Pla}/\text{Qp}$ , where Ppa is pulmonary arterial pressure, Pla is left atrial pressure and Qp is flow through the left pulmonary artery; Pla was assumed to be 9 mm Hg (16,38).

Ventilation parameters, including dynamic respiratory compliance and mean airway pressure were recorded every 10 min. Lung tissue samples were collected for determination of the wet/dry lung weight ratio as an index of interstitial tissue fluid content; samples were dried (at 60 °C) and weighed daily until there was no change in the dry weight over 3 d.

### Statistical Analysis

Concentrations of caffeine in plasma and urine, concentrations of electrolytes in urine and plasma, blood-gas values, and renal and pulmonary function values were analyzed using two-way repeated measures ANOVA with treatment (caffeine vs. saline infusion) and time as factors. If significant effects of treatment group and/or time were found, Fisher's test of least significant difference was used, *post hoc*, to identify the time points at which there were differences between the groups. Urine flow rates over the experimental period were assessed by measuring the area under the curve and differences between groups were compared using an unpaired *t* test. An unpaired *t* test was used to compare gestational age at birth, birth weight, wet lung weight, wet/dry lung weight ratio, and the volume of fluids infused during the experiment between the caffeine and saline control groups. Data are presented as mean  $\pm$  SEM. The level of statistical significance was  $P < 0.05$ .

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