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Reversal of Depressed Miduterine Arterial Flow during Hyperthermia-Induced Respiratory Alkalosis (42791)

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Abstract. The influence of ambient and arterial PCO_2 on miduterine arterial flow of pregnant sheep acutely exposed to hot environments was investigated. Five mixed-breed ewes between 120 and 130 days of gestation were subjected to hot environments (increasing from thermoneutral 23 to 40°C), and arterial blood pH, PCO_2, and PO_2 were determined at 5-min intervals. Respiratory rate, heart rate, rectal temperature, blood pressure, and miduterine arterial flow were continuously monitored prior to and during elevation of ambient air temperature. When miduterine arterial flow had decreased to 50% of thermoneutral control levels, ambient air CO_2 was increased to 2.5%. Elevated ambient inspired CO_2 caused a reversal in arterial pH and PCO_2 to near thermoneutral levels. Miduterine arterial flow increased to 77% of the control levels following the elevated ambient PCO_2 period. Respiratory rate also decreased when ambient CO_2 was increased but remained 136% greater than the thermoneutral control level. All other parameters remained near their heat stress (40°C) level during the elevation of ambient CO_2. These data indicate that heat-stress-induced depression of miduterine arterial flow is vasoactively regulated, and cause–effect related to both arterial pH and PCO_2, and thermoregulatory shunting of blood to heat-dissipating surfaces.

Heat stress during late gestation has led to intrauterine growth retardation (IUGR) in sheep (1–4) and cattle (5, 6). Ewes at thermoneutrality restricted to the feed intake of heat-stressed ewes produce normal birthweight lambs (3, 6). Fetal nutrition, however, may still be compromised during heat stress since Oakes *et al.* (7), Roman-Ponce *et al.* (8, 9), and Brown and Harrison (10, 11) have demonstrated that decreased miduterine arterial flow occurs during maternal hyperthermia.

Increased miduterine arterial flow accounts for all increased uterine nutrient uptake of substrates as gestation proceeds (12–14) and recent data in our laboratory suggest a lack of nutrient uptake compensation for decreased miduterine arterial flow in late gestation. It is thus imperative that we understand the mechanisms which alter miduterine arterial flow during heat stress in late pregnancy. α-Adrenergic vasoconstriction of the uterine vascular bed has been reported (9, 15, 16). β-Adrenergic receptors are present in the uterine circulation of sheep (17) but do not affect miduterine arterial flow at thermoneutrality. Heat-stress-induced suppression of uterine blood flow, however, occurs despite blockade of sympathetic ganglia or β-adrenergic control systems (10, 11).

Metabolically linked vasoactive compounds may be responsible for miduterine arterial flow control during maternal hyperthermia. Uterine vascular H^+ and/or CO_2 have been shown to alter uterine vascular resistances during normothermia (18) and during hyperthermia while rebreathing CO_2 (7). An experiment was thus conducted on unanesthetized chronically instrumented pregnant ewes not rebreathing expired air to determine if thermally induced hypocapnia is responsible for suppression of uterine blood flow.

Materials and Methods. Five sheep of mixed breeding with timed twin pregnancies were used between 120 and 130 days of gestation. Ewes were placed in a plexiglass calorimetry stall for 3 days of acclimation and

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fasted for 24 hr prior to surgery. Under continuous ketamine anesthesia (0.25 mg/kg/min, iv) and electromagnetic blood flow transducer (ER416, Carolina Medical Electronics, King, NC) was implanted around the left miduterine artery and a polyvinyl catheter was sutured into the left carotid artery as described previously (10).

Beginning 4 days after surgery, five ewes were utilized for 11 heat stress experiments in which inspired CO_2 gas was elevated to 2.5% in the calorimetry chamber for 15 min at the time when miduterine arterial flow had decreased 50%. Chamber O_2 and CO_2 were continuously monitored[2] at a chamber air exchange rate of 120 liter/min. All ewe heat stress exposures were separated by at least 48 hr.

Ewes were instrumented on the morning of a heat stress episode. Catheter and transducer cables were exteriorized through a chamber porthole and the ewes remained undisturbed in the chamber during heat exposure. Chamber air temperature and ewe rectal temperature recorded at a depth of 6 cm were recorded from a YSI 47 telethermometer.[3] Rectal temperature thermistors expelled from ewes during heat stress were not replaced in order to not disturb chamber gas status. Respiratory rate, heart rate, carotid artery blood pressure, and uterine blood flow[4] were recorded on a polygraph. Uterine vascular resistance was calculated by dividing mean arterial pressure by miduterine arterial blood flow. Venous blood pressure and intraamniotic pressures were assumed to remain relatively constant and low in relation to arterial pressure. Therefore, miduterine arterial vascular resistance was calculated as previously reported (19). Carotid artery blood samples were collected into glass syringes, stored on ice, and measured for oxygen (P_aO_2), carbon dioxide (P_aCO_2), and pH (pH_a) within 10 min of collection.[5] The pH/blood gas analyzer thermostat was set to maintain the blood analysis chamber bath near the recorded rectal temperature at 39 ± 1°C. At least 30 min after all instrument calibration, blood samples were taken initially and at 5-min intervals spanning a 15-min thermoneutral (22.8°C) control period. Control period mean values are the average of the four values spanning the 15-min period. A 58-cm, 600-W radiant heat tube in the chamber was then turned on and T_a allowed to reach 40°C at an air exchange rate of 120 liters/min. Each ewe was monitored for all parameters until miduterine arterial flow had decreased to 50% of the thermoneutral control period flow rate. At this time, gas from a CO_2 tank was directed into the chamber air intake port and mixed by an internal recirculating fan for complete mixing prior to reaching the ewe's face. The CO_2 of 2.5% was maintained in the inspired air by continuously monitoring exhaust CO_2 content. Equilibrium was reached within 2 min and maintained for 15 min at which time the CO_2 was turned off and inspired CO_2 concentration returned to less than 1% in the subsequent 10-min period.

The following multiple linear regression model was fitted to the data from each animal for uterine blood flow, heart rate, respiratory rate, blood pressure, blood pH, blood PCO_2, blood PO_2, inspired oxygen, and inspired carbon dioxide,

$$y_{ij} = \beta_O + \beta_1 t_j + \beta_2 t_j^2 + \beta_3 t_j^3 + \theta x_{i(j-1)} + e_{ij},$$

where

y_{ij} is the value of the dependent variable (e.g., miduterine arterial flow) in animal i at time t_j

t_j is time from elevation of inspired CO_2

β_O is an effect common to all observations

β_1, β_2, and β_3 are the linear, quadratic, and cubic effects of time (t_j)

$x_{i(j-1)}$ is the percentage CO_2 in the chamber for animal i at time t_{j-1}

θ is the linear regression of y_{ij} on $x_{i(j-1)}$

e_{ij} is a random residual.

Also, orthogonal polynomial regression coefficients for t_j and θ were estimated by multiple regression. The effects of temperature and CO_2 on the dependent variables were tested for statistical significance by performing t tests on these regression coefficients (20). The predicted values of the dependent variables during heat stress at time 0 (CO_2

[2] OM 11 gas analyzer, LB2 CO_2 gas analyzer, Beckman Instrument Co., Irvine, CA.

[3] Yellow Springs Instruments, Yellow Springs, OH.

[4] Model 501, Carolina Medical Electronics, King, NC.

[5] Micro 13, Instrumentation Labs, Lexington, MA.

on), 15 min (CO_2 off), and 30 min (+15) were compared to the thermoneutral values of these variables by paired t tests (Table I).

Results. Administration of CO_2 without reduction of P_aO_2 during chronic heat stress results in reversal of both heat-induced respiratory alkalosis and depressed uterine blood flow. Miduterine artery blood flow decreased 50% from an average of 467 to 234 ml/min during a 40°C acute heat exposure (Fig. 1). Maximum reduction in miduterine arterial flow occurred an average of 109 min from the onset of heat challenge. The respiratory system response to heat was evident as panting occurred. The respiratory rate increased from 63 to 220 breaths/min just prior to CO_2 administration. Arterial CO_2 decreased to 10.2 mm Hg and pH_a reached 7.85 at this time (Fig. 2). Chamber CO_2 was then raised to 2.4% within 5 min and maintained during the 15-min exposure period. Chamber O_2 was simultaneously diluted to 19.3% but ewe P_aO_2 was not significantly changed and remained greater than 90 mmHg during the time of elevated chamber CO_2. Reversal of the heat-induced respiratory alkalosis was nearly complete by the end of 15 min exposure to 2.5% CO_2. Predicted P_aCO_2 increased from 16 to 22 mm Hg ($P < 0.05$) and pH_a decreased from 7.78 to 7.64 (Table I). Both P_aCO_2 and pH_a had thus returned to values seen during thermoneutrality while heart rate and respiratory rate remained elevated during the continuous heat exposure (Table I). An increase in miduterine arterial flow ($P < 0.0001$) due primarily to a decrease in uterine vascular resistance ($P < 0.02$) was seen concurrent with reversal of respiratory alkalosis and exposure to 2.5% CO_2 (Fig. 1). Arterial blood pressure did not change during the 15-min elevation of chamber CO_2.

The thermally-induced elevation in respiratory rate was abruptly reduced ($P < 0.01$) following exposure to the 2.5% CO_2 (Fig. 2). However, the respiratory frequency remained greater ($P < 0.001$) than that during the thermoneutral period (Table I). The temperature-controlled chamber could not be opened during the period of increased ambient CO_2 and only two of the rectal thermistors were maintained in place. Therefore, the relative effect of the CO_2 suppression of respiratory panting on body temperature could not be ascertained. However, the two

TABLE I. PHYSIOLOGICAL RESPONSES OF PREGNANT EWES TO ELEVATED ATMOSPHERIC CO_2 DURING ACUTE HEAT STRESS[a]

Parameter	Thermoneutral	Heat period[b]		
		CO_2 on	CO_2 off	+15 min
Inspired O_2 (%)	20.5	20.0	19.2	19.9
Inspired CO_2 (%)	0.25	0.43	2.5	0.85
Air temp. (°C)	22.8	40.3	40.8	40.8
Rectal temp. (°C)	38.8	40.8	—	—
Heart rate (beats/min)	110	136 (0.003)	147 (0.000)	164 (0.009)
Resp. rate (breath/min)	63	174 (0.001)	156 (0.000)	180 (0.000)
P_aO_2 (mm Hg)	86.8	95 (0.000)	94 (0.000)	89 (0.494)
P_aCO_2 (mm Hg)	28.6	16 (0.000)	22 (0.072)	2.7 (0.000)
pH_a	7.53	7.78 (0.000)	7.64 (0.042)	7.91 (0.000)
Blood pressure (mm Hg)	105	112 (0.054)	122 (0.005)	102 (0.687)
Miduterine arterial flow (ml/min)	467	258 (0.000)	344 (0.000)	292 (0.001)
Uterine vascular resistance (mm Hg · ml^{-1} · min)	0.29	0.54 (0.000)	0.48 (0.002)	0.50 (0.003)

[a] Physiological parameters during the heat period (40°C) are the predicted values compared to the actual thermoneutral values. Values in parentheses indicate level of significance of these variables compared to thermoneutral by paired t tests.

[b] CO_2 on is the mean parameter measurement immediately prior to elevation of ambient CO_2 to 2.5%. CO_2 off is the mean parameter measurement immediately prior to returning ambient CO_2 toward control period levels. +15 min is the mean parameter measurement at 15 min after supplemental ambient CO_2 was turned off.

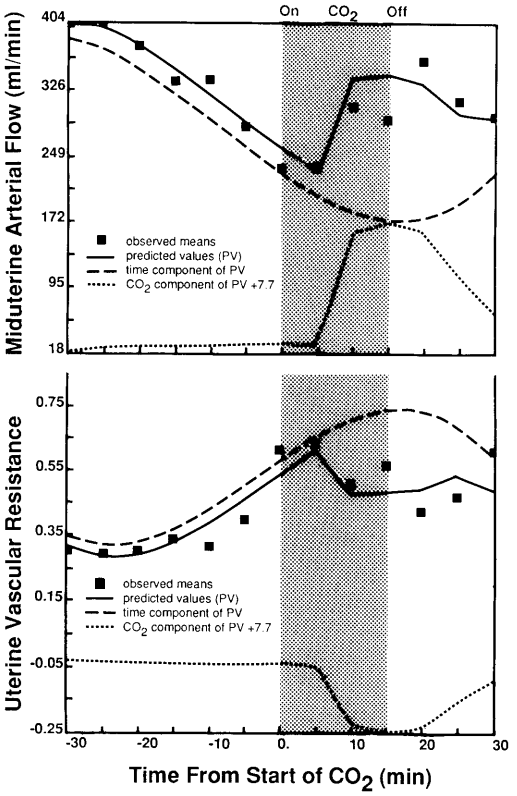

FIG. 1. Change in miduterine arterial flow and uterine vascular resistance (arterial blood pressure/miduterine arterial flow) of pregnant ewes during exposure to a hot ambient temperature (40°C) and changes in ambient inspired CO_2. The time component for change in blood flow and vascular resistance at 40°C was significant at $P < 0.0001$ and 0.005, respectively. The CO_2 component of change in blood flow and vascular resistance was significant at $P < 0.0001$ and 0.016, respectively.

ewes that were measured during this period showed an elevation in mean rectal temperature to 41.9°C.

Termination of elevation of inspired CO_2 resulted in the ewes returning toward respiratory alkalosis (Fig. 2), increased uterine vascular resistance, and decreased miduterine arterial flow (Fig. 1). During the first 5 min following reduction of chamber CO_2, miduterine arterial flow continued to increase (Fig. 1). At 10 min after elevation of inspired CO_2, ambient CO_2 was down to 1%, the blood gases were similar to pre-CO_2 levels, and miduterine arterial flow began decreasing again. The estimated value of θ for uterine blood was 65.59 ($P < 0.0001$), indicating a strong relationship between miduterine arterial flow at time t_j and percentage CO_2 in the chamber at time t_{j-1} (Fig. 1).

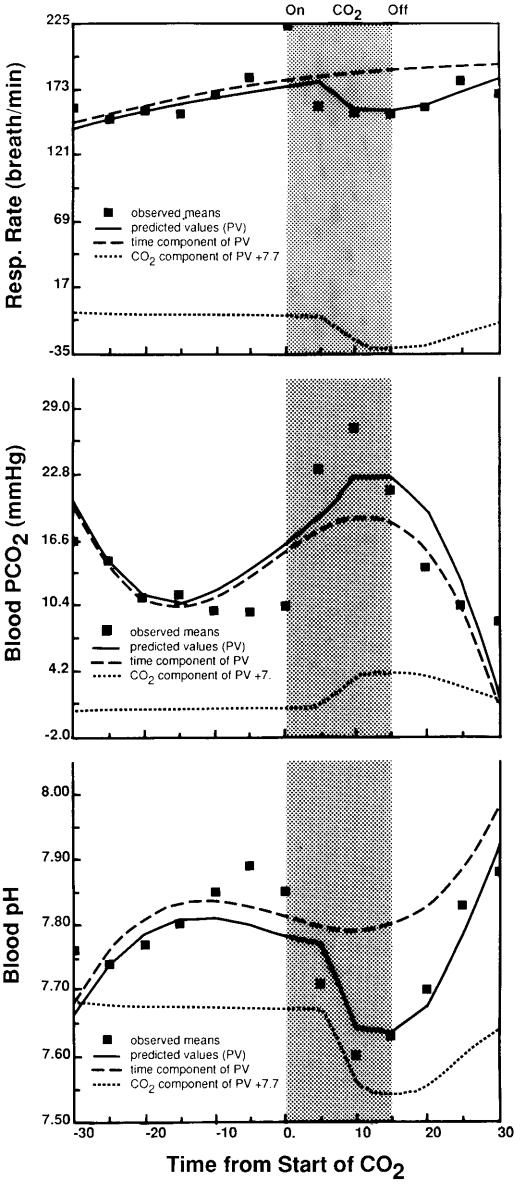

FIG. 2. Change in respiratory rate, P_aCO_2, and pH_a of pregnant ewes during exposure to a hot ambient temperature (40°C) and changes in ambient inspired CO_2. The time component for change in respiratory rate, P_aCO_2, and pH_a at 40°C was significant at $P < 0.069$, 0.001, and 0.027, respectively. The CO_2 component for change in respiratory rate, P_aCO_2, and pH_a was significant at $P < 0.014$, 0.016, and 0.001, respectively.

Discussion. Sheep respond to heat stress with a biphasic system of panting (21). Above a body temperature of 40.5°C ewes are generally in second-phase panting with up to a fivefold increase in alveolar ventilation. Pregnant ewes in the current experiment were definitely in second-phase panting at the time of altering chamber gases. The rectal temperature of 40.8°C, P_aCO_2 of 10.2 mm Hg, and pH_a of 7.85 all indicate severe respiratory alkalosis. Ewes responded with a 50% reduction in miduterine arterial flow as seen previously in both hyperthermic and normothermic procedures (7, 10, 11, 22). Maximum depression of miduterine arterial flow occurred despite a systemic blood pressure equal to or higher than that during the thermoneutral control period. The doubling of uterine vascular resistance thus implies active vasoconstriction in the uterine vasculature. Employing a similar heat stress protocol, we have previously observed a consistently suppressed miduterine arterial flow for over 2 hr of heat exposure and ruled out central and peripheral β-sympathetic vasoconstriction as the controlling mechanism (10, 11). This is consistent with the current concept of pregnancy-induced uterine sympathetic denervation. Little or no adrenergic transmitter is present by late pregnancy and considerable disappearance of adrenergic fibers around the growing conceptus occurs (23). It appears, however, that uterine vascular resistance is responsive to the pH and PCO_2 system.

The uterine vascular resistance, which had increased by the start of elevated CO_2, decreased with increased chamber CO_2 to a value not different from that of the thermoneutral period. The uterine vascular resistance increased again after CO_2 supplementation was discontinued. These changes in uterine vascular resistance all occurred with no decrease in P_aO_2, since a rebreathing bag was not employed and RR remained elevated. These findings fully support the results of Oakes *et al.* (7), who found that reversal of alkalosis accounted for one-half of heat-induced reduction of uterine blood flow. In the present study miduterine arterial flow decreased during heat stress to 49.7% of the control period and increased to 77% of the control period 5 min after the end of the elevated inspired CO_2. The question remains whether a longer period of elevated CO_2 gas during panting would have allowed miduterine arterial flow to fully return to thermoneutral flow rates or whether separate control mechanisms are simultaneously at work as suggested by Oakes *et al.* (7). However, the indication of severe hyperthermia caused by the CO_2 suppression of panting would not permit expanded exposure to the 2.5% CO_2 atmosphere. Vasoconstrictive H^+ and/or CO_2 receptors are present in the pregnant ovine uterine vasculature (24). However, the relative contribution of thermoregulatory blood shunts to heat exchange areas (21) and direct vascular pH and PCO_2 effects on miduterine arterial flow regulation in hot environments remain to be delineated.

Ovine fetal dwarfing is a consequence of untreated maternal heat stress. Fetal homeostasis is influenced by uteroplacental blood flow (25), which is directly affected by maternal respiratory alkalosis, rather than hyperthermia per se (7). It is imperative therefore that we determine whether uterine vasoconstrictive processes can be reversed by treatment with H^+ or CO_2 in order to best prevent impaired fetal development arising from hot-humid conditions during pregnancy.

1. Yeates NTM. The effect of high air temperature on reproduction in the ewe. J Agric Sci **43**:199–216, 1953.
2. Shelton M. Relation of birth weight to death losses and certain productive characteristics of fall born lambs. J Anim Sci **23**:355–359, 1964a.
3. Shelton M. Relation of environmental temperature during gestation to birth weight and mortality of lambs. J Anim Sci **23**:360–366, 1964b.
4. Brown DE, Harrison PC, Hinds FC, Lewis JA, Wallace MH. Heat stress effects on fetal development during late gestation in the ewe. J Anim Sci **44**:442–446, 1977.
5. Collier RJ, Doelger SG, Head HH, Thatcher WW, Wilcox CJ. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight, and postpartum milk yield of Holstein cows. J Anim Sci **54**:309–319, 1982.
6. Cartwright GA, Thwaites CJ. Foetal stunting in sheep. I. The influence of maternal nutrition and high ambient temperature on the growth and proportions of Merino foetuses. J Agric Sci **86**:573–580, 1976.
7. Oakes GK, Walker AM, Ehrenkranz RA, Cefalo

RC, Chez RA. Utero placental blood flow during hyperthermia with and without respiratory alkalosis. J Appl Physiol **41**:197–201, 1976.
8. Roman-Ponce HH, Thatcher WW, Caton D, Barron DH, Wilcox CJ. Effects of thermal stress and epinephrine on miduterine arterial flow in ewes. J Anim Sci **46**:167–174, 1978a.
9. Roman-Ponce HH, Thatcher WW, Caton D, Barron DH, Wilcox CJ. Thermal stress effects on miduterine arterial flow in dairy cows. J Anim Sci **46**:175–180, 1978b.
10. Brown DE, Harrison PC. Central sympathetic control of uterine blood flow during acute heat stress. J Anim Sci **52**:1114–1121, 1981.
11. Brown DE, Harrison PC. Lack of peripheral sympathetic control of miduterine arterial flow during acute heat stress. J Anim Sci **59**:182–188, 1984.
12. Comline RS, Silver M. Some aspects of foetal and uteroplacental metabolism in cows with indwelling umbilical and uterine vascular catheters. J Physiol **260**:571–586, 1976.
13. Clapp JF. Cardiac output and miduterine arterial flow in the pregnant ewe. Amer J Obstet Gynecol **130**:419–423, 1978.
14. Ferrell DL, Ford SP. Bloodflow, steroid secretion, and nutrient uptake of the gravid bovine uterus. J Anim Sci **50**:1113–1121, 1980.
15. Ladner C, Brinkman III CR, Weston P, Assali NS. Dynamics of uterine circulation in pregnant and nonpregnant sheep. Amer J Physiol **218**:257–263, 1970.
16. Rosenfeld CR, Barton MD, Meschia G. Effects of epinephrine on distribution of blood flow in the pregnant ewe. Amer J Obstet Gynecol **124**:156–163, 1976.
17. Greiss FC, Pick JR. The uterine vascular bed: Adrenergic receptors. Obstet Gynecol **23**:209–213, 1964.
18. Buss DD, Bisgard GE, Rawlings CA, Rankin JHG. Utero placental blood flow during alkalosis in the sheep. Amer J Physiol **228**:1497–1500, 1975.
19. Magness RR, Rosenfeld CR. Sympathetic and uterine responses to α-adrenergic stimulation in pregnant and nonpregnant ewes. Amer J Obstet Gynecol **155**:897–904, 1986.
20. Allen OB, Burton JH, Holt JD. Analysis of repeated measurements from animal experiments using polynomial regression. J Anim Sci **57**:765–770, 1983.
21. Hales JRS. Effects of exposure to hot environments on the regional distribution of blood flow and on cardiorespiratory function in sheep. Pfluegers Arch **344**:133–148, 1973.
22. Levinson G, Schnider SM, deLorimier AA, Steffenson JL. Effects of maternal hyperventilation on uterine bloodflow and fetal oxygenation and acid-base status. Anesthesiology **40**:340–347, 1974.
23. Owman C, Alm Per, Sjoberg N-O, Stjernquist M. Structural, biochemical, and pharmacological aspects of the uterine autonomic innervation and its remodeling during pregnancy. In: Huszar, G, Ed. The Physiology and Biochemistry of the Uterus in Pregnancy and Labor. Boca Raton, Florida, CRC Press, 1986.
24. Haddy FJ, Scott JB. Metabolically linked vasoactive chemicals in local regulation of uterine blood flow. Physiol Rev **48**:688–707, 1968.
25. Skillman CA, Plessinger MA, Woods JR, Clark KE. Effect of graded reductions in uteroplacental blood flow on the fetal lamb. Amer J Physiol **249**:H1098–H1105, 1985.

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