

HINDLIMB BLOOD FLOW AND OXYGEN CONSUMPTION  
IN THE LATE GESTATION FETAL LAMB

by

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B.Sc., The University of Lethbridge, 1991

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Faculty of Medicine

Department of Obstetrics and Gynecology

Reproductive and Developmental Sciences Program)

We accept this thesis as conforming  
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

November 1998

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Date December 14, 1998

## ABSTRACT

The purpose of this research was to examine hindlimb blood flow and oxygen supply in the late-gestation fetal lamb under normal and pathophysiological conditions. Although the carcass, or non-visceral tissue, accounts for a large proportion of fetal weight and oxygen demand, it has received little research attention. Because the hindlimb is composed of skin, bone, and skeletal muscle, it is representative of the carcass. We implanted vascular catheters in fetal lambs to permit sampling of blood entering and leaving the hindlimb, and a transit-time flow transducer on the external iliac artery to measure hindlimb blood flow.

In late-gestation, there are specific patterns of electrocortical (ECoG) and electro-ocular activity (EoG), which are associated with episodic fetal breathing and body movements. Hindlimb blood flow tends to be higher and more variable during high voltage ECoG activity. To determine whether this is due to the increased body movements during this state, the neuromuscular blocking agents gallamine and pancuronium were administered. Mean hindlimb blood flow fell  $8.3 \pm 3.5\%$  following blockade, but flow still tended to be higher during quiet sleep. As neuromuscular blockade did not appear to effect the behavioural state modulation of hindlimb blood flow, this relationship may be directly mediated by the central nervous system.

The effects of hypoxemia and ischemia on hindlimb metabolism were compared. No difference was seen in the relationship between oxygen delivery and consumption or in the overall magnitude of hindlimb lactate release for the two perturbations. Thus, for similar reductions in oxygen delivery, we found no difference in hindlimb oxygen consumption or lactate production between hypoxemia and ischemia. Therefore, there is no evidence that hypoxemia is less well tolerated than hindlimb ischemia.

As arginine vasopressin (AVP) has vasoconstrictor actions in the fetus and adult, the effect of exogenous AVP was examined in fetal and non-pregnant adult sheep. Both

groups exhibited similar degrees of hypertension, reduced hindlimb blood flow, and increased hindlimb vascular resistance. However, heart rate declined significantly more in the adults. Therefore, there is no evidence for a greater sensitivity of fetal vascular smooth muscle to vasopressin, but the fetus does appear to be less sensitive to the negative chronotropic effects of AVP.

In conclusion, our studies indicate that the hindlimb, and thus the carcass, plays a major role in the dynamic nature of the fetus under normal conditions as well as in its ability to adapt to pathophysiological situations.

## TABLE OF CONTENTS

ABSTRACT .....	ii
TABLE OF CONTENTS .....	iv
LIST OF TABLES .....	viii
LIST OF FIGURES .....	x
ABBREVIATIONS .....	xvii
ACKNOWLEDGEMENT .....	xx
DEDICATION .....	xxi
<b>1. INTRODUCTION.....</b>	<b>1</b>
1.1 STATEMENT OF PURPOSE.....	1
1.2 BACKGROUND.....	2
1.2.1 Sheep as a model system .....	2
1.2.2 Oxygen demand.....	4
1.2.3 Compensations for reduced oxygen supply.....	5
1.2.4 Hindlimb as representative of carcass tissue .....	6
1.3 RATIONALE.....	8
1.4 OBJECTIVES.....	10
1.5 HYPOTHESES.....	11
<b>2. GENERAL EXPERIMENTAL.....</b>	<b>12</b>
2.1 CASTING.....	12
2.1.1 Introduction .....	12
2.1.2 Methods.....	12
2.1.3 Results/Discussion .....	13

2.2 ANIMAL CARE AND MAINTENANCE.....	15
2.3 SURGICAL PREPARATION.....	17
2.4 PHYSIOLOGICAL RECORDING AND MONITORING PROCEDURES .....	26
2.5 CATHETER AND ELECTRODE PREPARATION.....	28
2.6 STATISTICAL ANALYSIS.....	33
<b>3. HINDLIMB BLOOD FLOW MEASUREMENT CORRELATION: MICROSPHERES VERSUS TRANSIT-TIME FLOW TRANSDUCER.....</b>	<b>34</b>
3.1 INTRODUCTION.....	34
3.2 METHODS.....	38
3.3 RESULTS AND DISCUSSION.....	39
<b>4. HINDLIMB BLOOD FLOW AND WEIGHT DISTRIBUTION.....</b>	<b>44</b>
4.1 INTRODUCTION.....	44
4.2 METHODS.....	44
4.3 RESULTS.....	45
4.4 DISCUSSION.....	49
<b>5. BEHAVIOURAL STATE MODULATION OF HINDLIMB BLOOD FLOW .....</b>	<b>53</b>
5.1 INTRODUCTION.....	53
5.2 METHODS.....	58
5.3 RESULTS.....	59
5.4 DISCUSSION.....	69

<b>6. HYPOXEMIA AND ISCHEMIA.....</b>	<b>78</b>
6.1 INTRODUCTION.....	78
6.2 METHODS.....	80
6.3 RESULTS.....	88
6.3.1 Animal information.....	88
6.3.2 Blood gas and acid-base status .....	90
6.3.3 Hindlimb blood flow, oxygen delivery, and oxygen consumption.....	93
6.3.4 Lactate and glucose concentrations and fluxes.....	95
6.3.5 Cardiovascular Responses .....	99
6.4 DISCUSSION.....	101
6.4.1 Hypoxemia and ischemia levels .....	101
6.4.2 Blood gas and acid-base responses.....	101
6.4.3 Hindlimb blood flow.....	102
6.4.4 Oxygen delivery and consumption.....	105
6.4.5 Lactate and glucose responses .....	110
6.4.6 Cardiovascular responses.....	117
<b>7. ARGININE VASOPRESSIN INFUSION.....</b>	<b>121</b>
7.1 INTRODUCTION.....	121
7.2 METHODS.....	122
7.2.1 Animal Preparation and Experimental Protocol.....	122
7.2.2 Arginine Vasopressin Analysis.....	125
7.2.3 Statistics.....	126

7.3 RESULTS.....	127
7.3.1 Arginine vasopressin effects on the fetus.....	127
7.3.2 Arginine vasopressin effects on the adult.....	141
7.3.3 Fetal versus adult responses to arginine vasopressin concentration..	151
7.4 DISCUSSION.....	158
7.4.1 AVP levels-normal and pathophysiological.....	158
7.4.2 Blood gas and metabolic responses .....	161
7.4.3 Cardiovascular responses.....	163
7.4.4 Fetal versus adult responses.....	167
<b>8. SUMMARY AND CONCLUSIONS.....</b>	<b>173</b>
<b>9. REFERENCES .....</b>	<b>185</b>
<b>10. APPENDIX. Unsuccessful fetal surgical preparations .....</b>	<b>201</b>

## LIST OF TABLES

<b>TABLE 1.</b> Fetal tissues supplied by the major branches of the terminal descending aorta and hindlimb arterial vessels, based on vascular casting results from eight fetal lambs at 118-139 days gestation.....	16
<b>TABLE 2.</b> Successful fetal surgical preparations.....	18
<b>TABLE 3.</b> Successful surgical preparations of non-pregnant adult ewes.....	21
<b>TABLE 4.</b> Inner and outer diameters of silicone rubber (Silastic®) catheters used in animal preparations.....	30
<b>TABLE 5.</b> Gestational age, fetal and hindlimb weights at autopsy for the six fetuses used in the hindlimb blood flow and weight distribution study. In the case of multiple lambs, weights are those of the operated fetus only.....	46
<b>TABLE 6.</b> Hindlimb tissue weight and blood flow distribution on the day of experimentation for the six fetal lambs used in the hindlimb blood flow and weight distribution study.....	47
<b>TABLE 7.</b> Effect of neuromuscular blockade on blood gas status and metabolic variables in the fetal lamb hindlimb (7 experiments).....	68
<b>TABLE 8.</b> Individual fetal age, weight, and study limb weight at autopsy and on day of study for the animals involved in the hypoxemia and ischemia studies. In the case of multiple lambs, weights are those of the operated fetus only. ....	89
<b>TABLE 9.</b> Arterial and venous blood gas responses to hypoxemia in the fetal lamb hindlimb (7 animals). Control data are presented as absolute values; other data are change from the control values .....	91
<b>TABLE 10.</b> Arterial and venous blood gas responses to hindlimb ischemia in six fetal lambs. Control data are presented as absolute values; other data are change from the control values .....	92

- TABLE 11.** Hindlimb metabolic variables during hypoxemia and ischemia experiments. Control data are presented as absolute values; other data are expressed as a percent of the control values .....94
- TABLE 12.** Hindlimb lactate and glucose responses to hypoxemia and ischemia. Control data and flux values are presented as absolute values; arterial and venous concentrations are expressed as a change from control values.....97
- TABLE 13.** Hindlimb blood flow and cardiovascular responses to hypoxemia and ischemia in the fetal lamb. Control data are presented as absolute values; other data are a percent of the control values .....100
- TABLE 14.** Blood gas and metabolic values from fetuses given either saline vehicle only (2 experiments) or exogenous arginine vasopressin (AVP) infusions at a dose of 4.5 mU/min (8 experiments).....131
- TABLE 15.** Cardiovascular and metabolic data obtained from fetal lambs prior to vehicle (saline) infusion (2 experiments) or infusion of 4.5 mU/min arginine vasopressin (8 experiments). Data are presented as absolute values (mean  $\pm$  SEM).....132
- TABLE 16.** Arterial blood gas and metabolic values from non-pregnant adult ewes given arginine vasopressin infusions of 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments). Data are presented as absolute values (mean  $\pm$  SEM) .....144
- TABLE 17.** Cardiovascular and metabolic data obtained from non-pregnant adult ewes prior to receiving arginine vasopressin infusions at doses of 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments). Data are presented as absolute values (mean  $\pm$  SEM).....146

## LIST OF FIGURES

- FIGURE 1.** Ventral view of a cast of the arterial vasculature in the hindquarters of a fetal lamb at 135 days gestation. Major blood vessels are identified.....14
- FIGURE 2.** Line drawing of fetal lamb hindquarters (ventral view) indicating location of catheters, transit-time flow transducer, and vascular occluder.....24
- FIGURE 3.** A typical polygraph recording obtained from a fetal lamb in late gestation. Episodes of fetal breathing, rapid eye movements, and high and low voltage electrocortical activity are indicated. Abrupt changes in amniotic, arterial, and tracheal pressures are due to position changes of the ewe. Hindlimb blood flow was obtained with a transit-time flow transducer positioned on the external iliac artery.....27
- FIGURE 4.** Construction of silicone rubber (Silastic®) catheters implanted in the late gestation fetal lamb preparations. Diagrams are not drawn to scale .....29
- FIGURE 5.** Construction of electrocortical and electro-ocular electrodes for the late gestation fetal lamb preparations. Diagrams are not drawn to scale .....32
- FIGURE 6.** Transonic® transit-time flow transducer and its operation (from Hartman *et al.*, 1985). The transducer is positioned around the external iliac artery and provides continuous measurements of hindlimb blood flow .....37
- FIGURE 7.** Comparison of fetal lamb hindlimb blood flow as measured with a transit-time flow transducer on the external iliac artery and radionuclide-labeled microspheres for five fetal lambs.....40
- FIGURE 8.** Individual animal comparisons of fetal lamb hindlimb blood flow as measured with transit-time flow transducers and radionuclide-labeled microspheres .....42
- FIGURE 9.** Comparison of tissue weights and blood flows for skin, bone, and skeletal muscle in six fetal lambs.....48

- FIGURE 10.** Polygraph recording obtained from a fetal lamb in late gestation prior to neuromuscular blockade. Episodes of fetal breathing and high and low voltage electrocortical activity are indicated. A uterine contracture is also labeled. Abrupt changes in amniotic, arterial, venous, and tracheal pressures are due to positional changes of the ewe. Hindlimb blood flow was obtained with a transit-time flow transducer on the external iliac artery .....60
- FIGURE 11.** Polygraph recording obtained from a fetal lamb in late gestation following the administration of a neuromuscular blocking agent (pancuronium). High and low voltage electrocortical activity and a uterine contracture are indicated. Fetal breathing movements are absent. Abrupt changes in amniotic, arterial, venous, and tracheal pressures are due to positional changes of the ewe. Hindlimb blood flow was obtained with a transit-time flow transducer on the external iliac artery.....61
- FIGURE 12.** Effect of neuromuscular blockade on fetal hindlimb blood flow (8 experiments). Mean hindlimb blood flow as well as that during high and low voltage ECoG cycles are presented for both before and after blockade. Blood flow was measured with a transit-time flow transducer on the external iliac artery .....63
- FIGURE 13.** Effect of neuromuscular blockade on fetal heart rate (8 experiments). Mean heart rate as well as that during high and low voltage ECoG cycles are presented for both before and after blockade.....64
- FIGURE 14.** Effect of neuromuscular blockade on fetal arterial pressure (8 experiments). Mean arterial pressure as well as that during high and low voltage ECoG cycles are presented for both before and after blockade .....65
- FIGURE 15.** Effect of neuromuscular blockade on fetal venous pressure (8 experiments). Mean venous pressure as well as that during high and low voltage ECoG cycles are presented for both before and after blockade .....66

- FIGURE 16.** Effect of neuromuscular blockade on fetal hindlimb vascular resistance (8 experiments). Mean resistance as well as that during high and low voltage ECoG cycles are presented for both before and after blockade.....67
- FIGURE 17.** Plexiglas chamber used to alter maternal inspired oxygen concentrations during hypoxemia experiments (figure from Rurak *et al.*, 1990). Ewes were able to eat, drink, and lay down normally when their heads were inside the chamber .....81
- FIGURE 18.** Protocol for hypoxemia experiments involving Plexiglas chamber. Arrows indicate fetal blood samples (taken at approximately 10 minute intervals).....83
- FIGURE 19.** Protocol for hypoxemia experiments in which hypoxic gas mixture was introduced through the maternal tracheal catheter. Arrows indicate fetal blood samples (taken at approximately 10 minute intervals during hypoxemia and 20 minute intervals during the control and recovery phases) .....84
- FIGURE 20.** Protocol for fetal hindlimb ischemia experiments. Ischemia was achieved by partial inflation of a vascular occluder on the fetal external iliac artery. In most experiments two episodes of ischemia were conducted separated by a recovery phase. At least 30 minutes were allowed between the end of the first recovery phase and the second ischemia episode. Arrows indicate fetal blood samples (taken at approximately 10 minute intervals) .....85
- FIGURE 21.** Polygraph recording of a hindlimb ischemia episode obtained from a late gestation fetal lamb. Ischemia was achieved by partial inflation of a vascular occluder positioned on the external iliac artery upstream to a transit-time flow transducer. In this figure, hindlimb blood flow was reduced to approximately 60% of control values. The onset of the occlusion episode is followed by abrupt alterations in blood flow as the inflation of the occluder is manually adjusted to achieve the desired level of ischemia. Release (or deflation) of the occluder results in an immediate, transient hyperemia followed by a rapid return of hindlimb blood flow to control values. Abrupt changes in pressures are due to position changes of the ewe.....87

- FIGURE 22.** Relationship between hindlimb oxygen consumption and delivery in fetal lambs exposed to hypoxemia (n=7) or ischemia (n=6).....96
- FIGURE 23.** Relationship between hindlimb blood flow and gestational age in the fetal lamb. The longitudinal study was conducted on 16 fetuses (each symbol represents an individual animal). Mean daily blood flow was determined with a transit-time flow transducer on the external iliac artery. Only days in which no other experiment was performed are included.....104
- FIGURE 24.** Protocol for exogenous arginine vasopressin infusion experiments in fetal and non-pregnant adult ewes. The arrows indicate blood samples which were taken at ~15 minute intervals during the 30 minute control period and the 90 minute infusion period and at 30 minute intervals during the 120 minute recovery period .....123
- FIGURE 25.** Plasma arginine vasopressin concentrations before, during, and after AVP infusion to fetal lambs. The lambs received either saline vehicle (2 experiments), or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment).....128
- FIGURE 26.** Plasma arginine vasopressin concentrations in fetal lambs before, during, and after either saline vehicle (n=2) or exogenous AVP infusion at 4.5 mU/min (n=8) .....129
- FIGURE 27.** Change in fetal arterial pressure during and after arginine vasopressin administration to fetal lambs. The lambs received either saline vehicle (n=1) or exogenous AVP at 4.5 mU/min (n=8).....134
- FIGURE 28.** Change in fetal heart rate during and after arginine vasopressin administration to fetal lambs. The lambs received either saline vehicle (n=2) or exogenous AVP at 4.5 mU/min (n=7).....135
- FIGURE 29.** Change in hindlimb blood flow during and after arginine vasopressin administration to fetal lambs. The lambs received either saline vehicle (n=2) or exogenous AVP at 4.5 mU/min (n=8). Blood flow was measured with a transit-time flow transducer on the external iliac artery .....136

- FIGURE 30.** Change in hindlimb oxygen delivery during and after arginine vasopressin infusion to fetal lambs. The lambs received either saline vehicle (2 experiments), or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment).....137
- FIGURE 31.** Change in hindlimb blood flow during and after arginine vasopressin infusion to fetal lambs. The lambs received either saline vehicle (2 experiments) or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment). Blood flow was measured with a transit-time flow transducer on the external iliac artery .....138
- FIGURE 32.** Change in hindlimb oxygen consumption during and after arginine vasopressin infusion to fetal lambs. The lambs received either saline vehicle (2 experiments), or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment).....139
- FIGURE 33.** Change in hindlimb oxygen extraction during and after arginine vasopressin infusion to fetal lambs. The lambs received either saline vehicle (2 experiments), or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment).....140
- FIGURE 34.** Plasma arginine vasopressin concentrations before, during, and after AVP infusion to non-pregnant adult ewes. The ewes received exogenous AVP at doses of 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments) .....142
- FIGURE 35.** Plasma arginine vasopressin concentrations before, during, and after AVP administration to non-pregnant adult ewes. The ewes received AVP at 25 mU/min (n=2), 45 mU/min (n=3), or 90 mU/min (n=3).....143
- FIGURE 36.** Change in arterial pressure during and after arginine vasopressin administration to non-pregnant adult ewes. The ewes received AVP at 25 mU/min (n=2), 45 mU/min (n=3), or 90 mU/min (n=3).....147

- FIGURE 37.** Change in heart rate during and after arginine vasopressin administration to non-pregnant adult ewes. The ewes received AVP at 25 mU/min (n=2), 45 mU/min (n=2), or 90 mU/min (n=3)..... 148
- FIGURE 38.** Change in hindlimb blood flow during and after arginine vasopressin administration to non-pregnant adult ewes. The ewes received AVP at 25 mU/min (n=2), 45 mU/min (n=3), or 90 mU/min (n=3). Blood flow was measured with a transit-time flow transducer on the external iliac artery ..... 149
- FIGURE 39.** Change in hindlimb oxygen delivery during and after arginine vasopressin infusion to non-pregnant adult ewes. Ewes received AVP at 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments)..... 150
- FIGURE 40.** Change in hindlimb blood flow during and after arginine vasopressin infusion to non-pregnant adult ewes. The ewes received AVP at a dose of 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments). Blood flow was measured with a transit-time flow transducer on the external iliac artery..... 152
- FIGURE 41.** Relationship between percentage change in arterial pressure and plasma arginine vasopressin concentration during the infusion of vasopressin to fetal (10 experiments) and non-pregnant adult (5 experiments) sheep. The regression equations are not significantly different..... 153
- FIGURE 42.** Relationship between percentage change in heart rate and plasma arginine vasopressin concentration during the infusion of vasopressin to fetal (9 experiments) and non-pregnant adult (5 experiments) sheep. The slopes are significantly different ( $p < 0.01$ ) ..... 155
- FIGURE 43.** Relationship between percentage change in hindlimb blood flow and plasma arginine vasopressin concentration during the infusion of vasopressin to fetal (10 experiments) and non-pregnant adult (5 experiments) sheep. Blood flow was measured with transit-time flow transducers on the external iliac arteries. The regression equations are not significantly different..... 156

**FIGURE 44.** Relationship between percentage change in estimated hindlimb vascular resistance and plasma arginine vasopressin concentrations during the infusion of vasopressin to fetal (10 experiments) and non-pregnant adult (5 experiments) sheep. Blood flow was measured with transit-time flow transducers on the external iliac arteries. The regression equations are not significantly different.....157

**ABBREVIATIONS**

A-V	arterial-venous difference
AMP	adenosine monophosphate
ANOVA	analysis of variance
AUC	area under the concentration versus time curve
AVP	arginine vasopressin
bpm	beats per minute
C/S	Cesarean-section
CIA	circumflex iliac artery
cm	centimeter
CNS	central nervous system
dL	deciliter
Do <sub>2</sub>	oxygen delivery
ECoG	electrocortical
Eo <sub>2</sub>	oxygen extraction
EoG	electro-ocular
FBM	fetal breathing movements
g	acceleration due to gravity
g	gram
ID	inner diameter
IIA	internal iliac artery

IU	international units
kg	kilogram
L	liter
m <sup>3</sup>	cubic meter
mEq/L	milliequivalents per liter
min	minute(s)
ml	milliliter
mm	millimeter
mM	millimolar
mmHg	millimeters of mercury
mU	milli-units
ng	nanogram
O <sub>2</sub>	oxygen
OD	outer diameter
p	probability factor
P <sub>a</sub> O <sub>2</sub>	arterial partial pressure of oxygen
P <sub>CO<sub>2</sub></sub>	partial pressure of carbon dioxide
pg	picogram
pH	negative logarithm of hydrogen ion concentration
P <sub>O<sub>2</sub></sub>	partial pressure of oxygen
r	correlation coefficient

REM.....	rapid eye movements
rpm .....	revolutions per minute
S/L.....	spontaneous labour
SEM .....	standard error of the mean
U.....	units
$\dot{V} O_2$ .....	oxygen consumption
[G].....	glucose concentration
[L].....	lactate concentration
$^{\circ}C$ .....	degrees Celsius
$\mu m$ .....	micrometer
$\mu mol$ .....	micromoles
$\mu U$ .....	micro-units
$\mu V$ .....	microvolts

## ACKNOWLEDGEMENT

I would like to express my appreciation to my research supervisor Dr. Dan Rurak for his guidance. Also, thank you to the other members of my supervisory committee: Drs. Wayne Riggs, Anthony Perks, Darlene Reid, and Joseph Skala. Their advice and interest in this project have been invaluable.

Also, I am grateful to our laboratory technicians, Caroline Hall and Eddie Kwan, and to my fellow students, Weiping Tan and Cindy Wu for their friendship and support. Many thanks to Becky Leopold for her friendship and secretarial skills.

Financial support in the form of scholarships from the Natural Sciences and Engineering Research Council of Canada and the British Columbia Research Institute for Child and Family Health is gratefully acknowledged. This research was also supported by the Medical Research Council of Canada.

## **DEDICATION**

This thesis is dedicated to my family: my Mom, Elizabeth; brother, Patrick; and  
to the memory of my Dad, Art.

Also, to my beloved husband Greg.

Thank you for your love, encouragement, understanding, and enthusiasm.

## 1. INTRODUCTION

### 1.1 Statement of Purpose

The purpose of this research was to examine hindlimb blood flow and oxygen supply in the late gestation fetal lamb under normal and pathophysiological conditions. Because the hindlimb is composed almost exclusively of skin, bone, and muscle, it is representative of the carcass (non-visceral tissues). In late gestation the carcass elements comprise 70% of fetal weight and account for 40% of oxygen demand. Thus, the response of these tissues to alterations in oxygen supply can have a profound effect on the fetus as a whole. To study the hindlimb required a surgical protocol which allowed sampling of hindlimb blood and continuous measurement of hindlimb blood flow. Vascular casting and comparison of hindlimb blood flows obtained with radionuclide-labeled microspheres and a transit-time flow transducer on the external iliac artery were used to establish a surgical protocol which minimized sources of collateral circulation and ensured accurate measurement of hindlimb blood flow. This led to an investigation of the effect of the catheterization protocol on hindlimb weight and blood flow.

The normal late gestation fetal lamb experiences a complicated pattern of behavioural states involving rapid eye movements, fetal breathing and body movements, and high and low voltage electrocortical activity. These cycles are associated with alterations in blood flow, metabolism, and cardiovascular variables. Hindlimb blood flow appears to be higher and more variable during high voltage activity which we attribute to the greater prevalence of body movements during this state. Thus, the administration of neuromuscular blocking agents, which inhibit fetal breathing and body movements, should allow us to determine if skeletal muscle activity is responsible for the fluctuations in hindlimb blood flow that occur in relation to behavioural state. In addition, we sought to determine the effect of behavioural state and neuromuscular blockade on hindlimb oxygen consumption. Inhibition of skeletal activity should reduce fetal oxygen requirements. Fetal hypoxemia is

associated with an inhibition of fetal breathing and rapid eye movements, and fewer incidences of low voltage electrocortical activity. Therefore, these may be compensatory mechanisms to lower oxygen consumption when the fetus faces a reduction in oxygen delivery.

We compared the cardiovascular and hindlimb metabolic responses to hypoxemia, in which fetal arterial  $P_{O_2}$  is reduced, and ischemia, where  $P_{O_2}$  is maintained. The hypothesis to be tested was that a fall in oxygen delivery achieved by ischemia would be better tolerated by the fetal lamb hindlimb than hypoxemia, and this latter perturbation would be associated with an earlier fall in oxygen consumption or greater release of lactate by the hindlimb.

Hypoxemia is associated with a rise in circulating levels of arginine vasopressin. A previous study observed a greater hypertensive effect of arginine vasopressin in the fetus compared to the adult ewe while another report found no difference in the pressor response but a greater degree of bradycardia in the adults. Thus, we examined the effect of exogenous arginine vasopressin infusion on cardiovascular and hindlimb metabolic responses in the fetus and non-pregnant ewe.

## **1.2 Background**

### **1.2.1 Sheep as a model system**

Sheep are used extensively in laboratory work due to their availability and docility. Pregnant ewes demonstrate similar changes in physiological parameters to those observed in pregnant humans, making ewes good models for human obstetrical research (Cissik *et al.*, 1991). Furthermore, in late gestation the fetal lamb is similar in weight to a human fetus (~3.5 kg). Thus, as technical and ethical constraints limit study of the human fetus, the fetal lamb is a useful alternative model. A major difference between these two species is in the type of placentation: humans possess a hemochorial placenta (fetal chorionic epithelium is bathed directly by maternal blood) whereas sheep have an epitheliochorial placenta (fetal

chorionic epithelium is in contact with maternal endometrial epithelium). Depending on the type of experiments being conducted, the advantages of the sheep model generally outweigh the disadvantage of placental dissimilarity (Assali *et al.*, 1974).

Early studies on the fetal lamb were usually acute studies on anesthetized animals. Reports using exteriorized lambs, in which the fetus was removed from the uterus but the umbilical circulation remained intact, are also prevalent in the early literature. Although much information was revealed by such work, anesthetized and exteriorized fetuses cannot be considered physiologically representative of the *in utero* state (Rurak and Taylor, 1986; Jones, 1977).

Meschia and colleagues (1966) first reported the use of chronically catheterized fetuses. As the sheep uterus is thinner and less muscular than that of many other species, most notably humans, surgery on fetal lambs carries a reduced risk of premature labour. Using aseptic techniques catheters can be inserted into fetal vessels, the ewe and fetus allowed to recover from surgery, and thereafter the fetus can be studied *in utero* in a non-anesthetized state approximating physiological normality. As chronic catheterization does not seem to adversely affect the fetus both acute and long-term studies can be conducted. In addition, advancements in radionuclide-labeled microspheres and transit-time flowprobes have allowed researchers to study blood flow and its distribution. These developments have made it possible to continuously study the fetus *in utero* for days or weeks at a time. Direct measurements of cardiovascular, metabolic, and behavioural state variables are possible in fetal lambs thus avoiding the subjectivity when observing the human fetus remotely i.e., by ultrasound.

Although the oxygen delivery systems of the sheep and human fetus seem to differ quantitatively, oxygen consumption seems comparable in the two species (Rurak *et al.*, 1987). As well human and sheep fetuses exhibit similar compensatory mechanisms when faced with a reduction in oxygen supply.

### 1.2.2 Oxygen demand

As the organ of gas exchange in the fetus is the placenta rather than the lungs, oxygen delivery ( $\text{Do}_2$ ) to the fetus is a function of umbilical blood flow and the oxygen content of the umbilical venous blood. Likewise, oxygen delivery to specific organs or tissues is the product of arterial oxygen content and blood flow to that particular tissue. By the Fick Principle the amount of oxygen taken up by fetal tissues or oxygen consumption ( $\dot{V} \text{O}_2$ ) can be calculated as the product of blood flow and the arterial-venous oxygen content difference. The ratio of oxygen consumption to delivery constitutes oxygen extraction ( $\text{Eo}_2$ ).

Uterine contractions, maternal or fetal hemorrhage, and constriction of uterine, umbilical, or other fetal vessels may reduce oxygen supply to fetal tissues. Hypoxemia by definition is a reduction in arterial  $\text{Po}_2$ . Experimental perturbations that reduce fetal systemic oxygen delivery primarily via fetal hypoxemia include reducing uterine blood flow (Jensen *et al.*, 1991) and decreasing maternal inspired oxygen concentration (Rurak *et al.*, 1990a; Rurak *et al.*, 1990b; Cohn *et al.*, 1974). Under normal conditions oxygen consumption by the placenta accounts for about half of the total uterine-umbilical venous  $\text{Po}_2$  difference (Wilkening and Meschia, 1983; Meschia *et al.*, 1980). Thus, when oxygen availability is decreased, this high rate of oxygen consumption can significantly reduce oxygen delivery to the fetus (Groome, 1991). Hypoxia occurs when tissue oxygen supply is not sufficient to meet normal metabolic demands. Due to the high rate of blood flow to fetal organs, hypoxemia is not necessarily associated with hypoxia (Richardson, 1989). Hypoxia ranges in severity with the more severe reductions in oxygen delivery accompanied by metabolic acidosis (Peeters *et al.*, 1979). If fetal  $\text{Po}_2$  drops below that required to maintain the diffusion gradient from the blood to the inner mitochondrial membrane (critical  $\text{Po}_2$ ), anaerobic glycolysis occurs producing lactic acidemia. This diffusion gradient is a particularly important factor when oxygen supply is limited (Kennedy and Jones, 1986; Jones and Kennedy, 1982). Oxygen supply can also be diminished by other means such as

fetal hemorrhage (Rurak *et al.*, 1989) or reduced umbilical blood flow (Itskovitz *et al.*, 1987; Itskovitz *et al.*, 1983). In these cases the fall in delivery is generally caused by alteration of perfusion while fetal  $P_{O_2}$  is generally maintained.

Lactate and glucose are the major substrates of normal fetal metabolism (Singh *et al.*, 1984). Severe hypoxemia, accompanied by hypoxia, results in a net lactate production by the fetus (Milley, 1988; Jones, 1977). Metabolic acidemia further reduces blood oxygen content probably by a rightward shift in the oxyhemoglobin dissociation curve or Bohr Shift (Rurak *et al.*, 1990b). The shift from pH optima as a consequence of the acidemia reduces intracellular enzyme activity and may lead to tissue necrosis. Compensatory mechanisms, primarily increased blood flow, attempt to maintain oxygen delivery to the heart, brain, and adrenal glands at the expense of less vital organs, often resulting in regional acidemia. When hypoxia exceeds the capacity of these compensatory mechanisms, even the vital organs face a severe reduction in oxygen supply with death usually imminent.

### 1.2.3 Compensations for reduced oxygen supply

The fetus exists in a state of physiological hypoxemia relative to the neonate and adult but has developed a number of adaptations which permit it to thrive in its unique environment. These include: circulatory shunts to direct oxygen-rich blood to the vital organs (Rudolph, 1985; Edelstone and Rudolph, 1979), overperfusion of organs relative to their oxygen requirements (Richardson, 1989), and a higher oxygen affinity hemoglobin (Itskovitz *et al.*, 1984). The main compensatory mechanisms which the fetus possesses to combat acute disturbances in oxygen supply are to maintain oxygen consumption by increasing extraction and to decrease total body oxygen demands. Below the critical  $P_{O_2}$ , acidemia develops which eventually limits the capacity of these mechanisms to sustain physiological well-being in the fetus.

In the fetal lamb the normal range of oxygen extraction is 25 to 35% (Rurak *et al.*, 1987; Rudolph, 1984). By increasing oxygen extraction, consumption can be maintained in

the face of reductions in oxygen delivery to about 50%. This compensation is termed the oxygen margin of safety or oxygen reserve (Richardson, 1989). Extraction increases in a curvilinear fashion as delivery falls (Richardson, 1989; Schumacker and Samsel, 1989), peaking at approximately 60-70% (Edelstone, 1984; Itskovitz *et al.*, 1983). Beyond approximately a 50% fall in delivery, oxygen consumption begins to decline precipitously. As extraction rises, the  $P_{O_2}$  of the blood perfusing tissues downstream will be reduced. Thus the need to maintain  $P_{O_2}$  above critical values imposes a limitation on the degree of oxygen delivery that can be tolerated.

In addition, when contending with reduced oxygen delivery, available oxygen is preferentially redistributed to the vital organs, such as the heart and brain, (Sheldon *et al.*, 1979; Cohn *et al.*, 1974) and away from peripheral tissues like the carcass (Jensen and Berger, 1991; Boyle *et al.*, 1990; Peeters *et al.*, 1979). This compensatory mechanism is limited by the development of systemic and tissue lactic acidemia. Arginine vasopressin (AVP) has been implicated in the increased peripheral vascular resistance (Iwamoto *et al.*, 1979). The late gestation fetal lamb also reduces or abolishes body movements (Natale *et al.*, 1981) and breathing activity (Hohimer and Bissonnette, 1991) in order to lower oxygen requirements when the supply is limited.

#### 1.2.4 Hindlimb as representative of carcass tissue

Carcass, defined as the non-visceral tissues, primarily includes bone, skin, and skeletal muscle. The carcass elements of the fetal lamb receives nearly 40% of combined ventricular output (Rudolph and Heymann, 1970), and these tissues constitute approximately 70% of fetal weight in late gestation (Wilkening *et al.*, 1988; Wallace, 1948). It has been estimated that these tissues account for 40% of total fetal oxygen demand (Rudolph, 1984). As a result, any change in substrate delivery, consumption, or extraction by the carcass elements can have a profound effect on the fetus as a whole. As the fetal lamb hindlimb consists almost exclusively of skin, bone, and muscle, a number of

researchers consider it to be representative of the carcass (Boyle *et al.*, 1990; Wilkening *et al.*, 1988; Singh *et al.*, 1984). Because the peripheral circulation in skin and muscle receives a large percentage of fetal cardiac output, vasomotor responses of these tissues can lead to a major redistribution of the fetal circulation (Rudolph and Heymann, 1970).

### 1.3 Rationale

1. In late gestation, the carcass (non-visceral tissues) comprises a large fraction of fetal body weight and receives a significant proportion of fetal cardiac output. However, there is limited information on carcass blood flow and metabolism under normal and pathophysiological conditions.
2. The hindlimb is composed of skin, bone, and skeletal muscle and is considered to be representative of the fetal carcass. Blood primarily enters and exits the hindlimb via the external iliac artery and vein. It should thus be possible to catheterize and instrument the hindlimb so as to determine its blood flow and metabolism.
3. Under normal conditions, the late gestation fetal lamb experiences cyclical variations in behavioural state, including electrocortical and electro-ocular activity, as well as breathing and body movements. Hindlimb blood flow fluctuates with behavioural state; however, the mechanisms involved in this relationship are not known.
4. When reductions in fetal oxygen delivery are achieved via hypoxemia, the rise in circulating lactate concentrations is greater than when oxygen delivery is reduced to a similar degree via decreased blood flow. This may be because hypoxemia results in greater falls in capillary and tissue  $P_{O_2}$  compared to ischemia, thereby limiting tissue oxygen utilization to a greater extent and resulting in an increased glycolytic rate with enhanced lactate production. The carcass could be involved in this difference; however, comparisons of fetal carcass metabolic responses to hypoxemia and ischemia are lacking.
5. During severe fetal hypoxemia, carcass blood flow falls which exacerbates the fall in  $P_{O_2}$ . Arginine vasopressin has been implicated in this response as well as in the

cardiovascular effects (hypertension and bradycardia) seen with hypoxemia.

Previous studies suggest that this hormone may be a more potent vasoconstrictor or a less potent negative chronotrope in the fetus compared to the adult. However, a direct comparison of the cardiovascular and vasoconstrictor effects in the fetus and adult has not been conducted.

#### 1.4 Objectives

1. To examine the vascular anatomy of the fetal lamb hindlimb through casting and dissection. These data were used to design a surgical protocol to permit measurement of solely hindlimb blood flow and metabolism.
2. To compare estimates of fetal hindlimb blood flow measured with a transit-time flow transducer on the external iliac artery to those obtained with radionuclide-labeled microspheres.
3. To compare the distribution of blood flow and weight in the study and non-study hindlimb tissues (skin, bone, and muscle) of the late gestation fetal lamb using radionuclide-labeled microspheres.
4. To measure hindlimb blood flow; blood gas status; cardiovascular responses; oxygen supply, consumption, and extraction; and glucose and lactate fluxes in the fetal lamb hindlimb before and after neuromuscular blockade. Also, to record hindlimb blood flow and oxygen consumption in relation to behavioural state.
5. To measure hindlimb blood flow; arterial and venous blood gas values and oxygen contents; oxygen consumption, delivery, and extraction; glucose and lactate fluxes, and cardiovascular variables of the fetal lamb in response to hypoxemia and ischemia. Hypoxemia was achieved by reducing maternal inspired oxygen concentrations and ischemia by partial inflation of a vascular occluder on the fetal external iliac artery.
6. To measure hindlimb blood flow; arterial and venous blood gas values and oxygen contents; oxygen consumption, delivery, and extraction; glucose and lactate fluxes; and cardiovascular variables of the fetal lamb in response to exogenous arginine vasopressin (AVP). Also, to compare the effects of vasopressin administration to fetal and non-pregnant adult sheep.

## 1.5 Hypotheses

1. Ligation of the circumflex iliac, internal iliac, and pudendoepigastric arteries will be necessary to enable a flow transducer located on the external iliac artery of the fetal hindlimb to measure solely hindlimb blood flow and metabolism.
2. With the proposed surgical protocol, a transit-time flow transducer positioned around the external iliac artery can be used to measure total hindlimb blood flow. This will be demonstrated by a good correlation with hindlimb blood flow measurements obtained with radionuclide-labeled microspheres.
3. Distribution of blood flow to and weight of hindlimb tissues will be similar between the study and non-study limbs.
4. Hindlimb blood flow and oxygen consumption will be greater during quiet sleep (high voltage ECoG). Administration of neuromuscular blocking agents to the fetus will abolish fetal breathing and body movements, including movements of the hindlimb. As a consequence, the metabolic demands of hindlimb muscles will not increase during quiet sleep so that hindlimb blood flow will not rise.
5. The hindlimb of the fetal lamb will be less able to tolerate a reduction in oxygen delivery resulting from hypoxemia than from ischemia as shown by an earlier fall in oxygen consumption and/or a greater release of lactate by the hindlimb, contributing to systemic lactic acidemia, with hypoxemia.
6. Exogenous arginine vasopressin administered to the fetal lamb will result in a rise in arterial pressure, a fall in heart rate, and a fall in hindlimb blood flow. Similar changes will be observed during vasopressin infusion to non-pregnant adult ewes.

## 2. GENERAL EXPERIMENTAL

### 2.1 Casting

#### 2.1.1 Introduction

Because this thesis focuses on the hindlimb and assumes that the external iliac artery serves only hindlimb tissue, it was necessary to first determine which tissues and blood vessels constitute the fetal lamb hindlimb. We employed the Batson casting technique (Polysciences Data Sheet #105), in which a partially polymerized monomer is injected into fetal blood vessels at autopsy, to help define the hindlimb tissues. Because radionuclide-labeled microspheres were used in some experiments to measure hindlimb blood flow, it was important to determine the bones, muscles, and skin which are served by the external iliac artery under our surgical protocol. By tracing the distribution of the main hindlimb vessels through the skin, bone, and muscles, it should be possible to eliminate potential sources of error in the calculation of hindlimb weight. For example, the inclusion of non-hindlimb tissue, especially from the gluteal region, would lead to an overestimate of total hindlimb weight and an underestimate of blood flow per unit weight of tissue. Furthermore, we also used a transit-time flow transducer on the external iliac artery to measure blood flow in the hindlimb. Thus, it was necessary to determine possible sites of collateral circulation. In their studies of the fetal lamb hindlimb, Wilkening (1988) and Boyle (1990) ligated the circumflex iliac artery and pudendoepigastric arterial vessels to minimize collateral circulation and ensure they were recording solely blood flow to the hindlimb.

#### 2.1.2 Methods

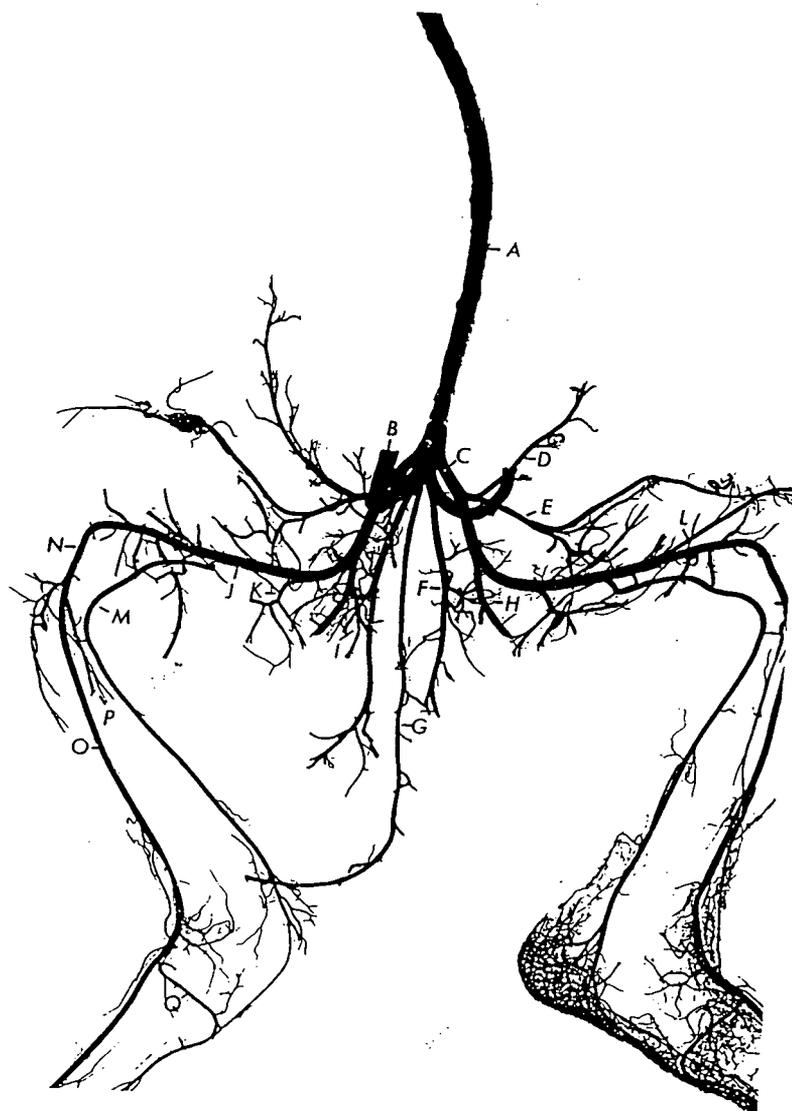
Vascular casting was performed on eight unoperated fetuses at 118–139 days gestation. Casting was performed at autopsy using Batson's No. 17 Replica and Corrosion Kit (Polysciences Inc., Warrington, Pennsylvania). In five animals, a midline incision was

made in the fetal lamb and the abdominal tissues carefully dissected to reveal the aorta. A small incision was then made in the abdominal aorta and Tygon flexible plastic tubing (R-3603, Norton Performance Plastics, Akron, Ohio; 2.38 mm ID, 3.97 mm OD) was inserted approximately 2 cm and secured. A 16 gauge needle hub was then secured at the end of the tubing and the vessels were gently flushed with a small amount of saline to clear them of any remaining blood. A coloured, partially polymerized polymer was prepared according to the manufacturer's directions and gently injected into the descending aorta. In one case, after allowing the polymer to harden for several hours, the fetus was placed into macerating solution (340 g KOH:1 L water) for several days until the tissues dissolved, leaving a coloured cast of the hindlimb vasculature (Figure 1).

In three fetuses, hindlimb vessels (external iliac artery and/or internal iliac artery, femoral artery) were catheterized with appropriate diameter Silastic® medical grade tubing (Dow Corning, Midland, Michigan). The polymer was injected into the individual vessels, allowed to harden, and the fetal hindlimbs were then dissected to determine which tissues were served by the catheterized vessels. In some animals several vessels were catheterized, and casting material containing different coloured pigments was simultaneously injected via each catheter. Finally, in the four remaining fetuses, the abdominal aorta was catheterized as above and the internal iliac, pudendoepigastric, and circumflex iliac arteries were ligated. The casting polymer was then injected and allowed to harden for several hours before the tissues of the hindlimbs were carefully dissected. These final animals represented the hindlimb vasculature as it would appear after our surgical intervention.

### 2.1.3 Results/Discussion

Figure 1 illustrates the arterial vascular cast of the hind quarters of a fetal lamb (135 days gestation); in this case the casting material was injected via the descending aorta. From our dissection of the tissues following casting, we defined the hindlimb as the muscles of the upper and lower pelvic limb as well as the gluteal and deep gluteal muscles and the skin




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**Major Blood Vessels**


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- A descending aorta
- B umbilical artery
- C external iliac artery
- D anterior circumflex iliac artery
- E posterior circumflex iliac artery
- F internal iliac artery
- G sagittal artery
- H deep femoral artery
- I pudendoepigastric artery
- J femoral artery
- K anterior femoral artery
- L posterior femoral artery
- M saphenous artery
- N popliteal artery
- O anterior tibial artery
- P posterior tibial artery
- Q recurrent tibial artery

**FIGURE 1.** Ventral view of a cast of the arterial vasculature in the hindquarters of a fetal lamb at 135 days gestation. Major blood vessels are identified.

overlying these tissues. Skin over the genitalia, rectum, and tail was excluded. Hindlimb bones included the femur, tibia, tarsals, and metatarsal, but not the iliosacral bones. The results from casting individual vessels indicate that the circumflex iliac artery, internal iliac artery, and pudendoepigastric artery serve primarily non-hindlimb tissues or are potential sources of collateral flow (Table 1). A flow transducer placed on the external iliac artery distal to the origin of the circumflex iliac artery would underestimate hindlimb blood flow unless the latter vessel was ligated. Furthermore, catheterization of the pudendoepigastric artery (and vein) permits sampling of hindlimb arterial and venous blood without compromising limb perfusion. These findings were incorporated into our surgical protocol. Our results were consistent with the findings of other researchers (Boyle *et al.*, 1990; Wilkening *et al.*, 1988).

## **2.2 Animal Care and Maintenance**

Ewes of Dorset-Suffolk breeds were housed at the University of British Columbia Farm where they underwent time-dated natural service mating. Groups of ewes (2 to 4 animals per group) were brought into synchronous estrus by implantation of vaginal pessaries (Veramix®, Tuco Products Company, Orangeville, Ontario) containing medroxyprogesterone acetate which inhibits ovulation. Two weeks later the pessaries were removed, follicle development was induced with intramuscular injections of 333 to 500 IU pregnant mare's serum gonadotropin (Equinex®, Ayerst Laboratories, Montreal, Quebec), and the ewes were placed in with a ram. Pregnancy was determined by measurement of maternal serum progesterone levels at approximately 17 days post-ovulation and was confirmed 50 to 90 days later by real-time ultrasound. Each group of ewes was brought to the Research Institute approximately one week prior to surgery where they were kept in large pens in view of other sheep. The animals had free access to water, food, and hay. All studies were approved by the University of British Columbia Committee on Animal Care and conformed to the guidelines of the Canadian Council on Animal Care.

**TABLE 1 .** Fetal tissues supplied by the major branches of the terminal descending aorta and hindlimb arterial vessels, based on vascular casting results from eight fetal lambs at 118-139 days gestation.

Blood Vessel	Major Tissue Served
Femoral and saphenous arteries	Skin over lower leg, thigh, and lower leg muscles
Deep femoral artery	Skin and muscles of thigh
Anterior femoral artery	Thigh and gluteal muscles
Pudendoepigastric artery	
anterior branch	Abdominal muscles
posterior branch	Skin of inguinal and mammary regions
Circumflex iliac artery	
anterior branch	Abdominal and gluteal muscles
posterior branch	Abdominal muscles, skin of rump and thigh, thigh and gluteal muscles
Internal iliac artery	Skin and muscle of inguinal and mammary regions
Sagittal artery	Tail

### 2.3 Surgical Preparation

Successful experiments were conducted on 22 fetal lambs (Table 2) and 3 adult non-pregnant ewes (Table 3). Unsuccessful preparations are presented in the Appendix. Surgery was conducted on the fetuses at 118–130 days gestation (term approximately 140 days). Food was withheld for about 18 hours prior to surgery. Ewes received 3 mg of atropine sulfate (Astra Pharma, Mississauga, Ontario) intravenously to control salivation followed by induction of anesthesia with 1 gram thiopental sodium (Pentathol®, Abbott Laboratories, Montreal, Quebec). The ewes were then placed in a supine position, intubated, and maintained on a ventilator for the duration of surgery. Anesthesia was maintained with 1.0–1.5% halothane (Fluothane®, Wyeth-Ayerst Canada Inc., St-Laurent, Quebec) and nitrous oxide (70%) in oxygen. Approximately 500 ml of 5% dextrose solution (Baxter Corporation, Mississauga, Ontario) was given at a rate of ~2 ml/min via the maternal jugular vein. Aseptic technique was observed throughout the surgical procedure. The ewe's abdomen was shaved, 1% lidocaine hydrochloride (Xylocaine®, Astra Pharma, Mississauga, Ontario) was injected subcutaneously along the midline, the surgical area was swabbed with 7.5% povidone-iodine (Betadine® surgical scrub, Purdue Frederick, Pickering, Ontario), and sterile sheets and drapes were positioned around the surgical field.

Avoiding major blood vessels and following the lidocaine injection site, a midline abdominal incision was made in the ewe to expose the uterus. An incision was then made in the uterus in a site free of cotyledons and major blood vessels and the fetal head was exteriorized. Silicone rubber catheters (Silastic®, Dow Corning, Midland, Michigan) with an outer diameter of 2.16 mm (1.02 mm ID) were placed in the trachea and amniotic cavity. The tracheal catheter was inserted through a small incision made between two rings of cartilage just below the larynx. It was advanced distally approximately 4 cm into the trachea and was anchored to the skin overlying the incision with 3-0 silk (Davis+Geck Cyanamid Canada Inc., Montreal, Quebec). A drop of surgical glue (Vetbond tissue adhesive, 3M

TABLE 2. Successful fetal surgical preparations

Ewe Number	Experiment	Gestational Age (days)		No. of Fetuses	Operated Fetus Weight (kg)	Fate
		Surgery	Experiment Delivery			
E1154	hypoxemia (trachea)	125	132	1	3.409	died in utero
E116	ischemia	125	139	2	3.206	ewe and fetus euthanized
E1223	hypoxemia (chamber)	118	124	1	3.471	S/L; fetus euthanized
	AVP (4.5 mU/min)	127				
	AVP (4.5 mU/min)	134				
E1245	neuromuscular blockade (gallamine)	124	131	3	3.225	ewe and fetus euthanized
	hypoxemia (trachea)	136				
	flow correlation	138				
	ischemia & weight/flow distribution	138				
E133x	flow correlation & weight/flow distribution	123	129	3	1.598	ewe and fetus euthanized
E136	ischemia & weight/flow distribution	124	136	1	3.158	ewe and fetus euthanized
E207HIN	hypoxemia (trachea)	118	124	2	2.218	ewe and fetus euthanized
	flow correlation	130				
	ischemia & weight/flow distribution	130				

trachea = hypoxemia achieved via maternal tracheal catheter

chamber = hypoxemia achieved via Plexiglas box

AVP = arginine vasopressin infusion

S/L = spontaneous labour

TABLE 2. Successful fetal surgical preparations (continued)

Ewe Number	Experiment	Gestational Age (days)		No. of Fetuses	Operated Fetus Weight (kg)	Fate
		Surgery	Experiment Delivery			
E2102	neuromuscular blockade (gallamine)	125	131	1	5.108	C/S; fetus euthanized
	AVP (0.72 mU/min)		133			
	AVP (4.5 mU/min)		135			
E2121	neuromuscular blockade (gallamine)	126	131	1	3.096	died in utero
E2123	flow correlation	118	130	2	3.098	ewe and fetus euthanized
E2149	neuromuscular blockade (gallamine)	120	126	1	3.218	died in utero
E2176	AVP (4.5 mU/min)	121	125	2	1.800	died in utero
E207HLM	weight/flow distribution	125	137	1	3.766	ewe and fetus euthanized
E2180	AVP (4.5 mU/min)	118	126	1	3.600	ewe and fetus euthanized
E2224	neuromuscular blockade (pancuronium)	126	130	1	3.944	S/L; fetus euthanized
	hypoxemia (chamber)		132			
	AVP (4.5 mU/min)		134			
	neuromuscular blockade (pancuronium)		136			

chamber = hypoxemia achieved via Plexiglas box

AVP = arginine vasopressin infusion

S/L = spontaneous labour

C/S = Cesarean-section

TABLE 2. Successful fetal surgical preparations (continued)

Ewe Number	Experiment	Gestational Age (days)		No. of Fetuses	Operated Fetus Weight (kg)	Fate
		Surgery	Experiment Delivery			
E238	neuromuscular blockade (gallamine)	125	130	2	2.604	died in utero
	flow correlation & weight/flow distribution		132			
E3210	AVP (4.5 mU/min)	120	142	1	5.294	S/L; fetus died
	hypoxemia (chamber)		128			
	AVP (vehicle only)		131			
E4152	AVP (4.5 mU/min)	127	132	1	3.133	S/L; fetus euthanized
	AVP (9.0 mU/min)		134			
	neuromuscular blockade (pancuronium)		137			
E540	ischemia	130	140	2	3.96	S/L; fetus euthanized
E722	ischemia	123	138	2	3.096	C/S; fetus euthanized
E953y	AVP (vehicle only)	119	125	1	1.815	died in utero
E967	hypoxemia (trachea)	130	136	2	2.979	C/S; fetus died

Weight = weight of operated fetus at delivery  
 chamber = hypoxemia achieved via Plexiglas box  
 trachea = hypoxemia achieved via maternal tracheal catheter  
 AVP = arginine vasopressin infusion  
 S/L = spontaneous labour  
 C/S = Cesarean-section

**TABLE 3.** Successful surgical preparations of non-pregnant adult ewes.

<b>Ewe Number</b>	<b>Experiment</b>	<b>Ewe Weight (kg)</b>	<b>Fate</b>
E0101z	arginine vasopressin infusion (90 mU/min)	79	returned to UBC for rebreeding
	arginine vasopressin infusion (45 mU/min)		
E4146	arginine vasopressin infusion (25 mU/min)	60	returned to UBC for rebreeding
	arginine vasopressin infusion (45 mU/min)		
	arginine vasopressin infusion (90 mU/min)		
E4215	arginine vasopressin infusion (25 mU/min)	52	returned to UBC for rebreeding
	arginine vasopressin infusion (90 mU/min)		
	arginine vasopressin infusion (45 mU/min)		

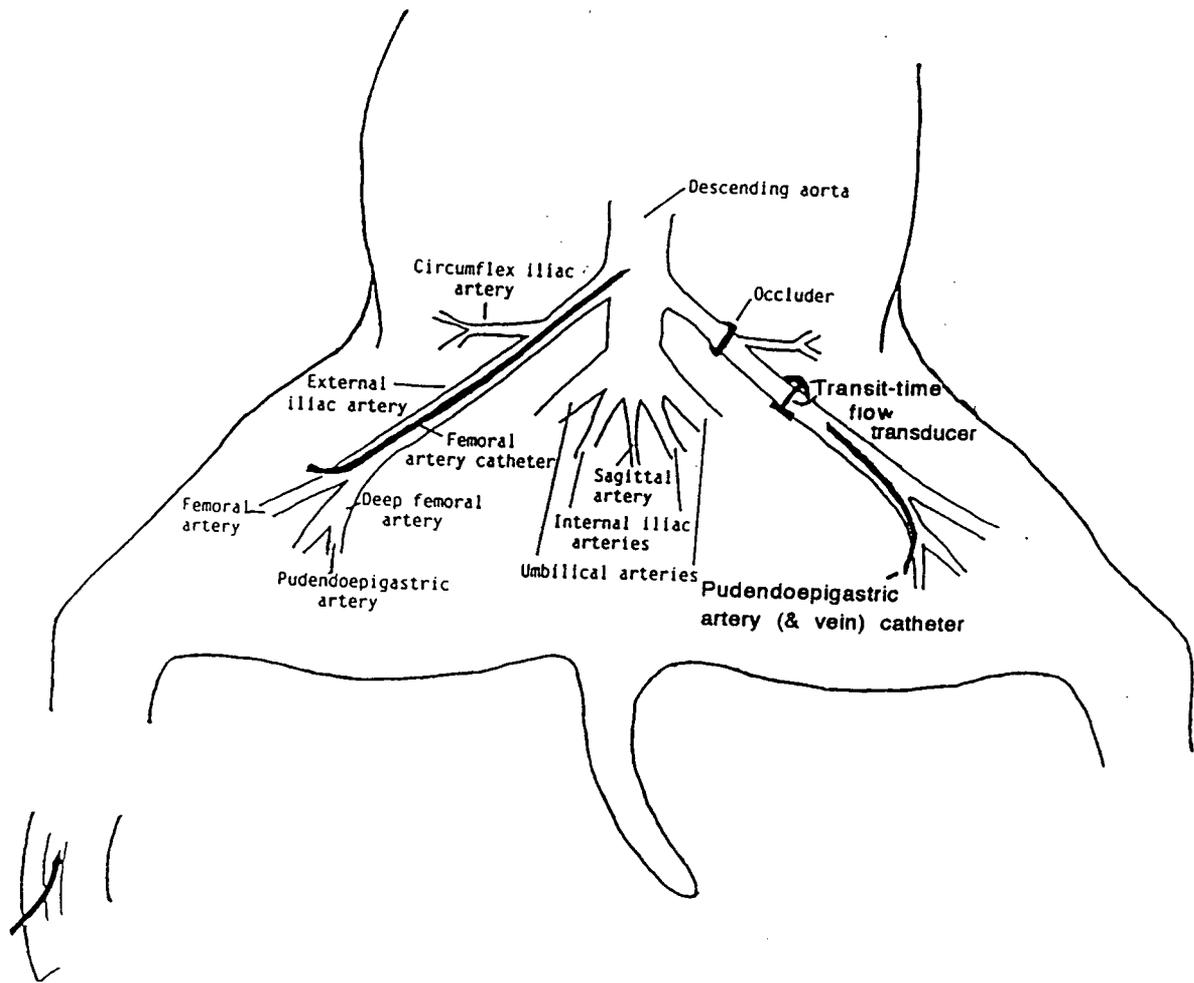
Animal Care Products, St. Paul, Minnesota) was applied to the catheter insertion point, and the incision was closed. The non-occlusive nature of this catheter did not interfere with lung fluid flux through the trachea. The amniotic catheter was simply anchored to the skin of the fetal neck.

While the fetal head was still exposed, electrodes for recording electrocortical (ECoG) and electro-ocular (EoG) activity were implanted in the skull. A transverse incision was made across the dorsal skull just rostral to the ears, the skin retracted, and two small holes (1.6 mm diameter) were drilled approximately 5 cm apart through the parietal region of the skull on either side of the midline. Electrodes for recording ECoG activity were positioned in the holes so as to rest on the parietal dura mater and adhered to the fetal skull with surgical glue applied to the plastic mounting disks. A grounding wire was sutured to the scalp skin. The scalp incision was then closed with 3-0 silk. Next, a 1 cm vertical incision was made over the orbital ridge of the zygomatic arch of each eye and, using a curved cutting needle, the EoG electrodes were passed through the bone. The stripped section of the wire was knotted in place and secured with tissue glue (Vetbond tissue adhesive, 3M Animal Care Products, St. Paul, Minnesota). After closing the incisions, the ECoG and EoG electrode catheters were secured to the fetal skin.

After completing the catheterization of the upper body, the uterine incision was closed and a second uterine incision was made through which the fetus' lower body could be exposed. Silicone rubber catheters were inserted into the femoral artery and lateral tarsal vein and advanced into the descending aorta and caudal vena cava, respectively. Both of these vascular catheters have an inner diameter of 0.63 mm (1.19 mm OD). This leg was designated the non-study or recording limb. In the contralateral leg, which serves as the sampling or study limb, smaller diameter catheters (0.51 mm ID, 0.94 mm OD) were inserted into the pudendoepigastric artery and vein and advanced 3–4 cm so that the catheter tips were in the external iliac artery and vein, respectively. Catheter placement involved making a small skin incision and then using blunt dissection to expose the vessel to be

catheterized. Three pieces of 3-0 silk were placed under the artery or vein. The blood vessel was tied off with the most distal piece of silk and the proximal silk was lifted up to temporarily constrict the vessel. A tiny incision was then made in the wall of the vessel between the distal and middle silks, the catheter inserted and advanced, and finally the three silk sutures were used to secure the catheter. A drop of surgical glue was also applied to the catheter entry point. Finally, the catheter was anchored to the adjacent muscle on either side of the incision with the silk tie attached to the catheter. A second amniotic catheter was also sutured to the fetal abdominal skin.

Next, a 5 cm posterior flank incision was made in the fetus and, using a lateral approach, the study limb's external iliac artery was exposed. Two pieces of Silastic® tubing were placed under this vessel and gently lifted so as to expose the vessel. A 4 mm (4S or 4R series) transit-time ultrasonic blood flow transducer (Transonic Systems, Ithaca, New York) and a 4 mm inflatable perivascular occluder (In Vivo Metric, Healdsburg, California) were positioned on this vessel, with the occluder placed proximal to the origin of the circumflex iliac artery and the transducer distally. The tubing was removed and the flow transducer and occluder were anchored to the adjacent muscle. To ensure accurate measurement of hindlimb blood flow, we ligated the circumflex iliac arterial trunk with hemoclips (Hemoclip®, Edward Weck and Company, Research Triangle Park, North Carolina). In addition, the internal iliac artery was ligated to minimize potential collateral circulation to the limb. The placement of an occlusive catheter in the pudendoepigastric artery effectively eliminated this vessel as a source of non-hindlimb blood flow, while permitting blood sampling without compromising limb perfusion. The location of fetal vascular catheters, transit-time flow transducer, and vascular occluder are illustrated in Figure 2. The fetus was then gently returned to the uterus and amniotic fluid lost during surgery was replaced with approximately 1500 ml of 0.9% irrigation saline (Baxter Corporation, Mississauga, Ontario). In the event that the ewe carried more than one lamb, only one fetus was instrumented (referred to as the operated fetus).



**FIGURE 2.** Line drawing of fetal lamb hindquarters (ventral view) indicating location of catheters, transit-time flow transducer, and vascular occluder.

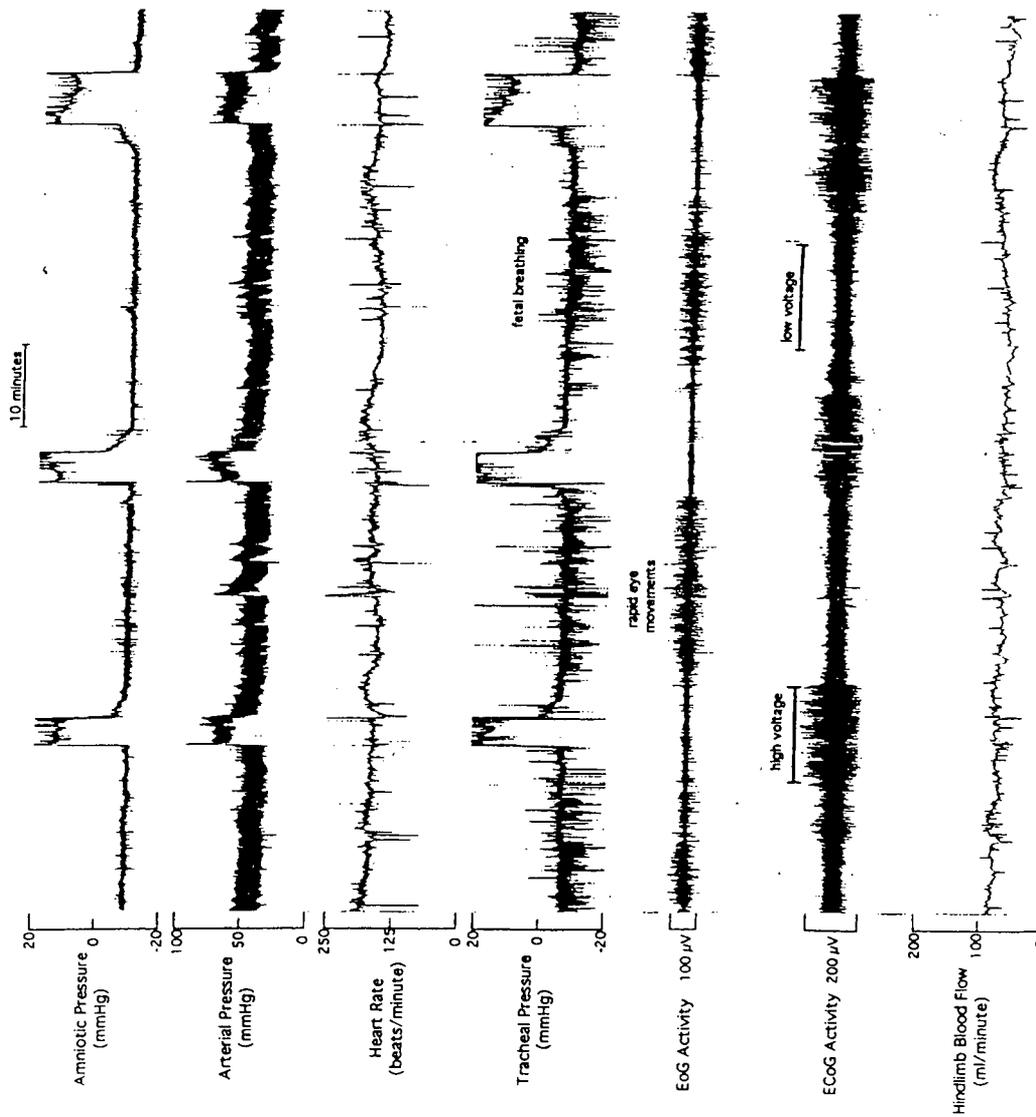
The uterine incision was closed with a continuous 2-0 gut chromic suture and then oversewn. Finally, using the same procedure as outlined above, catheters (1.02 mm ID, 2.16 mm OD) were placed in the maternal femoral artery and vein and advanced into the maternal descending aorta and caudal vena cava, respectively. Several ewes also had a transit-time flow transducer (4RB or 6RB series; Transonic Systems, Ithaca, New York) positioned on the external iliac artery for measurement of adult hindlimb blood flow. All catheters and cables were tunneled subcutaneously to emerge from an incision in the ewe's flank. The ewe's abdominal muscle layer was closed with discontinuous 3-0 Dexon (Davis+Geck Cyanamid Canada Inc., Montreal, Quebec) and Ethibond (Ethicon, Peterborough, Ontario) sutures. Finally the abdominal and flank skin incisions were closed with 3-0 Dexon. In some animals, a non-occlusive catheter (4.8 mm ID, 7.9 mm OD) made of Tygon tubing (Fisher Scientific, Vancouver, British Columbia) was also inserted into the maternal trachea for introduction of hypoxic gas mixtures.

All catheters were filled with heparinized saline (24 U/ml saline; Hepalean®, Organon Teknika, Toronto), capped, and stored in a denim pouch adhered to the ewe with medical adhesive. Elastic crepe bandages were wrapped around the ewe's midsection to keep the pouch and incision clean and to prevent the other animals from damaging the catheters. The patency of the catheters was maintained by daily flushing with heparinized saline. Prophylactic antibiotics consisting of 500 mg ampicillin sodium (Novopharm, Scarborough, Ontario) and 80 mg gentamicin (Garamycin®, Schering Canada Inc., Pointe-Claire, Quebec) were administered to the ewe intramuscularly for 5 days postoperatively. Ampicillin (500 mg) and gentamicin (20 mg) were injected daily into the amniotic cavity for the duration of the preparation. In order to monitor the condition of the fetus, blood samples were taken daily for determination of blood gas status, oxygen saturation, and hemoglobin, glucose, and lactate concentrations. The animals were allowed to recover from surgery for a minimum of three days before being used in experiments.

## 2.4 Physiological Recording and Monitoring Procedures

After several days to recover from surgery, the ewes were placed in monitoring pens and given free access to food and water. The fetal tracheal, amniotic, femoral arterial, and lateral tarsal venous catheters were connected to disposable DTX transducers (Spectramed, Oxnard, California) for continuous monitoring. Fetal heart rate was measured from the arterial pulse with a cardiometer (Gould Biotach amplifier model 13-4615-66, Gould, Cleveland, Ohio). The variables were recorded on either a Beckman R-711 polygraph recorder and on a computerized data acquisition system developed in our laboratory (Kwan *et al.*, 1995a) or on a Gould TA 4000 polygraph recorder combined with Labtech Notebook for Windows data acquisition program (Version 7.2.1 Advantec, Laboratory Technologies Corporation, Wilmington, Massachusetts). Figure 3 shows a typical polygraph recording obtained from a late gestation fetal lamb in which amniotic, arterial, and tracheal pressures, fetal heart rate, hindlimb blood flow, and electrocortical and electro-ocular activity were recorded. Episodes of fetal breathing, rapid eye movements (REM activity), and high and low voltage electrocortical activity (ECoG) are clearly evident. Venous pressure was not obtained from this animal. Abrupt changes in the pressure variables are due to position changes of the ewe. For data analysis, fetal pressures were corrected for amniotic pressure by the computer (eliminating artifacts due to the mother's position) and one minute average values were stored on disk.

Blood pH,  $P_{O_2}$ , and  $P_{CO_2}$  were measured with an IL 1306 pH/Blood gas analyzer (Allied Instrumentation Laboratory, Milan, Italy) corrected to 39°C for maternal samples and 39.5°C for fetal blood. An OSM2 Hemoximeter (Radiometer, Copenhagen, Denmark) was used to measure hemoglobin and oxygen saturation in triplicate. Blood glucose and lactate concentrations, also measured in triplicate, were determined with a 2300 STAT Plus glucose/lactate analyzer (YSI Bioanalytical Products, Yellow Springs, Ohio). Oxygen content was calculated as the product of hemoglobin concentration, oxygen saturation, and a



**FIGURE 3.** A typical polygraph recording obtained from a fetal lamb in late gestation. Episodes of fetal breathing, rapid eye movements, and high and low voltage electrocortical activity are indicated. Abrupt changes in amniotic, arterial, and tracheal pressures are due to position changes of the ewe. Hindlimb blood flow was obtained with a transit-time flow transducer positioned on the external iliac artery.

hemoglobin oxygen binding capacity of 1.34 ml/dL, and converted to mM. Other values were calculated as follows:

$$\text{Oxygen delivery} = \dot{V}O_2 = \text{hindlimb blood flow} \times \text{arterial } O_2 \text{ content}$$

$$\text{Oxygen consumption} = \dot{V}O_2 = \text{hindlimb blood flow} \times (\text{arterial} - \text{venous } O_2 \text{ content})$$

$$\text{Oxygen extraction (\%)} = E_{O_2} = \frac{\text{oxygen consumption}}{\text{oxygen delivery}} \times 100$$

$$\text{Lactate flux} = \text{hindlimb blood flow} \times (\text{arterial} - \text{venous lactate concentration})$$

$$\text{Glucose flux} = \text{hindlimb blood flow} \times (\text{arterial} - \text{venous glucose concentration})$$

$$\text{Hindlimb vascular resistance} = \frac{(\text{arterial pressure} - \text{hindlimb venous pressure})}{\text{hindlimb blood flow}}$$

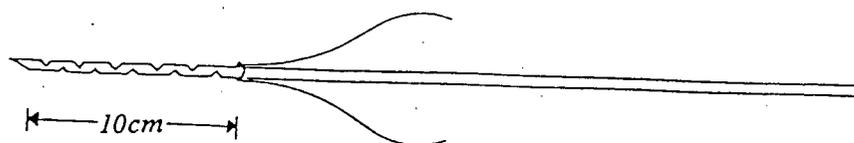
Fetal weight *in utero* was estimated using an equation for fetal growth in sheep (Koong and Rattray, 1975) that predicts an average rate of growth of about 2% per day which is similar to the value determined in our laboratory using blood volume estimates of weight *in utero* (Kwan *et al.*, 1995a). Fetal results were then normalized to hindlimb weight at the time of experimentation, with hindlimb weight calculated from the following equation:

$$\text{Hindlimb weight}_{in\ utero} = \text{fetal weight}_{in\ utero} \times \frac{\text{hindlimb weight}_{autopsy}}{\text{fetal weight}_{autopsy}}$$

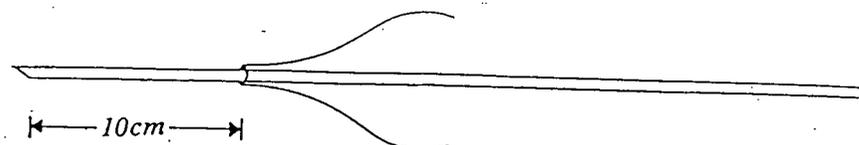
## 2.5 Catheter and Electrode Preparation

Catheter construction is illustrated in Figure 4. Catheters were made of silicone rubber (Silastic®, Dow Corning, Midland, Michigan) with outer diameters appropriate for the vessel size (Table 4). For ease of insertion into the blood vessel, the catheter tip was cut on an angle. Ties made of 3-0 silk were securely knotted around the catheter tip for securing of the catheter in the vessel. Catheters made for insertion into the amniotic cavity

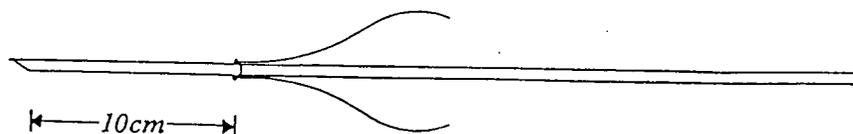
Amniotic catheter



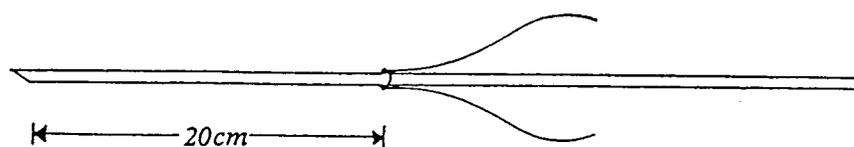
Tracheal catheter



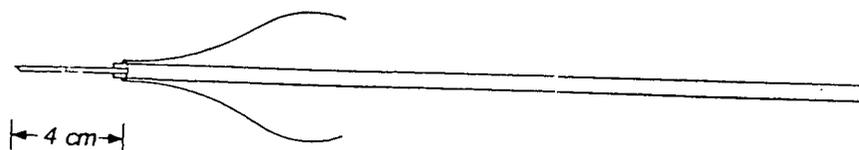
Femoral artery catheter



Tarsal vein catheter



Pudendoepigastric artery/vein catheters



**FIGURE 4.** Construction of silicone rubber (Silastic®) catheters implanted in the late-gestation fetal lamb preparations. Diagrams are not drawn to scale.

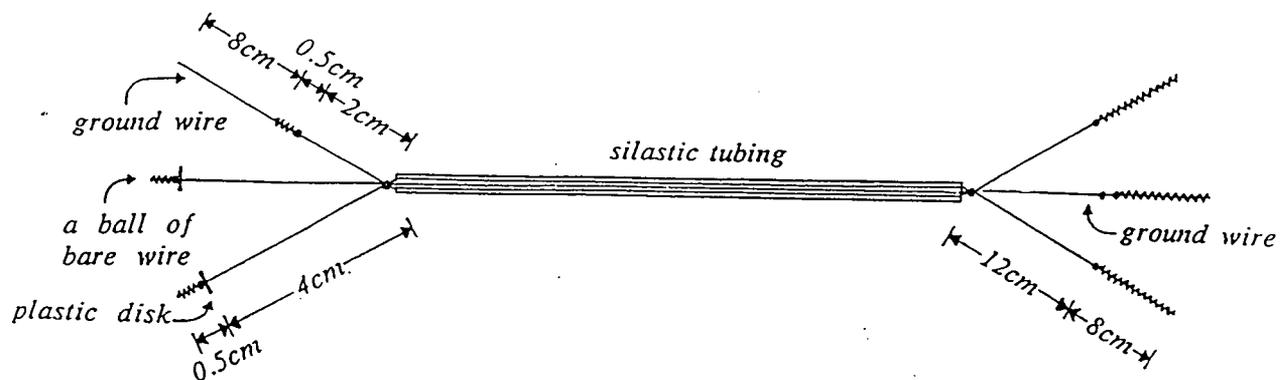
**TABLE 4.** Inner and outer diameters of silicone rubber (Silastic®) catheters used in animal preparations.

<b>Catheter</b>	<b>Inner Diameter (mm)</b>	<b>Outer Diameter (mm)</b>
Amniotic	1.02	2.16
Fetal trachea (non-occlusive)	1.02	2.16
Fetal femoral artery	0.63	1.19
Fetal lateral tarsal vein	0.63	1.19
Fetal pudendoepigastric artery	0.51	0.94
Fetal pudendoepigastric vein	0.51	0.94
Maternal femoral artery	1.02	2.16
Maternal femoral vein	1.02	2.16

were similarly prepared except that a series of small holes was cut along the length of the catheter distal to the ties. This design helped reduce the likelihood of catheter tip obstruction by the membranes lining the amniotic cavity. On the contralateral end of all catheters (i.e., end that emerges from the flank incision) bands of coloured silicone were applied to enable identification of the vessel in which each catheter was positioned. Catheters prepared for the pudendoepigastric vessels were modified slightly from the description above. In these catheters, the portion of the catheter actually inserted into the vessels was 0.94 mm outer diameter (0.51 mm ID). This was then telescoped into larger diameter tubing (1.02 mm ID; 2.16 mm OD). The small lumen section, approximately 3.75 to 4 cm in length, was appropriate for the small diameter of these vessels. However, because withdrawing blood samples through a long length of small lumen catheter is very slow, the addition of the larger lumen section was required.

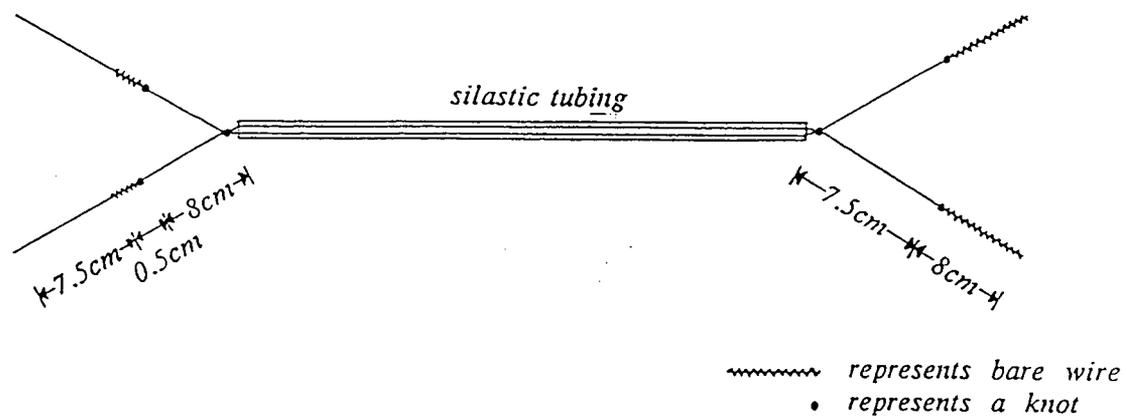
As illustrated in Figure 5 electrocortical and electro-ocular electrodes were prepared by threading Teflon sheathed multi-strand stainless steel wire (Cooner Wire, Chatsworth, California) through Silastic® tubing with an outer diameter of 1.95 mm (1.47 mm ID). The ECoG electrodes were composed of two lengths of wire whose ends were threaded through small plastic disks, knotted, and the stripped ends formed into a stiff oblong ball. The coiled ends were inserted through holes made in the skull for contact with the brain while the disks were used for securing the electrode to the skull with tissue glue. A third length of wire, used as a ground, was secured to the fetal scalp. Electro-ocular electrodes consisted of a total of two lengths of wire inserted through a silicone rubber catheter. Stripped sections of the end wires were secured next to each eye in the fetus. Small sections of the contralateral ends (i.e., ends emerging from ewe's flank) of the EoG and ECoG electrodes were stripped for connection to the computer cable which records their output.

## Electrocortical (ECoG) electrode



The plastic disk was 0.5cm in diameter.

## Electro-ocular (EoG) electrode



**FIGURE 5.** Construction of electrocortical and electro-ocular electrodes for the late gestation fetal lamb preparations. Diagrams are not drawn to scale.

## 2.6 Statistical Analysis

Data are presented as means  $\pm$  1 standard error of the mean unless otherwise indicated. Data were analyzed using StatView statistical software (Abacus Concepts Inc., Berkeley, California). Results were compared using two-tailed paired or unpaired t-tests as appropriate. For multiple comparisons, one-way (factorial) analysis of variance followed by Fisher's Protected Least Significant Difference (PLSD) post-hoc test were used. Differences were considered significant if  $p < 0.05$ .

### **3. HINDLIMB BLOOD FLOW MEASUREMENT CORRELATION: MICROSPHERES VERSUS TRANSIT-TIME FLOW TRANSDUCER**

#### **3.1 Introduction**

In order to study oxygen delivery and consumption in the fetal lamb hindlimb, continuous accurate measurements of hindlimb blood flow were necessary. This required the determination of total blood flow into and out of the hindlimb. Radionuclide-labeled microspheres are widely used to determine blood flows to specific tissues but do not enable continuous measurements. Thus, we elected to use a transit-time flow transducer positioned on the external iliac artery to provide these continuous measurements. By correlating the hindlimb blood flow measurements obtained with each of these techniques, we hoped to determine sources of error, including potential sources of collateral flow or blood vessels in the hindlimb that serve primarily non-hindlimb tissues. Based on these results and data from other researchers, we sought to develop a surgical protocol which would permit us to obtain continuous accurate measurements of hindlimb blood flow using a transit-time flow transducer positioned on the external iliac artery.

Radionuclide-labeled microspheres are non-biodegradable, inert, polystyrene spheres with a radioactive nuclide incorporated into the plastic as opposed to simply attached to the surface. Nuclide labels are chosen for well defined energy peaks with a minimum overlap of energy spectra. This permits the injection of up to five different radionuclide labels into a single animal. Computer software integrated with a gamma counter differentiates the radioactivity emitted when multiple nuclides are present in each sample.

Microspheres are available in a number of diameters and approximate the specific gravity of blood. To prevent aggregation of the spheres, they are generally suspended in isotonic saline with Tween-80 (polyoxyethylene 80 sorbital monooleate) added. Theory dictates that the microspheres are distributed in proportion to blood flow and will be trapped

in capillary beds on the first circulation; obviously the microspheres must remain entrapped until counting can be completed. In sheep, 15  $\mu\text{m}$  diameter microspheres are generally used to satisfy the criteria of distribution similar to erythrocytes and the absence of recirculation or non-entrapment (Heymann *et al.*, 1977). Similar counts obtained for paired organs such as the kidneys indicate good mixing and even distribution of microspheres.

Radionuclide-labeled microspheres have been widely used to measure the distribution of cardiac output and organ blood flow since the pioneering work of Rudolph and Heymann (1967) and subsequent introduction of the reference sample method (Makowski *et al.*, 1968). For adequate mixing of the microspheres in blood, they should pass through the left side of the heart before reaching the systemic circulation. In newborns and adults this requires left atrial or ventricular injection. In the fetus it is possible to inject the microspheres into the systemic venous circulation. Because of shunts in the fetal circulation, in particular the foramen ovale and ductus arteriosus, it is possible to inject the microspheres into the systemic venous circulation; most of the spheres bypass the lungs and become entrapped in the systemic circulation. Rudolph and Heymann (1967) concluded that microspheres do not significantly recirculate, are distributed in proportion to blood flow, and do not alter circulatory physiology.

Calculation of blood flow to individual organs is simplified by the reference sample method introduced by Makowski and colleagues (Makowski *et al.*, 1968). In this technique, an arterial blood sample is collected at a known, constant rate into a glass syringe which thus acts as an artificial organ. Blood flow to a specific organ can then be calculated as the product of this reference blood flow rate and the ratio of the radioactivity in the organ to that in the reference sample. In order to keep errors  $\pm 10\%$ , it is estimated that a minimum of 400 microspheres should be present in each sample (Buckberg *et al.*, 1971).

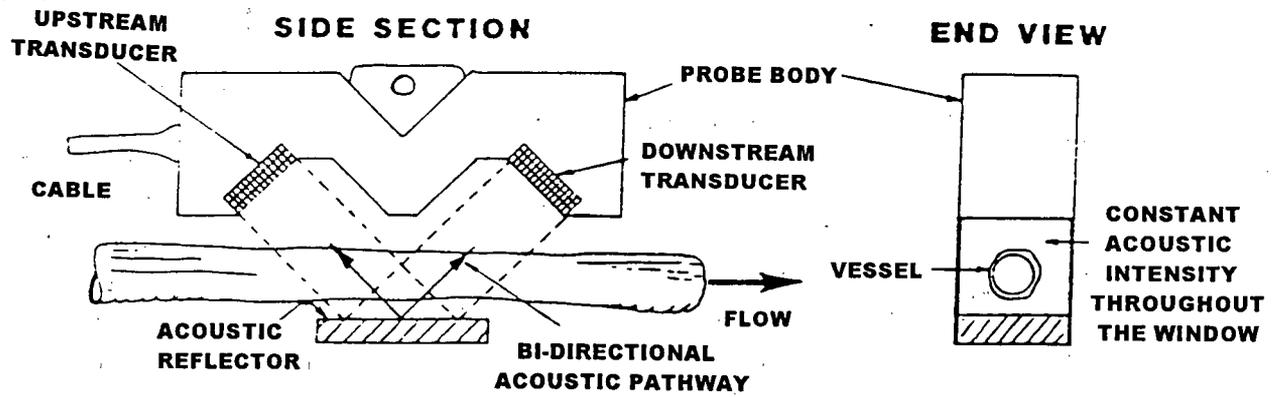
Archie *et al.* (1973) used a calibrated pump during heart bypass in lambs and dogs to create known cardiac outputs. Microspheres were found to give cardiac output measurements within 10 to 15% of the known values. The microsphere method also

compares favourably with blood flow measurements obtained with electromagnetic flowprobes (Buckberg *et al.*, 1971; Rudolph and Heymann, 1967). Furthermore, a number of experimenters have used serial injections of microspheres for multiple flow measurements with little loss of precision or alteration of hemodynamics due to embolization of vascular beds (Jansson, 1991; Baer *et al.*, 1984).

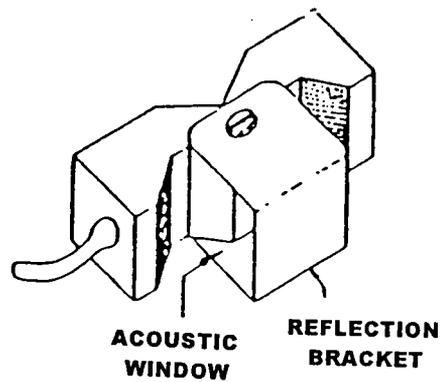
The Transonic® transit-time flow transducer, unlike other widely used *in vivo* flowmeters, is largely unaffected by its positioning on the vessel, is not limited by electrical interference, and, as a tight fit is not required, the flow transducer is non-constrictive. The transducer body houses upstream and downstream ultrasonic transducers with a fixed acoustic reflector positioned opposite (Figure 6). The flow transducer is placed on a blood vessel such that the transducers are on one side of the vessel and the reflector is located on the opposite side midway between the transducers. A plane wave of ultrasound emitted by each of the transducers bounces off the acoustic reflector and is received by the other transducer. The sound wave emitted by the upstream transducer travels with a vector component of the blood flow. As the downstream transducer's sound wave travels against the direction of blood flow, its transit time will be prolonged relative to the transit-receive sequence of the upstream transducer. Flowmeter circuitry calculates the difference in total transit time of the two cycles to record a signal proportional to the rate of blood flow. This signal, scaled with the predetermined calibration factor of the flow transducer, is displayed as the absolute volume rate of flow through the vessel. As the Transonic® flow transducer relies solely on the difference of the transit times it is independent of vessel diameter or alignment of the flow transducer.

Transit time flowmeters have been calibrated *in vivo* against established methods of measuring blood flow. When measuring ascending aorta blood flow in dogs Hartman and coworkers report correlations of over 0.97 between transit time flowmeters and electromagnetic flowprobes, thermodilution, and exsanguination (Hartman *et al.*, 1985).

Transonic® transit-time flow transducer



Schematic view of a transit-time flow transducer



**FIGURE 6.** Transonic® transit-time flow transducer and its operation (from Hartman *et al*, 1985). The transducer is positioned around the external iliac artery and provides continuous measurements of hindlimb blood flow.

Radionuclide-labeled microspheres allow the determination of organ blood flow *in vivo* with little disturbance to the animal; however, they are limited to measuring blood flow at only brief moments in time and require the animal to be sacrificed. In contrast, transit-time ultrasonic flow transducers measure blood flow continuously *in vivo*, but they do not give specific organ blood flows. In the present study, hindlimb blood flow in the fetal lamb was measured with a transit-time flow transducer on the external iliac artery and compared with measurements obtained with radionuclide-labeled microspheres.

### 3.2 Methods

Five animals underwent surgery between 118–125 days gestation (mean  $122 \pm 2$  days) using the techniques previously described (Section 2.3). Tracheal, amniotic, femoral arterial, lateral tarsal venous, and pudendoepigastric arterial and venous catheters were inserted. Hindlimb blood flow was continuously measured with a transit-time flowmeter on the external iliac artery distal to the circumflex iliac artery. Flow through this vessel was altered by partial inflation of the vascular occluder achieved by injecting saline into the occluder through a 3 cc syringe mounted on a Harvard infusion/withdrawal pump. Blood flow was maintained at each level for 10-15 minutes. A total of 19 observations were made in the 5 animals.

The distribution of hindlimb blood flow was measured with 15  $\mu\text{m}$  diameter radionuclide-labeled microspheres using established technique (Rurak *et al.*, 1990a). Briefly, a pre-weighed 10 cc glass syringe mounted on a Harvard Pump was set to withdraw blood from the femoral artery at 2.2 ml/min. After 30 seconds, approximately  $1.2 \times 10^6$  sonicated microspheres (3M Health Care, St. Paul, Minnesota), labeled with one of  $^{141}\text{Ce}$ ,  $^{85}\text{Sr}$ ,  $^{153}\text{Gd}$ ,  $^{51}\text{Cr}$ , and  $^{95}\text{Nb}$ , were injected into the tarsal vein over 1 minute. The pump continued to withdraw blood for 2 minutes after microsphere injection. The syringe was then reweighed and the blood transferred into counting tubes. The availability of five different radionuclide-labels permitted up to five observations, at different degrees of

external iliac artery occlusion, on each animal. After completion of the serial microsphere injections, the animals were euthanized, tissues were collected from both hindlimbs and other lower body (distal to the diaphragm) sites, weighed, and catheter placement was verified. The tissues were carbonized in a 300°C oven, packed into counting tubes, and analyzed in a gamma counter (Micrad Inc., Knoxville, Tennessee). Tissue blood flow was calculated as follows:

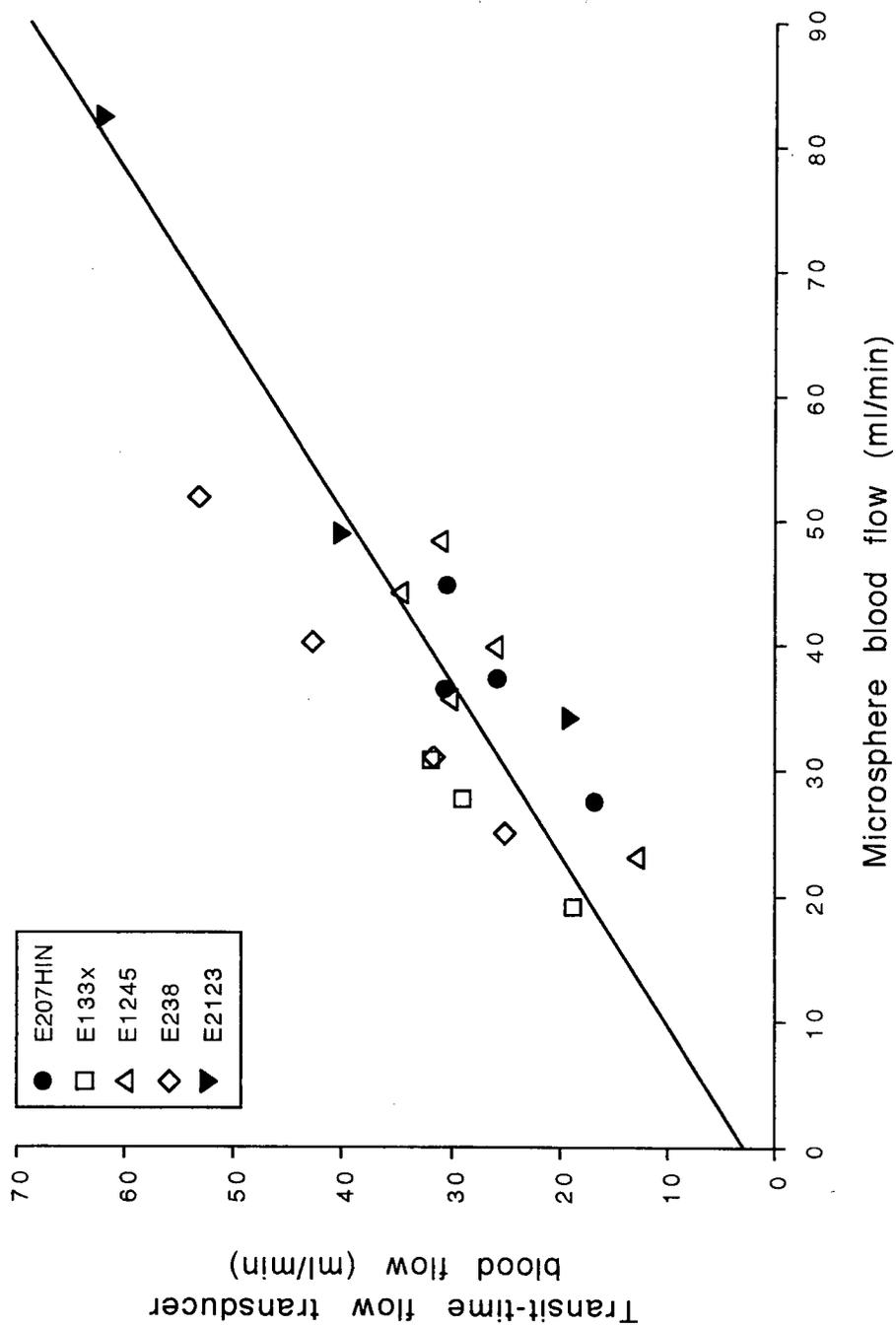
$$\text{Tissue blood flow (ml/min)} = \text{withdrawal rate (ml/min)} \times \frac{\text{tissue radioactivity}}{\text{reference sample radioactivity}}$$

### 3.3 Results and Discussion

In all the microsphere observations presented, similar numbers of microspheres were obtained for the right ( $13407 \pm 882$ ) and left kidneys ( $13569 \pm 698$ ), indicating good mixing and even distribution of microspheres. In addition, the blood and tissue samples contained at least 400 microspheres, the minimum number stipulated by Buckberg for blood flow errors to be  $\pm 10\%$  in sheep (Buckberg *et al.*, 1971).

The calculated hindlimb blood flow obtained from the microspheres was compared with the average transit-time flowmeter reading during the same interval. The overall regression line for the 19 observations is presented in Figure 7. The regression data does not go through the origin (y-intercept = 3.02) indicating that the flow transducer is measuring some blood flow that is not accounted for in the microsphere calculations, probably reflecting the fact that data was collected from animals that did not undergo identical surgical procedures. The difference is within the inherent inaccuracies ( $\pm 5-15\%$ ) of the two methods for determining blood flow (Burton and Gorewit, 1984; Archie *et al.*, 1973; Buckberg *et al.*, 1971).

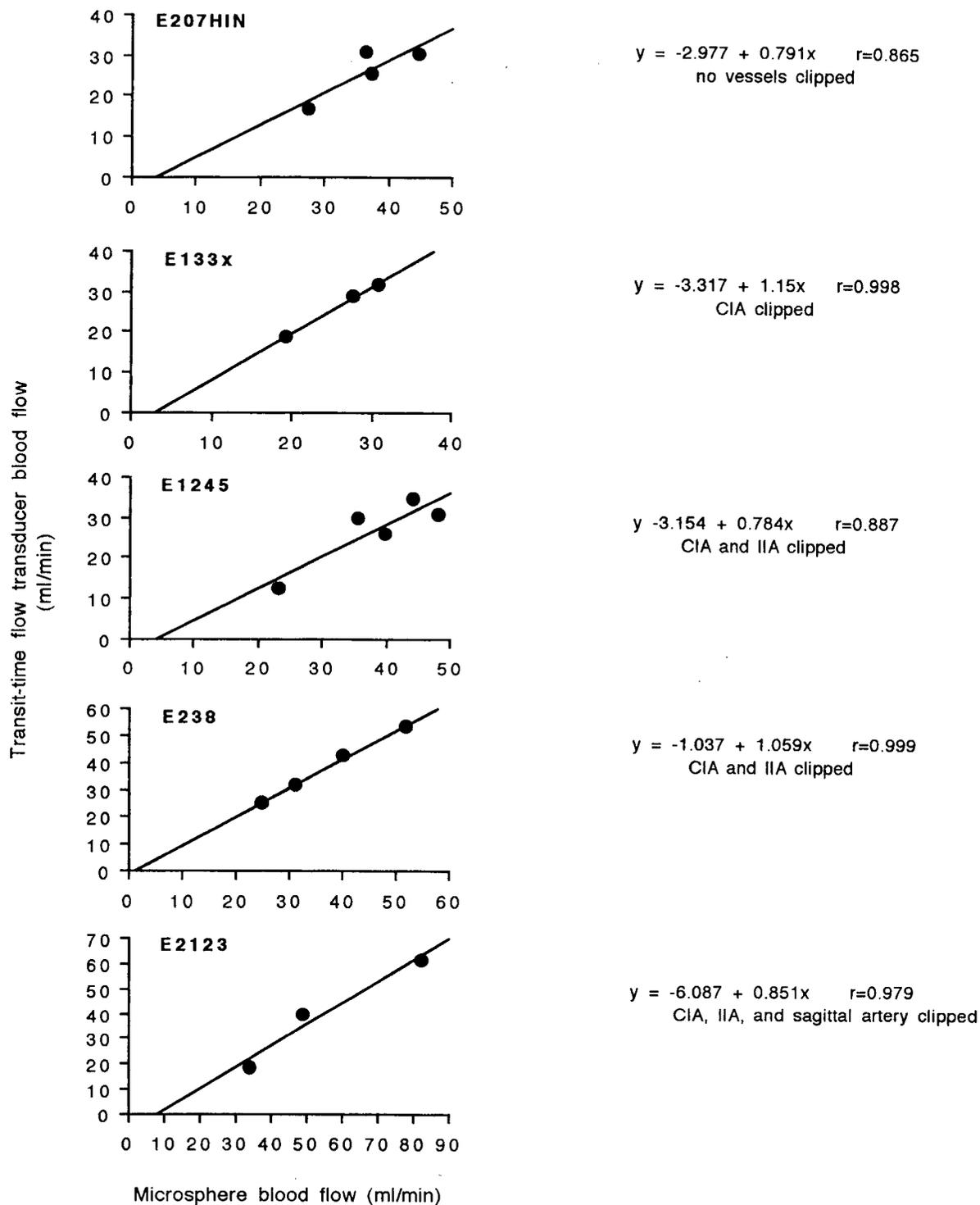
For the individual animals the correlation coefficients ( $r$ ) ranged from 0.865 to 0.999 indicating a very good agreement between microsphere and transit-time flowmeter



**FIGURE 7.** Comparison of fetal lamb hindlimb blood flow as measured with a transit-time flow transducer and radionucleide-labeled microspheres for five fetal lambs. Regression equation:  $y = 3.023 + 0.731x$   $r = 0.854$

measurements of hindlimb blood flow (Figure 8). The casting results indicated that the circumflex iliac (CIA) and internal iliac arteries (IIA) are potential sources of error in determining hindlimb blood flow. This was also suggested by the above results as animal E207HIN, in which no hindlimb vessels were hemoclipped, demonstrated a lower correlation coefficient compared to the animals in which the CIA and IIA were ligated. Animal E133x, in which only the CIA was hemoclipped, had a very high correlation coefficient ( $r = 0.998$ ) indicating that, because the CIA serves both hindlimb and non-hindlimb tissue, it must be ligated to ensure accurate measurements of blood flow. The internal iliac artery, a potential source of collateral flow, plays a less important role. Thus, hindlimb blood flow would be overestimated by a flow transducer on the external iliac artery proximal to the origin of the CIA unless the latter vessel was ligated. Considering these data and the casting results, ligation of both the CIA and IIA was incorporated into the surgical protocol. In one animal (E2123) we attempted to catheterize the sagittal artery and advance the catheter into the descending aorta; however, the vessel was too small and had to be ligated. As the sagittal artery was found to serve only the tissues of the tail, it was not hemoclipped in subsequent animals.

To further confirm the casting results, total occlusion of the external iliac artery followed by injection of microspheres was carried out on several animals to determine potential sources of collateral blood flow. However, although complete occlusion was achieved as indicated by a flowmeter reading of 0 ml/min, microspheres appeared in the hindlimb tissue in normal or above normal numbers. We speculated that microspheres in the systemic circulation accumulated in the length of the external iliac artery proximal to the occluder during total occlusion and when the occluder was subsequently released, these microspheres flowed into the hindlimb tissues as normal. Considering these results, any data points in which hindlimb blood flow was reduced to less than 20% of normal blood flow were considered inaccurate and excluded from subsequent analyses.



**FIGURE 8.** Individual animal comparisons of fetal lamb hindlimb blood flow as measured with transit-time flow transducers and radionuclide-labeled microspheres. The ewes' numbers are indicated on each graph (i.e., E133x). Each symbol represents a separate blood flow measurement (different radionuclide label). The regression equations and the blood vessels that were ligated are also indicated. CIA = circumflex iliac artery; IIA = internal iliac artery

In conclusion, the circumflex iliac, internal iliac, and pudendoepigastric arteries are potential sources of error when measuring total hindlimb blood flow with a transit-time flow transducer on the external iliac artery. When used in conjunction with a surgical protocol that ligates these vessels, blood flow measured with a flow transducer provides a good correlation to measurements obtained with radionuclide-labeled microspheres. Thus, a transit-time flow transducer can be used to provide continuous accurate determinations of hindlimb blood flow in the late gestation fetal lamb.

## 4. HINDLIMB BLOOD FLOW AND WEIGHT DISTRIBUTION

### 4.1 Introduction

Although the use of occlusive-type catheterization is commonly used in hindlimb vessels (Rudolph and Heymann, 1984), there is limited information about its effect on hindlimb blood flow and growth. In the present study, we compared weight and blood flow distribution in the late gestation fetal lamb hindlimb under normal conditions. We implanted catheters in the femoral artery and lateral tarsal vein of one hindlimb (non-study limb) and smaller diameter, shorter catheters in the more peripheral pudendoepigastric artery and vein of the other (study) limb. The effect of these different catheterization protocols on the weight and blood flow distribution of the study and non-study hindlimbs was assessed. This research has been previously published by our laboratory (Knight *et al.*, 1996).

### 4.2 Methods

Surgery was performed on 6 sheep between 118 and 125 days gestation (mean =  $123 \pm 1$  days, term ~145 days), using the techniques previously described (Section 2.3). Briefly, silicone rubber catheters (2.26 mm OD; Dow Corning, Midland, Michigan) were placed in the fetal trachea and amniotic cavity. Occlusive catheters (1.19 mm OD) were placed in the femoral artery and lateral tarsal vein of one hindlimb (non-study limb) and advanced into the descending aorta and caudal vena cava, respectively. In the other limb (study limb), smaller catheters (0.94 mm OD) were inserted into the pudendoepigastric artery and vein and advanced 3–4 cm so that the catheter tips lay in the external iliac artery and vein, respectively. For the purposes of other experiments, a transit-time ultrasonic blood flow transducer (Transonic Systems, Inc., Ithaca, New York) and a 4 mm vascular occluder (In Vivo Metric, Healdsburg, California) were placed around the external iliac artery of the study limb, with the transducer being distal to the origin of the circumflex iliac artery of the study limb, and the occluder being proximal. The circumflex iliac arterial trunk

and the internal iliac artery were ligated. The right leg was instrumented as the study limb in 2 fetuses while the left limb was used in the remaining 4 animals. Finally, catheters (2.16 mm OD) were inserted into a maternal femoral artery and vein.

The studies were conducted between 129 and 139 days gestation (mean =  $134 \pm 2$  days), 6–14 days (mean  $10 \pm 1$  days) following surgery. Hindlimb blood flow distribution was measured using radionuclide-labeled microspheres during the control periods of other experiments. Only the microsphere flow data were used and only that obtained prior to flow manipulation in the hindlimbs. The ewes and fetuses were euthanized with pentobarbital sodium (Euthanyl Forte, M.T.C. Pharmaceuticals, Cambridge, Ontario) 1–2 days after experiment completion (8–15 days post-surgery). Data are presented as mean  $\pm$  standard error of the mean. Results were compared using two-tailed paired t-tests with differences considered significant if  $p < 0.05$  (StatView statistical software, Abacus Concepts Inc., Berkeley, California).

### 4.3 Results

The values for arterial  $P_{O_2}$  ( $18.4 \pm 1.2$  mmHg),  $P_{CO_2}$  ( $51.0 \pm 1.0$  mmHg), pH ( $7.284 \pm 0.012$ ), glucose ( $0.61 \pm 0.11$  mM) and lactate concentrations ( $2.07 \pm 0.84$  mM), arterial blood pressure ( $46.9 \pm 0.8$  mmHg) and heart rate ( $150 \pm 1$  bpm) on the day of blood flow estimation were within the normal range for our laboratory.

Table 5 presents the weights of the study and non-study hindlimbs for the 6 animals studied, as well as fetal weight. Although non-study limb weight was  $2.8 \pm 1.3\%$  less than that of the study limb, the difference was not statistically significant. In Table 6, the hindlimb weight data are broken down into skin, bone, and muscle components, and again there was no significant weight differences between the study and non-study limb tissue components. In addition, the distribution of hindlimb blood flow to skin, bone, and muscle in the study and non-study limbs is given in Table 6. The values for hindlimb blood flow to each tissue, expressed as a percentage of total hindlimb flow, are plotted in Figure 9, along

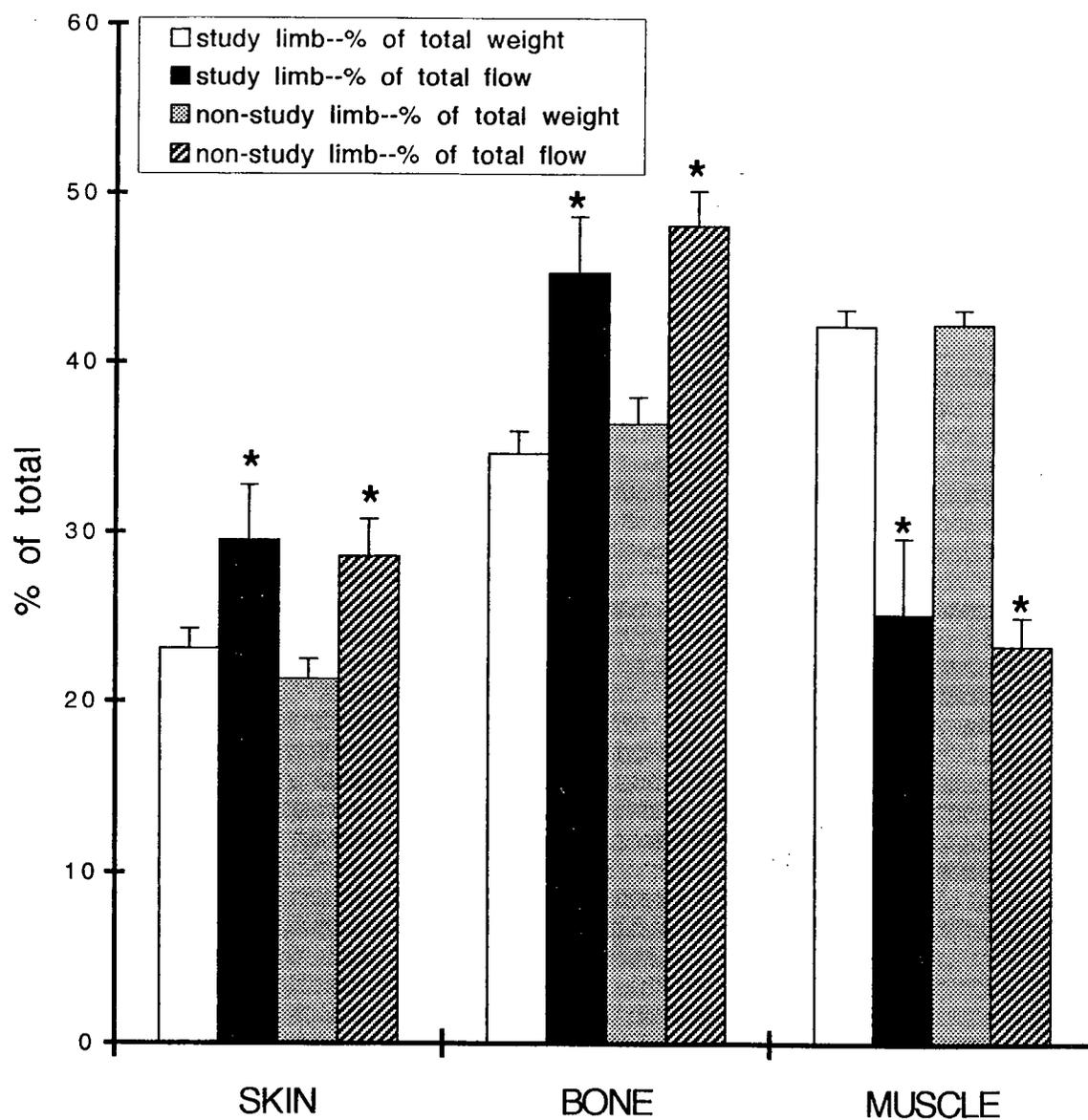
**TABLE 5.** Gestational age, fetal and hindlimb weights at autopsy for the six fetuses used in the hindlimb blood flow and weight distribution study. In the case of multiple lambs, weights are those of the operated fetus only.

Ewe Number	# of fetuses	Gestational age (days)	Fetal weight (g)	Study limb		Nonstudy limb	
				Weight (g)	% of fetal weight	Weight (g)	% of fetal weight
E207HIN	2	132	2218	184.2	8.30	173.4	7.82
E238	2	133	2604	169.0	6.49	169.5	6.51
E136	1	137	3158	252.5	7.99	242.9	7.69
E207HLIM	1	137	3766	261.6	6.95	263.5	7.00
E1245	3	139	3225	225.4	6.99	210.6	6.53
E133	3	131	1598	117.1	7.33	115.3	7.22
Mean $\pm$ SEM		135 $\pm$ 1	2762 $\pm$ 319	201.6 $\pm$ 22.6	7.34 $\pm$ 0.28	195.9 $\pm$ 22.1	7.13 $\pm$ 0.23

**TABLE 6.** Hindlimb tissue weight and blood flow distribution on the day of experimentation for the six fetal lambs used in the hindlimb blood flow and weight distribution study.

Tissue	Study Limb			Non-Study Limb		
	weight* (g)	absolute flow (ml/min)	relative flow (ml•min <sup>-1</sup> •100g <sup>-1</sup> )	weight* (g)	absolute flow (ml/min)	relative flow (ml•min <sup>-1</sup> •100g <sup>-1</sup> )
Skin	44.9±4.5 <sup>a</sup>	12.7±2.3	30.0±4.9 <sup>a</sup>	41.5±5.2 <sup>a</sup>	11.0±2.3 <sup>a</sup>	28.3±3.9 <sup>a</sup>
Bone	68.3±8.8 <sup>b</sup>	19.8±4.2	30.1±3.3 <sup>a</sup>	68.1±8.1 <sup>b</sup>	17.3±2.4 <sup>b</sup>	27.1±2.0 <sup>a</sup>
Muscle	83.6±11.1 <sup>c</sup>	9.5±1.0	14.0±3.1 <sup>b</sup>	81.7±10.9 <sup>b</sup>	8.3±1.0 <sup>a</sup>	11.6±1.7 <sup>b</sup>
Leg	196.8±22.9	42.0±6.0	23.2±2.1	191.2±22.5	36.6±5.4†	20.7±2.0†

Values are mean ± SEM. \* indicates weight in utero (estimated). † indicates significant difference from study limb. Different letter superscripts within each column indicate a significant difference.



**FIGURE 9.** Comparison of tissue weights (open columns, study limb; stippled columns, non-study limb) and blood flows (closed columns, study limb, diagonal-hatched columns, non-study limb) for skin, bone, and skeletal muscle in six fetal lambs. Bars are mean  $\pm$  SEM. \* = significant difference between hindlimb blood flow to each tissue, expressed as a percentage of total limb flow, and the weight of each tissue, expressed as a percentage of total limb weight ( $p < 0.05$ ).

with the weight of the tissues expressed as a percentage of total limb weight. In every animal, total absolute flow and flow per 100 g tissue were less in the non-study limb, with the mean weight normalized flow being  $13.4 \pm 1.8\%$  less than in the study limb. Blood flows to muscle, skin, and bone also tended to be lower in the non-study limb, but the differences were not statistically significant. In both limbs, skin and bone blood flows, expressed per 100 g weight, were similar, and both were significantly higher than the flow to muscle. This indicates that, compared to hindlimb muscle, the skin and bone components are more highly perfused in relation to the proportion of total limb weight that they comprise (Table 6, Figure 9). Bone received the highest fraction ( $45.3 \pm 3.6\%$ ) of total flow, compared to skin ( $29.5 \pm 3.3\%$ ) and muscle ( $25.2 \pm 4.5\%$ ).

#### 4.4 Discussion

Several authors have reported that the hindlimb comprises approximately 10% of fetal weight in late gestation (Boyle *et al.*, 1992; Boyle *et al.*, 1990; Wilkening *et al.*, 1988; Dawes *et al.*, 1968), while in our animals the mean value for the study limb was  $7.34 \pm 0.28\%$ . This discrepancy may be due to differences in the tissues, particularly muscle, considered as hindlimb, variations in the breed of sheep used, or differences in the number of fetuses per ewe. Previous estimates of hindlimb blood flow in the fetal lamb average  $\sim 18 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ , while estimates of flow to the general carcass range from 19 to  $26 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  (Boyle *et al.*, 1992; Davis and Hohimer, 1991; Jensen *et al.*, 1991; Wilkening *et al.*, 1988; Court *et al.*, 1984). This compares to our estimates of  $23.2 \pm 2.1$  and  $20.7 \pm 2.0 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  for the study and non-study limbs, respectively. Similarly, our estimates of study limb blood flow to the skin, muscle, and bone (Table 6) fall in the range of values reported by others: skin, 19–32  $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ , bone, 18–30  $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ , and muscle, 10–17  $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  (Davis and Hohimer, 1991; Rurak *et al.*, 1990a; Wilkening *et al.*, 1988; Jensen *et al.*, 1987; Jensen *et al.*, 1986). However, we found that in relation to weight, the skin and bone of the hindlimb have higher perfusion rates compared to muscle (Figure

9), with bone receiving the highest fraction of total limb flow. Similar results can be extracted from data given in Wilkening *et al.* (1988), but this was not commented on in that paper nor apparently elsewhere.

The rate of bone blood flow in the fetus ( $\sim 30 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ) is 2 to 3 fold higher than the values reported in the adult pig, dog, and rabbit, which range from 9–14  $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  (Kiaer *et al.*, 1993). This is also the case for muscle perfusion, which averages  $\sim 7 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  in adult sheep hindlimb (Oddy *et al.*, 1981), compared to our estimates in the fetus of  $\sim 14 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ . The higher tissue perfusion rates in the fetus likely reflect the need to compensate for the lower oxygen content in fetal blood compared to the adult (Rurak, 1994). In both the fetus and adult there appears to be a higher rate of blood flow to bone than to muscle in the hindlimb. In adult rat hindlimb, blood flow to marrow is higher than to bone, and marrow perfusion is increased by about 3 fold when hematopoiesis is stimulated by hemorrhage, normovolemic anemia, or erythropoietin administration (Iverson *et al.*, 1992). The high perfusion rate that we have observed in fetal bone may also result from hematopoiesis. In the last 2 weeks of gestation, there is a rapid switch from fetal to adult hemoglobin (Bard *et al.*, 1972). This, plus the rapid growth rate ( $\sim 2\%$ /day) in late gestation (Kwan *et al.*, 1995a), likely results in a high rate of hematopoiesis. Skin perfusion is elevated probably due to vasodilation in response to the high ambient temperature in the amniotic cavity. When amniotic fluid and fetal temperature are decreased, skin blood flow, particularly in the lower body, decreases significantly (Van Bel *et al.*, 1993). Skeletal muscle blood flow in the fetal hindlimb may be lower than that to skin and bone because of the limited extent of limb movement and the lack of any requirement for thermogenesis. It increases about three-fold 2 hours after Cesarean delivery probably due to shivering thermogenesis, increased limb movement, and postural muscle activity (Richardson *et al.*, 1989).

Although chronically catheterized fetal sheep have been utilized for more than 25 years (Meschia *et al.*, 1965), there have been few studies of the effects of occlusive

catheterization on the organs and tissues normally perfused by the catheterized vessel. Wilkening *et al.* (1988) attributed a 4% difference between hindlimb weights to the effect of arterial catheterization of the non-study limb, although they found no difference in study and non-study hindlimb blood flows. In contrast, we found no significant difference in study and non-study hindlimb weights (although non-study limb weight tended to be lower), whereas blood flow was  $13.4 \pm 1.8\%$  less in the non-study limb. We attribute the difference between the two studies to variations in the surgical protocols. Wilkening *et al.* (1988) catheterized the non-study limb pedal artery with a 1.38 mm OD polyvinyl catheter. As there are few potential sources of collateral flow in the lower hindlimb, ligation of the pedal artery could have impaired both flow and growth of the lower limb while not greatly affecting blood flow measured in the external iliac artery. We catheterized the non-study limb femoral artery with a 1.19 mm OD silicone rubber catheter. The use of a smaller catheter in a larger vessel (i.e. femoral artery versus pedal artery) may have less effect on flow in the catheterized vessel upstream from the site of occlusion; moreover, in the upper leg there are more sources for collateral flow than in the lower limb. As a result hindlimb growth was not retarded, but we did find diminished blood flow probably reflecting local interference in tissue perfusion of the upper limb. That the reduced flow did not lead to a significant effect on weight may be due to the relatively small reduction in flow and the short time (6–14 days) between fetal surgery and the study. However, it is possible that the initial reduction in flow in the non-study limb may have been greater than the ~13% observed, particularly if there were a gradual post-surgical increase in hindlimb perfusion via existing or newly developed collateral vessels. Furthermore, in both our study and that of Wilkening *et al.* (1988) the effect of vascular catheterization on the hindlimb may have been underestimated since the study or reference limb was also catheterized, albeit with smaller diameter catheters in peripheral branches of the hindlimb vasculature. In view of the common employment of fore and hindlimb vascular catheters in studies of chronically

instrumented fetal lambs, the impact of catheterization on both limb perfusion and growth seem worthy of further investigation.

## 5. BEHAVIOURAL STATE MODULATION OF HINDLIMB BLOOD FLOW

### 5.1 Introduction

Szeto describes a behavioural state as the concurrence of a certain set of physiological measures that is recurrent over time (Szeto, 1992). Sleep cycles consisting of a quiet and an active or rapid eye movement (REM) phase have a long phylogeny, having been observed in all terrestrial placental mammals (Campbell and Tobler, 1984; Tobler, 1984) including humans (Nijhuis *et al.*, 1982). In species born with a relatively well developed central nervous system, organized fetal behavioural states are evident in late gestation. Cyclic alterations in behavioural state are a normal component of pregnancy and are very obvious features of fetal life particularly in the last third of gestation. Prior to about 110 days gestation in the fetal lamb, these associations of electrocortical activity, eye movements, and skeletal muscle activity are random, with the transition to more organized behavioural states developing between 115 to 120 days gestation (Szeto and Hinman, 1985; Clewlow *et al.*, 1983). In humans, this transition occurs after approximately 36 weeks gestation (Nijhuis *et al.*, 1982). As an animal matures, less time is spent in rapid eye movement sleep. In sheep, REM sleep occurs approximately 40% of the time in late gestation, less than 20% of the time in the neonate (Ruckebusch, 1972a), and about 2.5% of the time in adults (15% of total sleep time) (Ruckebusch, 1972b). Time spent in quiet sleep (high voltage ECoG) changes from 55% of the time in the fetus, 43% in the newborn (Ruckebusch, 1972a), to 14% of the time in the adult (Ruckebusch, 1972b). Thus there is no correlation between maternal and fetal REM or ECoG activity.

In the late gestation fetal lamb, skeletal muscle activity, in the form of fetal breathing and body movements, is associated with specific patterns of electrocortical (ECoG) and electro-ocular (EoG) activity (Walker, 1984). Sleep state is usually classified into three patterns. Quiet or non-REM sleep, is characterized by high voltage slow frequency electrocortical activity, no breathing or rapid eye movements, and sluggish body movements.

This state occurs approximately 55% of the time. Active or REM sleep, which occurs about 40% of the time, consists of low voltage fast frequency ECoG activity with breathing and rapid eye movements, but with fetal activity limited to abrupt twitches. A third category represents an awake or aroused state in which electrocortical activity is of low voltage fast frequency without REM and is associated with breathing and vigorous body movements. This state of responsiveness is of short duration, lasting a few moments at most and occupying approximately 5% of the time. In the late gestation fetal lamb, a complete sleep cycle lasts 30 to 40 minutes (Clapp *et al.*, 1980; Ruckebusch *et al.*, 1977).

Fetal breathing movements in late gestation are episodic, involving rhythmic movement of the diaphragm and movements of small amounts of fluid in the trachea. Their incidence is about 40% (Dawes *et al.*, 1972) with a greater prevalence during the early-morning hours (Boddy and Dawes, 1975). Body movements include twitches, isolated limb movements, and complex changes in limb and trunk orientation. They, too, are more prevalent in the early morning. As indicated, fetal breathing movements (FBM) occur during low voltage high frequency electrocortical activity with REM (Dawes *et al.*, 1972), while body movements are most vigorous during the transitions between electrocortical states and are very infrequent during low voltage activity with REM (Natale *et al.*, 1981). It has been suggested that breathing and body movements *in utero* develop the musculature in preparation for post-natal life (Boddy and Dawes, 1975). Fetal breathing movements appear to be necessary for normal lung development although the mechanism is unclear. Fetal lambs that have undergone high spinal cord transection (integrity of phrenic nerves and diaphragm retained) exhibit marked decreases in lung growth and distensibility (Harding *et al.*, 1993; Kitterman, 1988). Long-term inhibition of body movements in fetal rats resulted in joint abnormalities, pulmonary hypoplasia, and growth retardation involving reduced bone and muscle mass (Moessinger, 1983).

An association between ECoG state transition and maternal contractures has been reported (Nathanielsz *et al.*, 1980). Uterine contractures, or non-labour contractions of the

uterus, occur at 30 to 60 minute intervals in the pregnant sheep (Harding *et al.*, 1982). The initial minutes of a contracture are associated with increased fetal carcass blood flow (Llanos *et al.*, 1986; Parer *et al.*, 1984) and oxygen consumption (Llanos *et al.*, 1988) suggesting that there is increased fetal muscle activity. Nathanielsz (1982) has shown that the duration, frequency, and associated rise in uterine pressure of contractures are unchanged when the fetus is paralyzed with pancuronium. Thus fetal skeletal muscle activity does not appear to be a factor in the initiation of contractures or the associated change in sleep state. Uterine contractures result in mild hypoxemia as evidenced by a fall in  $P_aO_2$ , rise in arterial and venous pressures, and bradycardia (Brace and Brittingham, 1986; Harding *et al.*, 1983; Jansen *et al.*, 1979). Cessation of fetal breathing and rapid eye movements have been observed with the onset of a contracture (Harding *et al.*, 1981). This abolition of activity may be a result of the mild hypoxemia.

Many reports emphasize the significance of behavioural state on fetal functions. Fetal micturition or voiding is more prevalent during low voltage ECoG activity; however, bladder drainage to abolish spontaneous voiding did not alter the duration or number of low and high voltage ECoG episodes (Wlodek *et al.*, 1989). Thus voiding appears to be linked to electrocortical state but bladder contractions and voiding do not alter the normal cycling of ECoG states. Episodes of fetal swallowing are associated with low voltage ECoG and periods of arousal (Harding *et al.*, 1984). Fetal heart rate and arterial pressure are lower during the low voltage state in comparison to observations made during high voltage activity (Clapp *et al.*, 1980). In addition, cerebral blood flow is greater during low voltage ECoG episodes (Jensen *et al.*, 1986; Richardson *et al.*, 1985). Fetal animals exhibit a high proportion of low voltage ECoG with REM activity which, as this state is associated with increased cerebral metabolism, may contribute to the rapid prenatal growth and development of the brain (Richardson *et al.*, 1985). Walker *et al.* (1984) did not find a relationship between ECoG state and fetal oxygen consumption, but oxygen consumption does increase significantly during fetal breathing episodes (Rurak and Gruber, 1983a).

Bocking *et al.* (1985) have demonstrated a very close relationship between gross body movements and fetal heart rate accelerations in sheep. This relationship has been used clinically as an assessment of fetal well-being. The non-stress test monitors fetal movements and heart rate with Doppler ultrasound. A healthy fetus will generally exhibit at least two heart rate accelerations in a 20 minute interval, defined as a reactive non-stress test (Hacker and Moore, 1986). Some women also undergo a contraction stress test in which a low dose of oxytocin is given to induce several uterine contractions. A healthy fetus will respond to these contractions with heart rate decelerations. Fetal breathing and body movements may also be assessed by ultrasound.

The biological significance of alterations in behavioural state is unknown (Szeto, 1992). However, many forms of fetal distress are associated with reductions in skeletal muscle activity. Acute hypoxemia, for example, is associated with a reduced incidence of low voltage electrocortical activity, a virtual abolition of REM, and an inhibition of fetal breathing (Hohimer and Bissonnette, 1991) and limb movements (Natale *et al.*, 1981). It has been suggested that this reduction in movements and breathing activity is a compensatory mechanism that lowers oxygen requirements when the fetus is faced with a reduction in oxygen supply (Richardson, 1989; Harding *et al.*, 1983; Rurak and Gruber, 1983a). This is supported by evidence showing a reduction in oxygen consumption when gallamine triethiodide or pancuronium bromide, neuromuscular blocking agents which inhibit both breathing and body movements, are administered to the fetus (Wilkening *et al.*, 1988; Rurak and Gruber, 1983a).

Blood flow to the umbilical and abdominal veins may be modulated by fetal breathing movements, suggesting that peripheral blood flow may be modulated by behavioural state in both sheep (Hassaart and de Haan, 1985) and humans (Marsal *et al.*, 1984). However this may not be a direct relationship to behavioural state but rather to the phasic changes in intrathoracic pressure with breathing activity (Hassaart and de Haan, 1985). We have previously shown in our laboratory that high voltage ECoG activity is

associated with higher fetal hindlimb blood flow and heart rate as well as decreased hindlimb vascular resistance when compared to the low voltage ECoG state (Rurak *et al.*, 1992). The mechanism responsible for the relationship between blood flow and behavioural state may be a direct central nervous system one. This is suggested by evidence that the fall in umbilical blood flow with quiet sleep precedes the switch from low to high voltage (Slotten *et al.*, 1989; Walker *et al.*, 1984). A second explanation for this relationship may be that skeletal muscle activity, and thus increased oxygen demand, is modulating blood flow. As quiet sleep is associated with body movements of longer duration and more complexity than during REM sleep (Natale *et al.*, 1981), it seems likely that quiet sleep would be associated with a greater oxygen demand by the tissues involved. This leads to the postulate that such movements would elicit an increase in blood flow in the fetal lamb hindlimb during quiet sleep. The administration of a neuromuscular blocking agent, which would abolish skeletal muscle activity, can be used to help elucidate whether the relationship between state and blood flow is a direct one or a result of skeletal muscle activity. Previous studies have shown that neuromuscular blockade decreases total body and hindlimb oxygen consumption and hindlimb blood flow (Wilkening *et al.*, 1989; Wilkening *et al.*, 1988; Rurak and Gruber, 1983a). However, the effect of neuromuscular blockade on hindlimb blood flow modulation with electrocortical state does not appear to have been examined previously.

One of the objectives of our study was to administer neuromuscular blocking agents to the fetus in order to abolish skeletal muscle activity. This will allow us to determine if these movements are responsible for the fluctuations in hindlimb blood flow that occur in relation to fetal behavioural state. In addition, we attempted to obtain blood samples during high and low voltage ECoG state both pre- and post-drug administration in order to determine the effect of behavioural state and neuromuscular blockade on hindlimb oxygen consumption.

## 5.2 Methods

Surgery was performed on 7 fetal lambs between 120–127 days gestation (mean =  $125 \pm 1$  days) using the techniques previously described (Section 2.3). Catheters were placed in the amniotic cavity, and in the fetal femoral artery, lateral tarsal vein, and pudendoepigastric artery and vein. Hindlimb blood flow was continuously measured with a transit-time flow transducer on the external iliac artery. Electrocortical (ECoG) and electro-ocular (EoG) electrodes were implanted for determination of fetal behavioural state.

A total of 8 experiments were performed at a mean gestational age of  $132 \pm 1$  days (range = 126–137 days). Neuromuscular blockade was achieved by a bolus injection of either 15 mg gallamine triethiodide (Flaxedil®, Rhône-Poulenc Rorer Canada, Ville St-Laurent, Quebec) (n=5) or 2–4 mg pancuronium bromide (Pavulon®, Organon Canada, Scarborough, Ontario) (n=3) with the drug administered around noon (range 08:30 to 16:49). Blood samples were collected from the pudendoepigastric artery and vein during both quiet and active sleep prior to and following the drug bolus. Data were analyzed from 3 complete paired high and low voltage ECoG patterns (three sleep cycles) before and after drug administration for each animal. The data used were generally collected between 6:00 a.m. and 7:00 p.m. Electrocortical state was determined by visual inspection of the polygraph record with high voltage ranging from 125 to 175 microvolts and low voltage from 25 to 50 microvolts. Arousal or awake episodes (low voltage ECoG without REM), which are most prevalent between 1:00 a.m. and 7:00 a.m., were not clearly evident on the polygraph recordings and thus were not included in the behavioural state analysis. Electro-ocular activity was classified by the presence or absence of rapid eye movements. Fetal breathing movements were detected from the tracheal pressure polygraph trace. Alterations in tracheal pressure were considered fetal breathing episodes if they were at least 1 mmHg (1.36 cm water) in amplitude and had a minimum duration of 10 seconds. As the results from gallamine and pancuronium were similar, the data from both drugs were combined.

Data were compared using paired t-tests with values considered significant if  $p < 0.05$  (StatView statistical software, Abacus Concepts Inc., Berkeley, California).

### 5.3 Results

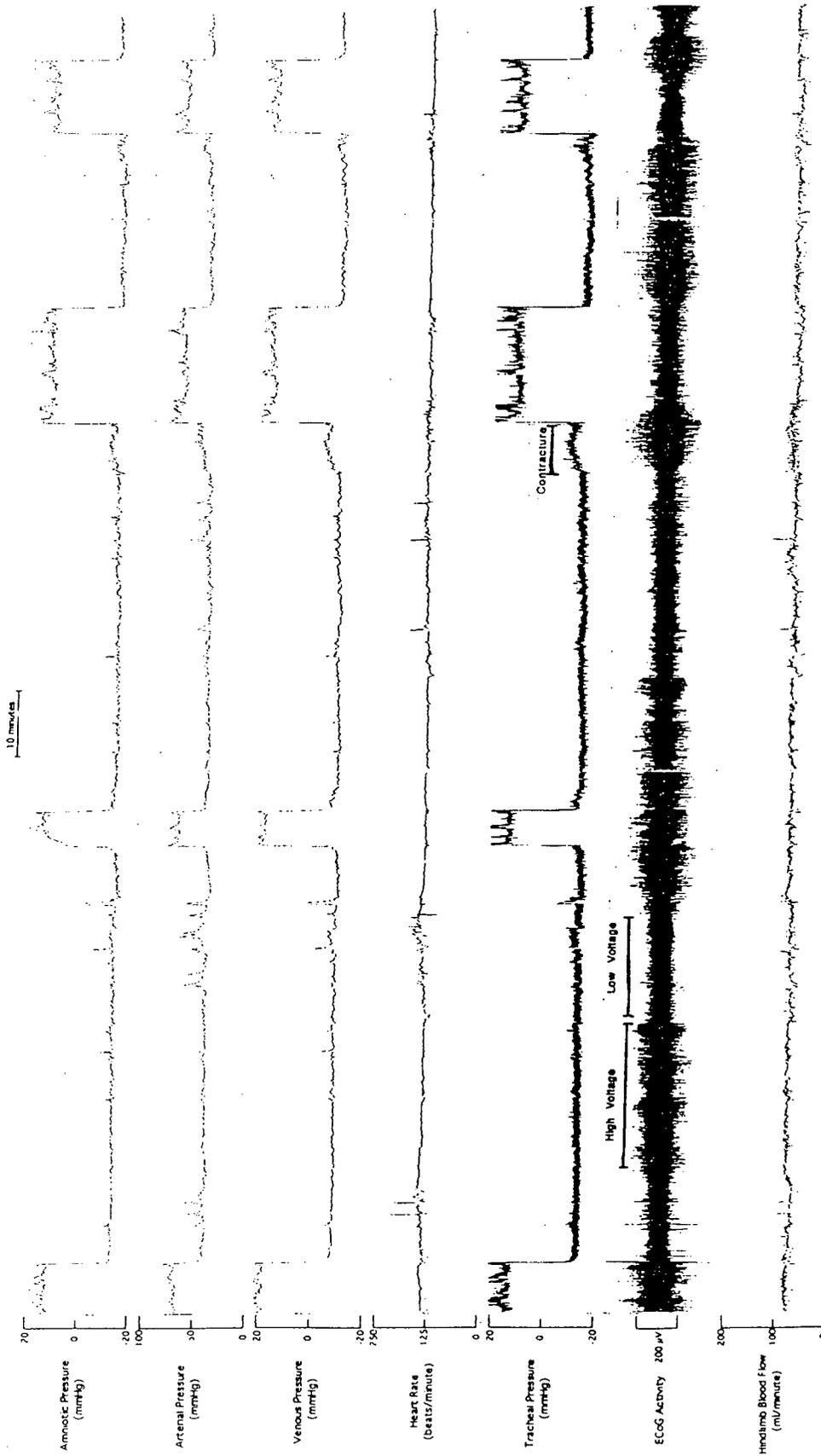
Polygraph recordings obtained prior to (Figure 10) and following neuromuscular blockade (Figure 11) show clear episodes of high and low voltage ECoG activity. The fetus spent  $46.1 \pm 2.0\%$  of the sleep cycle in the high voltage state prior to neuromuscular blockade compared to  $49.9 \pm 1.8\%$  following blockade; this difference was not significant. Similarly, there was no difference in cycle length ( $34.7 \pm 2.0$  minutes pre-blockade;  $28.8 \pm 1.5$  minutes post-blockade). Thus neuromuscular blockade does not appear to alter fetal ECoG patterns.

Prior to blockade, the association between fetal breathing movements, obtained from the tracheal pressure trace, and low voltage activity is evident (Figure 10). During episodes of high voltage activity hindlimb blood flow appears to be higher and more variable. A recording from the same fetus following the administration of neuromuscular blockade (pancuronium) suggests that mean hindlimb blood flow and its variability, particularly during high voltage, are reduced (Figure 11). As expected, no fetal breathing movements are evident. The effectiveness of the neuromuscular blockade was demonstrated by the total lack of fetal breathing movements evident on the polygraph recordings for at least 6 hours post-administration. It should be noted that on the polygraph charts arterial, venous, and tracheal pressures have not been corrected for changes in amniotic pressure. The sudden changes in these pressures on the polygraph are due to position changes of the ewe. In addition, uterine contractures are indicated in Figures 10 and 11. The contractures are associated with a change in state from low to high voltage and hindlimb blood flow is increased in the initial moments of the uterine activity.

In the pre-blockade phase, average hindlimb blood flow was  $33.6 \pm 2.4$   $\text{ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  with flow tending to be higher during high voltage ( $34.3 \pm 3.8$  versus



**FIGURE 10.** Polygraph recording obtained from a fetal lamb in late gestation prior to neuromuscular blockade. Episodes of fetal breathing and high and low voltage electrocortical activity are indicated. A uterine contracture is also labeled. Abrupt changes in amniotic, arterial, venous, and tracheal pressures are due to positional changes of the ewe. Hindlimb blood flow was obtained with a transit-time flow transducer on the external iliac artery.

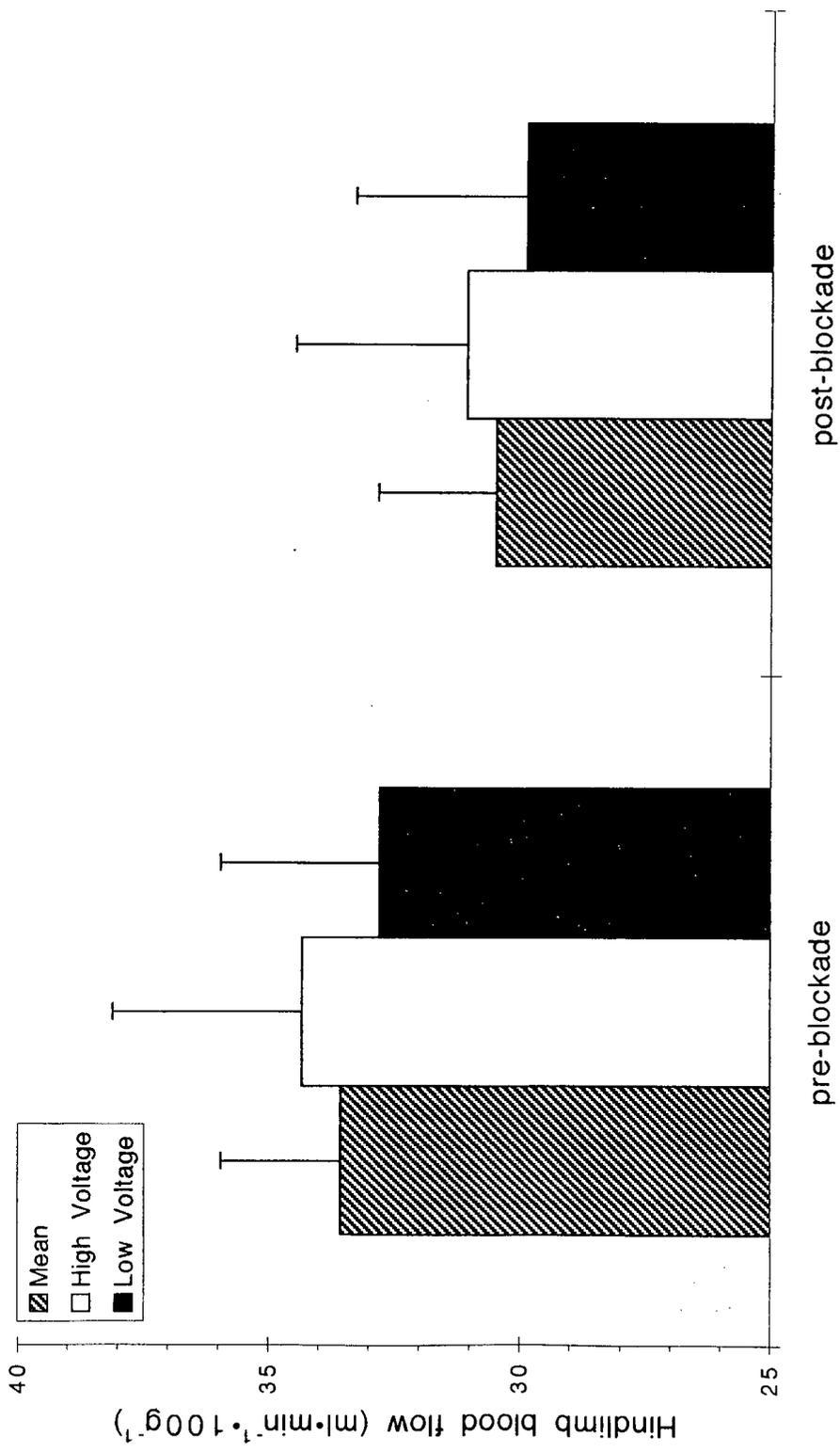


**FIGURE 11.** Polygraph recording obtained from a fetal lamb in late gestation following the administration of a neuromuscular blocking agent (pancuronium). High and low voltage electrocortical activity and a uterine contracture are indicated. Fetal breathing movements are absent. Abrupt changes in amniotic, arterial, venous, and tracheal pressures are due to positional changes of the ewe. Hindlimb blood flow was obtained with a transit-time flow transducer on the external iliac artery.

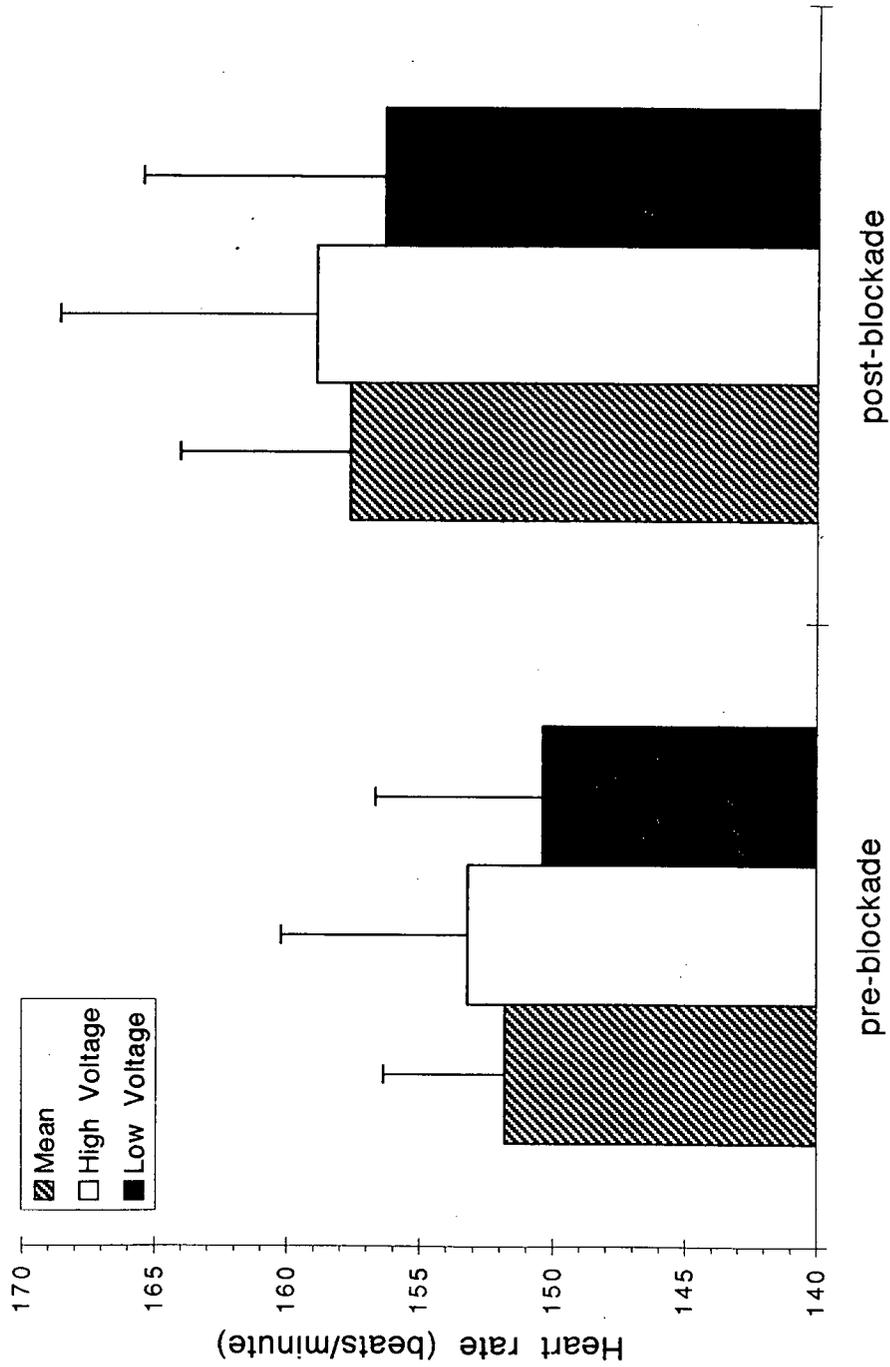
$32.8 \pm 3.2 \text{ ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$  in REM sleep) (Figure 12). Following drug administration, mean flow fell  $8.3 \pm 3.5\%$  to  $30.5 \pm 2.3 \text{ ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ , but the decrease just failed to be statistically significant ( $p = 0.0510$ ). After the elimination of skeletal muscle activity, hindlimb blood flow still tended to be greater in the high voltage state, but the difference was again not significant. Variability in hindlimb blood flow, expressed as coefficient of variation, was reduced from pre-blockade ( $7.2 \pm 0.4\%$ ) to post-blockade ( $7.0 \pm 0.5\%$ ), but this difference was not significant.

Figures 13 through 16 illustrate the change in fetal cardiovascular variables prior to and following the administration of neuromuscular blockade. The values for the high (white bars) and low voltage (solid bars) electrocortical states as well as the average of these two states (diagonal pattern) are presented for before and after blockade. The data are given as mean  $\pm$  standard error of the mean. Fetal heart rate was not different in high voltage ( $153.2 \pm 7.0 \text{ bpm}$ ) compared to the low voltage ECoG state ( $150.4 \pm 6.3 \text{ bpm}$ ) and, although heart rate tended to rise following the administration of gallamine or pancuronium, the changes were not significant (Figure 13). No difference in arterial pressure was seen between high and low voltage activity; however, mean arterial pressure did increase significantly following administration of the drugs (Figure 14). In the pre-blockade period, fetal hindlimb venous pressure was significantly lower in low voltage ( $5.1 \pm 0.9 \text{ mmHg}$ ) relative to the high voltage state ( $5.9 \pm 0.7 \text{ mmHg}$ ). Venous pressure fell significantly following neuromuscular blockade (Figure 15). As shown in Figure 16, hindlimb vascular resistance averaged  $1.3 \pm 0.1 \text{ mmHg/ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$  in the control period, and although resistance rose to  $1.5 \pm 0.3 \text{ mmHg/ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$  with neuromuscular blockade, this change was not statistically significant.

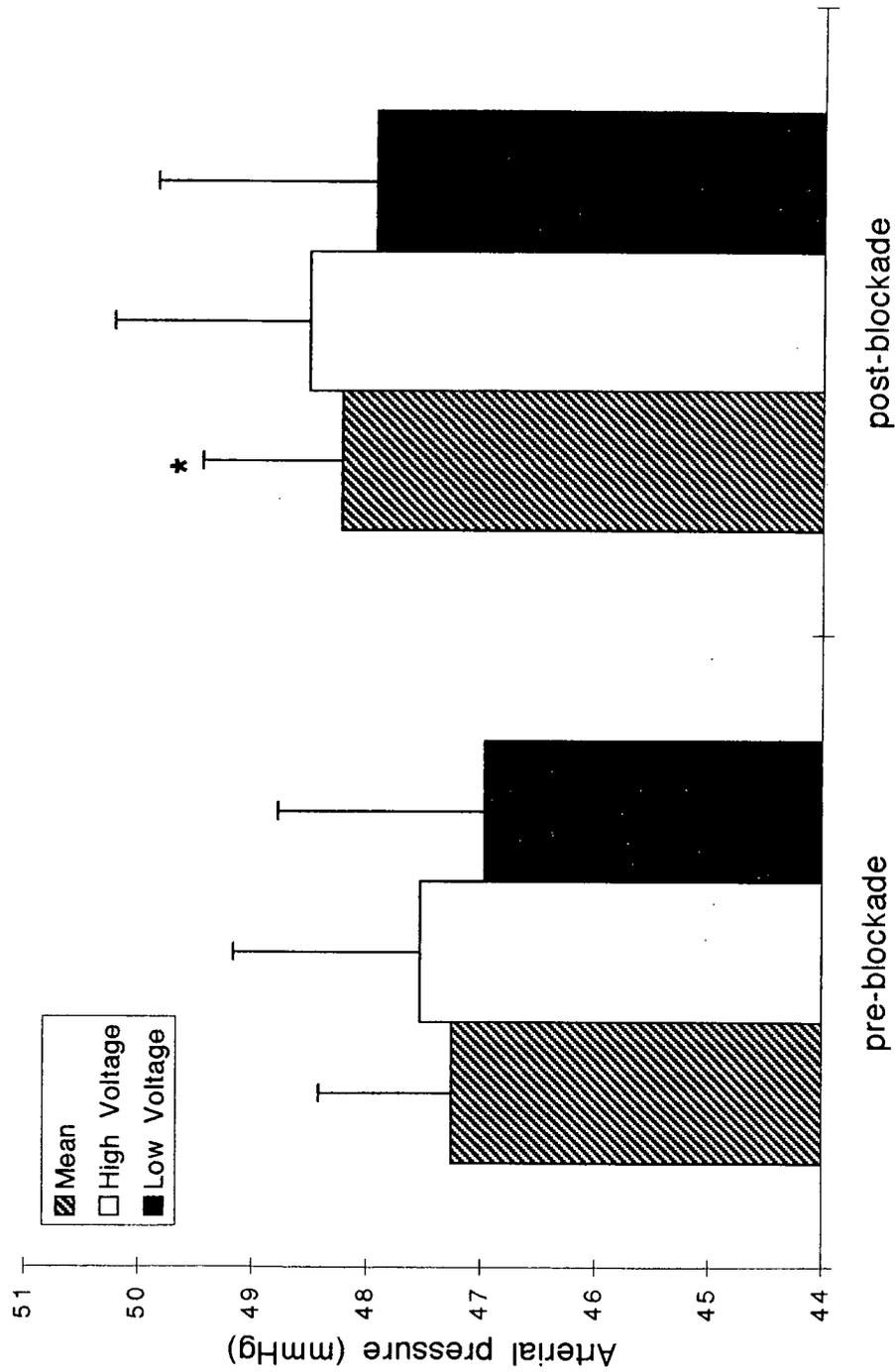
Table 7 presents the blood gas status and metabolic variables obtained from the fetal hindlimb as a baseline as well as that obtained after the administration of neuromuscular blockade. The data represent only 7 experiments due to failure of the arterial sampling catheter during one experiment. All pre-blockade values were within the normal range seen



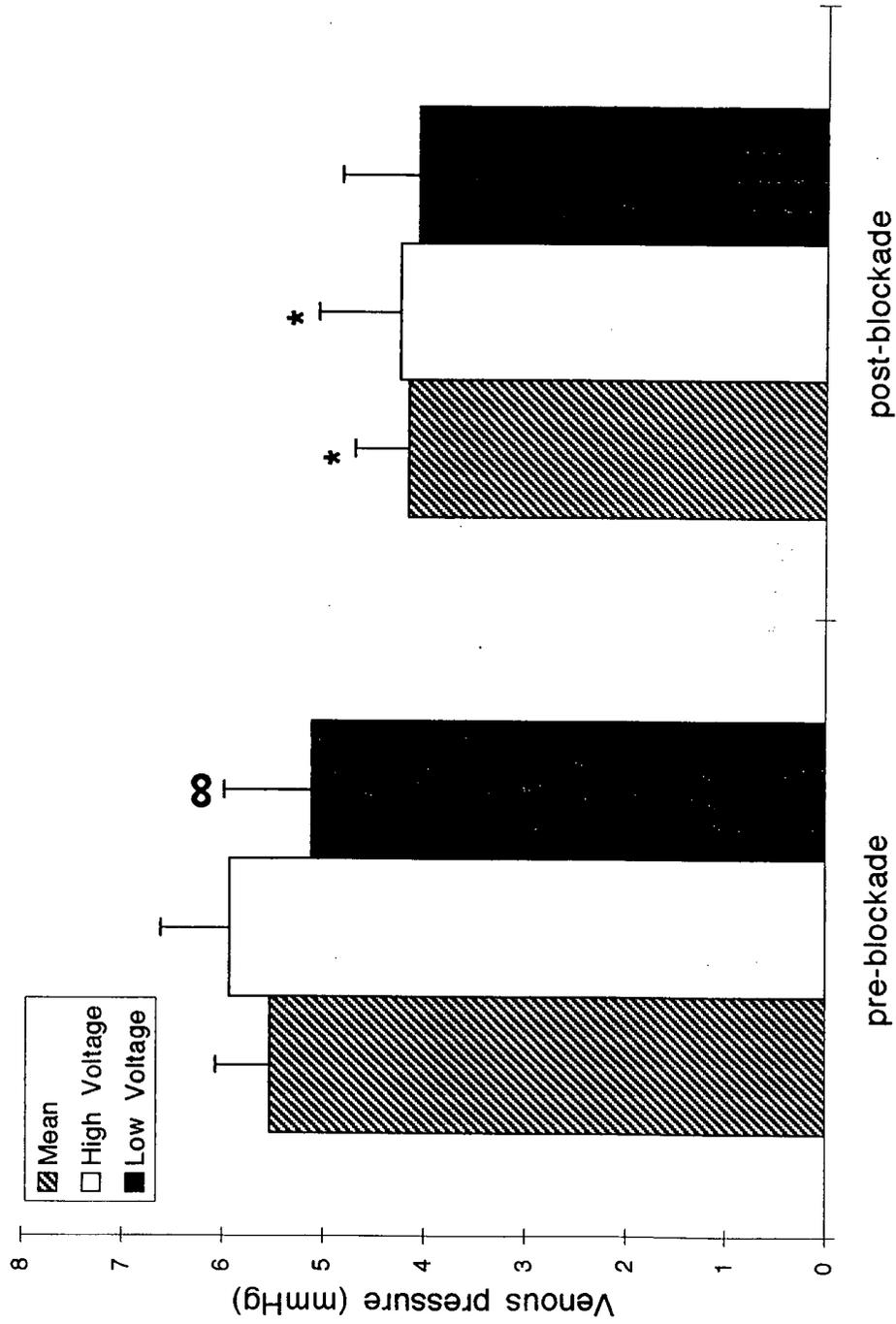
**FIGURE 12.** Effect of neuromuscular blockade on fetal hindlimb blood flow (8 experiments). Mean hindlimb blood flow as well as that during high and low voltage ECoG cycles are presented for both before and after blockade. Blood flow was measured with a transit-time flow transducer on the external iliac artery. Values are mean  $\pm$  SEM.



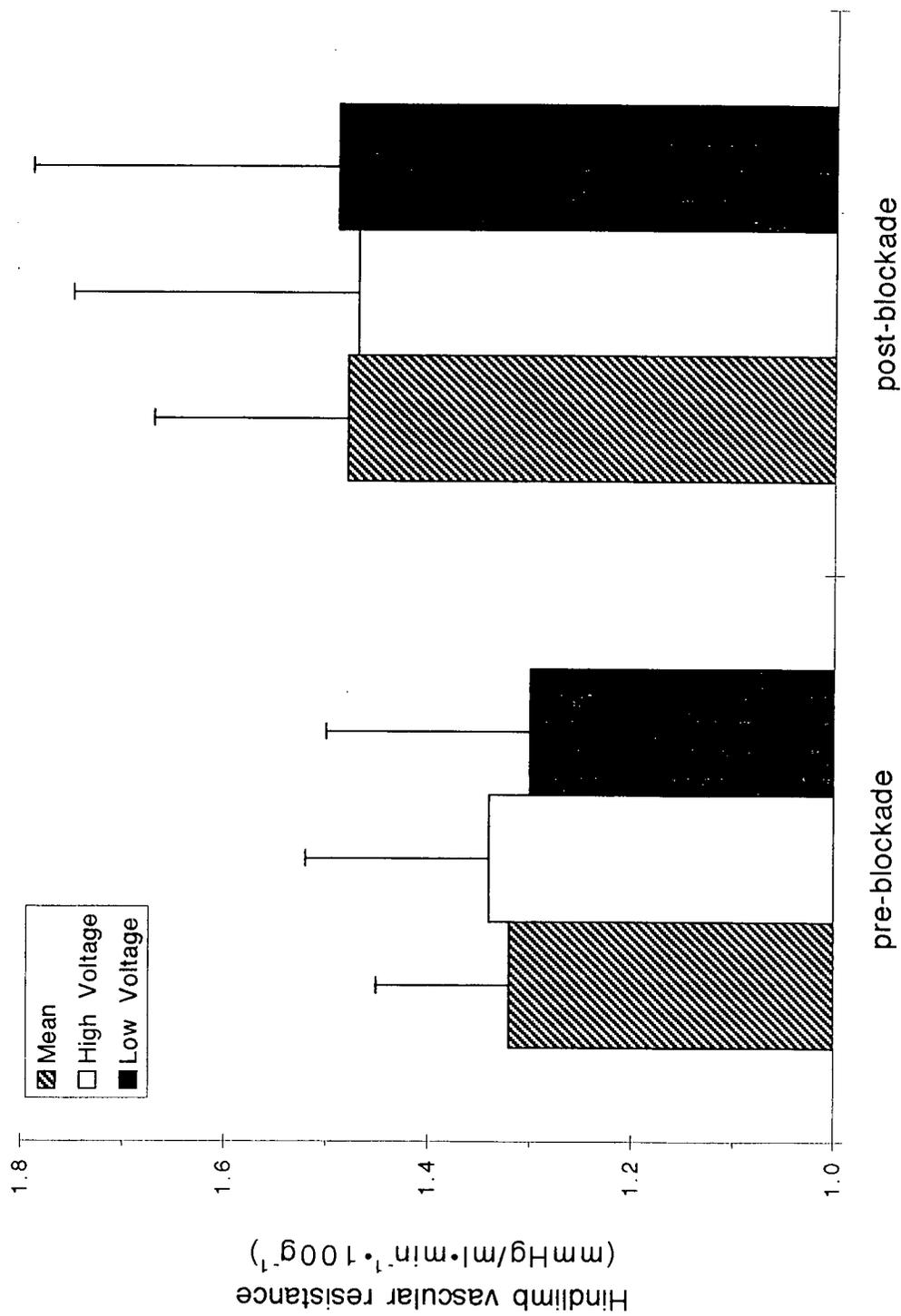
**FIGURE 13.** Effect of neuromuscular blockade on fetal heart rate (8 experiments). Mean heart rate as well as that during high and low voltage ECoG cycles are presented for both before and after blockade. Values are mean  $\pm$  SEM.



**FIGURE 14.** Effect of neuromuscular blockade on fetal arterial pressure (8 experiments). Mean arterial pressure as well as that during high and low voltage ECoG cycles are presented for both before and after blockade. Values are mean  $\pm$  SEM. \* = significant difference from pre-blockade value ( $p < 0.05$ ).



**FIGURE 15.** Effect of neuromuscular blockade on fetal venous pressure (8 experiments). Mean venous pressure as well as that during high and low voltage ECoG cycles are presented for both before and after blockade. Values are mean  $\pm$  SEM.  $\infty$  indicates a significant difference from high voltage ECoG values ( $P < 0.05$ ). \* indicates significant difference from pre-blockade value ( $p < 0.05$ ).



**FIGURE 16.** Effect of neuromuscular blockade on fetal hindlimb vascular resistance (8 experiments). Mean resistance as well as that during high and low voltage ECoG cycles are presented for both before and after blockade. Values are mean  $\pm$  SEM.

**TABLE 7.** Effect of neuromuscular blockade on blood gas status and metabolic variables in the fetal lamb hindlimb (7 experiments).

	<b>Pre- Neuromuscular Blockade</b>	<b>Post- Neuromuscular Blockade</b>
pH	7.333 ± 0.010	7.346 ± 0.009
$P_{O_2}$ (mmHg)	17.6 ± 1.4	20.1 ± 1.5†
$P_{CO_2}$ (mmHg)	52.0 ± 1.6	51.0 ± 1.1
Base excess (mEq/L)	2.2 ± 0.9	2.4 ± 0.8
Hemoglobin concentration (g/dL)	10.2 ± 0.6	10.2 ± 0.6
$O_2$ saturation (%)	38.9 ± 4.3	45.4 ± 5.3†
$O_2$ content (mM)	2.48 ± 0.36	2.92 ± 0.44†
Lactate flux ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	-2.78 ± 0.80	-0.05 ± 1.21
Glucose flux ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	4.35 ± 0.60	4.11 ± 0.62
$O_2$ delivery--absolute value ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	75.2 ± 6.5	72.3 ± 6.4
--percentage of pre-blockade value		(96.8 ± 5.6)
$O_2$ consumption--absolute value ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	18.6 ± 1.6	16.4 ± 1.6
--percentage of pre-blockade value		(88.2 ± 4.6)†
$O_2$ extraction--absolute value (%)	26.0 ± 2.9	24.0 ± 3.2
--percentage of pre-blockade value		(91.1 ± 2.2)†

Values are mean ± SEM. Data are presented as absolute values except those in parentheses which are expressed as a percentage of the pre-blockade values. † indicates a significant difference from pre-blockade data ( $p < 0.05$ ).

in our laboratory. After gallamine or pancuronium injection, significant increases in fetal  $P_{O_2}$ , oxygen saturation, and oxygen content were observed. Similar changes following neuromuscular blockade have been reported by other researchers (Chestnut *et al.*, 1989; Wilkening *et al.*, 1989; Wilkening *et al.*, 1988; Rurak and Gruber, 1983b). Lactate and glucose fluxes were unaltered by gallamine or pancuronium administration. Although oxygen delivery did not change following neuromuscular blockade, oxygen consumption fell  $11.8 \pm 4.6\%$  from pre-blockade values (Table 7). There was also a significant fall in oxygen extraction to  $8.9 \pm 3.2\%$  below the baseline value. No difference was found in oxygen consumption or extraction values between high and low voltage ECoG activity either before or after neuromuscular blockade.

#### 5.4 Discussion

Gallamine triethiodide and pancuronium bromide are competitive neuromuscular blocking agents and thus abolish skeletal muscle activity including fetal breathing and body movements. During the control phase, our fetuses spent  $46.1 \pm 2.0\%$  of their time in high voltage ECoG activity with an average sleep cycle length of  $34.7 \pm 2.0$  minutes. These values are similar to values previously reported for the late gestation fetal lamb (Ruckebusch *et al.*, 1977; Dawes *et al.*, 1972). The drugs did not alter normal ECoG patterns as shown by the lack of effect on sleep cycle length and the amount of time spent in each electrocortical state.

Gallamine generally exhibits more ganglionic blockade effects than pancuronium although the latter drug may also cause some tachycardia and hypertension (Gilman *et al.*, 1990). At the doses used in the current study, both drugs caused an increase in arterial pressure but heart rate was not changed. The ganglionic effects of the neuromuscular blocking agents thus appear to have been similar and the results from both drugs were combined. Mean arterial pressure rose from  $47.3 \pm 1.2$  mmHg in the pre-blockade period to  $48.2 \pm 1.2$  mmHg post-blockade ( $p = 0.0164$ ) while venous pressure was significantly

reduced from  $5.5 \pm 0.5$  to  $4.2 \pm 0.5$  mmHg ( $p = 0.0059$ ). Fetal heart rate and hindlimb vascular resistance were not altered following neuromuscular blockade. Our results then are similar to previous studies which have reported a slight but insignificant trend for increased fetal heart rate and arterial pressure following gallamine (Rurak and Gruber, 1983a) or pancuronium (Wilkening *et al.*, 1988) administration. Following administration of either gallamine triethiodide or pancuronium bromide, mean hindlimb blood flow fell  $8.3 \pm 3.5\%$  which just failed to reach statistical significance ( $p = 0.0510$ ). Hindlimb blood flow was reduced following blockade in 6 of the 8 animals. Wilkening's group (1988) reported a significant (12%) reduction in hindlimb blood flow after pancuronium administration. These data suggest that skeletal muscle activity is associated with greater hindlimb blood flow.

Clapp *et al.* (1980) reported high voltage ECoG activity to be associated with significantly greater fetal heart rate, arterial pressure, and umbilical blood flow relative to the low voltage state. In a previous study in our laboratory, fetal heart rate was  $\sim 7\%$  higher, arterial pressure  $\sim 3\%$  greater, and hindlimb blood flow  $\sim 17\%$  higher in the high voltage ECoG state compared to low voltage (Rurak *et al.*, 1992). In the current study, during the baseline or pre-blockade period, fetal heart rate, arterial and venous pressures, and hindlimb blood flow were  $150.4 \pm 6.3$  bpm,  $47.0 \pm 1.8$  mmHg,  $5.1 \pm 0.9$  mmHg, and  $32.8 \pm 3.2$  ml·min<sup>-1</sup>·100g<sup>-1</sup> during low voltage electrocortical activity. During high voltage ECoG, heart rate rose 2%, arterial pressure 1%, hindlimb blood flow 4%, and hindlimb vascular resistance 4%; however, the only significant change was in venous pressure which was 21% greater in high voltage ( $p = 0.0472$ ). Thus, compared to previous studies, the direction of our changes were the same but the magnitude was not as great. The degree of difference between these results may reflect the way data were obtained. In our initial work (Rurak *et al.*, 1992), the data were quite crudely obtained i.e., by direct visual analysis of the polygraph recordings without the benefit of a computerized data acquisition system. In addition, the episodes analyzed were selected so as to avoid events like postural changes by

the ewe and uterine contractures. We had the benefit of computer analysis for the data currently presented which increased the precision of our measurements. However, we did not specifically avoid contractures and postural changes, and these may be possible confounding factors in our results.

As stated, in a previous study in our laboratory, we found hindlimb blood flow to be significantly greater during quiet sleep (Rurak *et al.*, 1992), and we attributed this to a greater oxygen demand required for the increased body movements that are associated with this state. This modulation of hindlimb blood flow with sleep state is also suggested by the polygraph recording (Figure 10). In the current study, pre-blockade hindlimb blood flow tended to be greater during high voltage ECoG activity than low ( $34.3 \pm 3.8$  versus  $32.8 \pm 3.2$  ml·min<sup>-1</sup>·100g<sup>-1</sup>;  $p = 0.2200$ ). Because of the failure of the current data to reach statistical significance, more data is required before we can conclude that hindlimb blood flow is higher during high voltage ECoG activity. However, in the pre-blockade period, 5 of the 8 experiments demonstrated greater hindlimb blood flow during high voltage. A power analysis indicated that 13 pairs of data would be required to demonstrate a 10% difference in hindlimb blood flow between high and low voltage activity, assuming that variance remained the same. Given that the trend for hindlimb blood flow to be higher during high voltage activity has been observed in many polygraph recordings made during this and other experiments, this trend appears to be consistent. Following blockade, hindlimb blood flow still tended to be greater with quiet sleep ( $31.1 \pm 3.4$  versus  $29.9 \pm 3.4$  ml·min<sup>-1</sup>·100g<sup>-1</sup>;  $p = 0.0607$ ). Again, the difference just failed to be significant, but this relationship was seen in 6 of the 8 experiments. Thus, although the data were not statistically significant, the results suggest that neuromuscular blockade did not alter the apparent behavioural state modulation of flow in the fetal lamb hindlimb.

Hindlimb blood flow demonstrates marked inter-animal variability, i.e., in these experiments pre-blockade flow ranged from ~17 to 49 ml·min<sup>-1</sup>·100g<sup>-1</sup>. The use of paired comparisons largely overcomes this, but the conservative nature of this method also makes it

more difficult for differences to reach statistical significance. The observed changes in hindlimb blood flow, expressed in absolute or percentage terms, are quite small. For example, the average flow difference between electrocortical states was  $\sim 1.5 \text{ ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$  or  $\sim 5\%$ , and the inhibition of skeletal muscle activity with neuromuscular blockade resulted in a  $3.1 \text{ ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$  or  $\sim 8\%$  fall in flow. With such small differences, animal condition, measurement variability of the flow transducer, and artifacts during data collection can easily sway the results. Thus, such differences may be physiologically relevant but fail to demonstrate statistical significance.

The inability to demonstrate statistically significant differences in hindlimb blood flow between high and low voltage ECoG activity may also reflect the difficulty of precisely delineating high and low voltage episodes. Another possible factor is that, although quiet sleep is associated with more body movements (Natale *et al.*, 1981), fetal breathing movements occur during low voltage electrocortical activity (Dawes *et al.*, 1972). Both these forms of skeletal muscle activity could contribute to blood flow changes (Wilkening *et al.*, 1989; Wilkening *et al.*, 1988; Hassaart and de Haan, 1985; Rurak and Gruber, 1983a), and as neuromuscular blockade abolishes both breathing and body movements, their relative effects are difficult to discern. Thus, inhibition of fetal breathing movements may also contribute to the reduction in hindlimb blood flow following neuromuscular blockade. Such modulation of peripheral blood flow with fetal breathing activity, likely due to the changes in intrathoracic pressure, has been previously reported (Hassaart and de Haan, 1985; Marsal *et al.*, 1984; Dawes *et al.*, 1972). In addition, behavioural state was determined by visual analysis of the polygraph recording. The subjective nature of this method is prone to error in precisely delineating the beginning and end of each behavioural state episode. As well, the awake (or arousal) state, which is characterized by low voltage ECoG activity without REM and is associated with large amounts of fetal body movements, were not clearly evident on the recordings. The difficulty in detecting the awake state reflects its very short duration and the fact that it occurs only 5% of the time; thus it was not

identified in the behavioural state analysis. The awake state is most prevalent between 1:00 a.m. and 7:00 a.m., so we attempted to minimize the effect of this exclusion by analyzing data from the daylight hours. However, as the awake state occurs during low voltage electrocortical activity (albeit without REM), this state may have been included with the active sleep state (low voltage with REM), and thus would have the effect of masking the difference in hindlimb blood flow between true quiet and active sleep episodes. Elimination of this potential source of error would require the development of a computer program capable of objectively identifying quiet sleep (high voltage ECoG), active sleep (low voltage ECoG with REM), and awake periods (low voltage without REM). Such a system is currently not available.

The polygraph records also suggest that hindlimb blood flow is more variable during quiet sleep (Figure 10) and that the amount of variability is reduced following blockade (Figure 11). In Wilkening's study (1988), although the average coefficient of variation in hindlimb blood flow fell from 7% to 6% following blockade, this was not statistically significant. Likewise, in our study, hindlimb blood flow variability fell from  $7.2 \pm 0.4\%$  pre-blockade to  $7.0 \pm 0.5\%$  post-blockade, and in particular, the coefficient of variation for the high voltage ECoG episodes fell from  $7.1 \pm 0.6\%$  pre-blockade to  $6.1 \pm 0.5\%$  after drug administration; however, none of these changes were statistically significant. The lack of significance may reflect limitations in the way data were collected. Our computer acquisition system collects data in 10 second intervals and records the one minute average. Rapid variations in flow due to limb movements thus may not be adequately captured by this system.

The current study suggests that with neuromuscular blockade, hindlimb blood flow still tended to be higher in quiet sleep. Thus, because hindlimb skeletal muscle activity does not appear to be responsible for the apparent modulation in flow with behavioural state, this relationship may be an effect directly mediated by the central nervous system (CNS). This is also suggested by the abrupt fall in common umbilical arterial blood flow that occurs

upon the transition from low to high voltage (Walker *et al.*, 1984). In their study the depression in umbilical blood flow with the high voltage state was transient, with flow reduced 14.3% at two minutes into the high voltage state and then slowly returning to low voltage flow values. Slotten and colleagues (1989) also found a transient but significant fall in flow measured at the common umbilical vein during the high voltage ECoG state. The decrease in umbilical blood flow preceded the transition from low to high voltage by about one minute. These changes, as well as our own findings, are consistent with a direct CNS effect on blood flow. The rapid nature of these flow changes argues against a response to altered metabolic demands of the ECoG states.

It is difficult to suggest a logical explanation as to why umbilical and hindlimb blood flow would exhibit reciprocal changes with behavioural state. Both flows derive from the descending aorta making it unlikely that vasoactive agents could be responsible for the opposing effects on the umbilical and hindlimb vascular resistances. In contrast to the findings of Walker (1984) and Slotten (1989), Clapp *et al.* (1980) reported a 5 to 6% increase in common umbilical arterial blood flow during high voltage activity. The reason for these contradictory reports in the direction of umbilical blood flow change are not clear. All these studies employed electromagnetic flow probes and were conducted at similar gestational ages at which well differentiated behavioural state patterns had developed. The study by Clapp (1980) was done between 4 and 10 days post-operatively while Walker (1984) and Slotten (1989) began studies at 3 to 4 days after surgery. Thus in all cases the animals should have had sufficient time to recover from surgery. Until a consensus is reached as to the direction of umbilical blood flow change with ECoG activity, it will be difficult to reconcile the hindlimb and umbilical blood flow studies.

The nuclei or group of nuclei responsible for electrocortical state is still uncertain (Dawes, 1992). It has been difficult to study the particular functions that make up behavioural states because many of these activities are not independent. For example, fetal breathing and body movements can alter blood flow and heart rate patterns making it hard to

discern which aspects involve direct input from the central nervous system (CNS). Following transection of the brain stem above the level of the pons, fetal electrocortical activity and breathing movements become dissociated and breathing movements become continuous (Dawes *et al.*, 1983). Pharmacological agents including atropine (Moore *et al.*, 1989), L-5-hydroxytryptophan (Fletcher *et al.*, 1988), and ethanol (Smith *et al.*, 1989) have also been used to disrupt the synchrony between electrocortical state, eye movements, and breathing activity. Thus it appears that electrocortical state, REM, and breathing activity are predominantly independent and may be controlled separately by an as yet unknown "state-generator" (Moore and Hanson, 1992). Our present study also suggests that the modulation of hindlimb blood flow observed with alterations in ECoG activity may be a direct action of the central nervous system rather than linkage of ECoG and skeletal muscle activity.

In the present study, neuromuscular blockade, achieved either by the administration of gallamine triethiodide or pancuronium bromide, resulted in better fetal oxygenation. We observed increases of 15.3% in arterial  $P_{O_2}$  and 17.3% in oxygen content compared to pre-blockade values. In addition, oxygen saturation increased from an average of  $38.9 \pm 4.3$  to  $45.4 \pm 5.3\%$  (a mean change of 17.1%). In a similar experiment involving gallamine administration, Rurak and Gruber (1983a) reported increases in arterial and venous umbilical  $P_{O_2}$  values of 14% and 18%, respectively. In studies specifically looking at the fetal lamb hindlimb, a 17% increase in hindlimb arterial oxygen content was observed following gallamine (Wilkening *et al.*, 1988); while with pancuronium, oxygen saturation rose 11.1% and hindlimb arterial  $P_{O_2}$  increased 9.5% (Wilkening *et al.*, 1989). Therefore, the increase in oxygenation of the hindlimb following neuromuscular blockade that we observed is similar to that obtained in other hindlimb studies as well as in the fetus as a whole.

The increase in oxygenation following neuromuscular blockade appears to be due to a reduction in total fetal oxygen demands. Rurak and Gruber (1983b) reported an average

increase in oxygen consumption of 30% during fetal breathing movements with the increase ranging from 13.9% to 69.0% in individual experiments. Administration of gallamine, which inhibits fetal breathing and body movements, led to a 17% reduction in total body oxygen consumption (Rurak and Gruber, 1983a). In the hindlimb, oxygen consumption was reduced 7.5% by pancuronium (Wilkening *et al.*, 1989) and 12% with gallamine (Wilkening *et al.*, 1988). In the present study, neuromuscular blockade was associated with an  $11.8 \pm 4.6\%$  reduction in oxygen consumption. As demonstrated by Rurak and Gruber (1983a), the fall in consumption appears to be due to a reduction in oxygen extraction, which in our study fell  $8.9 \pm 3.2\%$  from the pre-blockade value.

No difference in hindlimb oxygen consumption was found between quiet and active sleep ( $16.3 \pm 1.4 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  versus  $15.6 \pm 1.4 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ;  $p = 0.4389$ ) in the pre-blockade period. This may reflect the small number of paired samples ( $n = 4$ ) that were collected due to difficulty in obtaining blood gas samples during clear episodes of high and low voltage activity. Often a fetus would appear to be in a quiet or REM sleep episode but by the time the blood gas sample could be collected, the sleep state had ended or a contracture caused a switch in state. Movements of the fetal forelimbs, including simple flexion or extension and more complex, longer duration activity, have been quantified as occurring during  $14.3 \pm 0.9\%$  of high voltage activity with each movement averaging  $4.55 \pm 0.59$  seconds (Natale *et al.*, 1981). Bursts of activity occur every 20 to 35 minutes (Natale *et al.*, 1981). Our observed lack of difference in hindlimb oxygen consumption between ECoG states could also mean that movements of the hindlimb during the high voltage episodes were either of insufficient duration or complexity to increase oxygen consumption.

In conclusion, the late gestation fetal lamb normally exhibits cyclical alterations in behavioural state including electrocortical activity, electro-ocular activity or eye movements, and breathing and body movements. Neuromuscular blockade, achieved either with gallamine triethiodide or pancuronium bromide, abolishes fetal breathing and body

movements. These drugs did not alter sleep cycle length or the amount of time the fetus spent in the high voltage ECoG state. Following neuromuscular blockade, mean hindlimb blood flow was reduced by  $8.3 \pm 3.5\%$  suggesting that skeletal muscle activity is associated with greater hindlimb blood flow. Although the differences were not statistically significant, blood flow to the hindlimb tended to be greater during quiet sleep both before and after the administration of gallamine or pancuronium. These data suggest that abolition of skeletal muscle activity by neuromuscular blockade did not alter the apparent behavioural state modulation of hindlimb blood flow, and thus, this may be an effect directly mediated by the central nervous system.

The increased variability in flow during quiet sleep observed on the polygraph recordings appeared to be reduced following drug administration. However, this decline in variability was not found to be statistically significant, perhaps because our data acquisition system did not record data frequently enough to capture the rapid variations in blood flow associated with hindlimb activity.

Neuromuscular blockade was associated with a significant increase in fetal oxygenation and an 11.8% fall in oxygen consumption. This suggests that inhibition of skeletal muscle activity, such as occurs during fetal hypoxemia, may be a compensatory mechanism that lowers fetal oxygen demands when faced with a reduction in oxygen supply. We were unable to show a statistical difference in oxygen consumption between high and low voltage electrocortical activity. This may reflect the low sample size or that the duration and complexity of the hindlimb movements during the high voltage episodes were insufficient to significantly increase oxygen consumption.

## 6. HYPOXEMIA AND ISCHEMIA

### 6.1 Introduction

The relationship between systemic oxygen delivery ( $Do_2$ ) and oxygen consumption ( $\dot{V} O_2$ ) in the fetal lamb has been examined in numerous studies in which oxygen delivery was acutely reduced by various methods, including: decreasing maternal inspired oxygen concentration (Rurak *et al.*, 1990a; Rurak *et al.*, 1990b; Cohn *et al.*, 1974), reducing uterine blood flow (Jensen *et al.*, 1991; Boyle *et al.*, 1990; Wilkening and Meschia, 1983), and diminishing umbilical blood flow (Itskovitz *et al.*, 1987; Itskovitz *et al.*, 1983). Generally a curvilinear relationship is seen between the two variables with  $\dot{V} O_2$  being maintained via an increase in oxygen extraction over a fairly wide range of oxygen delivery (Jensen and Berger, 1991 ; Carter, 1989). This compensation has been termed the oxygen margin of safety or oxygen reserve (Richardson, 1989). However, when oxygen delivery is reduced below ~50%, consumption begins to decline precipitously. As extraction rises, the  $PO_2$  of blood perfusing tissues downstream is reduced thereby imposing a limitation on this compensation. As well, when the fetus is compromised blood flow is redirected to the heart, brain, and adrenal glands at the expense of less vital tissues like gastrointestinal tract, skin, and muscle. When this occurs, significant fetal lactic acidemia develops which is thought to be due to anaerobic lactate production in fetal tissues and organs (Boyle *et al.*, 1992; Iwamoto and Rudolph, 1985).

In a recent study of acute fetal hemorrhage Kwan *et al.* (1995b) found a linear relationship between  $\dot{V} O_2$  and  $Do_2$  with consumption falling significantly when oxygen supply was reduced by only ~15%. As delivery was further reduced, consumption continued to fall. However, the rise in fetal blood lactate concentration (to ~2.9 mM) was low in comparison to that seen in other studies in which oxygen delivery was reduced by lowering maternal inspired oxygen levels or uterine artery compression (lactate values of

~7–15 mM) (Boyle *et al.*, 1992; Rurak *et al.*, 1990b). They concluded that a reduction in fetal  $\text{Do}_2$  achieved via acute fetal hemorrhage is better tolerated than when caused by other means. This may be because with fetal blood loss,  $\text{Do}_2$  is reduced primarily by a fall in cardiac output and umbilical blood flow with minimal changes in vascular  $\text{Po}_2$ . With the other methods of reducing  $\text{Do}_2$  the effect was primarily achieved via fetal hypoxemia. Since fetal arterial  $\text{Po}_2$  is low by postnatal standards, the oxygen gradient from blood to mitochondria may also be lower. Factors which reduce arterial  $\text{Po}_2$  nearer to that required to maintain the oxygen diffusion gradient between the blood and the inner mitochondrial membrane may jeopardize the fetus' ability to maintain normal oxygen consumption. Thus, during hypoxemia, which by definition is a reduction in arterial  $\text{Po}_2$ , oxygen diffusion into fetal tissues may become a limiting factor. In contrast, with fetal hemorrhage this would not be as great a problem since the main factor reducing systemic and tissue  $\text{O}_2$  delivery is decreased perfusion.

The greater degree of lactic acidemia that occurs with hypoxemia may be due to increased anaerobic glycolysis in those tissues in which  $\text{O}_2$  diffusion is impaired. As the carcass components receive ~40% of combined ventricular output (Rudolph and Heymann, 1970) and are subject to decreased perfusion with severe hypoxemia (Jensen *et al.*, 1991; Boyle *et al.*, 1990; Peeters *et al.*, 1979), they could contribute to the systemic lactic acidemia. Another potential site of increased lactate production is the placenta. Under normal conditions, the sheep placenta is a substantial lactate producer (Sparks *et al.*, 1982). Although umbilical blood flow is maintained or even transiently increased during hypoxemia (Rurak *et al.*, 1990b; Richardson, 1989; Peeters *et al.*, 1979), the placenta increases the amount of lactate it releases into the fetal circulation (Milley, 1988). This also occurs during labour (Tan, 1997). Thus both fetal and placental tissues could be sites of increased lactate production during hypoxemia. The lesser tolerance of the fetal lamb to hypoxemia compared to hemorrhage may be due to the marked lactic acidemia that occurs

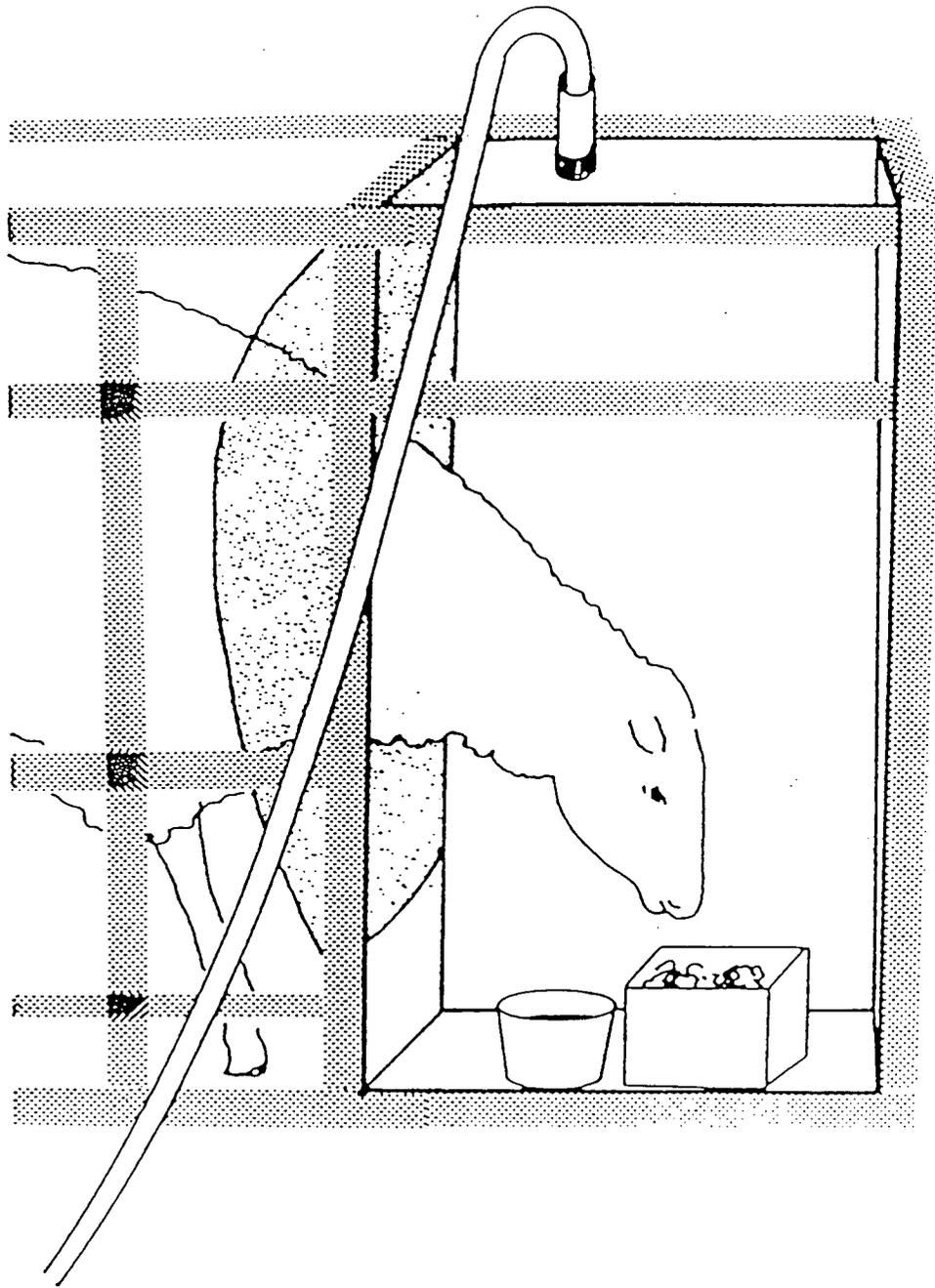
in the former situation. This further reduces blood oxygen content via a rightward shift in the oxyhemoglobin dissociation curve (Bohr Shift) (Rurak *et al.*, 1990b).

The objective of the present study was to measure the fetal cardiovascular and metabolic responses to hypoxemia, which reduces arterial  $P_{O_2}$ , and to hindlimb ischemia, in which  $P_{O_2}$  is maintained. The hypothesis to be tested was that a fall in oxygen delivery achieved via hypoxemia will be tolerated less well than ischemia, and this will be reflected by an earlier fall in hindlimb oxygen consumption and/or greater release of lactate, and that the latter process will contribute to the systemic lactic acidemia.

## 6.2 Methods

Surgery was performed on 11 sheep between 118–130 days gestation (mean = 124  $\pm$  1 days), using the techniques previously described (Section 2.3). Briefly, catheters were placed in the amniotic cavity and in the fetal trachea, femoral artery, lateral tarsal vein, and pudendoepigastric artery and vein. A transit-time flow transducer on the external iliac artery continuously measured blood flow to the hindlimb. Periods of graded ischemia were achieved by partial inflation of the vascular occluder located proximal to the flow transducer. In some ewes a non-occlusive catheter was inserted into the maternal trachea for introduction of hypoxic gas mixtures.

Hypoxemia studies were conducted on 7 animals between 124–136 days gestation (mean = 130  $\pm$  2). Hypoxemia was achieved by reducing maternal inspired oxygen content either by introducing nitrogen directly through a maternal tracheal catheter ( $n=4$ ) using the technique of Gleed (1986) or by placing the ewe's head in a 0.25 m<sup>3</sup> Plexiglas chamber (Figure 17) and introducing a hypoxic gas mixture of oxygen, 1.2% carbon dioxide, and nitrogen ( $n=3$ ) as utilized by Rurak (1990a). The use of the Plexiglas chamber was introduced as we found it difficult to maintain low arterial  $P_{O_2}$  values using the maternal tracheal catheter and, during one experiment, a ewe suddenly died upon introduction of the nitrogen into her tracheal catheter (see E1116 in Appendix).

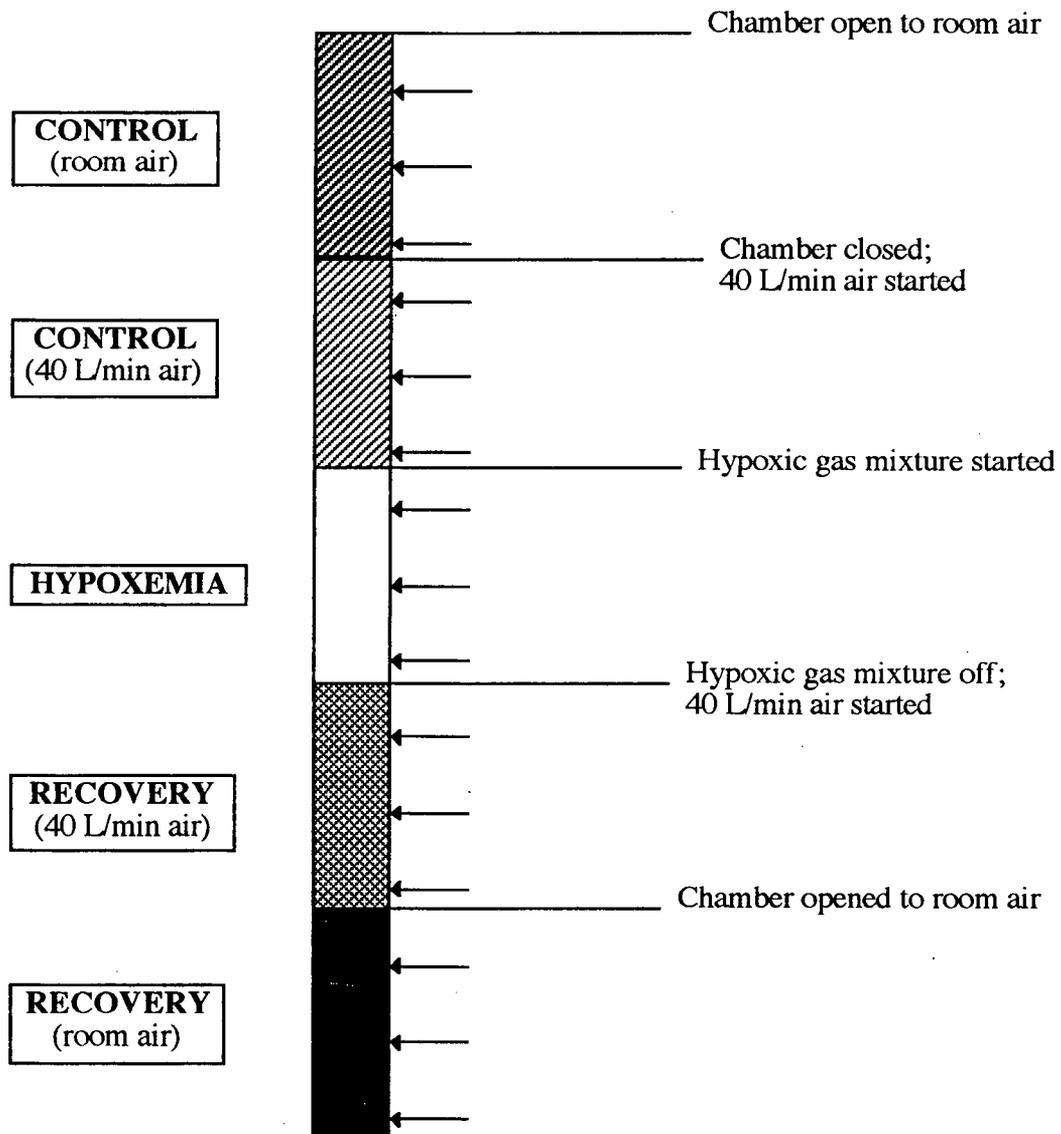


**FIGURE 17.** Plexiglas chamber used to alter maternal inspired oxygen concentrations during hypoxemia experiments (figure from Rurak *et al*, 1990). Ewes were able to eat, drink, and lay down normally when their heads were inside the chamber.

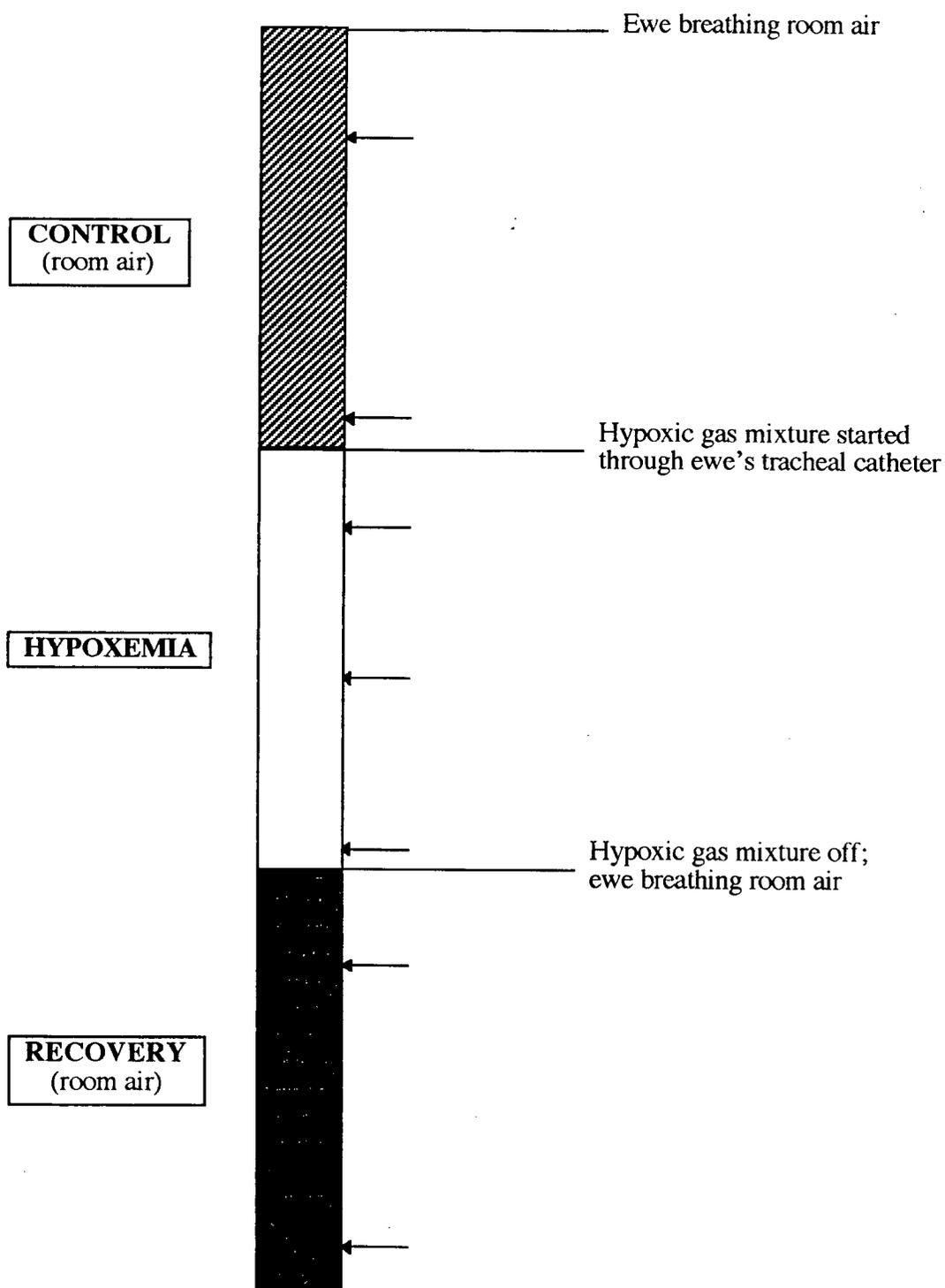
During all experiments the behaviour of the ewes did not seem altered, and they continued to eat and drink as usual. The hypoxemia experiments were divided into a control period, a hypoxemia period of approximately 30 minutes, followed by a recovery period during which the ewe's breathed normoxic air (Figures 18 and 19). In experiments utilizing the Plexiglas chamber, the ewes first breathed room air (chamber open) for 30 minutes followed by a 30 minute period in which the chamber was closed and 40 L/min of air was circulated through it. Similarly, in these experiments, the initial recovery phase involved the ewes breathing 40 L/min air from the chamber for 30 minutes, followed by opening of the chamber and the ewes breathing room air.

In all experiments, gas mixtures were allowed to equilibrate ten minutes before paired blood samples (~1 ml each) were taken from the pudendoepigastric artery and vein. For practical purposes we used change in fetal  $P_{aO_2}$  as an estimate of the level of fetal hypoxemia, following the example of Akagi and Challis (1990b). We attempted to produce three levels of hypoxemia by reducing fetal arterial  $PO_2$  to approximately 3, 5, or 7 mmHg below control values. After each blood sample was taken, the fetus was transfused via the tarsal vein with an equivalent amount of maternal blood. The replacement blood was collected from the ewes in heparinized syringes prior to the start of the experiment.

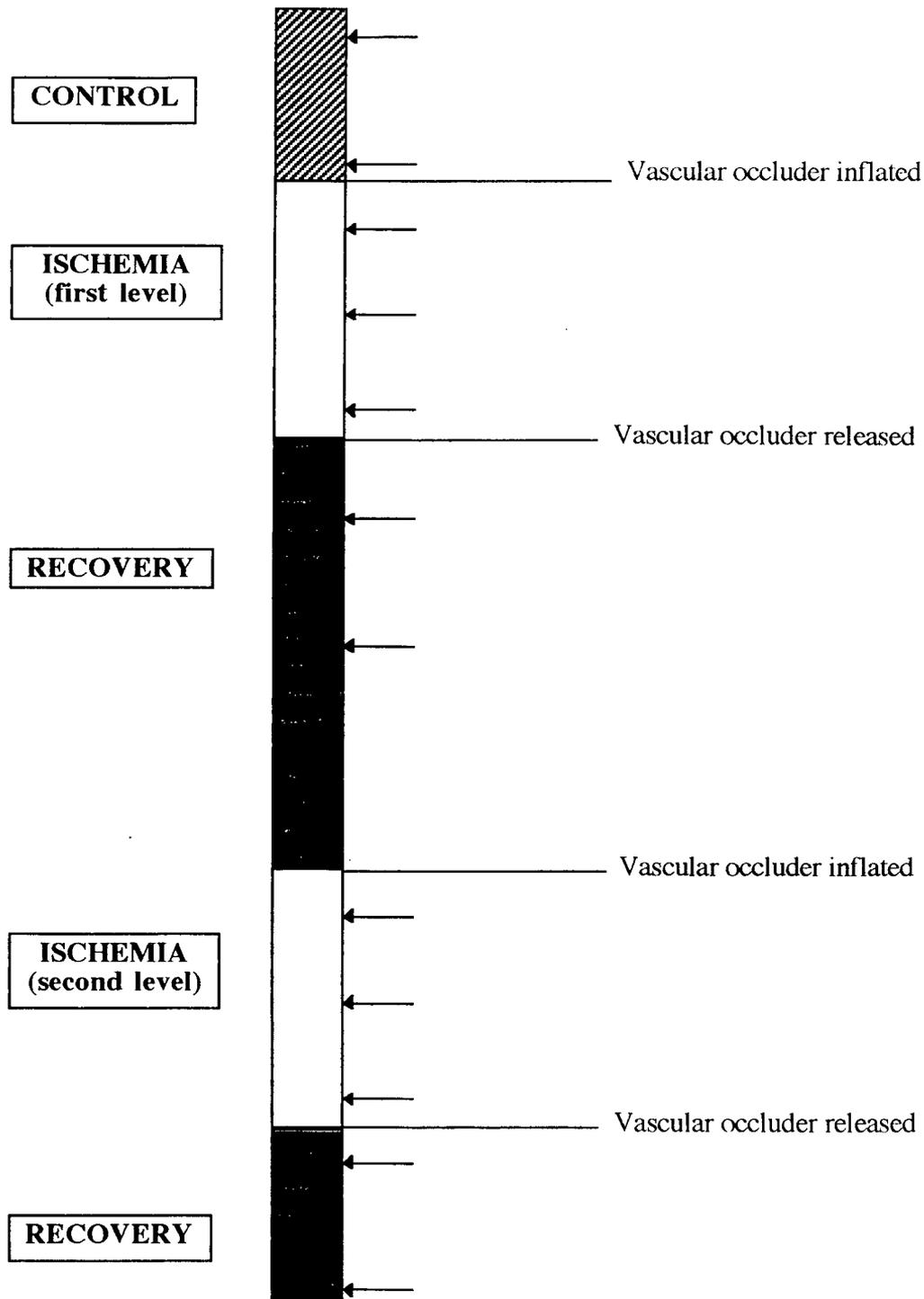
Ischemia experiments were conducted on 6 animals between 130–140 days gestation (mean =  $137 \pm 1$  days). Following a control period, hindlimb ischemia was achieved by injecting saline into the vascular occluder through a 3 cc syringe mounted on a Harvard infusion/withdrawal pump. By continuously monitoring hindlimb blood flow with the downstream flow transducer, the infusion rate into the occluder could be adjusted to achieve the desired reductions in hindlimb blood flow. We attempted to achieve three groupings of ischemia by reducing hindlimb blood flow by 25, 50, or 75%. Once the desired level of occlusion was achieved, blood samples were taken at 10 minute intervals for approximately 30 minutes (Figure 20). This was followed by a 20 minute recovery period with the first recovery sample taken 10 minutes after release of the occluder. In 5 of the 6



**FIGURE 18.** Protocol for hypoxemia experiments involving Plexiglas chamber. Arrows indicate fetal blood samples (taken at approximately 10 minute intervals).



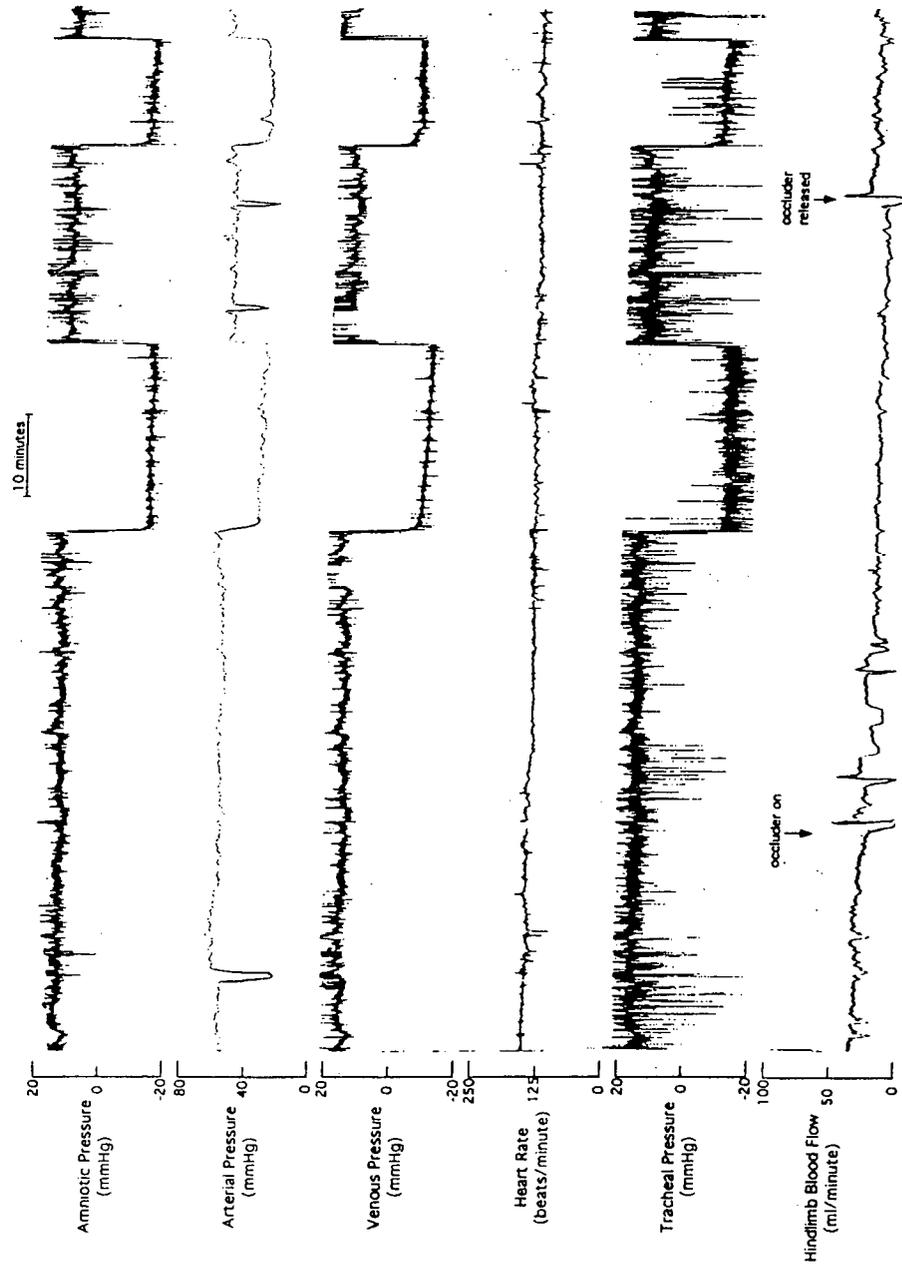
**FIGURE 19.** Protocol for hypoxemia experiments in which hypoxic gas mixture was introduced through the maternal tracheal catheter. Arrows indicate fetal blood samples (taken at approximately 10 minute intervals during hypoxemia and 20 minute intervals during the control and recovery phases).



**FIGURE 20.** Protocol for fetal hindlimb ischemia experiments. Ischemia was achieved by partial inflation of a vascular occluder on the fetal external iliac artery. In most experiments two episodes of ischemia were conducted separated by a recovery phase. At least 30 minutes was allowed between the end of the first recovery phase and the second ischemia episode. Arrows indicate fetal blood samples (taken at approximately 10 minute intervals).

experiments, this recovery phase was followed by a second 30 minute episode of ischemia (at a different level of occlusion) followed by another recovery phase. Thus a total of 11 ischemia episodes were studied. At least 30 minutes were allowed between the end of the first recovery phase and the beginning of the second ischemia trial. As with the hypoxemia experiments, an equivalent amount of maternal blood was used to replace fetal blood lost to sampling. Figure 21 illustrates a polygraph recording of a typical ischemia episode in which hindlimb blood flow was reduced to ~60% of the control value. Initially, blood flow is quite variable as the occluder is manually adjusted to achieve the desired degree of compression. Release of the occluder results in a transient hyperemia followed by a rapid return to normal flow values.

Data from both the hypoxemia and ischemia experiments were analyzed by grouping the results based on change in hindlimb oxygen delivery ( $Do_2$ ), expressed as a percentage of control values. The interventions were classified as mild if oxygen delivery fell to 67–100% of control values; moderate when  $Do_2$  was between 34–66% of baseline; and severe as a  $Do_2$  below 34% of control values. For the hypoxemia study, 26 samples were collected during the control and recovery phases, 14 during mild (from 4 different fetuses), and 10 during both moderate (6 fetuses) and severe hypoxemia (3 fetuses). The hypoxemia levels were somewhat difficult to maintain; thus, in 2 of the 7 experiments samples corresponding to 1 level of hypoxemia were obtained, in 4 experiments samples were from 2 different levels of hypoxemia (the levels were adjacent i.e., either mild and moderate or moderate and severe), and from 1 fetus mild, moderate, and severe hypoxemia samples were collected during a single episode. From the ischemia group, a total of 12 control samples were obtained, 3 during mild ischemia (from 2 different fetuses), 9 during moderate (from 5 fetuses), 6 during severe (from 4 fetuses), and 18 during the recovery phase. The ischemia levels were easier to sustain as in only 1 episode were the samples collected equivalent to 2 different ischemia levels (moderate and severe).



**FIGURE 21.** Polygraph recording of a hindlimb ischemia episode obtained from a late gestation fetal lamb. Ischemia was achieved by partial inflation of a vascular occluder positioned on the external iliac artery upstream to a transit-time flow transducer. In this figure, hindlimb blood flow was reduced to approximately 60% of control values. The onset of the occlusion episode is followed by abrupt alterations in blood flow as the inflation of the occluder is manually adjusted to achieve the desired level of ischemia. Release (or deflation) of the occluder results in an immediate, transient hyperemia followed by a rapid return of hindlimb blood flow to control values. Abrupt changes in pressures are due to position changes of the ewe.

Data collected prior to the introduction of the hypoxic gas mixture or inflation of the vascular occluder were considered baseline or control data with each fetus serving as its own control. Control data are generally presented as absolute values, with other data calculated as either a change from or a percentage of the individual animal's control values. For analysis of the cardiovascular variables, the fetal cardiovascular data from 3 minutes before to 3 minutes after each blood sample (total of 7 minutes of data) were used. Data are generally presented as mean  $\pm$  standard error of the mean. Data were analyzed with StatView statistical software (Abacus Concepts Inc., Berkeley, California). Changes in the variables during experiments were analyzed with one-way (factorial) analysis of variance (ANOVA) and Fisher's Protected Least Significant Difference (PLSD) post-hoc test. Repeated measures ANOVA could not be used as not all animals were exposed to the three levels of intervention. Differences between the hypoxemia and ischemia groups were compared using two-tailed unpaired t-tests. Differences in regression equations were compared by applying unpaired t-tests to the slopes and y-intercepts. In all cases, differences were considered significant if  $p < 0.05$ .

## **6.3 Results**

### **6.3.1 Animal information**

Table 8 presents individual animal gestational age, fetal weight, and study limb weight at the time of experiment and at autopsy for the animals involved in the hypoxemia and ischemia studies. The hypoxemia experiments were conducted at a significantly earlier gestational age compared to the ischemia studies ( $130 \pm 2$  days versus  $137 \pm 1$  days). The number of fetuses carried by each ewe was the same in both groups. Furthermore, no difference was found between the two groups in terms of fetal weight or hindlimb weight at autopsy and on the day of study.

**TABLE 8.** Individual fetal age, weight, and study limb weight at autopsy and on day of study for the animals involved in the hypoxemia and ischemia studies. In the case of multiple lambs, weights are those of the operated fetus only.

Ewe Number	No. of Fetuses	Experiment			Autopsy		
		gestational age (days)	estimated fetal weight (g)	estimated hindlimb weight (g)	gestational age (days)	fetal weight (g)	hindlimb weight (g)
<b>HYPOXEMIA</b>							
E3210	1	128	3708	273	142	5294	389
E2224	1	132	3466	255	137	3944	290
E1223	1	124	2444	180	136	3471	255
E1245	3	136	3005	210	139	3225	225
E967	2	136	2776	204	139	2979	219
E207HIN	2	124	1734	144	132	2218	184
E1154	1	132	3409	251	132	3409	251
Mean±SEM	1.6±0.3	130±2	2935±259	217±17	137±1	3506±359	259±25
<b>ISCHEMIA</b>							
E1245	3	138	3152	220	139	3225	225
E722	2	138	3026	222	139	3096	228
E116	2	139	3206	236	139	3206	236
E540	2	140	3798	279	142	3960	291
E136	1	136	3082	246	137	3158	252
E207HIN	2	130	2095	174	132	2218	184
Mean±SEM	2.0±0.3	137±1†	3060±224	230±14	138±1	3144±226	236±14

† = significantly different from hypoxemia value ( $p < 0.05$ ).

### 6.3.2 Blood gas and acid-base status

Prior to the introduction of the hypoxic gas mixture for the 3 experiments using the Plexiglas chamber, blood samples were collected with the chamber open and the ewe breathing room air as well as with the box closed and the ewe breathing air administered at 40 L/min into the chamber. As there were no differences in the blood gas values between these two situations, the data were pooled as control samples. Similarly, we grouped together as recovery samples the data collected after the completion of the hypoxemia phase when the ewe breathed 40 L/min of air circulated through the Plexiglas chamber and when she breathed room air with the chamber open.

The blood gas values and acid-base status for the 7 animals studied in the hypoxemia experiments and the 6 animals involved in the ischemia studies are presented in Tables 9 and 10. The control data (presented as absolute values) indicated no significant blood gas or acid base status differences between the animals which were to be subjected to hypoxemia or ischemia except for higher baseline arterial ( $3.27 \pm 0.17$  versus  $2.70 \pm 0.16$  mM;  $p = 0.0384$ ) and venous ( $2.47 \pm 0.16$  versus  $1.94 \pm 0.14$  mM;  $p = 0.0310$ ) oxygen contents in the ischemia study group. During hypoxemia, fetal arterial  $P_{O_2}$  fell by  $3.4 \pm 0.5$  mmHg during mild,  $5.3 \pm 0.5$  mmHg with moderate, and  $6.8 \pm 0.3$  mmHg during severe hypoxemia. During severe hypoxemia fetal arterial  $P_{O_2}$  averaged  $13.0 \pm 1.0$  mmHg compared to the control value of  $20.2 \pm 0.6$  mmHg. In addition, arterial oxygen saturation and content were reduced at each level of hypoxemia, falling by  $19.0 \pm 0.9\%$  and  $1.00 \pm 0.04$  mM, respectively, during severe hypoxemia. Fetal arterial  $P_{CO_2}$  also fell with each level of hypoxemia. Arterial pH and base excess were maintained until severe hypoxemia when they fell significantly with pH falling by  $0.094 \pm 0.016$  and base excess decreasing by  $7.5 \pm 1.0$  mEq/L. As with the arterial values, with each level of hypoxemia venous  $P_{O_2}$  was significantly reduced reaching values  $2.8 \pm 0.5$ ,  $5.5 \pm 0.6$ , and  $8.3 \pm 0.4$  mmHg below control (to  $14.9 \pm 0.4$ ,  $12.6 \pm 0.9$ , and  $7.4 \pm 1.2$  mmHg). Venous oxygen saturation and

**TABLE 9.** Arterial and venous blood gas responses to hypoxemia in the fetal lamb hindlimb (7 animals). Control data are presented as absolute values; other data are change from the control values.

	CONTROL	MILD	MODERATE	SEVERE	RECOVERY
<b>ARTERIAL</b>					
$P_{O_2}$ (mmHg)	20.2 ± 0.6	-3.4 ± 0.5†	-5.3 ± 0.5†	-6.8 ± 0.3†	0.0 ± 0.4
$P_{CO_2}$ (mmHg)	49.0 ± 0.5	-1.1 ± 0.3†	-1.4 ± 0.7†	-3.8 ± 0.5†	-1.1 ± 0.3†
pH	7.330 ± 0.010	0.003 ± 0.003	-0.018 ± 0.009	-0.094 ± 0.016†	-0.105 ± 0.016†
Base excess (mEq/L)	0.5 ± 0.7	-0.3 ± 0.2	-1.9 ± 0.6	-7.5 ± 1.0†	-7.0 ± 1.0†
Hemoglobin concentration(g/dL)	10.0 ± 0.1	0.0 ± 0.0	0.5 ± 0.2†	1.0 ± 0.1†	-0.1 ± 0.1
O <sub>2</sub> saturation (%)	43.5 ± 2.4	-10.3 ± 1.3†	-16.6 ± 1.8†	-19.0 ± 0.9†	-5.8 ± 0.9†
O <sub>2</sub> content (mM)	2.70 ± 0.16	-0.67 ± 0.10†	-0.93 ± 0.13†	-1.00 ± 0.04†	-0.41 ± 0.05†
<b>VENOUS</b>					
$P_{O_2}$ (mmHg)	16.4 ± 0.5	-2.8 ± 0.5†	-5.5 ± 0.6†	-8.3 ± 0.4†	-0.6 ± 0.4
$P_{CO_2}$ (mmHg)	51.8 ± 0.6	-0.7 ± 0.3	-0.2 ± 1.0	1.7 ± 0.8†	-1.4 ± 0.4†
pH	7.312 ± 0.010	0.000 ± 0.000	-0.003 ± 0.009	-0.142 ± 0.022†	-0.108 ± 0.017†
Base excess (mEq/L)	0.3 ± 0.7	-0.6 ± 0.2	-2.2 ± 0.6	-8.3 ± 1.2†	-7.3 ± 1.0†
Hemoglobin concentration(g/dL)	10.0 ± 0.1	-0.1 ± 0.1	0.5 ± 0.2†	1.1 ± 0.1†	-0.2 ± 0.1
O <sub>2</sub> saturation (%)	31.1 ± 2.1	-9.6 ± 1.2†	-16.7 ± 1.7†	-16.0 ± 1.4†	-4.8 ± 0.8†
O <sub>2</sub> content (mM)	1.94 ± 0.14	-0.64 ± 0.08†	-0.99 ± 0.12†	-0.91 ± 0.07†	-0.35 ± 0.04†

Values are mean ± SEM; † = significant change from control (p<0.05).

**TABLE 10.** Arterial and venous blood gas responses to hindlimb ischemia in six fetal lambs. Control data are presented as absolute values; other data are change from the control values.

	CONTROL	MILD	MODERATE	SEVERE	RECOVERY
<b>ARTERIAL</b>					
$P_{O_2}$ (mmHg)	21.2 ± 1.0	-1.1 ± 0.6	-0.2 ± 0.3	-0.8 ± 0.6	-0.9 ± 0.3
$P_{CO_2}$ (mmHg)	48.7 ± 0.6	0.7 ± 1.2	-0.2 ± 1.0	1.2 ± 1.0	0.2 ± 0.5
pH	7.313 ± 0.011	-0.004 ± 0.007	-0.007 ± 0.007	-0.014 ± 0.009	-0.005 ± 0.005
Base excess (mEq/L)	-0.9 ± 0.7	1.0 ± 0.9	-0.5 ± 0.4	-0.5 ± 0.7	-0.2 ± 0.3
Hemoglobin concentration(g/dL)	10.8 ± 0.5	-0.3 ± 0.2	-0.2 ± 0.2	-0.2 ± 0.1	-0.3 ± 0.1
$O_2$ saturation (%)	49.3 ± 2.9	-1.7 ± 1.1	-0.9 ± 0.9	-5.0 ± 2.5	-4.3 ± 1.8
$O_2$ content (mM)	3.27 ± 0.17	-0.21 ± 0.13	-0.14 ± 0.03	-0.41 ± 0.16†	-0.38 ± 0.11†
<b>VENOUS</b>					
$P_{O_2}$ (mmHg)	17.6 ± 0.9	-2.1 ± 0.6†	-1.8 ± 0.6†	-2.1 ± 0.5†	-1.6 ± 0.2†
$P_{CO_2}$ (mmHg)	52.0 ± 0.8	1.1 ± 1.0	1.4 ± 0.8	3.0 ± 1.0	-0.5 ± 1.9
pH	7.290 ± 0.013	0.003 ± 0.003	-0.015 ± 0.004	-0.019 ± 0.005	-0.006 ± 0.005
Base excess (mEq/L)	-1.1 ± 0.8	0.6 ± 0.5	-0.3 ± 0.3	-0.3 ± 0.2	0.0 ± 0.3
Hemoglobin concentration(g/dL)	10.9 ± 0.5	-0.2 ± 0.1	-0.1 ± 0.1	-0.1 ± 0.1	-0.2 ± 0.1
$O_2$ saturation (%)	37.3 ± 3.0	-6.5 ± 3.2†	-7.4 ± 1.7†	-8.5 ± 2.0†	-5.8 ± 1.5†
$O_2$ content (mM)	2.47 ± 0.16	-0.44 ± 0.21†	-0.49 ± 0.12†	-0.62 ± 0.12†	-0.43 ± 0.09†

Values are mean ± SEM; † = significant change from control (p<0.05).

O<sub>2</sub> content also fell with each level of hypoxemia reaching values of  $16.0 \pm 1.4\%$  and  $0.91 \pm 0.07$  mM below the control values, respectively, during severe hypoxemia. The severe perturbation was also associated with a significant rise in venous *P*co<sub>2</sub> (by  $1.7 \pm 0.8$  mmHg), and reductions in pH (by  $0.142 \pm 0.022$ ) and base excess (by  $8.3 \pm 1.2$  mEq/L). During the recovery phase mean arterial and venous *P*O<sub>2</sub> values returned to baseline levels; however, the other variables remained significantly different from the control levels.

In contrast to hypoxemia, fetal arterial *P*O<sub>2</sub>, pH, base excess, and O<sub>2</sub> saturation declined with increasing levels of ischemia but none of the changes were significant. Venous pH and base excess were also not statistically affected by reducing hindlimb blood flow. Arterial oxygen content did fall significantly with severe ischemia reaching  $0.41 \pm 0.16$  mM below the control value and remained low during recovery. Venous *P*O<sub>2</sub> was reduced by  $2.1 \pm 0.6$  mmHg during mild,  $1.8 \pm 0.6$  mmHg during moderate, and  $2.1 \pm 0.5$  mmHg during severe ischemia (to  $16.7 \pm 1.3$ ,  $16.2 \pm 0.7$ , and  $14.3 \pm 1.2$  mmHg, respectively). Similarly, drops in venous O<sub>2</sub> saturation and O<sub>2</sub> content were observed, with average reductions of  $8.5 \pm 2.0\%$  and  $0.62 \pm 0.12$  mM, respectively, during severe ischemia. During the recovery phase, these latter 3 variables remained below baseline levels.

### 6.3.3 Hindlimb blood flow, oxygen delivery, and oxygen consumption

The data on hindlimb blood flow, and oxygen delivery and consumption are given in Table 11. Data collected during the control periods are expressed as absolute values. In order to facilitate comparisons, the remaining data (mild, moderate, severe, and recovery phases) are expressed as mean ( $\pm$  SEM) percentages of the individual animal control values.

In the control period, blood flow in the ischemia animals ( $13.8 \pm 0.9$  ml·min<sup>-1</sup>·100g<sup>-1</sup>) was significantly lower than in the hypoxemia group ( $28.9 \pm 1.8$  ml·min<sup>-1</sup>·100g<sup>-1</sup>). This difference was accompanied by significantly lower values for O<sub>2</sub> delivery ( $44.3 \pm 3.1$  versus  $71.4 \pm 1.7$  μmol·min<sup>-1</sup>·100g<sup>-1</sup>) and consumption ( $10.7 \pm 1.3$

**TABLE 11.** Hindlimb metabolic variables during hypoxemia and ischemia experiments. Control data are presented as absolute values; other data are expressed as a percent of the control values.

	CONTROL	MILD	MODERATE	SEVERE	RECOVERY
<b>HYPOXEMIA</b>					
Hindlimb blood flow (ml·min <sup>-1</sup> ·100g <sup>-1</sup> )	28.9 ± 1.8	99.6 ± 3.2	71.2 ± 4.1†	38.5 ± 4.6†	110.9 ± 3.5†
Oxygen delivery (μmol·min <sup>-1</sup> ·100g <sup>-1</sup> )	71.4 ± 1.7	79.4 ± 2.5†	50.8 ± 2.8†	20.5 ± 2.3†	92.6 ± 3.0
Oxygen consumption (μmol·min <sup>-1</sup> ·100g <sup>-1</sup> )	21.1 ± 1.4	91.3 ± 6.2	75.4 ± 5.2†	30.3 ± 4.5†	103.4 ± 6.1
Oxygen extraction (%)	29.3 ± 1.7	114.5 ± 6.7†	148.0 ± 6.3†	143.3 ± 9.1†	111.0 ± 5.0
<b>ISCHEMIA</b>					
Hindlimb blood flow (ml·min <sup>-1</sup> ·100g <sup>-1</sup> )	13.8 ± 0.9*	77.4 ± 0.3†	50.4 ± 2.6†	29.5 ± 1.8†	94.8 ± 4.2
Oxygen delivery (μmol·min <sup>-1</sup> ·100g <sup>-1</sup> )	44.3 ± 3.1*	73.1 ± 3.1†	48.0 ± 2.3†	26.0 ± 1.8†	83.1 ± 4.1†
Oxygen consumption (μmol·min <sup>-1</sup> ·100g <sup>-1</sup> )	10.7 ± 1.3*	111.1 ± 11.0	74.3 ± 8.6†	42.6 ± 7.9†	103.9 ± 7.5
Oxygen extraction (%)	24.4 ± 2.6	149.1 ± 15.9†	155.3 ± 17.7†	160.6 ± 21.3†	124.8 ± 6.9

Values are mean ± SEM

\* = significant difference from hypoxemia values (p<0.05)

† = significant change from control (p<0.05)

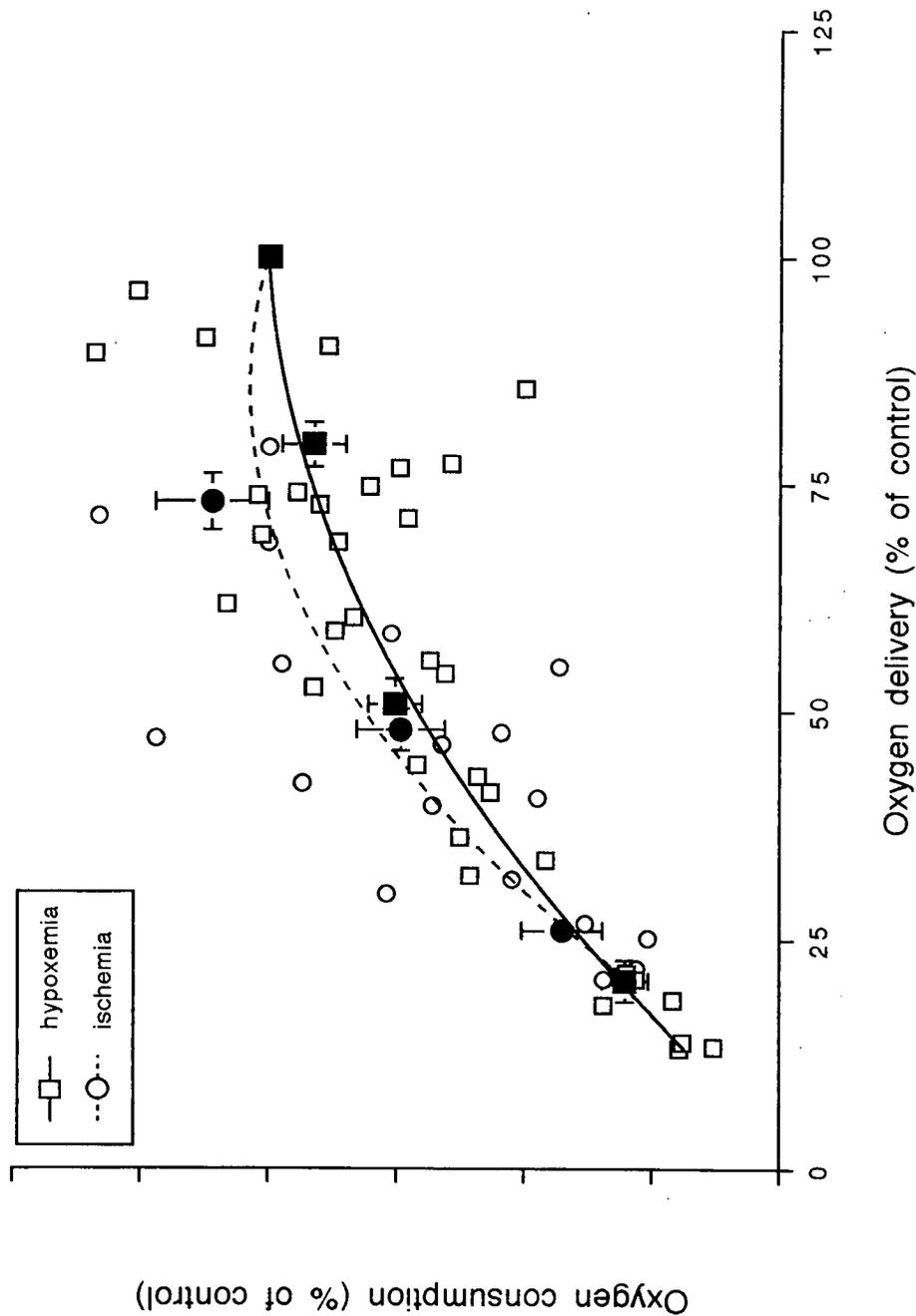
versus  $21.4 \pm 1.4 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ). However, oxygen extraction was similar in both groups ( $24.4 \pm 2.6$  versus  $29.3 \pm 1.7\%$ ).

During the ischemia experiments, hindlimb blood flow fell as expected to  $77.4 \pm 0.3\%$  of control values for mild,  $50.4 \pm 2.6\%$  for moderate, and  $29.5 \pm 1.8\%$  for severe ischemia. In most cases, there was a transient hyperemia following release of the occluder (Figure 21). The hyperemia was too brief to be reflected in the mean flow values during the recovery period which did not significantly differ from the control data. With mild hypoxemia, hindlimb blood flow was not altered. However, with moderate and severe levels, flow was reduced to  $71.2 \pm 4.1$  and  $38.5 \pm 4.6\%$  of the control level respectively.

With mild hypoxemia and ischemia, the  $\sim 25\%$  reduction in  $\text{Do}_2$  was not associated with a fall in  $\text{O}_2$  consumption. However, with moderate levels of perturbation, the reduction in delivery to  $50.8 \pm 2.8$  and  $48.0 \pm 2.3\%$  of control values for hypoxemia and ischemia, respectively, was accompanied by  $\dot{V} \text{O}_2$  values that were  $75.4 \pm 5.2$  and  $74.3 \pm 8.6\%$  of control. With severe hypoxemia and ischemia, there were further reductions in both oxygen delivery and consumption. Although both variables were reduced to lower values with hypoxemia ( $20.5 \pm 2.3$  and  $30.3 \pm 4.5\%$  for  $\text{Do}_2$  and  $\dot{V} \text{O}_2$ , respectively), compared to ischemia ( $26.0 \pm 1.8$  and  $42.6 \pm 7.9\%$ ), the differences between these 2 groups was not statistically significant. In both groups, oxygen extraction rose progressively as  $\text{O}_2$  delivery was reduced. Although the rise tended to be greater with ischemia (to  $160.6 \pm 21.3\%$  of control) than with hypoxemia ( $143.3 \pm 9.1\%$ ), again the difference was not statistically significant. Figure 22 plots the relationship between oxygen delivery and consumption, with both the individual measurements and the mean values for mild, moderate, and severe reductions presented. There was no difference in this relationship for the two perturbations.

#### 6.3.4 Lactate and glucose concentrations and fluxes

Arterial and venous glucose and lactate concentrations as well as hindlimb glucose and lactate fluxes are presented in Table 12. The control values and the flux data are



**FIGURE 22.** Relationship between hindlimb oxygen consumption and delivery in fetal lambs exposed to hypoxemia (n=7) or ischemia (n=6). Open symbols represent individual samples; solid symbols represent mean values ( $\pm$  SEM) for mild, moderate, and severe reductions in oxygen delivery. The regression equations are based on the individual sample points (not mean values).

Hypoxemia (squares):  $y = -0.010x^2 + 2.029x - 6.264$   $r = 0.913$

Ischemia (circles):  $y = -0.017x^2 + 2.990x - 24.519$   $r = 0.820$

**TABLE 12.** Hindlimb lactate and glucose responses to hypoxemia and ischemia. Control data and flux values are presented as absolute values; arterial and venous concentrations are expressed as a change from control values.

	CONTROL	MILD	MODERATE	SEVERE	RECOVERY
<b>HYPOXEMIA</b>					
Arterial [L] (mM)	1.42 ± 0.09	0.51 ± 0.15	1.44 ± 0.36†	3.70 ± 0.54†	3.69 ± 0.37†
Venous [L] (mM)	1.38 ± 0.08	0.70 ± 0.21	1.64 ± 0.34†	3.88 ± 0.60†	3.80 ± 0.35†
<i>Lactate flux</i> ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	1.15 ± 0.81	-4.08 ± 1.87	-2.65 ± 1.13	-2.24 ± 0.92	-2.00 ± 1.95
Arterial [G] (mM)	1.08 ± 0.06	-0.08 ± 0.04	0.01 ± 0.09	0.33 ± 0.10†	0.08 ± 0.05
Venous [G] (mM)	0.95 ± 0.06	-0.11 ± 0.04	-0.02 ± 0.07	0.07 ± 0.11	0.04 ± 0.05
<i>Glucose flux</i> ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	3.50 ± 0.33	2.72 ± 0.42	3.11 ± 0.68	4.66 ± 0.59	4.38 ± 0.32
<b>ISCHEMIA</b>					
Arterial [L] (mM)	2.02 ± 0.12*	-0.41 ± 0.22	0.50 ± 0.27	0.71 ± 0.24†	0.29 ± 0.16
Venous [L] (mM)	1.77 ± 0.10*	0.25 ± 0.18	0.64 ± 0.22†	1.01 ± 0.37†	0.84 ± 0.18†
<i>Lactate flux</i> ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	3.37 ± 1.24	-3.16 ± 1.75	0.73 ± 1.31	-0.25 ± 1.62	-4.28 ± 1.75†
Arterial [G] (mM)	0.74 ± 0.04*	0.02 ± 0.03	0.07 ± 0.06	0.10 ± 0.06	0.06 ± 0.04
Venous [G] (mM)	0.65 ± 0.05*	0.09 ± 0.06	0.05 ± 0.11	-0.06 ± 0.05	0.08 ± 0.07
<i>Glucose flux</i> ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	1.07 ± 0.72*	0.73 ± 0.57	0.77 ± 0.72	0.93 ± 0.32	1.07 ± 0.68

Values are mean ± SEM

[L] = lactate concentration; [G] = glucose concentration

\* = significant difference from hypoxemia values ( $p < 0.05$ )

† = significant change from control ( $p < 0.05$ )

presented as absolute values while the remaining data are expressed as the mean ( $\pm$  SEM) change from the individual animal control values. During the control period, arterial glucose concentrations were significantly higher in the hypoxemia experiments than in the ischemia trials ( $1.08 \pm 0.06$  versus  $0.74 \pm 0.04$  mM;  $p = 0.0005$ ), while lactate levels were lower ( $1.42 \pm 0.09$  versus  $2.02 \pm 0.12$  mM;  $p = 0.0005$ ). With increasing severity of hypoxemia, both arterial and venous lactate levels rose progressively to values of  $3.70 \pm 0.54$  and  $3.88 \pm 0.60$  mM above control levels, respectively, during severe hypoxemia. There was also a rise in arterial glucose concentration during severe hypoxemia (to  $0.33 \pm 0.10$  mM above control), but venous levels were not significantly altered. With ischemia, the arterial lactate concentration was minimally altered, reaching  $0.71 \pm 0.24$  above control values, while hindlimb venous lactate concentrations peaked at  $1.01 \pm 0.37$  mM above control levels during severe ischemia.

In both groups there was a net uptake of lactate and glucose during the control period. Control glucose uptake was higher in the hypoxemia group compared to the ischemia animals ( $3.50 \pm 0.33$  versus  $1.07 \pm 0.72 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ), but control lactate uptake was not different between the groups ( $1.15 \pm 0.81$  for hypoxemia versus  $3.37 \pm 1.24$  for ischemia). Glucose continued to be taken up by the hindlimb during both hypoxemia and ischemia at rates that were not different from the control values. Glucose flux was higher during moderate and severe hypoxemia than the corresponding levels of ischemia.

In contrast, lactate tended to be released from the hindlimb during and following both hypoxemia and ischemia. This net release of lactate from the hindlimb was observed at all levels of hypoxemia, although the lactate efflux did not increase with the severity of hypoxemia. Moreover, none of the changes were significantly different from the control value. However, when the pooled mean lactate flux during hypoxemia ( $-3.12 \pm 0.87 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ) was compared to the control value ( $1.15 \pm 0.81 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ), the difference was statistically significant. During ischemia, there was not consistent lactate

release from the hindlimb, and none of the changes were different from control. As with hypoxemia, the pooled mean lactate flux during ischemia ( $-0.25 \pm 0.41 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) was significantly different from the control value ( $3.37 \pm 1.24 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ). At each level of perturbation (mild, moderate, and severe) there was no significant difference in lactate flux between the hypoxemia and ischemia experiments. For example, lactate efflux during severe hypoxemia ( $-2.24 \pm 0.92 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) was not different from that seen with severe ischemia ( $-0.25 \pm 1.62 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ). However, when the pooled mean lactate fluxes were compared, lactate efflux was significantly greater with hypoxemia than with ischemia. During the ischemia recovery period, there was significant lactate release from the hindlimb ( $-4.28 \pm 1.75 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ), whereas in the hypoxemia recovery phase, although there was net lactate efflux ( $-2.00 \pm 1.95 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ), it was not different from the control value.

### 6.3.5 Cardiovascular Responses

The cardiovascular responses to hypoxemia and ischemia are presented in Table 13. Once again, the control data are expressed as absolute values while the remaining data are presented as mean percentages ( $\pm$  SEM) of the individual control values. During the control period, hindlimb vascular resistance was greater in the ischemia animals compared to the hypoxemia group ( $3.6 \pm 0.5$  versus  $1.7 \pm 0.1 \text{ mmHg/ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ). In contrast, hindlimb blood flow and heart rate in the ischemia animals ( $13.8 \pm 0.9 \text{ ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$  and  $147.3 \pm 3.7 \text{ bpm}$ , respectively) were lower than in the hypoxemia group ( $28.9 \pm 1.8 \text{ ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$  and  $162.8 \pm 2.5 \text{ bpm}$ ).

Fetal heart rate fell slightly with moderate hypoxemia (to  $91.6 \pm 3.8\%$  of control). Bradycardia ( $77.0 \pm 2.4\%$  of control) was also observed with severe hypoxemia along with increases in vascular pressures ( $119.9 \pm 3.4$  and  $153.3 \pm 6.5\%$  above control for arterial and venous, respectively) and hindlimb vascular resistance (to  $347.6 \pm 49.6\%$  above control). No change in fetal heart rate or arterial pressure occurred with ischemia, but

**TABLE 13.** Hindlimb blood flow and cardiovascular responses to hypoxemia and ischemia in the fetal lamb. Control data are presented as absolute values; other data are a percent of the control values.

	CONTROL	MILD	MODERATE	SEVERE	RECOVERY
<b>HYPOXEMIA</b>					
Blood flow ( $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	28.9 $\pm$ 1.8	99.6 $\pm$ 3.2	71.2 $\pm$ 4.1†	38.5 $\pm$ 4.6†	110.9 $\pm$ 3.5†
Heart rate (beats/minute)	162.8 $\pm$ 2.5	102.3 $\pm$ 1.2	91.6 $\pm$ 3.8†	77.0 $\pm$ 2.4†	109.2 $\pm$ 1.8†
Arterial pressure (mmHg)	50.1 $\pm$ 1.1	100.9 $\pm$ 2.1	104.1 $\pm$ 2.8	119.9 $\pm$ 3.4†	107.4 $\pm$ 2.0†
Venous pressure (mmHg)	4.4 $\pm$ 0.2	89.4 $\pm$ 5.7	107.0 $\pm$ 7.8	155.3 $\pm$ 6.5†	93.8 $\pm$ 3.6
Vascular resistance ( $\text{mmHg}/\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	1.7 $\pm$ 0.1	104.8 $\pm$ 6.4	145.4 $\pm$ 12.9	347.6 $\pm$ 49.3†	101.2 $\pm$ 5.6
<b>ISCHEMIA</b>					
Blood flow ( $\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	13.8 $\pm$ 0.9*	77.4 $\pm$ 0.3†	50.4 $\pm$ 2.6†	29.5 $\pm$ 1.8†	94.8 $\pm$ 4.2
Heart rate (beats/minute)	147.3 $\pm$ 3.7*	101.3 $\pm$ 4.7	103.0 $\pm$ 2.9	98.9 $\pm$ 2.0	100.4 $\pm$ 2.1
Arterial pressure (mmHg)	49.9 $\pm$ 2.2	93.6 $\pm$ 4.7	103.3 $\pm$ 1.7	102.8 $\pm$ 2.4	102.3 $\pm$ 1.8
Venous pressure (mmHg)	4.4 $\pm$ 0.7	—	113.2 $\pm$ 10.5	123.0 $\pm$ 9.9	115.0 $\pm$ 7.7
Vascular resistance ( $\text{mmHg}/\text{ml}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	3.6 $\pm$ 0.5*	—	212.1 $\pm$ 31.5†	385.7 $\pm$ 37.7†	112.6 $\pm$ 4.2

Values are mean  $\pm$  SEM

\* = significant difference from hypoxemia values ( $p < 0.05$ )

† = significant change from control ( $p < 0.05$ )

— = data not available

hindlimb resistance increased markedly, reaching levels  $385.7 \pm 37.7\%$  above control values during severe ischemia. The increase in resistance seen with hypoxemia and ischemia appears to be mainly a reflection of decreased hindlimb blood flow.

## 6.4 Discussion

### 6.4.1 Hypoxemia and ischemia levels

In 10 of the 11 ischemia episodes only 1 level of ischemia was tested while during the remaining episode samples from both moderate and severe ischemia were collected. However, in only 2 of the 7 hypoxemia experiments were samples from a single level of hypoxemia obtained (in 4 experiments samples were from 2 levels of hypoxemia while in 1 experiment all 3 levels of hypoxemia were examined). Thus, fetal hindlimb ischemia, achieved by partial inflation of the external iliac artery occluder, was easier to sustain than hypoxemia, whether achieved via a maternal tracheal catheter or the ewe breathing a hypoxic gas mixture in a Plexiglas chamber. This difference reflects the local nature of the ischemia i.e., it is limited to one hindlimb of the fetus. In contrast, hypoxemia affects the whole body and, furthermore, both the ewe and fetus experience reduced levels of oxygenation. Thus, alterations in uterine blood flow, umbilical blood flow, skeletal muscle activity, fetal or maternal cardiac output or oxygen content are all variables which could influence the level of fetal hypoxemia achieved at each sampling time.

### 6.4.2 Blood gas and acid-base responses

The control blood gas results obtained prior to hypoxemia (Table 9) or ischemia (Table 10) are typical of those obtained in our laboratory and in others. Although arterial oxygen content was significantly lower in the hypoxemia group ( $2.7 \pm 0.2$  mM) compared to the ischemia animals ( $3.3 \pm 0.2$  mM), both of these values fall in the range of values previously reported for the descending aorta/femoral artery (Kwan *et al.*, 1995b; Boyle *et al.*, 1992; Boyle *et al.*, 1990; Rurak *et al.*, 1990b; Rurak *et al.*, 1990a; Wilkening *et al.*,

1988). As expected from our experimental protocol, fetal arterial  $P_{O_2}$  fell significantly with each level of hypoxemia reaching values of  $6.8 \pm 0.3$  mmHg below the control level during severe hypoxemia. These changes were associated with reductions in fetal arterial and venous oxygen saturation and content. Severe hypoxemia was also associated with reductions in arterial pH and base excess values, the latter falling  $7.5 \pm 1.0$  mEq/L below the control values. As arterial  $P_{CO_2}$  levels were unchanged or fell slightly there was no evidence of fetal asphyxia. Blood gases collected following hypoxemia indicate that  $P_{O_2}$  quickly returned to control values and the other variables were returning more slowly to the pre-insult levels. In contrast to the hypoxemia animals, arterial  $P_{O_2}$  did not change with ischemia. Furthermore, arterial and venous  $P_{CO_2}$ , pH, and base excess levels were unaltered even with severe ischemia. A minor fall in arterial oxygen content was observed during severe ischemia (to  $0.4 \pm 0.2$  mM below the control value). This change is likely a reflection of the slight, but not significantly different, reductions in hemoglobin concentration and oxygen saturation from which  $O_2$  content is derived.

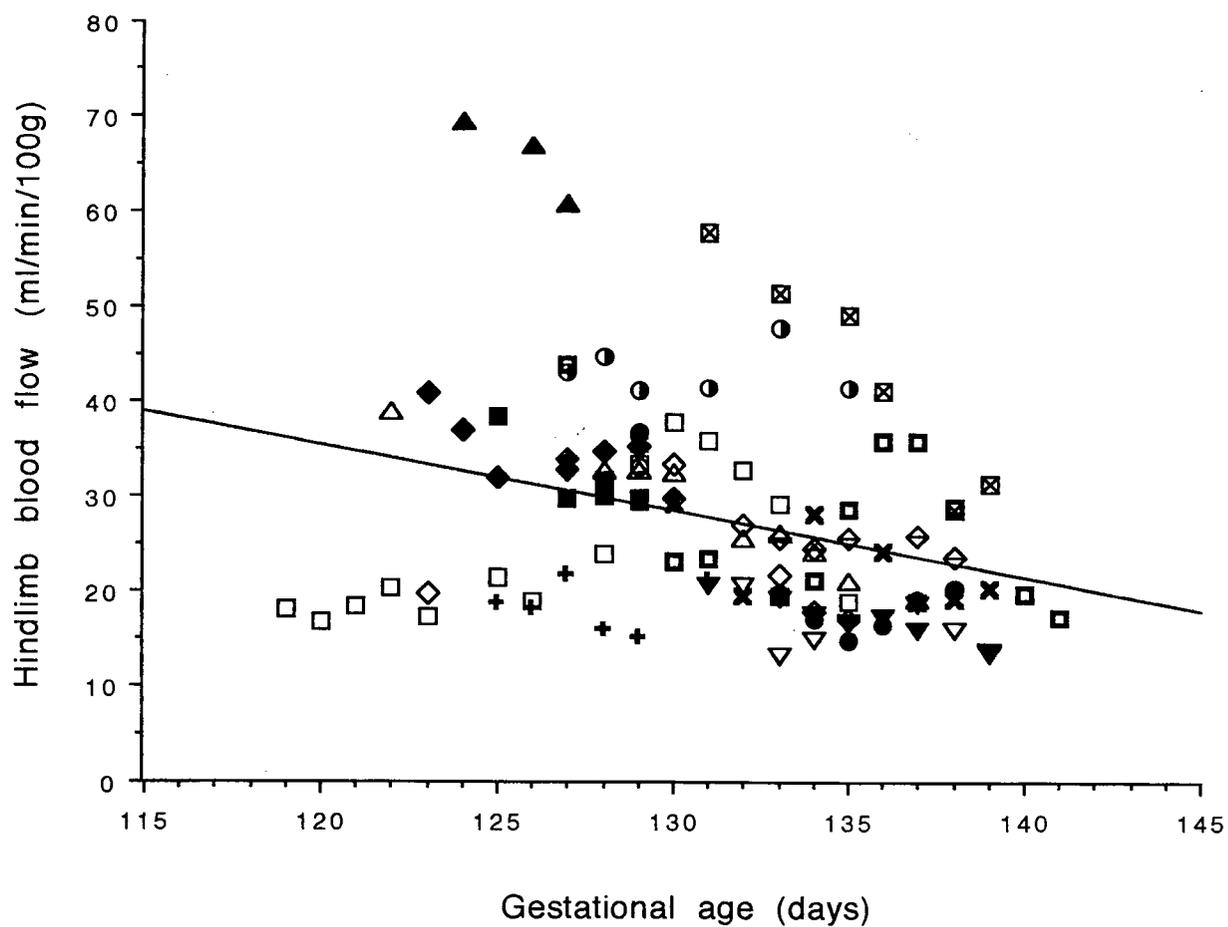
The total amount of blood taken from the fetus during each experiment was about 20 ml or, assuming a fetal blood volume of 110 ml/kg in late gestation (Brace, 1983), approximately 6% of fetal blood volume. However, the fetal volume lost during each sampling (2 ml or ~0.7% of total fetal blood volume) was replaced with maternal blood after each withdrawal. Fetal hemoglobin concentrations were unchanged during the ischemia experiments and rose only slightly as the hypoxemia experiments progressed. Thus, there is no evidence that transfusion of small amounts of maternal blood into the fetus had an overall effect on the experimental outcomes.

#### 6.4.3 Hindlimb blood flow

We found that hindlimb blood flow was less in the ischemia control group compared to the hypoxemia animals (Table 11). Previous studies have reported estimates of fetal lamb hindlimb blood flow in late gestation (127 to 142 days) ranging from 9 to 30

ml·min<sup>-1</sup>·100g<sup>-1</sup> with average values of approximately 18 ml·min<sup>-1</sup>·100g<sup>-1</sup> (Knight *et al.*, 1997; Boyle *et al.*, 1990; Wilkening *et al.*, 1988). Thus, the control hindlimb blood flows observed in both the hypoxemia (28.9 ± 1.8 ml·min<sup>-1</sup>·100g<sup>-1</sup>) and ischemia (13.8 ± 0.9 ml·min<sup>-1</sup>·100g<sup>-1</sup>) groups fall within this range of values. That our mean blood flows were on either end of this range may explain why the difference was significant.

The mean gestational age of the animals subjected to ischemia was approximately a week greater than those in the hypoxemia experiments (137 ± 1 versus 130 ± 2 days). Two of these animals were involved in both hypoxemia and ischemia experiments. As part of the hindlimb weight and blood flow distribution study (Section 4), 3 of the fetuses involved in the ischemia experiments received radionuclide-labelled microspheres. As this required the fetuses to then be euthanized, these experiments were conducted later in gestation so as to permit the fetuses to also be used for other experiments (such as hypoxemia). Thus, the order in which the fetuses were exposed to the hypoxemia and ischemia interventions could not be randomized. This is reflected in the difference in the average gestational ages of the hypoxemia and ischemia fetuses. To further investigate whether this difference could be a factor in the discrepancy between the two groups in terms of blood flow, we did a retrospective, longitudinal study of the relationship between hindlimb blood flow (measured with a transit-time flow transducer on the external iliac artery) and gestational age. The results, collected from 16 fetuses in our laboratory between 119 and 141 days gestation, demonstrated a significant fall in blood flow with increasing gestational age (Figure 23). Previous studies in which hindlimb or carcass blood flows and gestational ages have been reported have not found a significant relationship between these variables (Boyle *et al.*, 1990; Wilkening *et al.*, 1988; Rudolph and Heymann, 1970). In contrast, Bendeck and Langille (1992), who measured organ blood flows at 120 and 140 days gestation, found a significant (50%) reduction in hindlimb bone blood flow and, although skeletal muscle flow fell from 17.2 to 12.8 ml·min<sup>-1</sup>·100g<sup>-1</sup>, neither this nor blood flow to the skin was significantly different. Similar trends for fetal skeletal muscle and skin blood flow were



**FIGURE 23.** Relationship between hindlimb blood flow and gestational age in the fetal lamb. The longitudinal study was conducted on 16 fetuses (each symbol represents an individual animal). Mean daily blood flow was determined with a transit-time flow transducer on the external iliac artery. Only days in which no other experiment was performed are included. The relationship is statistically significant:  $y = 118.8 - 0.69x$   $r = 0.30$

reported by Rosenfeld (1977) when comparing sheep at 60–100 versus 130–140 days gestation. Using the regression equation from our data (hindlimb blood flow =  $118.8 - 0.69 \times \text{gestational age}$ ), the gestational age difference between the hypoxemia and ischemia groups would only account for approximately a 17% difference in flow. The presence of the vascular occluder on the external iliac artery of the fetuses in the ischemia group is unlikely to be responsible for the reduced control hindlimb blood flow in this group as 3 of the 7 hypoxemia fetuses also had vascular occluders implanted. Furthermore, the occluders were only connected to the infusion pumps during the experiments, and the correct positioning of the occluders and flow transducers were verified at autopsy. Thus, it is unlikely that these devices were responsible for the lower control blood flow observed in the ischemia group. Thus, although the hindlimb blood flow values for both the hypoxemia and ischemia control groups fall within the normal range reported in the literature, a complete explanation for the significant difference between these two groups is not clear.

#### 6.4.4 Oxygen delivery and consumption

It is often difficult to compare studies in which  $\text{Do}_2$  is reduced because, depending on the goal of the experiment, different variables are used to define the decline in oxygen supply. For example, oxygen supply levels may be based on the percent reduction in umbilical blood flow in experiments of umbilical cord compression, change in maternal or fetal  $\text{Po}_2$  or oxygen contents when maternal inspired oxygen levels are altered, etcetera. However, it is important to consider both oxygen content and blood flow as it is both of these variables that constitute tissue oxygen supply. Thus, in the current study, we defined levels of hypoxemia and ischemia based on oxygen delivery rather than on arterial  $\text{Po}_2$  or hindlimb blood flow.

In other studies of the fetal lamb hindlimb in late gestation, oxygen delivery has ranged from 50 to  $86 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  and oxygen consumption from 12 to  $28 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  with approximate averages of 60 and  $17 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ , respectively

(Boyle *et al.*, 1992; Boyle *et al.*, 1990; Wilkening *et al.*, 1988). Our control  $\text{Do}_2$  and  $\dot{V}\text{O}_2$  in the hypoxemia group ( $71.4 \pm 1.7$  and  $21.1 \pm 1.4 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ , respectively) fall within these ranges but those of the ischemia group ( $44.3 \pm 3.1$  and  $10.7 \pm 1.3 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ), appear to be low. These differences between the hypoxemia and ischemia groups appear to be consistent i.e., no one or two animals contributed unusually high or low delivery and consumption values. Fetal arterial oxygen content was actually greater in the ischemia control group, and the arterial-venous oxygen content difference was similar for the hypoxemia ( $0.76 \pm 0.05 \text{ mM}$ ) and ischemia ( $0.79 \pm 0.09 \text{ mM}$ ) control groups. Thus the difference in control hindlimb blood flows between the hypoxemia and ischemia groups was responsible for the significant difference seen in the baseline oxygen delivery and consumption values. To facilitate comparison of the results, oxygen delivery and consumption values for the interventions are expressed as a percentage of their respective control values.

Several researchers have pointed out a problem in relating values that are calculated from a common set of directly measured variables: coupled errors in these measured variables may introduce a bias that leads to incorrect conclusions (Stratton *et al.*, 1987; Moreno *et al.*, 1986). In our case, oxygen delivery and consumption are calculated using hindlimb blood flow and arterial oxygen content values and thus, experimental errors in either of these values could create artifactual correlations between oxygen consumption and delivery. For example, an erroneous arterial content value would lead to the calculation of delivery and consumption values that were both high or both low, leading to a positive correlation between consumption and delivery that may be unrelated to any physiological phenomenon (Stratton *et al.*, 1987). Stratton and colleagues (1987) specifically investigated the problem of coupling errors in oxygen consumption and delivery studies. They concluded that, unless the measurement errors are very large or the range of deliveries used is very small, the influence of coupled error on oxygen consumption and delivery relationships is quantitatively small enough to be ignored. As the oxygen content values are

similar to those reported in the literature and oxygen delivery values ranged from approximately 13% to 100% of control values, in the current study the influence of coupled errors was considered to be negligible.

In both our hypoxemia and ischemia experiments, hindlimb oxygen consumption was maintained when delivery was reduced to approximately 75% of control and fell when delivery was reduced by more than ~50%. At these levels of oxygen supply, our hypoxemia animals demonstrated reductions in  $P_{O_2}$  and oxygen content while no such changes occurred with ischemia. In our ischemia experiments, the fall in hindlimb oxygen delivery mirrored the reduction in blood flow. During hypoxemia, blood flow did not change with mild hypoxemia but fell with moderate and severe reductions in oxygen delivery. With severe levels of hypoxemia and ischemia, both arterial oxygen content and hindlimb blood flow were significantly reduced exacerbating the reduction in  $Do_2$ .

Jensen *et al.* (1991) have studied the effect of altering fetal oxygen delivery by reducing uterine blood flow (thereby reducing fetal arterial oxygen saturation and content) and compared their results with previous work done in their laboratory in which  $Do_2$  was diminished by umbilical cord compression (Itskovitz *et al.*, 1987). Radionuclide-labeled microspheres were used to determine blood flow distribution. Oxygen delivery was reduced to 67% and 50% of control values with uterine artery compression and to 77% and 46% of control with umbilical cord occlusion. Graded reductions in uterine blood flow were associated with a fall in fetal descending aorta  $P_{O_2}$  and oxygen content whereas these variables did not change until oxygen delivery fell by ~50% during cord compression. Uterine blood flow reduction also resulted in a rise in  $P_{CO_2}$  in the descending aorta which was not evident with cord compression. Blood flow to the fetal brain and heart greatly increased with each level of uterine artery occlusion but with umbilical cord compression, the only significant increase was in brain blood flow at a 50% reduction in  $Do_2$ . In both studies, total body  $\dot{V}O_2$  was maintained, primarily by an increase in oxygen extraction, until delivery was reduced by ~50%. With the more severe reduction in  $Do_2$ , oxygen

consumption by the lower carcass fell in the uterine artery experiment but did not change when faced with umbilical cord compression.

With reduced maternal inspired oxygen levels and uterine artery occlusion, fetal  $P_{O_2}$  values fall whereas during fetal hemorrhage, hindlimb ischemia, and umbilical vein compression fetal  $P_{O_2}$  values are generally maintained. In the latter cases, oxygen delivery is altered primarily by decreases in blood flow. When we reduced  $Do_2$  by approximately 50% (moderate intervention),  $O_2$  consumption fell to  $75.4 \pm 5.2\%$  of control during hypoxemia versus  $74.3 \pm 8.6\%$  with hindlimb ischemia. These data compare favourably with the reductions in oxygen consumption seen with uterine artery occlusion (to ~70% of control) (Jensen *et al.*, 1991) and in studies in which fetal  $P_{O_2}$  is maintained (to ~75% of control) (Kwan *et al.*, 1995b; Itskovitz *et al.*, 1987; Itskovitz *et al.*, 1983). Furthermore, when other researchers have reduced oxygen delivery to 20–25% of control values by altering fetal  $P_{O_2}$  or blood flow, consumption was approximately 33% (Boyle *et al.*, 1990) and 50% of control values (Itskovitz *et al.*, 1983), respectively. These results are similar to those seen in the current study when the reductions in maternal inspired oxygen level or hindlimb blood flow were categorized as severe ( $30.3 \pm 43.5\%$  versus  $42.6 \pm 7.9\%$  of control, respectively). Thus, although our work as well as that of Boyle *et al.* (1990) focused on the hindlimb, the results aligned well with those of other researchers who measured whole body responses to reductions in oxygen supply.

A curvilinear relationship between oxygen consumption and delivery has been reported in many other studies (Carter, 1989; Edelstone *et al.*, 1989; Itskovitz *et al.*, 1983; Wilkening and Meschia, 1983). We also found a curvilinear relationship between these two variables with  $\dot{V} O_2$  declining gradually until  $Do_2$  fell by ~50% when the decline in consumption fell significantly (Figure 22). Although hindlimb  $\dot{V} O_2$  fell with a lesser fall in delivery during hypoxemia compared to ischemia, the difference was not statistically significant. As acute hypoxemia is associated with an inhibition of fetal breathing (Hohimer and Bissonnette, 1991; Koos *et al.*, 1987b) and limb movements (Natale *et al.*, 1981), the

earlier, though not significant, fall in oxygen consumption may reflect hypoxemia-elicited inhibition of muscle activity. Koos *et al.* (1987b) observed significant reductions in fetal breathing movements and REM activity when fetal brachial artery  $P_{O_2}$  was lowered beyond  $\sim 17.8$  mmHg by decreasing the inspired  $O_2$  concentration of the ewe. In a subsequent study the same researchers (Koos *et al.*, 1987a) noted reductions in fetal breathing and rapid eye movements in response to fetal anemia, a condition in which fetal  $P_aO_2$  is generally maintained. They concluded that eye and breathing movements were inhibited not by fetal arterial  $P_{O_2}$  but by tissue  $P_{O_2}$ , particularly in the pons region of the brain. This inhibition of fetal activity occurred with reductions in jugular venous  $P_{O_2}$  from 20.8 mmHg to 15.0 mmHg during hypoxemia (Koos *et al.*, 1987b) and from 19.5 mmHg to 17.8 mmHg with fetal anemia (Koos *et al.*, 1987a). In our study, fetal venous  $P_{O_2}$  (measured in the external iliac vein) ranged from  $14.9 \pm 0.4$  mmHg with mild hypoxemia to  $7.4 \pm 1.2$  mmHg during severe hypoxemia (control =  $16.4 \pm 0.5$  mmHg). Thus, even allowing for  $P_{O_2}$  values to be slightly greater in the upper body, the venous  $P_{O_2}$  values we observed with each of our hypoxemia levels (mild, moderate, and severe) should have been sufficient to reduce the incidence of fetal breathing movements and REM activity and thus reduce oxygen consumption. This suggestion is supported by the observation that during mild hypoxemia  $\dot{V}O_2$  was reduced by 8.7%. This is similar to our previous results (Section 5) in which neuromuscular blockade, which abolishes fetal breathing and body movements, was associated with an  $11.8 \pm 4.6\%$  reduction in hindlimb oxygen consumption.

During ischemia, arterial  $P_{O_2}$  was unchanged but venous  $P_{O_2}$  declined significantly (falling from  $17.6 \pm 0.9$  mmHg at control to  $16.2 \pm 0.7$  mmHg with mild ischemia and  $14.3 \pm 1.2$  mmHg during severe ischemia). Thus hindlimb tissue  $P_{O_2}$  would also have been reduced. However, inhibition of skeletal muscle activity would not be expected with ischemia as this perturbation causes only local reductions in oxygen delivery rather than the whole body effects of hypoxemia.

For comparable reductions in oxygen delivery, oxygen consumption fell to a similar degree with both hypoxemia and ischemia. We had hypothesized that hypoxemia would result in greater reductions in oxygen consumption as this intervention reduces an already low fetal arterial  $PO_2$  which could then diminish oxygen diffusion into the tissues. In the current experiments, compared to ischemia, hypoxemia did result in significantly greater reductions in  $PO_2$  of the arterial blood and in the hindlimb tissues (assuming venous  $PO_2$  is reflective of tissue  $PO_2$ ). However, no difference was seen in the arterial-venous  $PO_2$  gradients for the two perturbations. For example, with severe hypoxemia the arterial-venous  $PO_2$  difference was  $5.6 \pm 0.5$  mmHg compared to  $4.5 \pm 0.6$  mmHg with severe ischemia ( $p = 0.1800$ ). Thus, hypoxemia was associated with lower arterial and tissue  $PO_2$  values, but the oxygen diffusion gradient from blood to hindlimb tissue was not different for the two interventions. Although hypoxemia and ischemia reduce oxygen delivery by two different ways, oxygen diffusion into the tissues of the hindlimb was similar and thus hindlimb oxygen consumption did not differ for the two perturbations. As a result, hypoxemia and ischemia were similarly tolerated by the hindlimb and our hypothesis was rejected.

#### 6.4.5 Lactate and glucose responses

Glucose and lactate are important substrates for oxidative metabolism in the late gestation ovine fetus with amino acids providing an alternate fuel source. Under normal conditions, approximately 61% of glucose and 72% of lactate is oxidized accounting for about 50% of fetal oxygen consumption (Hay *et al.*, 1983). Lactate does not diffuse from the mother to fetus as fetal lactate concentrations are higher than those in the maternal circulation (Burd *et al.*, 1975). In contrast, maternal glucose concentrations are much higher than in the fetal lamb largely due to substantial glucose consumption by the uteroplacental tissues (Meschia *et al.*, 1980).

During the control period, arterial and venous glucose concentrations and glucose uptake were significantly lower in the ischemia fetuses while lactate concentrations were

higher. As the ewes were allowed free access to grain and hay, these differences likely reflect the recent nutritional intake of the mothers. No significant change in glucose concentrations was seen with either hypoxemia or ischemia with the exception of a rise in arterial glucose levels during severe hypoxemia. Net hindlimb uptake of glucose was seen during the control and experimental conditions, and there were no significant changes in glucose uptake during or after either intervention. In contrast, arterial lactate levels rose during moderate and severe hypoxemia reaching  $5.07 \pm 0.48$  mM. Severe hypoxemia was also associated with a reduction in pH (to  $7.234 \pm 0.031$ ) and a marked fall in base excess (to  $-6.8 \pm 1.8$  mEq/L). Arterial lactate levels remained high during the recovery phase. Similar pH, base excess, and arterial lactate levels (to  $\sim 7$  mM) have been previously reported in response to reductions in maternal inspired oxygen concentration (Rurak *et al.*, 1990b) and uterine artery compression (Boyle *et al.*, 1992; Jensen *et al.*, 1991). Because hypoxemia affects the whole fetus and the placenta, these higher lactate levels probably reflect the larger amount of tissue responding to the reduction in oxygen delivery. Unfortunately Itskovitz and colleagues (1987; 1983) did not report lactate values in their studies of umbilical cord compression; however, only modest reductions in descending aorta pH (from 7.39 during control to  $\sim 7.35$ ) were seen. Because blood flow is maintained or even increased to the carcass and peripheral tissues during cord occlusion, regional lactic acidemia is not likely. In the current study, no change in fetal pH, base excess, or lactate flux were seen and, even with severe ischemia, only a small increase in arterial lactate concentration was observed. This implies that this intervention had little adverse effect on the placental or systemic circulation. Ischemia, unlike hypoxemia, is primarily a local phenomenon and thus is unlikely to generate whole body responses.

During hypoxemia there was release of lactate from the hindlimb in contrast to net lactate uptake during the control period. Although the changes during mild, moderate, and severe hypoxemia were not different from the control level ( $1.15 \pm 0.81 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ), the pooled mean lactate flux ( $-3.12 \pm 0.87 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ) was statistically different. In

a study of prolonged (~4 hours) severe, nonlethal hypoxia, achieved by reducing uterine blood flow, Boyle *et al.* (1992) also observed net hindlimb lactate output ( $6.7 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) although the systemic lactate concentrations (~15 mM) were more markedly elevated than in our study. However, in their study, lactate was also released by the hindlimb during the control or normoxemic period ( $\sim 3.1 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ). Singh *et al.* (1984) have also reported hindlimb lactate efflux from both maternal and fetal sheep under normoxemic conditions, whereas Yeleswaram (1993), as in the current study, observed net lactate uptake by the fetal hindlimb. The reason for these differing results is not clear; however, it may relate to differences in fetal endocrine status in the various studies, particularly in terms of catecholamine concentrations. Another possible explanation relates to analytical variability in lactate measurements. As indicated in Table 12, the hindlimb arterio-venous (A-V) difference in lactate concentration is small; thus, relatively small measurement errors could result in an artifactual reversal of the A-V difference. This would be most problematic with elevated arterial and venous lactate concentrations.

During ischemia, there was also a tendency for lactate efflux. As with hypoxemia, at each level of ischemia lactate flux was not statistically different from the control value ( $3.37 \pm 1.24 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ), but the pooled mean lactate flux ( $-0.25 \pm 0.41 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) did reach statistical significance. The pooled mean lactate flux during hypoxemia was statistically greater than during ischemia. In contrast, the situation was reversed during the recovery phase, with hindlimb lactate efflux following ischemia ( $4.28 \pm 1.75 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) over twice as great as following hypoxemia ( $2.00 \pm 1.95 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ). As a result, the overall lactate efflux was not different between the two perturbations. The apparent difference in the time course of the lactate flux in the two sets of experiments may relate to the difference in blood flow responses. During ischemia blood flow was reduced to a greater extent than during hypoxemia. This could have limited lactate efflux so that a greater proportion of lactate was released during the recovery period when blood flow was restored.

The hindlimb lactate release that occurred with both hypoxemia and ischemia was not associated with any obvious increase in hindlimb glucose uptake. This suggests that the released lactate was derived from a source of glucose within the hindlimb. One likely source is skeletal muscle glycogen which accumulates in the fetus during late gestation (Shelley, 1961). Increased glycogenolysis in the hindlimb could be elicited by the rise in circulating catecholamines that occurs with hypoxemia (Cohen *et al.*, 1982; Jones and Robinson, 1975). Circulating catecholamines increase the liberation of glucose-1-phosphate from skeletal muscle glycogen, which can then be converted to lactate. Infusion of adrenaline to fetal lambs results in hyperglycemia and lactic acidemia, actions that can be attenuated by  $\beta$ -adrenergic antagonists (Jones and Ritchie, 1978), as can the rise in plasma lactate concentrations during hypoxemia (Jones and Ritchie, 1983). Yeleswaram *et al.* (1993) found that labetalol, a combined  $\alpha$  and  $\beta$  adrenergic blocker with significant  $\beta_2$  agonist activity, elicits prolonged lactic acidemia in the fetal lamb associated with sustained hindlimb lactate release. A similar mechanism could have operated during hypoxemia. However, as such systemic effects are unlikely to have been stimulated by hindlimb ischemia, local factors may have acted in this situation to cause the lactate efflux.

During hypoxemia, there was both systemic lactic acidemia and lactate release from the hindlimb. An obvious question is the extent to which lactate efflux from the hindlimb and the overall carcass contributed to the rise in circulating lactate concentrations. Yeleswaram *et al.* (1993) addressed this question in their study of labetalol by extrapolating hindlimb lactate release to the entire carcass and comparing this to an estimate of systemic lactate production. The latter value was obtained by multiplying the incremental increase in the area under the arterial lactate concentration versus time curve (lactate AUC) times a published value for lactate clearance in the fetal lamb. A similar approach can be used with our hypoxemia data. Overall carcass lactate efflux can be estimated by multiplying weight normalized hindlimb lactate efflux by 70% of fetal body weight to yield 1.92 mmol. Arterial lactate AUC is estimated as the average of the rise in arterial lactate concentration

during hypoxemia ( $1.72 \mu\text{mol/ml}$ ) times the duration of the hypoxemia period (30 minutes), with the resulting value being  $51.6 \mu\text{mol}\cdot\text{ml}^{-1}\cdot\text{min}$ . This value is then multiplied by fetal lactate clearance; however, it is not clear which lactate clearance value should be used. Boyle *et al.* (1992) obtained a normoxemia lactate clearance value of  $43.5 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ , which is similar to the estimate of  $35.5 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  published by Sparks (1982). Using the former value and the average fetal weight of our hypoxemia animals (2.935 kg) yields an estimate of  $127.7 \text{ ml}\cdot\text{min}^{-1}$ . This value times the lactate AUC produced a value of 6.59 mmol for total body lactate production. The carcass contribution to the total body lactate production would then be  $1.92 \text{ mmol}/6.59 \text{ mmol}$  or 29%. However, Boyle *et al.* (1992) found that during ~4 hours of severe hypoxemia, fetal lactate clearance fell dramatically although it could not be precisely calculated because arterial lactate specific activity was not at steady state as it had been during the normoxia portion of their protocol. The clearance value they calculated by neglecting the accumulation of tracer lactate was  $10.5 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  or  $30.8 \text{ ml}\cdot\text{min}^{-1}$  when calculated for the average body weight of our animals. This value multiplied by the arterial lactate AUC gives an estimate of 1.59 mmol for total body lactate production. The carcass contribution in this situation is thus  $1.92\text{mmol}/1.59 \text{ mmol}$  or 121%. As the level of hypoxemia we obtained was not as severe nor as prolonged as that of Boyle, fetal lactate clearance by our animals was likely greater than  $10.5 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ . Regardless of the exact values, it appears that lactate release from the carcass does contribute to the systemic lactic acidemia seen during hypoxemia, with the contribution ranging between approximately 29 and 100%. The precise contribution depends on the magnitude of the fetal lactate clearance after ~30 minutes of hypoxemia which is currently unknown.

If the carcass is not the sole source for the rise in fetal circulating lactate concentrations during hypoxemia, other sites must be considered. One such potential site is the placenta. As stated earlier, under normal conditions the placenta is a net producer of lactate, and the fetus is a net consumer of this substance (Sparks *et al.*, 1982). This placental release of lactate is illustrated by the higher lactate concentrations found in the

umbilical and uterine veins compared to the corresponding arteries (Burd *et al.*, 1975). The lactate produced by the placenta appears to be asymmetrically distributed with greater amounts entering the umbilical compared to the uterine circulation (Sparks *et al.*, 1982). The amount of lactate released into the fetal circulation increases during hypoxemia (Milley, 1988) and labour (Tan, 1997). Under normal conditions, fetal lactate utilization exceeds the umbilical uptake of lactate from the placenta indicating that fetal tissues must produce lactate endogenously (Hay *et al.*, 1983). Even with increased placental lactate release during hypoxemia, other fetal tissues likely must also contribute to the elevated plasma lactate concentrations.

Fisher *et al.* (1982) have examined the response of the fetal myocardium to hypoxemia, achieved by reducing maternal inspired oxygen levels. The fetal heart normally consumes lactate. In response to a 50% reduction in ascending aorta oxygen content, myocardial blood flow rose markedly to 160% above control values and cardiac oxygen consumption was unchanged. Arterial lactate levels were significantly increased but myocardial lactate consumption was unchanged. Thus, the fetal heart does not appear to be responsible for the increased systemic lactate observed during hypoxemia.

Lactate flux across the fetal liver has also been examined. Lactate appears to be an important substrate for the liver, as there is a net hepatic uptake of lactate under normal conditions in fetal, newborn, and adult sheep (Apatu and Barnes, 1991; Gleason *et al.*, 1985a; Gleason *et al.*, 1985b). This is supported by the observation that there is no net fetal hepatic uptake or release of glucose indicating that other carbon sources, such as lactate and amino acids, are used for oxidative metabolism by the liver (Gleason and Rudolph, 1985; Bristow *et al.*, 1983). Abnormal conditions, such as hypoxemia, could alter glucose and lactate production, extraction, utilization, and flux. Reducing maternal inspired oxygen levels (Bristow *et al.*, 1983) or severe umbilical cord compression (Rudolph *et al.*, 1989) decreases blood flow to the liver leading to lower hepatic  $Do_2$  and either maintained or slightly reduced  $V_{O_2}$ . However, even under such conditions, hepatic oxygen content,  $Do_2$ ,

and  $P_{O_2}$  exceed that of other organs due to receiving well-oxygenated umbilical venous blood. Thus, it is unlikely that the liver experiences tissue hypoxia. Although lactate continues to be consumed by the liver during cord compression, net hepatic lactate uptake declines significantly (Rudolph *et al.*, 1989). This likely contributes to the observed rise in arterial lactate concentrations and may be in part responsible for the fall in lactate clearance during severe hypoxemia reported by Boyle *et al.* (1992). However, other factors such as changes in lactate consumption by other organs or increased fetal lactate production may also contribute to the increase in circulating lactate.

Unlike during control conditions, glucose is released from the liver during hypoxemia (Rudolph *et al.*, 1989) and cord compression (Bristow *et al.*, 1983). Lactate is unlikely to be the source of this glucose as gluconeogenesis, in which non-carbohydrate substrates like lactate and amino acids are converted to pyruvate and then to glucose, does not appear to occur to an appreciable amount in the fetal liver under normal conditions (Townsend *et al.*, 1989; Gleason and Rudolph, 1985) or with acute reductions in umbilical blood flow (Rudolph *et al.*, 1989). Hepatic glycogen content is reduced during hypoxemia suggesting that glycogenolysis may be responsible (Stratford and Hooper, 1997). In the current study, hindlimb glucose consumption tended to increase, particularly during hypoxemia, but did not reach statistical significance. Hepatic glucose release may have contributed to the increased arterial glucose concentration seen with severe hypoxemia.

Carbohydrate metabolism has also been examined in the fetal kidney (Iwamoto and Rudolph, 1985) and lungs (Simmons and Charlton, 1988). During normoxia, the fetal lamb kidney consumes lactate but releases glucose into the systemic circulation. In response to reduced maternal inspired oxygen levels, the situation is reversed: there is net glucose uptake and net release of lactate from the renal circulation. This resulted in significant increases in lactate levels in the descending aorta (from  $1.53 \pm 0.32$  mM during control to  $4.92 \pm 1.66$  during hypoxemia). This rise in arterial lactate values is similar to what we observed with severe hypoxemia (from  $1.42 \pm 0.09$  mM during control to  $5.07 \pm 0.48$

mM). Furthermore, under normal conditions, measurements of lactate concentrations in the pulmonary arteries and veins of fetal lambs (119–141 days) indicate that the lung releases lactate into the circulation and that this does not change over the gestational ages examined (Simmons and Charlton, 1988). Thus, the fetal lung is another potential source of plasma lactate, although whether this production continues during conditions of reduced fetal oxygen delivery does not appear to have been examined.

In conclusion, there was lactate efflux from the fetal hindlimb during both hypoxemia and ischemia. With hypoxemia, most of the lactate was released during the intervention, while in the ischemia experiments, the majority was released during the recovery or post-ischemia period. However, there was no difference in the overall magnitude of the response between the two perturbations. During hypoxemia, lactate release from the carcass may contribute to the systemic lactic acidemia, with the estimated contribution ranging from approximately 29–100%. The increase in circulating lactate concentrations may involve other sources as well. The fetal liver, for example, although a net consumer of lactate, reduces its lactate uptake during hypoxemia, thereby contributing to the systemic levels. In addition, the placenta and fetal kidney appear to release lactate into the circulation in response to hypoxemia. Lactate flux across the fetal lung is another potential source of systemic lactate, although its metabolism has not been examined during hypoxemia.

#### 6.4.6 Cardiovascular responses

As stated earlier, control hindlimb blood flow was lower in the ischemia group of animals. As there was no difference in control arterial and venous pressures between the two groups, this difference in blood flow was responsible for the greater hindlimb vascular resistance seen in the ischemia animals. Control fetal heart rate was also significantly lower in the ischemia group ( $147.3 \pm 3.7$  bpm) compared to the hypoxemia animals ( $162.8 \pm 2.5$

bpm). This may reflect the greater gestational age of the ischemia animals as previous work has shown this variable to decline with increasing gestational age (Tan, 1997; Dawes, 1985).

Bradycardia, hypertension, and redistribution of blood flow away from peripheral tissue have been described by many previous researchers in response to hypoxemia achieved either by decreasing maternal inspired oxygen levels (Reuss *et al.*, 1982; Parer, 1980; Cohn *et al.*, 1974) or reducing uterine blood flow (Boyle *et al.*, 1992; Jensen *et al.*, 1991). We observed similar responses in hindlimb tissue particularly with severe hypoxemia. Hanson (1988) reviewed the complex interaction of factors affecting the fetal cardiovascular system during hypoxemia. Vascular responses to reductions in oxygen delivery may result from chemoreceptor stimulation and/or circulating hormones including catecholamines and arginine vasopressin. Hypoxemia-related bradycardia is attributed to a vagally mediated chemoreceptor reflex. Stimulation of the sympathetic nervous system in response to hypoxemia results in a redistribution of blood flow with vasoconstriction in peripheral tissues including the carcass and gut combined with vasodilation in the vital organs like the heart and brain (Iwamoto *et al.*, 1983; Reuss *et al.*, 1982). The hypertensive response to hypoxemia is probably the net effect of these changes in vascular resistance (Hanson, 1988).

In our ischemia animals, no change in fetal heart rate or vascular pressures were observed. Hindlimb blood flow and vascular resistance underwent reciprocal changes. For example, with severe ischemia blood flow fell to nearly a quarter of the control value while vascular resistance across the hindlimb increased approximately four fold. Local release of endothelium-derived contracting factors, such as endothelin-1, may have contributed to hindlimb vasoconstriction (Luscher and Wenzel, 1995) but was insufficient to cause a hypertensive response measurable in the descending aorta. Thus, partial occlusion of the external iliac artery resulted in mainly local effects such as vasoconstriction without the systemic responses evident with hypoxemia.

In summary, a curvilinear relationship was seen between oxygen delivery and consumption in the hindlimb with no significant difference between the curves for hypoxemia or ischemia. An earlier, but not significant, fall in consumption seen with hypoxemia may reflect an inhibition of muscle activity. Arterial and venous  $P_{O_2}$  values were significantly lower during hypoxemia than during ischemia. Thus hindlimb tissue  $P_{O_2}$  would be lower with hypoxemia. Hypoxemia affects the whole body, so that reduced tissue  $P_{O_2}$  in the brain would inhibit skeletal muscle activity and thus lower oxygen consumption. Although venous, and therefore, hindlimb tissue  $P_{O_2}$  were also reduced with ischemia, only local tissues would be affected and thus muscle activity is unlikely to be altered. Despite the significantly greater reductions in arterial and venous  $P_{O_2}$  with hypoxemia compared to ischemia, no significant difference was seen in the arterial-venous  $P_{O_2}$  gradient between the two interventions. Thus, although oxygen delivery is reduced by two different ways with hypoxemia and ischemia, oxygen diffusion into the hindlimb tissues was similar and thus hindlimb oxygen consumption did not differ for the two perturbations.

The change in hindlimb lactate flux from net uptake during control to net release with both perturbations indicates that tissue  $P_{O_2}$ , in both cases, was sufficiently reduced to stimulate anaerobic metabolism or reduce lactate oxidation in the hindlimb tissues. The overall magnitude of hindlimb lactate efflux was not significantly different between the hypoxemia and ischemia experiments. A greater rise in plasma lactate levels was seen with hypoxemia, and carcass lactate release may contribute to this. Other organs, including the placenta, liver, kidney, and lung may also have contributed to this rise in circulating lactate.

Although there was apparent lactate efflux from the fetal hindlimb during hypoxemia, the magnitude of the lactate flux was small compared to the decrement in oxygen consumption. For example, during severe hypoxemia, lactate efflux was  $2.24 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  compared to a decrease in  $\dot{V} O_2$  of  $\sim 14.2 \mu\text{mol} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ . This is similar to the results of Boyle *et al.* (1992). They suggested that the marked reduction in hindlimb (and overall carcass) oxygen consumption during severe hypoxemia limits the fall

in systemic  $O_2$  content thereby maintaining the supply of oxygen to the heart, brain, and adrenal glands, while the relatively small increase in lactate efflux is a protective mechanism to limit the rate of glucose breakdown and systemic lactic acid accumulation. This implies a suppression of hindlimb metabolic rate during hypoxemia, in that the fall in oxidative metabolic rate is not linked to an increase in glycolytic rate. In the current study, the lack of any obvious increase in lactate efflux with increasing severity of hypoxemia (and increasing decrement in  $\dot{V} O_2$ ) is consistent with this view.

The role of the various hindlimb tissue components (skin, bone, and skeletal muscle) in the response to hypoxemia and hindlimb ischemia is not known. As indicated in Section 4, muscle comprises the largest proportion of hindlimb weight. Because of its glycogen content, skeletal muscle is likely important in terms of lactate production. However, bone receives the highest fraction of hindlimb blood flow, and, compared to muscle, both skin and bone are more highly perfused in relation to the total hindlimb weight that they comprise. As a result, when oxygen delivery is reduced, bone and skin may be more greatly affected in terms of oxygen consumption. These potential differences in the response of the hindlimb components to a reduction in oxygen supply may contribute to the lack of relationship between the decrement in oxygen consumption and the magnitude of hindlimb lactate efflux.

Our results illustrate that whether oxygen delivery is reduced by hypoxemia or by reducing blood flow,  $\dot{V} O_2$  can generally be maintained until oxygen delivery is reduced by ~50%. Fetal hypoxemia did result in greater plasma lactate concentrations reflecting the whole body effects of this perturbation versus the local nature of the ischemia. However, there was no difference between hypoxemia and ischemia in terms of the overall magnitude of hindlimb lactate efflux. Thus, for similar reductions in oxygen delivery, there is no evidence that fetal hypoxemia is less well tolerated than hindlimb ischemia. Our hypothesis is not supported by the results obtained and must, therefore, be rejected.

## 7. ARGININE VASOPRESSIN INFUSION

### 7.1 Introduction

Arginine vasopressin (AVP) is a cyclic nine amino acid peptide produced in the supraoptic and paraventricular nuclei of the hypothalamus and released into the circulation from the posterior pituitary. Vasopressin, one of the first hormones to be identified, was first noted in 1895 for its pressor actions although most research has focused on its later-established antidiuretic action (Share, 1988). Vasopressin acts on vascular smooth muscle receptors classified as  $V_1$  receptors, with calcium as a second messenger, to elicit vasoconstriction;  $V_2$  receptors in the kidney, employing the adenylate cyclase-cyclic AMP system as the second messenger, are responsible for the antidiuretic action of this hormone (Share, 1988). In the adult, the main role of vasopressin appears to be the regulation of renal water excretion. It causes increased water resorption in the distal tubule resulting in increased urine osmolarity. The antidiuretic action of AVP in the fetus occurs over a very low range of hormone values. Under normal circumstances vasopressin levels are very low (<5 pg/ml) and fetal urine is hypotonic to plasma. At plasma AVP levels of ~13 pg/ml maximal urine concentrating occurs and urine osmolalities at or only slightly hypertonic to plasma are achieved (Woods *et al.*, 1986; Robillard and Weitzman, 1980). This indicates that fetal urine concentrating ability is only 20–30% of the adult potential (Ervin, 1988; Woods *et al.*, 1986; Robillard and Weitzman, 1980).

In the ovine fetus, AVP is released in response to stresses such as fetal hypoxemia (Rurak, 1978), hemorrhage (Ross *et al.*, 1986b; Drummond *et al.*, 1980; Robillard *et al.*, 1979), hypotension (Ross *et al.*, 1986a), and vena caval obstruction (Wood *et al.*, 1982). The administration of AVP results in a fall in fetal heart rate and a rise in arterial pressure (Wiriathian *et al.*, 1983; Iwamoto *et al.*, 1979; Rurak, 1978), as well as maintained (Iwamoto *et al.*, 1979) or increased umbilical blood flow (Rurak and Gruber, 1984). In addition, dramatic falls in blood flow to the peripheral tissues, particularly skin and skeletal

muscle, have been reported in dogs (Liard *et al.*, 1982) and fetal lambs (Iwamoto *et al.*, 1979) while perfusion of the heart and brain increases (Iwamoto *et al.*, 1979). This redistribution of cardiac output is similar to the pattern seen during fetal hypoxemia (Peeters *et al.*, 1979; Cohn *et al.*, 1974). These findings suggest that the release of arginine vasopressin in response to hypoxemia may play a role in the cardiovascular changes associated with this condition.

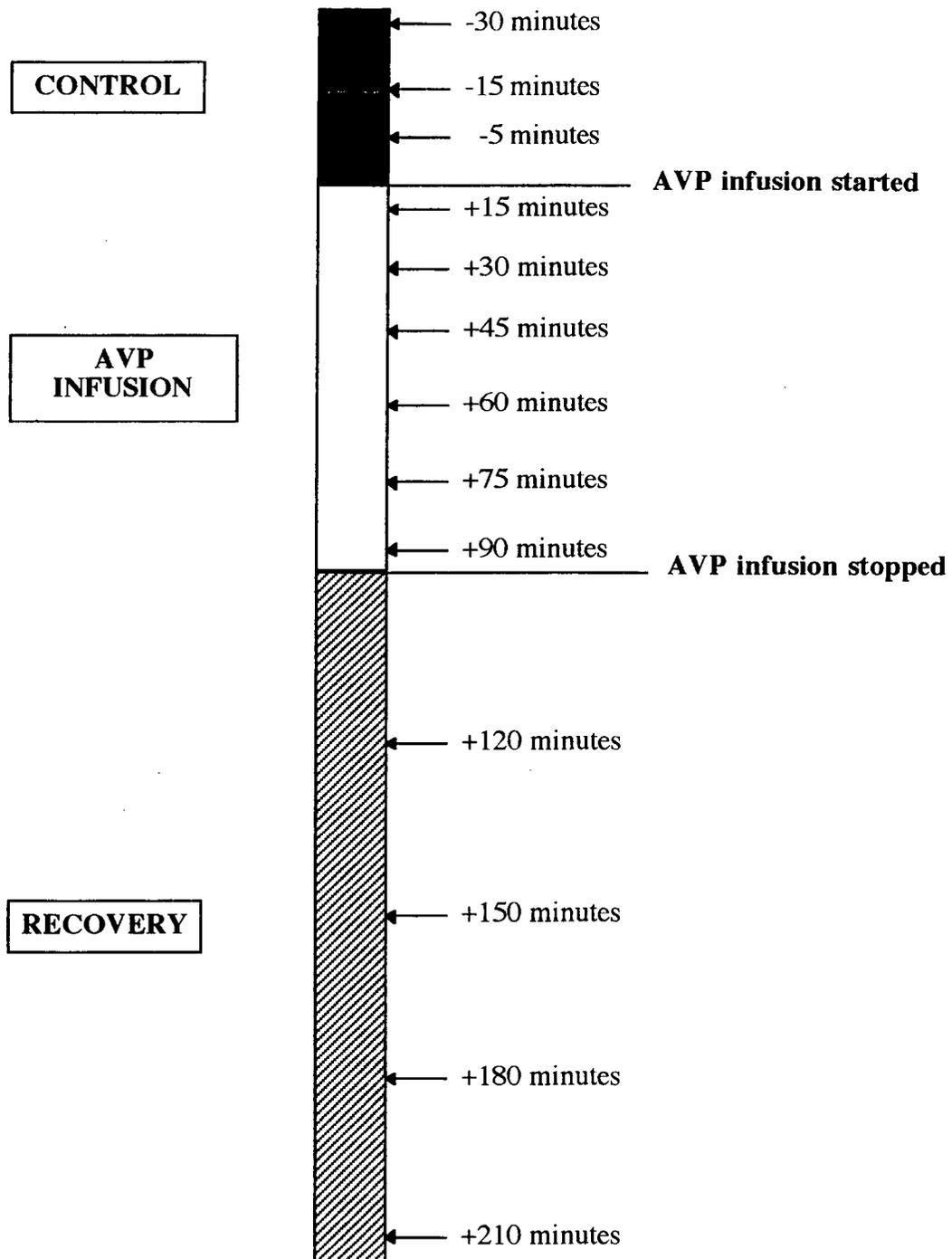
Few reports in the literature have compared the effect of arginine vasopressin infusion in fetal and adult animals. Rurak (1978) demonstrated a greater increase in arterial pressure in fetal lambs given exogenous arginine vasopressin compared to adult sheep. This was speculated to be due to a greater vasoconstrictor effect of AVP on the fetal systemic circulation. Conversely, Harper and Rose (1987) observed similar pressor responses to exogenous arginine vasopressin in fetal and adult sheep but the degree of bradycardia was greater in the ewes. The purpose of the current study was to examine the effect of exogenous arginine vasopressin infusion on blood gas status, hindlimb blood flow, and cardiovascular variables in fetal lambs and non-pregnant ewes.

## 7.2 Methods

### 7.2.1 Animal Preparation and Experimental Protocol

Surgery was performed on eight fetal lambs between 118–127 days gestation (mean =  $122 \pm 1$  days) using the techniques previously described (Section 2.3). Catheters were placed in the amniotic cavity, fetal trachea, femoral artery, lateral tarsal vein, and pudendoepigastric artery and vein. A transit-time flow transducer positioned on the external iliac artery provided measurements of hindlimb blood flow.

Experiments took place at a mean gestational age of  $130 \pm 1$  day (range = 125–135 days). A total of 12 experiments were conducted consisting of a 30 minute control period, a 90 minute infusion of arginine vasopressin (Pitressin, Parke-Davis, Scarborough, Ontario) or saline vehicle, followed by a 2 hour recovery phase (Figure 24). All experiments



**FIGURE 24.** Protocol for exogenous arginine vasopressin infusion experiments in fetal and non-pregnant adult ewes. The arrows indicate blood samples which were taken at ~15 minute intervals during the 30 minute control period and the 90 minute infusion period and at 30 minute intervals during the 120 minute recovery period.

conducted on the same animal were separated by at least 24 hours. Paired fetal pudendoepigastric arterial and venous samples (~1 ml each) were taken at 15 minute intervals during the control and infusion phases and at 30 minute intervals during the recovery phase. Each sample was immediately followed by the collection of approximately 3 ml of blood from the pudendoepigastric artery for subsequent analysis of plasma AVP concentrations. After each set of samples, the fetal blood lost was replaced with an equivalent amount (~5 ml) of maternal blood that had been collected from the mother prior to the experiment. Two fetuses served as controls receiving vehicle (0.9% saline) only, while the others received arginine vasopressin at 0.72 mU/min (1.8 ng/min) (n=1), 4.5 mU/min (11.25 ng/min) (n=8), or 9.0 mU/min (22.5 ng/min) (n=1) yielding a range of 0.2 to 3.2 mU·min<sup>-1</sup>·kg<sup>-1</sup> fetal weight (0.5–8 ng·min<sup>-1</sup>·kg<sup>-1</sup>). The AVP doses were made up with non-heparinized saline in 60 ml sterile syringes and administered intravenously using a Harvard infusion pump at an infusion rate of 0.16 ml/minute. Over the 90 minute infusion period, fetuses thus received approximately 14.4 ml of infusate.

In addition, three non-pregnant adult ewes underwent surgery to implant catheters in the femoral artery and vein and to position a transit-time flow transducer on the external iliac artery. A total of 8 adult experiments were conducted following the same protocol outlined for the fetus (Figure 24). The animals were randomly assigned to receive arginine vasopressin administered at 25 mU/min (62.5 ng/min) (n=2), 45 mU/min (112.5 ng/min) (n=3), or 90 mU/min (225 ng/min) (n=3) yielding a range of 0.3 to 1.7 mU·min<sup>-1</sup>·kg<sup>-1</sup> adult weight (0.75–4.25 ng·min<sup>-1</sup>·kg<sup>-1</sup>). The Harvard pump infusion rate was the same as used in the fetal experiments; thus, the adult sheep also received approximately 14.4 ml of infusate. As with the fetal lambs, at least 24 hours elapsed between experiments conducted on the same animal.

### 7.2.2 Arginine Vasopressin Analysis

Arterial blood samples for AVP analysis were collected in sterile syringes then transferred to EDTA Vacutainer® tubes (Becton Dickinson, Franklin Lakes, New Jersey) and kept on ice. The samples were centrifuged at 4°C for 20 minutes at 760xg and the plasma was transferred to plastic vials for storage at -70°C. Samples were analyzed using a commercial vasopressin <sup>125</sup>I radioimmunoassay kit (Incstar Corporation, Stillwater, Minnesota) that has been used previously in our laboratory. The kit's sensitivity or minimum detectable amount for arginine vasopressin is 2.5 pg/ml. The vasopressin antibody demonstrates <0.01 % cross-reactivity with oxytocin, 0.14% with vasotocin, and 600% with lysine vasopressin (found in pigs and related species).

The blood samples were assayed according to the guidelines of the Incstar kit. The plasma samples (1 ml) were first extracted using methanol and ODS-silica (octadecasilyl-silica) Sep-Pak columns. Briefly, this involved attaching each Sep-Pak column to a polypropylene syringe and then washing it with 5 ml methanol followed by 20 ml deionized water. The plasma was then acidified with 0.1 ml 1M HCl and pushed slowly, over a period of 1 minute, through the column. The column was then rinsed with 20 ml of 4% (v/v) acetic acid. Finally, 3 ml of methanol was passed through the column very slowly, such that it was left in contact with the ODS-silica for at least 3 minutes, and the eluate collected in a test tube. A final 1 ml of methanol was passed through the column to ensure complete elution of the peptide. After being evaporated to dryness under nitrogen in a 37°C waterbath, the samples were reconstituted with 0.5 ml 1% BSA (bovine serum albumin)-borate buffer (a greater volume of buffer was used for reconstitution if the plasma sample was expected to read greater than the highest standard). The samples were vortexed, incubated in a 37°C waterbath for 10 minutes, and then revortexed to ensure reconstitution of the sample. Aliquots of 0.2 ml of each sample were then assayed in duplicate. To each sample, 0.1 ml of the primary antibody, raised in guinea pigs against vasopressin (guinea pig anti-vasopressin), was added and the samples incubated for 18–24 hours in a shaker at 4°C. The

$^{125}\text{I}$  vasopressin tracer (0.1 ml) was then added to each sample and again incubated in the shaker overnight at 4°C. On the final day, phase separation was achieved in 20 minutes at room temperature with the addition of 0.5 ml goat anti-guinea pig precipitating complex or GAGP-PPT (pre-precipitated complex of second antibody, raised in goats against guinea pig serum, plus polyethylene glycol). After a 30 minute centrifugation at 4000 rpm, the supernatant was aspirated and the tubes were counted in a gamma-counter (Micrad Inc., Knoxville, Tennessee).

A calibration curve was prepared by plotting the natural logarithm of extent of binding ( $\text{Logit } B/B_0$ ) against stated log concentrations of the calibration standards included in the Incstar kit. This resulted in a linear response curve. The levels of AVP in the plasma samples (in pg/ml) were then interpolated from this plot. Following the example of Rurak (1978), samples whose arginine vasopressin levels registered as undetectable by the assay were assigned a value of one half the kit's sensitivity (1.25 pg/ml).

### 7.2.3 Statistics

Data are generally presented as mean  $\pm$  standard error of the mean. Data were analyzed with StatView statistical software (Abacus Concepts Inc., Berkeley, California). Arginine vasopressin and blood gas levels are presented as absolute values. Mean hindlimb blood flow, oxygen delivery, consumption, and extraction during the infusion and recovery periods are presented as a percentage change from the control values. For analysis of the cardiovascular variables, data from 3 minutes before to 3 minutes after each blood sample (total of 7 minutes of data) were used. Fetal hindlimb venous pressure was not available as the pudendoepigastric venous catheter was utilized for blood sampling and the infusate was administered via the lateral tarsal vein catheter. Likewise, because only one venous catheter was implanted and this was used for the drug infusion, no venous data were collected from the adult animals. The cardiovascular data, presented at each blood sampling point, are shown as a percentage change from the control values. In all cases, multiple comparisons

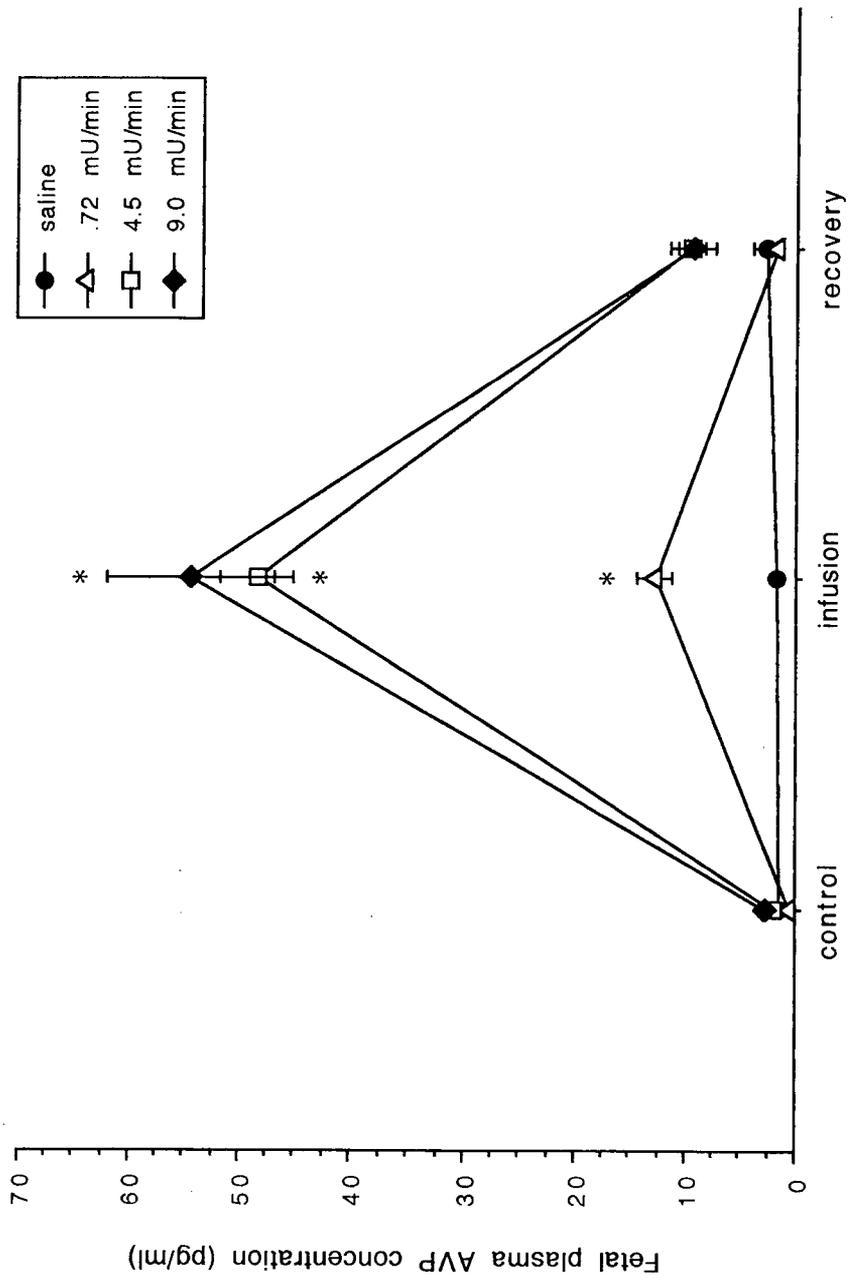
were analyzed with one-way (factorial) analysis of variance (ANOVA) and Fisher's Protected Least Significant Difference (PLSD) post-hoc test. Repeated measures ANOVA could not be used due to incomplete data sets (i.e., inability to draw some blood samples or record cardiovascular variables due to catheter failure, etc.). Differences were considered significant if  $p < 0.05$ .

Fetal and adult hindlimb blood flow and cardiovascular variables were compared by applying lines of best fit to the two groups of data. Differences in the regression equations were compared by applying unpaired t-tests to the slopes and y-intercepts. As with the previous data, differences were considered statistically significant if  $p < 0.05$ .

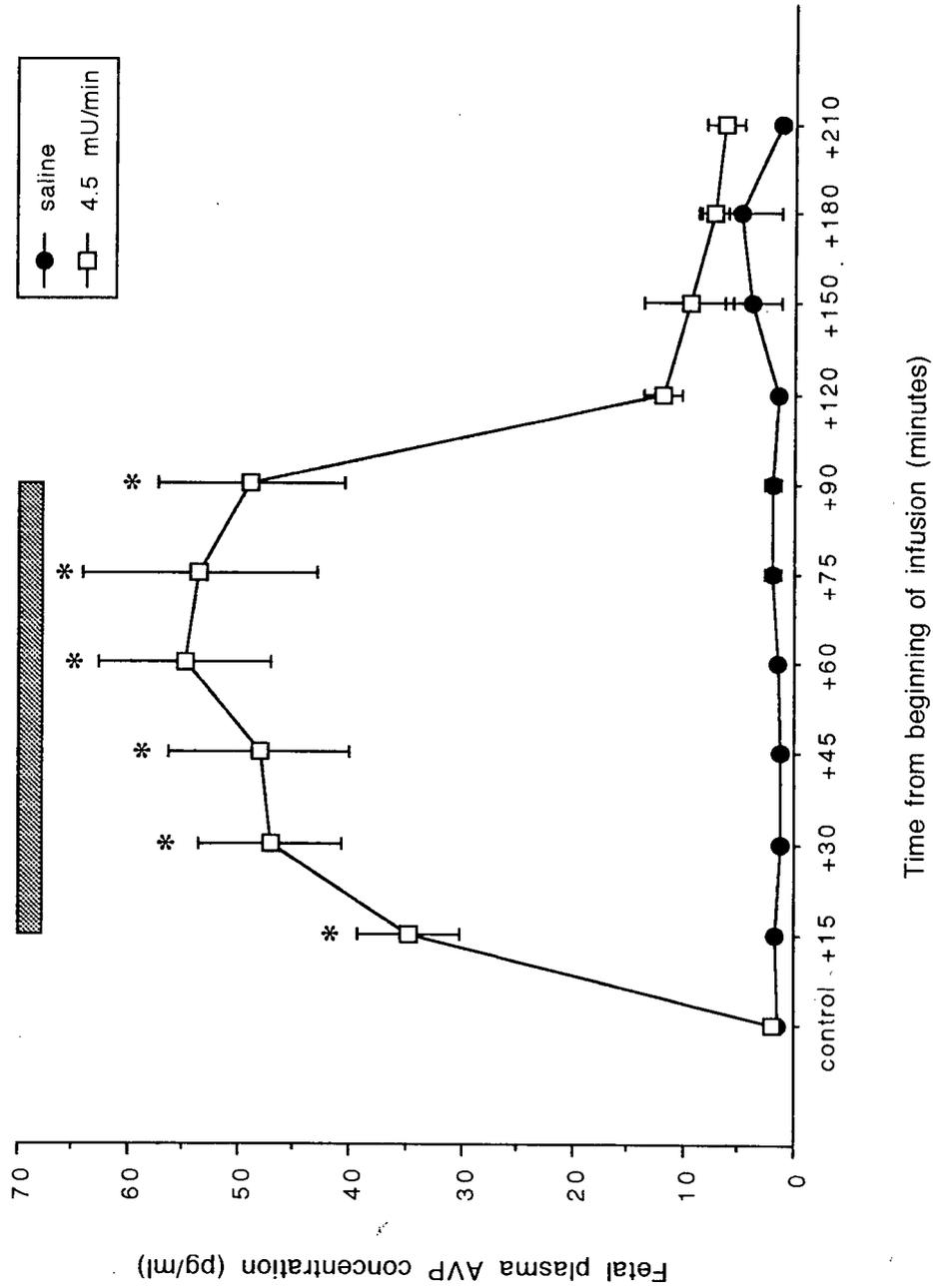
### 7.3 Results

#### 7.3.1 Arginine vasopressin effects on the fetus

Figure 25 shows the mean plasma arginine vasopressin levels for fetuses that received either vehicle (saline) or exogenous arginine vasopressin infusions. The overall control AVP concentration for the 12 experiments was  $1.8 \pm 0.2$  pg/ml. No change in AVP levels was observed for the two animals that received vehicle (saline) only. Infusion of arginine vasopressin resulted in mean plasma concentrations of  $12.7 \pm 1.6$ ,  $48.4 \pm 3.2$ , and  $54.4 \pm 7.5$  pg/ml for the animals receiving doses of 0.72 mU/min (1.8 ng/min), 4.5 mU/min (11.25 ng/min), and 9.0 mU/min (22.5 ng/min), respectively, all of which were significantly different from the control values. The mean AVP concentrations were not different for the 4.5 and 9.0 mU/min doses but this may reflect that the higher dose was only administered to one animal. As seen in Figure 26 plasma AVP levels rose rapidly during administration of the 4.5 mU/min dose, with a significant difference from control seen at 15 minutes into the infusion. Similarly, cessation of the infusion was associated with a dramatic decrease in plasma arginine vasopressin concentrations with levels returning to the control level at 30 minutes post-infusion (+120 sample). Plasma clearance of arginine vasopressin in the fetus was determined for the 4.5 mU/min (11.25 ng/min) infusion rate. Clearance rate was



**FIGURE 25.** Plasma arginine vasopressin concentrations before, during, and after AVP infusion to fetal lambs. The lambs received either saline vehicle (2 experiments), or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment). Values are expressed as mean  $\pm$  SEM. Asterisks indicate a significant difference from the control value ( $p < 0.05$ ).



**FIGURE 26.** Plasma arginine vasopressin concentrations in fetal lambs before, during, and after saline vehicle (n=2) or exogenous AVP infusion at 4.5 mU/min (n=8). Bar indicates infusion period. Values are expressed as mean  $\pm$  SEM. Asterisks indicate a significant difference from control values ( $p < 0.05$ ).

calculated as the rate of hormone infusion (per kg body weight) divided by the average of the 75 and 90 minute arterial plasma AVP concentrations. The 75 and 90 minutes samples were chosen as the hormone levels at these points appeared to be at steady state. Fetal clearance of the hormone was  $85.3 \pm 11.4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ .

Table 14 presents the blood gas and metabolic values for the fetuses given either vehicle or 4.5 mU/min (11.25 ng/min) of exogenous arginine vasopressin. As the 0.72 and 9.0 mU/min doses were only administered to 1 animal, the blood gas data for these infusion rates are not presented. All control values are within the normal range for our laboratory. Infusion of saline did not alter the blood gas or metabolic values, but the recovery phase was associated with significant reductions in arterial pH (to  $7.328 \pm 0.004$ ) and base excess (to  $-0.1 \pm 0.2 \text{ mEq/L}$ ). However, there were no alterations in lactate concentration or flux. Likewise, glucose parameters were unchanged during infusion of the vehicle. During infusion of AVP at 4.5 mU/min, no changes in fetal blood gases or metabolic values were observed. Arterial lactate values rose significantly during the recovery phase reaching a mean value of  $1.82 \pm 0.17 \text{ mM}$ . Although hindlimb venous lactate concentrations tended to rise during and following AVP infusion, the changes were not significant and there was no significant change in lactate flux. No changes in glucose levels or flux were observed.

Cardiovascular and metabolic data obtained prior to the infusions of either vehicle (saline) or 4.5 mU/min AVP to fetal lambs are given in Table 15. The results are presented as absolute values (mean  $\pm$  SEM). There were no significant differences between the two sets of data with the exception of hindlimb blood flow which was significantly higher in the AVP group of animals ( $23.7 \pm 2.5$  versus  $38.3 \pm 4.0 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$ ). Results from the 0.72 and 9.0 mU/min AVP doses are not shown in this table or the subsequent cardiovascular figures as only one experiment was performed at each of these doses and thus there were insufficient samples to determine if any changes observed were statistically significant.

**TABLE 14.** Blood gas and metabolic values from fetuses given either saline vehicle only (2 experiments) or exogenous arginine vasopressin (AVP) infusions at a dose of 4.5 mU/min (8 experiments).

	vehicle (saline)			4.5 mU/min arginine vasopressin		
	control	infusion	recovery	control	infusion	recovery
Arterial pH	7.349 ± 0.008	7.342 ± 0.002	7.328 ± 0.004†	7.316 ± 0.011	7.311 ± 0.007	7.312 ± 0.008
Arterial $P_{O_2}$ (mmHg)	23.0 ± 0.4	23.6 ± 0.4	21.7 ± 0.5	19.2 ± 0.6	20.0 ± 0.5	18.7 ± 0.6
Venous $P_{O_2}$ (mmHg)	17.3 ± 0.5	17.9 ± 0.4	16.4 ± 0.9	14.9 ± 0.6	14.1 ± 0.5	15.1 ± 0.8
Arterial $P_{CO_2}$ (mmHg)	46.9 ± 0.6	47.0 ± 0.2	47.9 ± 0.4	52.1 ± 0.4	50.9 ± 0.3	51.3 ± 0.4
Base excess (mEq/L)	0.8 ± 0.5	0.4 ± 0.1	-0.1 ± 0.2†	0.7 ± 0.7	-0.1 ± 0.5	0.1 ± 0.5
Hemoglobin level (g/dL)	11.0 ± 0.5	10.8 ± 0.4	11.4 ± 0.5	10.3 ± 0.3	10.0 ± 0.2	9.9 ± 0.3
A-V $O_2$ content difference (mM)	1.16 ± 0.11	1.08 ± 0.05	0.99 ± 0.06	0.58 ± 0.05	0.79 ± 0.06†	0.47 ± 0.06
Arterial [L] (mM)	1.12 ± 0.19	1.05 ± 0.09	1.29 ± 0.06	1.23 ± 0.10	1.54 ± 0.10	1.82 ± 0.17†
Venous [L] (mM)	1.07 ± 0.17	1.00 ± 0.08	1.35 ± 0.09	1.31 ± 0.13	1.66 ± 0.12	1.96 ± 0.23
Arterial [G] (mM)	0.89 ± 0.19	0.82 ± 0.10	0.94 ± 0.14	0.87 ± 0.07	0.98 ± 0.05	1.04 ± 0.08
Venous [G] (mM)	0.75 ± 0.20	0.71 ± 0.10	0.78 ± 0.13	0.78 ± 0.09	0.89 ± 0.06	1.03 ± 0.09
Lactate flux ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	1.17 ± 0.99	1.42 ± 0.46	-1.23 ± 1.25	-0.35 ± 0.92	-0.64 ± 0.53	-0.37 ± 0.79
Glucose flux ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ )	3.34 ± 0.83	2.90 ± 0.30	3.97 ± 0.43	4.77 ± 0.68	4.92 ± 0.34	4.74 ± 0.59

A-V = arterial-venous; [L] = lactate concentration; [G] = glucose concentration  
 Values are mean ± SEM.

† = significantly different from control value ( $p < 0.05$ ).

**TABLE 15.** Cardiovascular and metabolic data obtained from fetal lambs prior to vehicle (saline) infusion (2 experiments) or infusion of 4.5 mU/min arginine vasopressin (8 experiments). Data are presented as absolute values (mean  $\pm$  SEM).

	Vehicle (saline only)	4.5 mU/min AVP
Arterial pressure (mmHg)	55.2*	51.2 $\pm$ 1.4
Heart rate (beats/min)	144.9 $\pm$ 17.8	172.3 $\pm$ 5.0
Hindlimb blood flow (ml/min <sup>-1</sup> ·100g <sup>-1</sup> )	23.7 $\pm$ 2.5	38.3 $\pm$ 4.0†
Oxygen delivery ( $\mu$ mol/min <sup>-1</sup> ·100g <sup>-1</sup> )	87.2 $\pm$ 13.3	96.9 $\pm$ 16.1
Oxygen consumption ( $\mu$ mol/min <sup>-1</sup> ·100g <sup>-1</sup> )	27.8 $\pm$ 4.5	20.0 $\pm$ 2.3
Oxygen extraction (%)	32.2 $\pm$ 2.4	27.1 $\pm$ 2.0

\* only 1 data point available

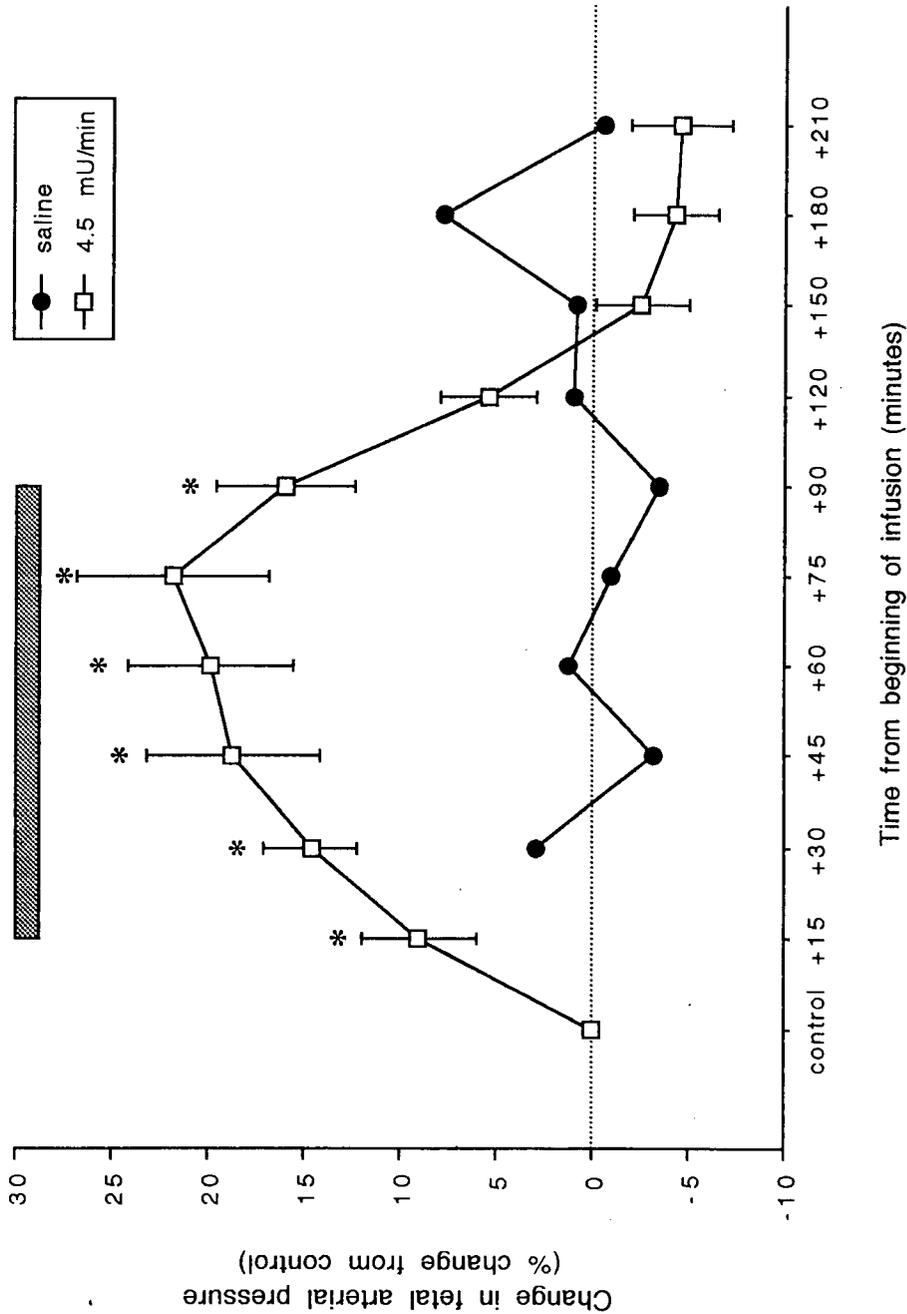
† = significant difference from vehicle value (p<0.05)

Fetal arterial pressure was unaltered by the administration of the saline vehicle (Figure 27). During the infusion of 4.5 mU/min AVP, arterial pressure rose rapidly and remained elevated for the duration of the infusion. The mean increase during infusion was  $16.5 \pm 1.6\%$  above control levels. During the recovery phase arterial pressure rapidly returned to pre-infusion values.

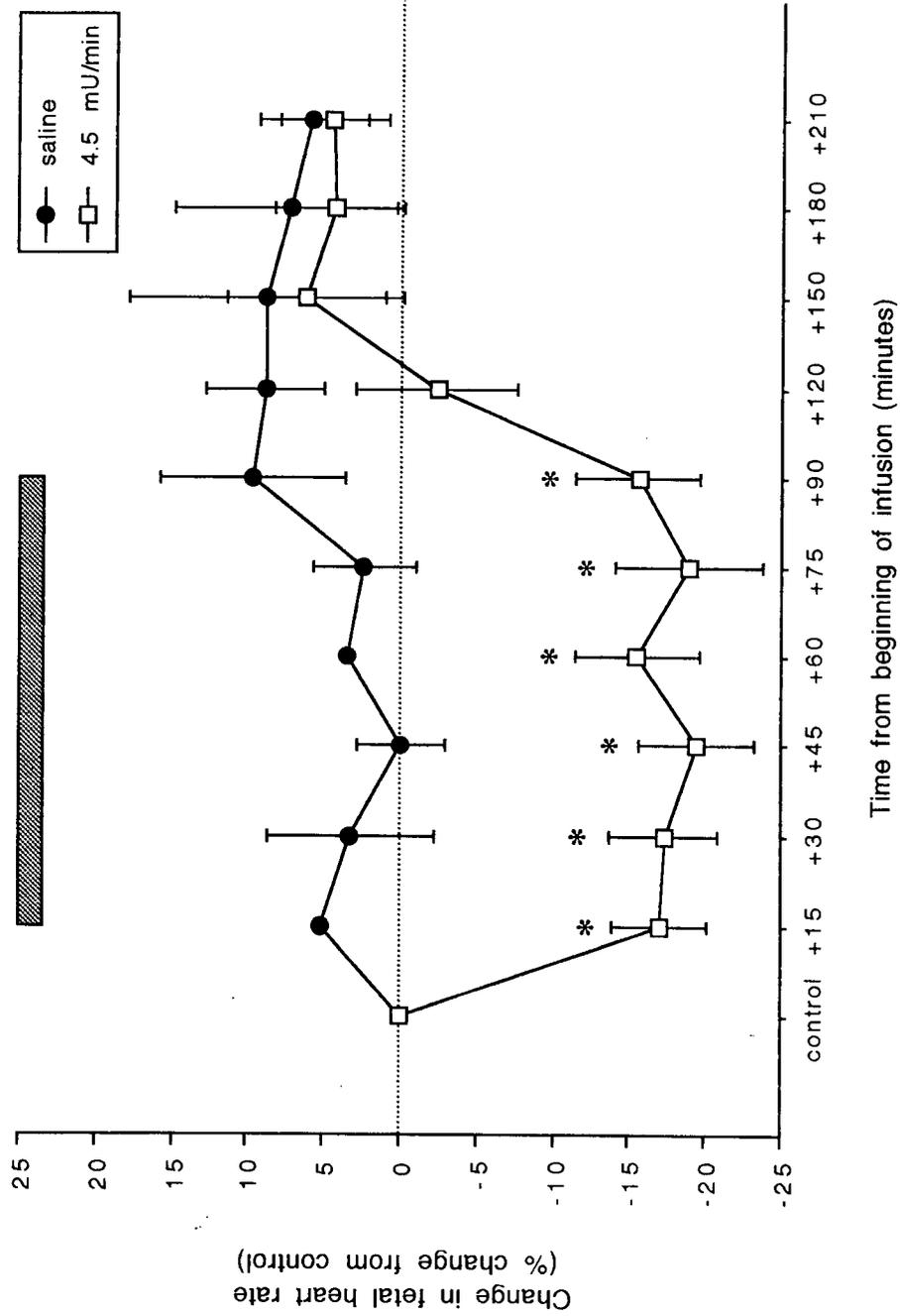
Figure 28 presents fetal heart rate responses to saline or arginine vasopressin at 4.5 mU/min. No change in heart rate was observed with the saline infusion. AVP was associated with rapid reductions in fetal heart rate, averaging  $17.2 \pm 1.5\%$  below the control values. The bradycardia persisted for the duration of the infusion with heart rate quickly returning to control values when the infusion was stopped.

The changes in fetal hindlimb blood flow that occurred during and following infusion of either vehicle (saline) or arginine vasopressin at 4.5 mU/min are illustrated in Figure 29. Infusion of saline alone did not alter hindlimb blood flow. AVP at 4.5 mU/min resulted in an average  $28.2 \pm 1.6\%$  reduction in flow. This fall in flow was statistically significant after 15 minutes of AVP infusion and remained low for the duration of the infusion. Flow had returned to control values 30 minutes after cessation of infusion.

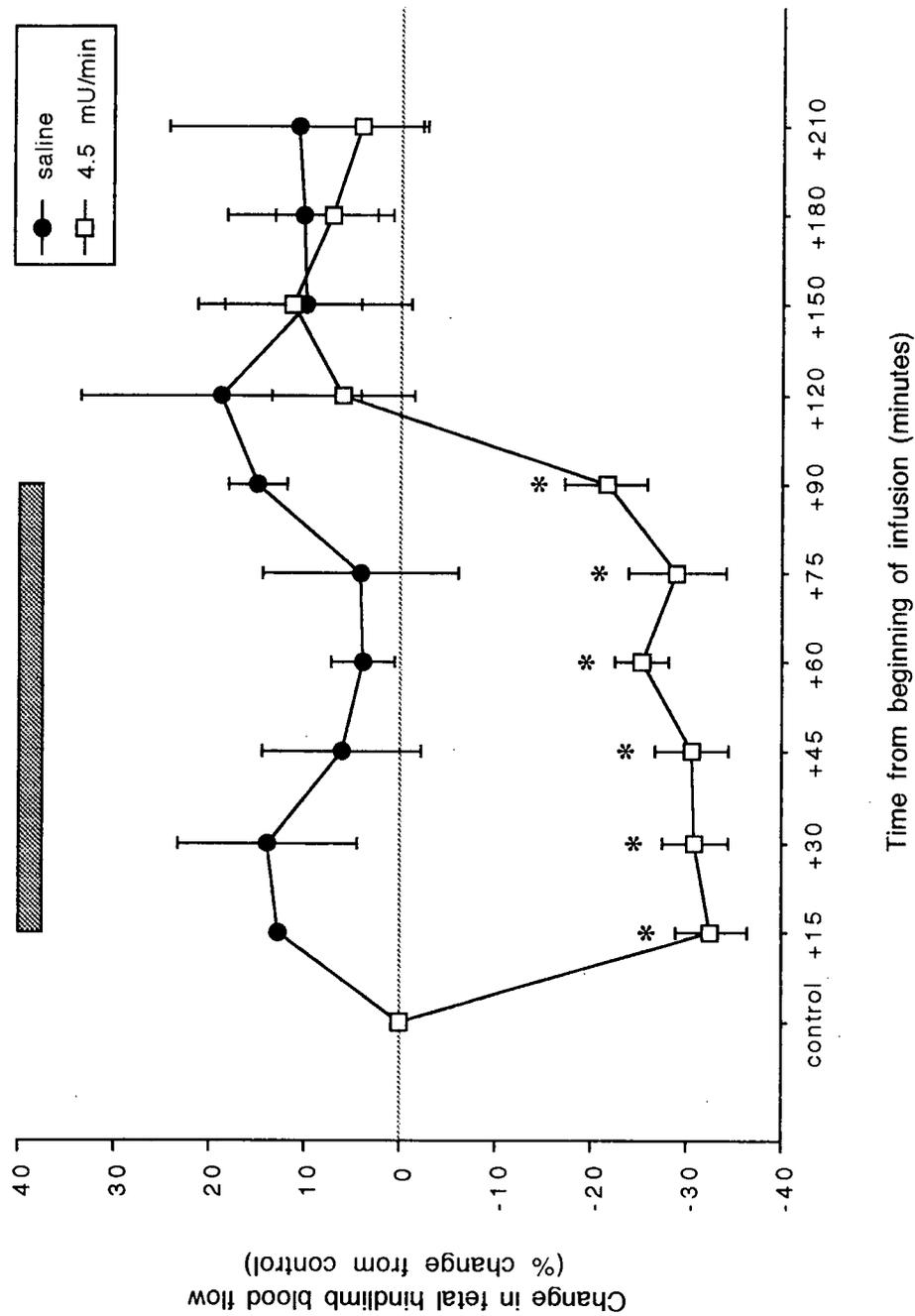
Fetal hindlimb oxygen delivery ( $\text{Do}_2$ ) fell significantly with arginine vasopressin infusions of 4.5 and 9.0 mU/min, but was unchanged during the 0.72 mU/min dose (Figure 30). All  $\text{Do}_2$  values returned to control levels during the recovery period. The fall in hindlimb oxygen delivery at the medium and high doses (to  $72.5 \pm 2.0\%$  and  $69.9 \pm 4.2\%$  of control, respectively) mirrors the reductions in mean hindlimb blood flow seen with these infusions (to  $71.8 \pm 1.9\%$  and  $67.7 \pm 3.2\%$  of control) (Figure 31). During the AVP infusions oxygen consumption ( $\dot{V} \text{O}_2$ ) was maintained (Figure 32) due to a rise in oxygen extraction (to  $126.9 \pm 5.4\%$  and  $168.0 \pm 8.5\%$  of the control values for the medium and high doses, respectively) (Figure 33). Mean  $\dot{V} \text{O}_2$  was significantly reduced during the recovery phase following the 4.5 mU/min AVP infusions. This is the effect of the small, but not significant, reductions in both hindlimb oxygen delivery ( $95.9 \pm 3.5\%$  of control)



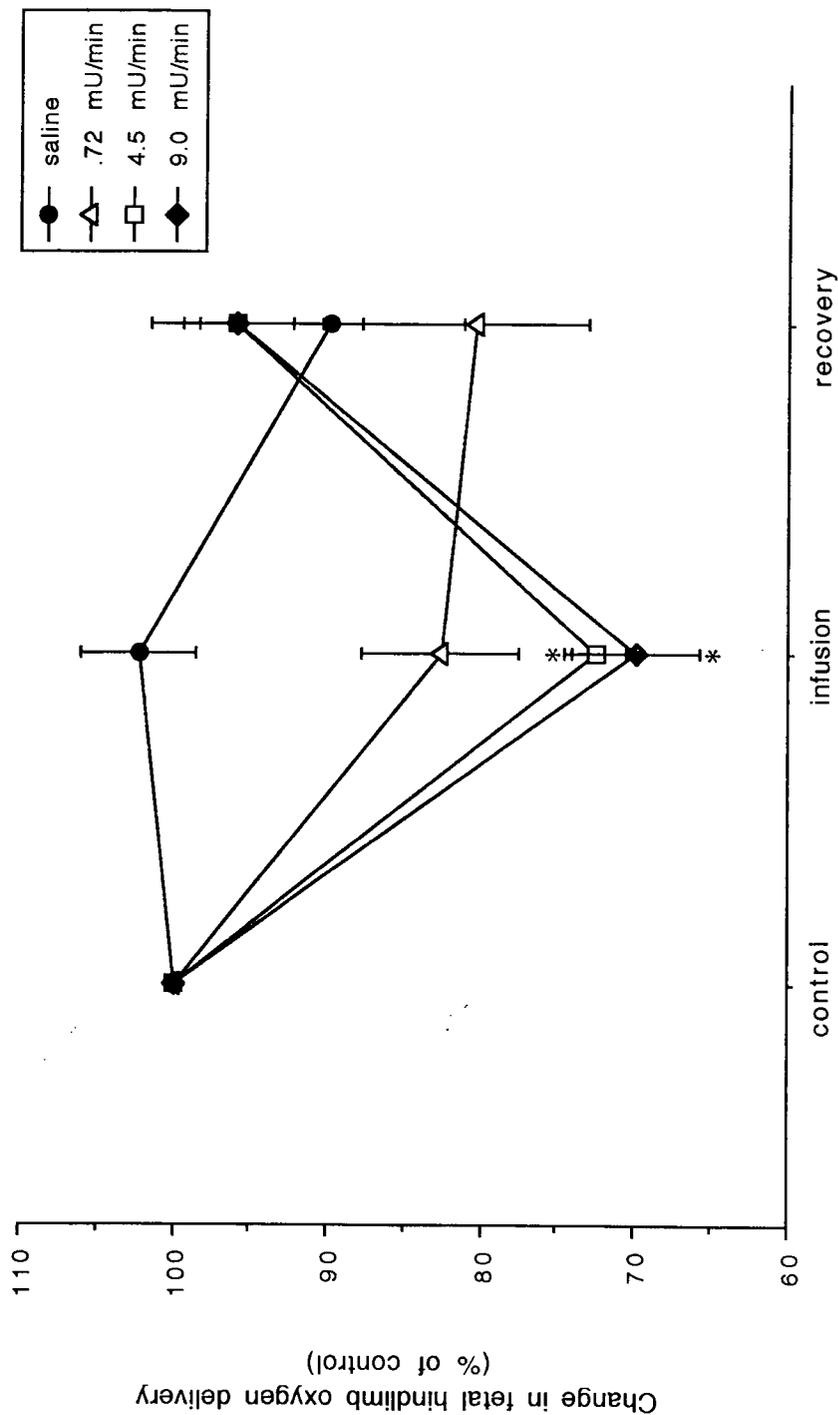
**FIGURE 27.** Change in fetal arterial pressure during and after arginine vasopressin administration to fetal lambs. The lambs received either saline vehicle (n=1) or exogenous AVP at 4.5 mU/min (n=8). Bar indicates infusion period. Values are expressed as mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ). No sample was obtained at 15 minutes into the saline infusion.



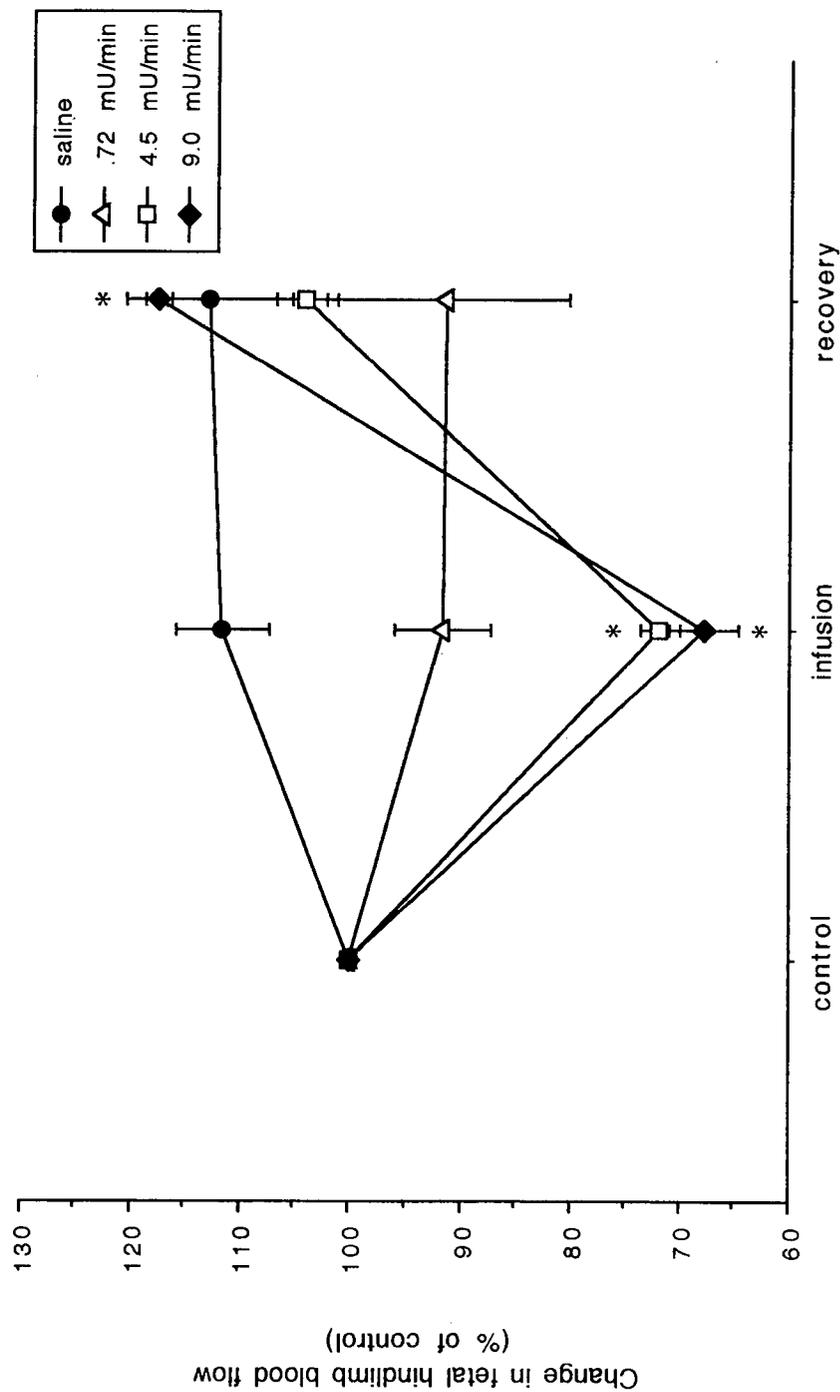
**FIGURE 28.** Change in fetal heart rate during and after arginine vasopressin administration to fetal lambs. The lambs received either saline vehicle (n=2) or exogenous AVP at 4.5 mU/min (n=7). Bar indicates infusion period. Values are expressed as mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ).



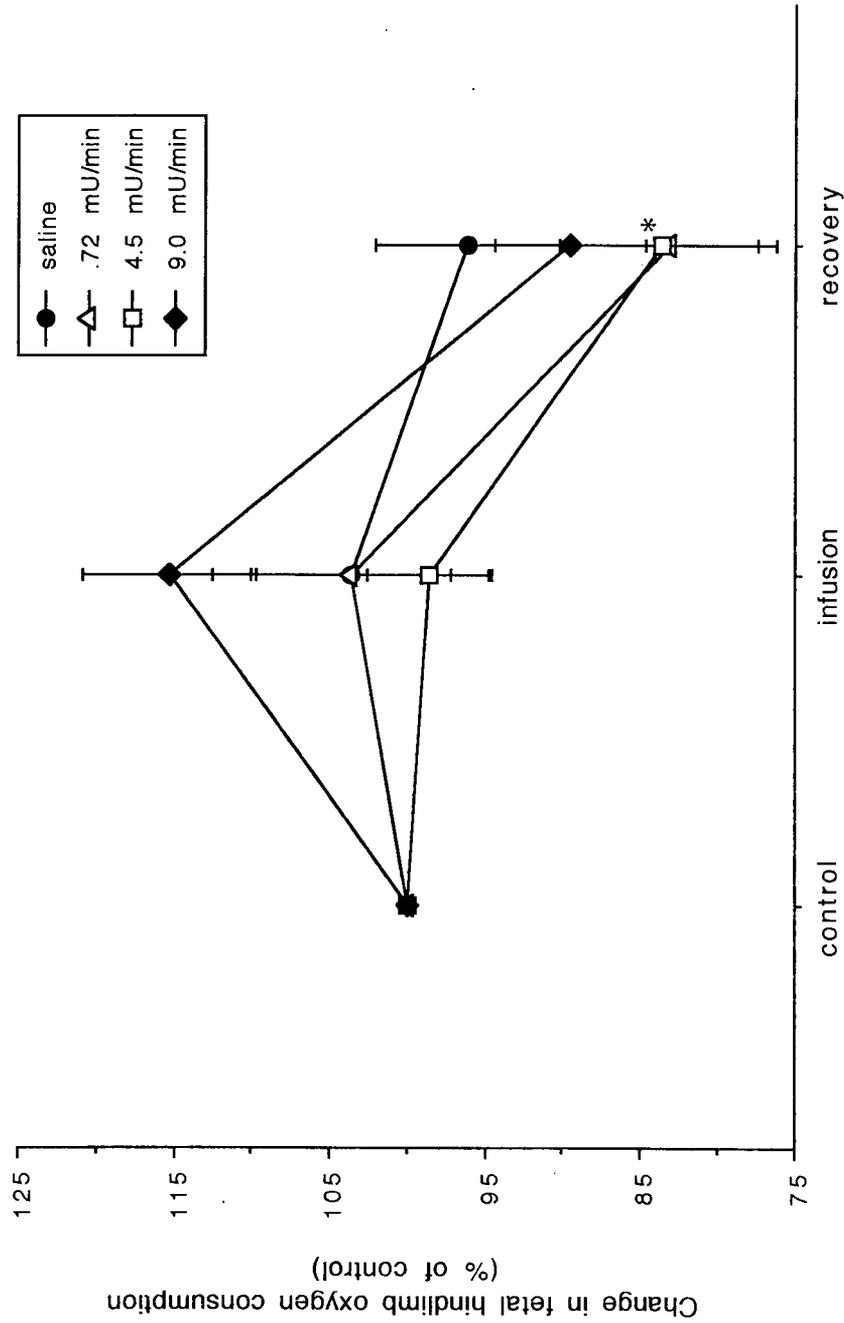
**FIGURE 29.** Change in hindlimb blood flow during and after arginine vasopressin administration to fetal lambs. The lambs received either saline vehicle (n=2) or exogenous AVP at 4.5 mU/min (n=8). Blood flow was measured with a transit-time flow transducer on the external iliac artery. Bar indicates infusion period. Values are expressed as mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ).



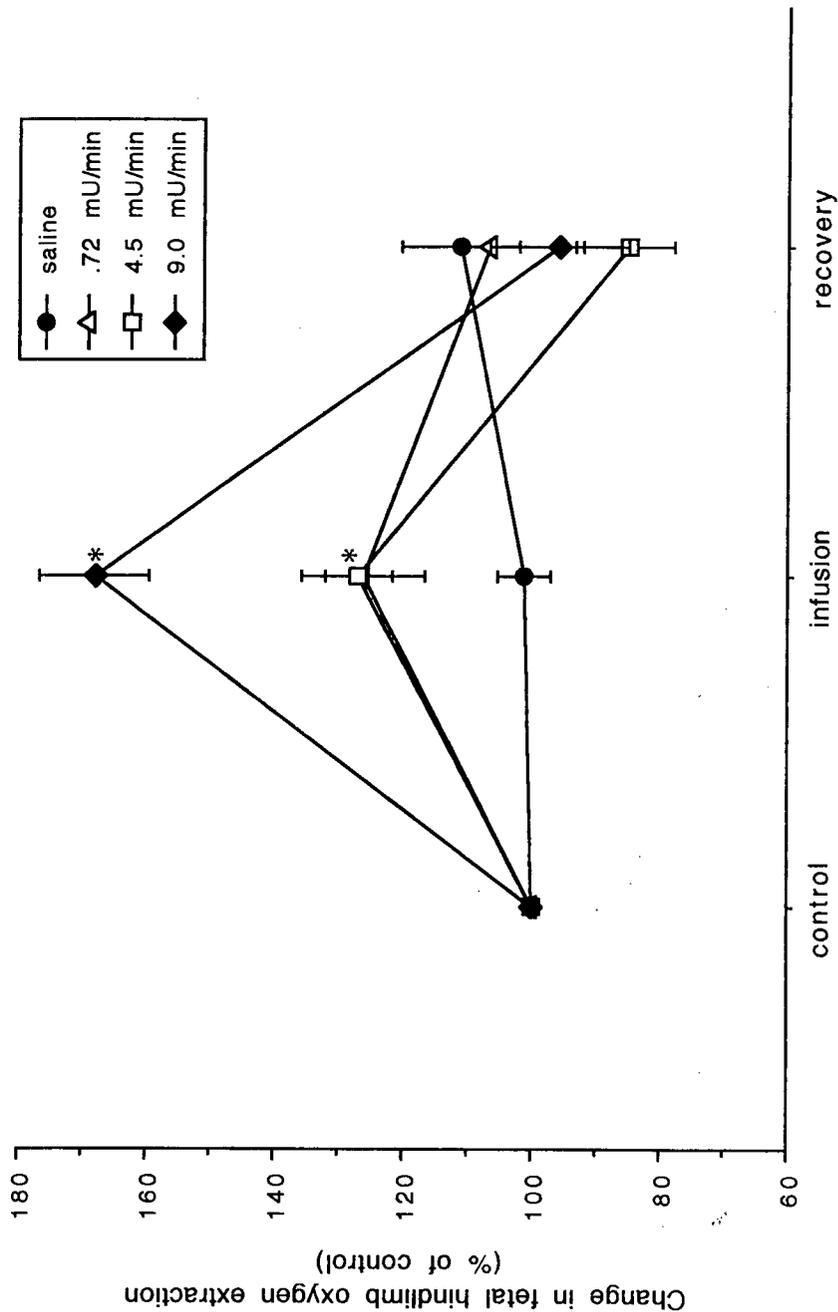
**FIGURE 30.** Change in hindlimb oxygen delivery during and after arginine vasopressin infusion to fetal lambs. The lambs received either saline vehicle (2 experiments), or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment). Values are expressed as mean  $\pm$  SEM. Asterisks indicate a significant difference from the control value ( $p < 0.05$ ).



**FIGURE 31.** Change in hindlimb blood flow during and after arginine vasopressin infusion to fetal lambs. The lambs received either saline vehicle (2 experiments) or exogenous A VP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment). Blood flow was measured with a transit-time flow transducer on the external iliac artery. Values are expressed as mean  $\pm$  SEM. Asterisks indicate a significant difference from the control value ( $p < 0.05$ ).



**FIGURE 32.** Change in hindlimb oxygen consumption during and after arginine vasopressin infusion to fetal lambs. The lambs received either saline vehicle (2 experiments), or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment). Values are expressed as mean  $\pm$  SEM. Asterisks indicate a significant difference from the control value ( $p < 0.05$ ).



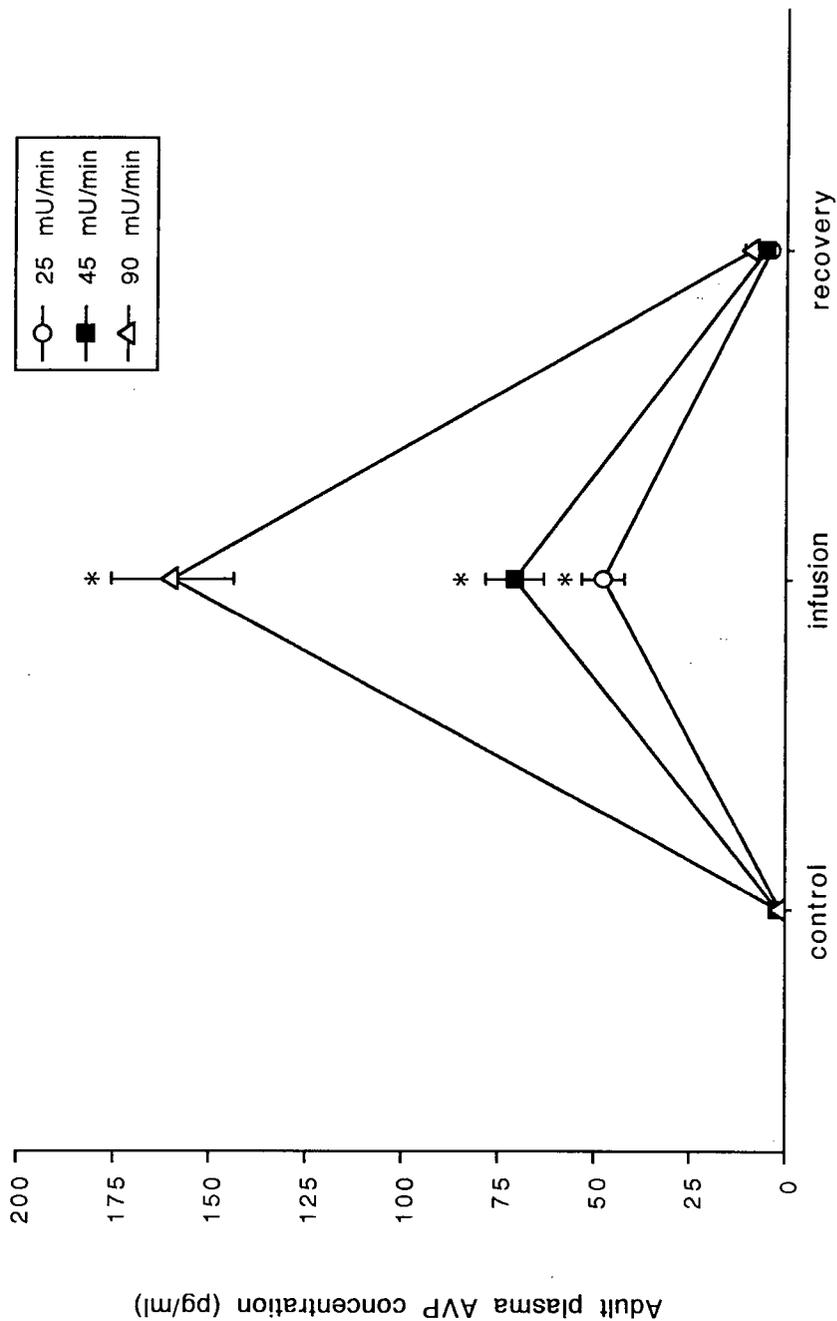
**FIGURE 33.** Change in hindlimb oxygen extraction during and after arginine vasopressin infusion to fetal lambs. The lambs received either saline vehicle (2 experiments), or exogenous AVP at 0.72 mU/min (1 experiment), 4.5 mU/min (8 experiments), or 9.0 mU/min (1 experiment). Values are expressed as mean  $\pm$  SEM. Asterisks indicate a significant difference from the control value ( $p < 0.05$ ).

and extraction ( $84.9 \pm 7.1\%$  of control) which were observed during recovery from the 4.5 mU/min dose.

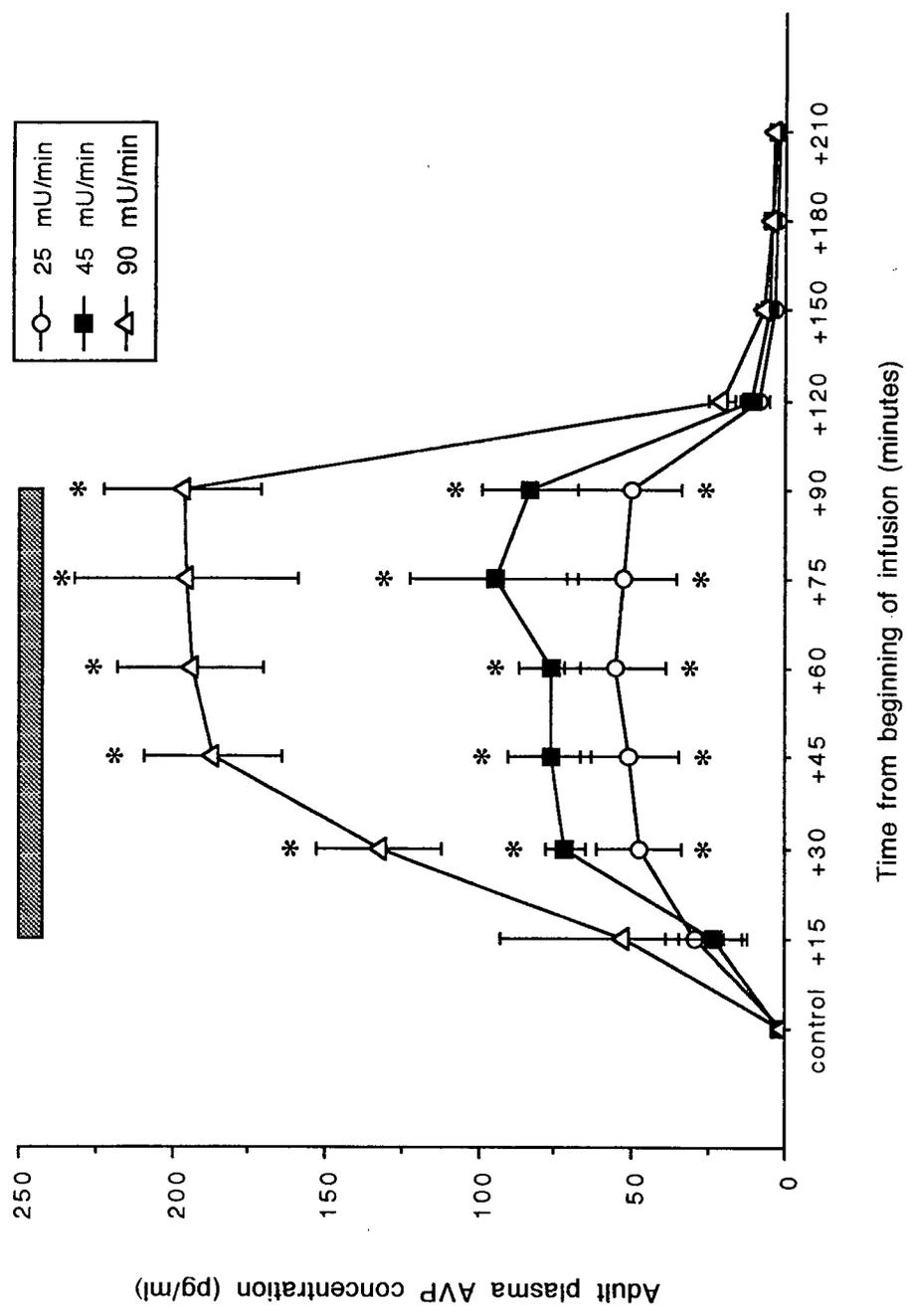
### 7.3.2 Arginine vasopressin effects on the adult

Significant increases in plasma arginine vasopressin levels were also found in adult sheep given exogenous AVP. From an overall control value of  $1.3 \pm 0.2$  pg/ml, plasma AVP levels reached mean concentrations of  $47.9 \pm 5.3$ ,  $71.1 \pm 7.6$ , and  $159.7 \pm 16.2$  pg/ml for infusion dosages of 25 mU/min (62.5 ng/min), 45 mU/min (112.5 ng/min), and 90 mU/min (225 ng/min) doses, respectively (Figure 34). Plasma AVP levels increased rapidly, achieving a significant difference from the control values after 30 minutes and remaining elevated throughout the infusion period (Figure 35). Arginine vasopressin levels returned to pre-infusion levels within 30 minutes after the infusion was ended. Clearance rates were determined in the adult ewes using the same methods as were used with the fetal animals: rate of hormone infusion (per kg body weight) divided by the average of the 75 and 90 minute arterial plasma AVP concentrations. Clearance values for the 25 mU/min (62.5 ng/min), 45 mU/min (112.5 ng/min), and 90 mU/min (225 ng/min) doses were  $23.9 \pm 6.6$ ,  $21.6 \pm 2.4$ , and  $19.0 \pm 2.4$  ml·min<sup>-1</sup>·kg<sup>-1</sup>, respectively. Thus fetal clearance of AVP was 3.6 to 4.5 times greater than in the adult.

No changes in maternal pH,  $P_{O_2}$ , or  $P_{CO_2}$  were observed during arginine vasopressin infusion (Table 16). Blood base excess tended to rise during the recovery phase, achieving a significant difference from the control values for the 25 and 90 mU/min doses (to  $6.1 \pm 0.4$  and  $5.9 \pm 0.4$  mEq/L, respectively). Similarly, hemoglobin concentrations were lower during vasopressin infusion with changes significant at the 25 and 90 mU/min doses. Arterial lactate concentrations were significantly elevated during the medium (45 mU/min) and high (90 mU/min) dose AVP infusions and, although lactate values decreased during the recovery phase, they remained high relative to the control values. Little change in arterial



**FIGURE 34.** Plasma arginine vasopressin concentrations before, during, and after AVP infusion to non-pregnant adult ewes. The ewes received exogenous AVP at doses of 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments). Values are expressed as mean  $\pm$  SEM. Asterisks indicate a significant difference from the control values ( $p < 0.05$ ).



**FIGURE 35.** Plasma arginine vasopressin concentrations before, during, and after AVP administration to non-pregnant adult ewes. The ewes received AVP at 25 mU/min (n=2), 45 mU/min (n=3), or 90 mU/min (n=3). Bar indicates infusion period. Values are expressed as mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ).



glucose values was observed except for a significant increase during the 90 mU/min infusion period.

Table 17 presents the cardiovascular and metabolic data obtained from the non-pregnant adult ewes prior to infusing arginine vasopressin. These data are presented as absolute values (mean  $\pm$  SEM). No significant differences were found between the control data of the three groups of animals.

The low dose arginine vasopressin did not alter arterial pressure in the adult animals but at the medium and high dosages arterial pressure rose an average of  $17.9 \pm 2.6$  and  $35.9 \pm 3.8\%$  above control values, respectively (Figure 36). The hypertension was evident by 30 to 45 minutes into the infusion period. Arterial pressure had returned to pre-infusion values by 30 minutes into the recovery phase.

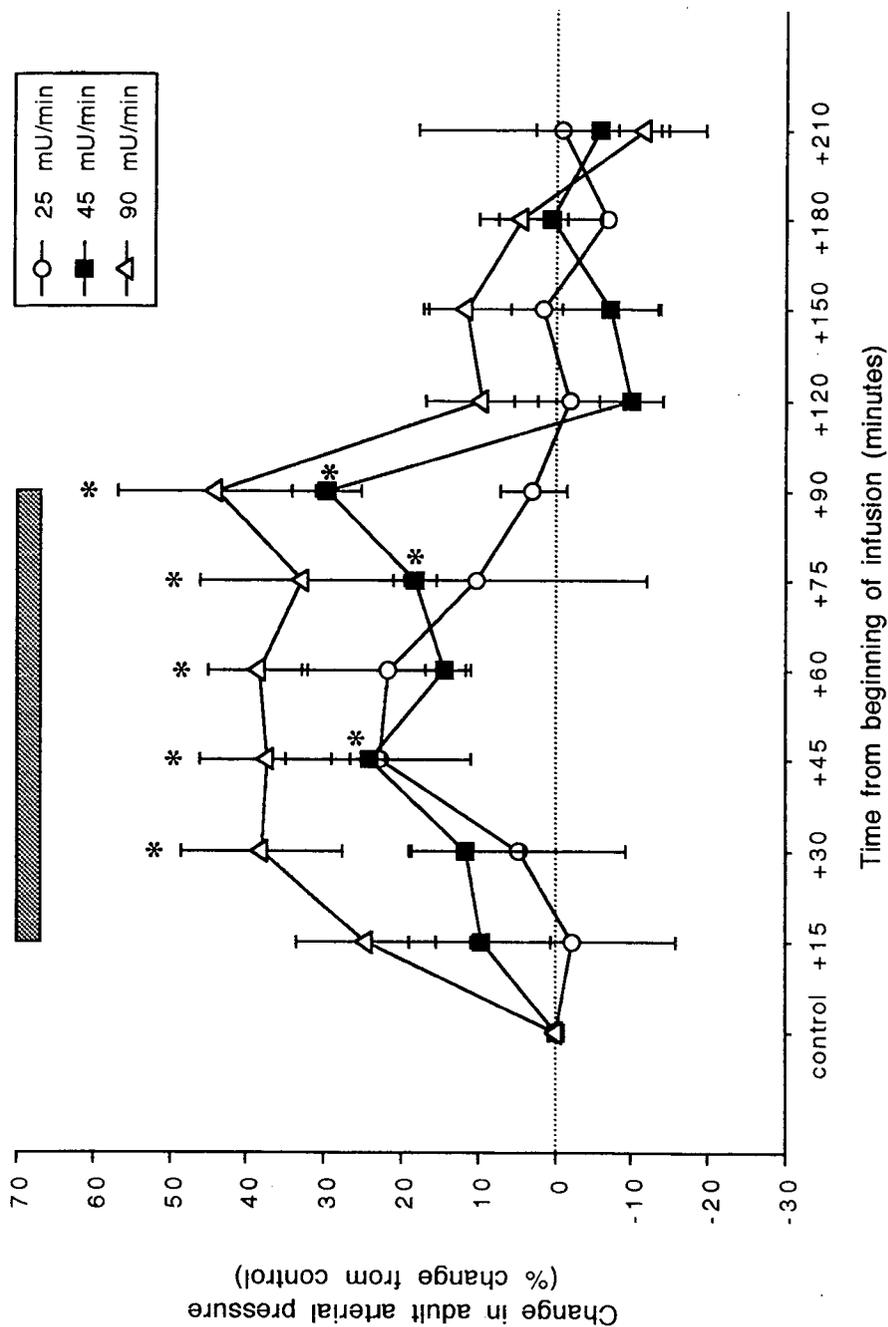
At all three doses of the drug, arginine vasopressin infusion to the adult ewes caused a very rapid bradycardia with heart rate remaining low for the duration of the infusion period (Figure 37). Mean heart rate fell by  $28.5 \pm 1.9$ ,  $39.9 \pm 1.6$ , and  $38.0 \pm 3.4\%$  for the 25, 45, and 90 mU/min AVP doses, respectively. The recovery phase was marked by a rapid return of heart rate to the pre-infusion values.

Figure 38 shows the changes in adult hindlimb blood flow at each sampling point during the infusion and recovery phases. Although blood flow tended to be less during all three doses of AVP, no significant changes were seen at the 25 mU/min level. The 45 and 90 mU/min doses were associated with reductions in flow that were statistically significant after 45 to 60 minutes of infusion. Regardless of the AVP regimen, during the recovery phase blood flow quickly returned to pre-infusion values.

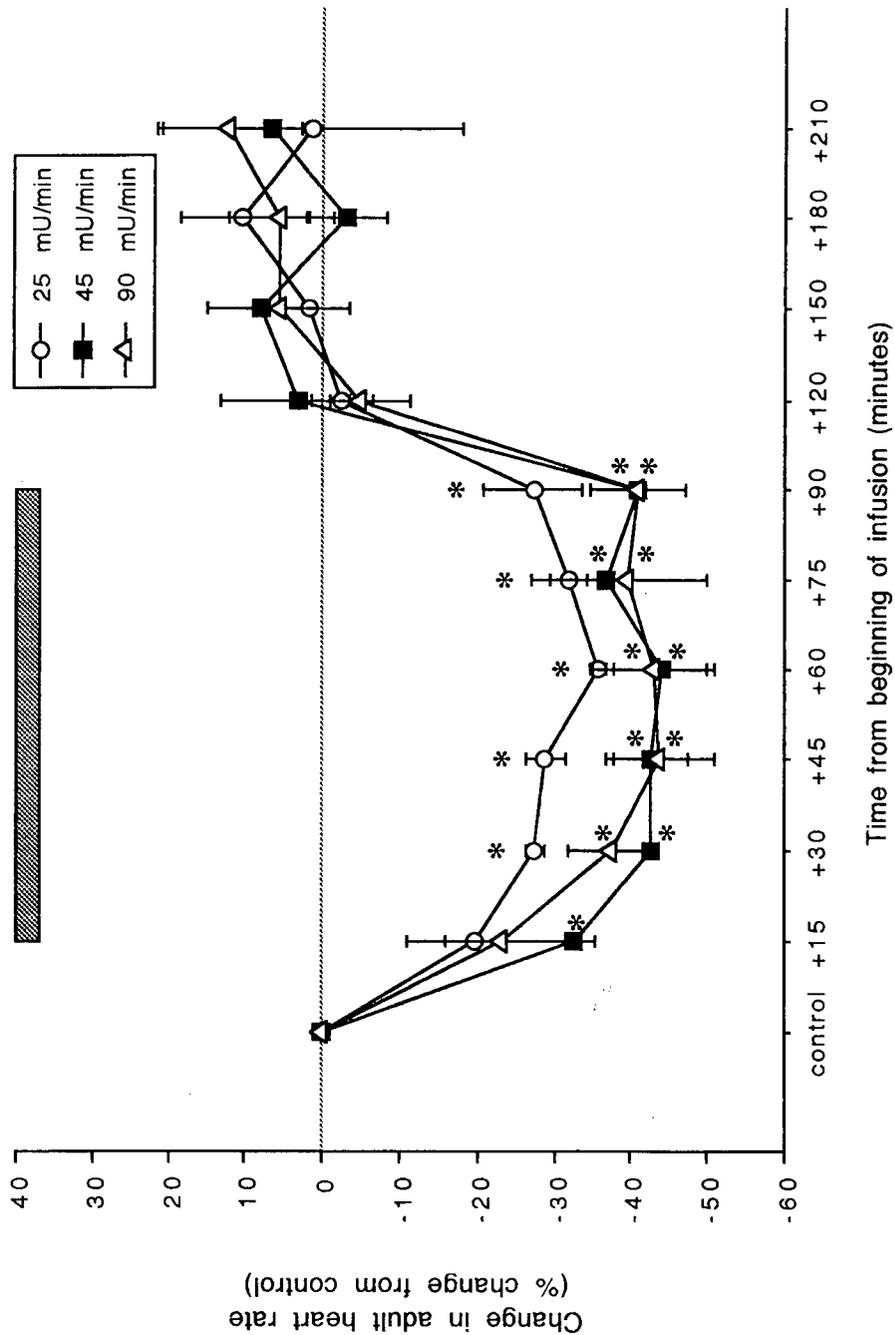
Mean hindlimb oxygen delivery was unchanged for the animals given 25 mU/min of arginine vasopressin whereas this variable fell to  $72.5 \pm 4.5$  and  $68.6 \pm 7.6\%$  of control values during the 45 and 90 mU/min AVP infusions (Figure 39). During the recovery phase,  $Do_2$  returned to control values for the low and medium doses but was significantly elevated ( $111.5 \pm 8.4\%$  of control) for the ewes that had received the 45 mU/min dose. As

**TABLE 17.** Cardiovascular and metabolic data obtained from non-pregnant adult ewes prior to receiving arginine vasopressin infusions at doses of 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments). Data are presented as absolute values (mean  $\pm$  SEM).

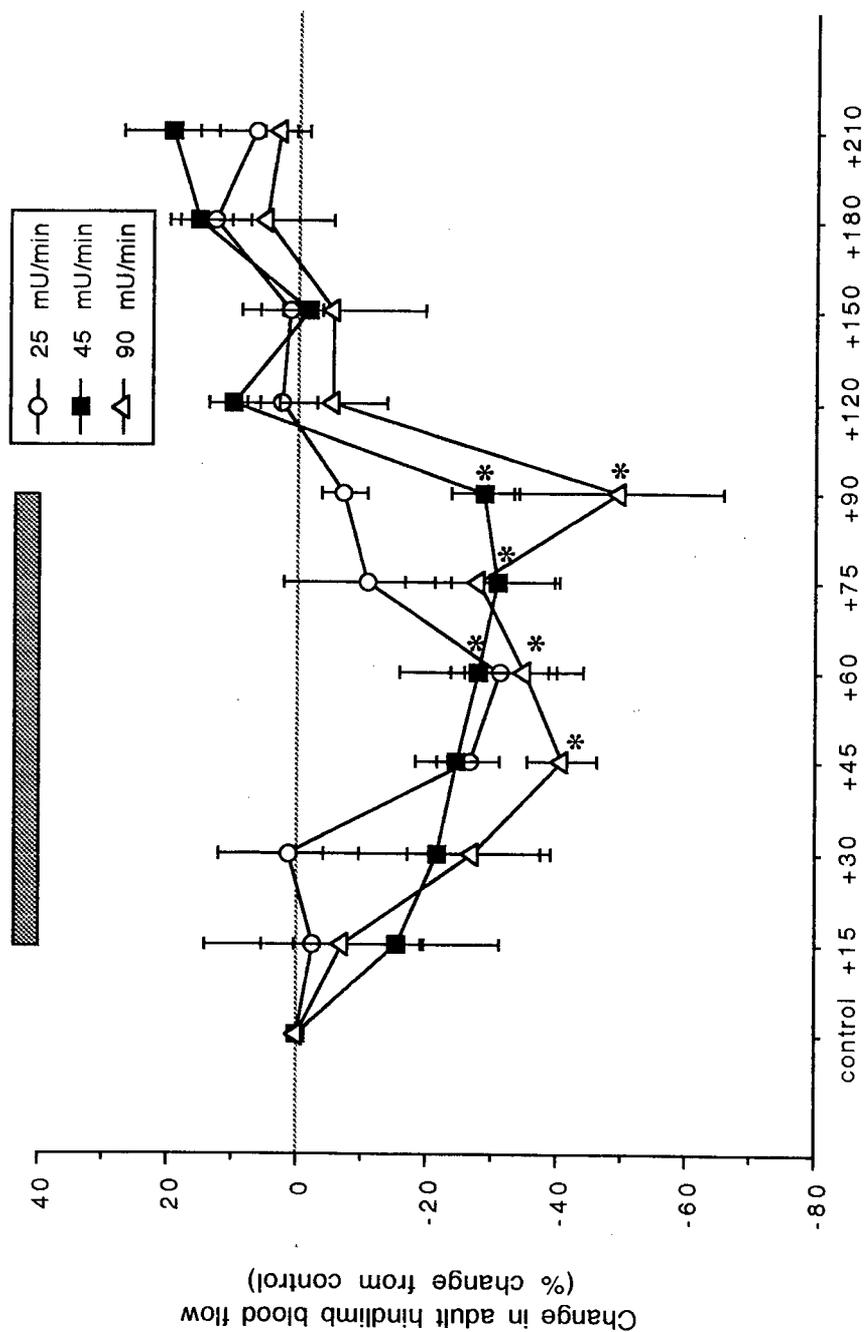
	ARGININE VASOPRESSIN DOSE		
	25 mU/min	45 mU/min	90 mU/min
Arterial pressure (mmHg)	76.3 $\pm$ 0.4	76.2 $\pm$ 6.5	69.3 $\pm$ 10.0
Heart rate (beats/minute)	91.3 $\pm$ 3.7	104.2 $\pm$ 15.8	102.0 $\pm$ 10.8
Hindlimb blood flow (ml·min <sup>-1</sup> )	98.0 $\pm$ 3.4	76.6 $\pm$ 3.9	65.0 $\pm$ 6.0
Oxygen delivery ( $\mu$ mol·min <sup>-1</sup> )	632.9 $\pm$ 29.3	578.2 $\pm$ 47.8	545.2 $\pm$ 71.7



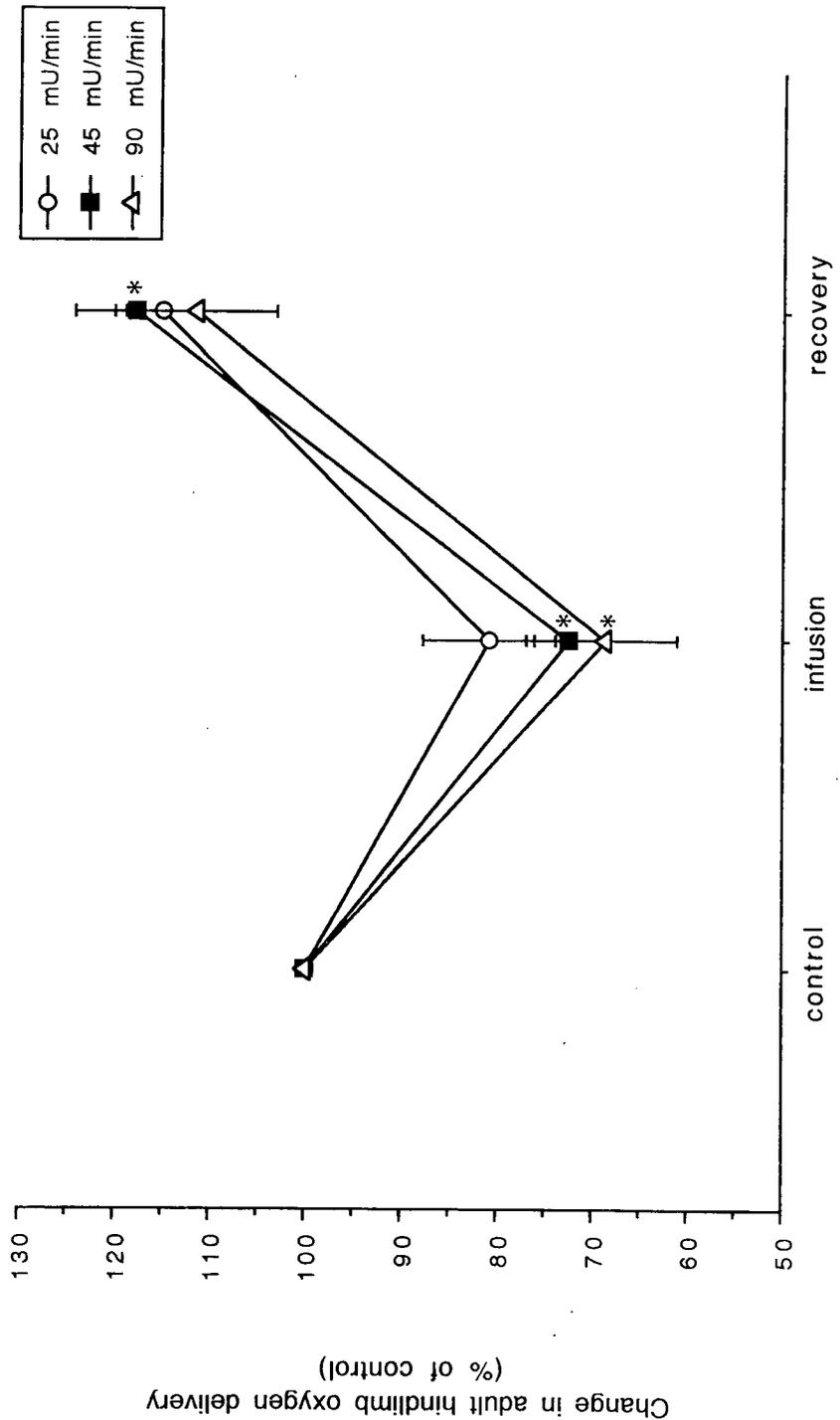
**FIGURE 36.** Change in arterial pressure during and after arginine vasopressin administration to non-pregnant adult ewes. The ewes received AVP at 25 mU/min (n=2), 45 mU/min (n=3), or 90 mU/min (n=3). Bar indicates infusion period. Values are expressed as mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ).



**FIGURE 37.** Change in heart rate during and after arginine vasopressin administration to non-pregnant adult ewes. The ewes received AVP at 25 mU/min (n=2), 45 mU/min (n=2), or 90 mU/min (n=3). Bar indicates infusion period. Values are expressed as mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ).



**FIGURE 38.** Change in hindlimb blood flow during and after arginine vasopressin administration to non-pregnant adult ewes. The ewes received AVP at 25 mU/min (n=2), 45 mU/min (n=3), or 90 mU/min (n=3). Blood flow was measured with a transit-time flow transducer on the external iliac artery. Bar indicates infusion period. Values are expressed as mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ).



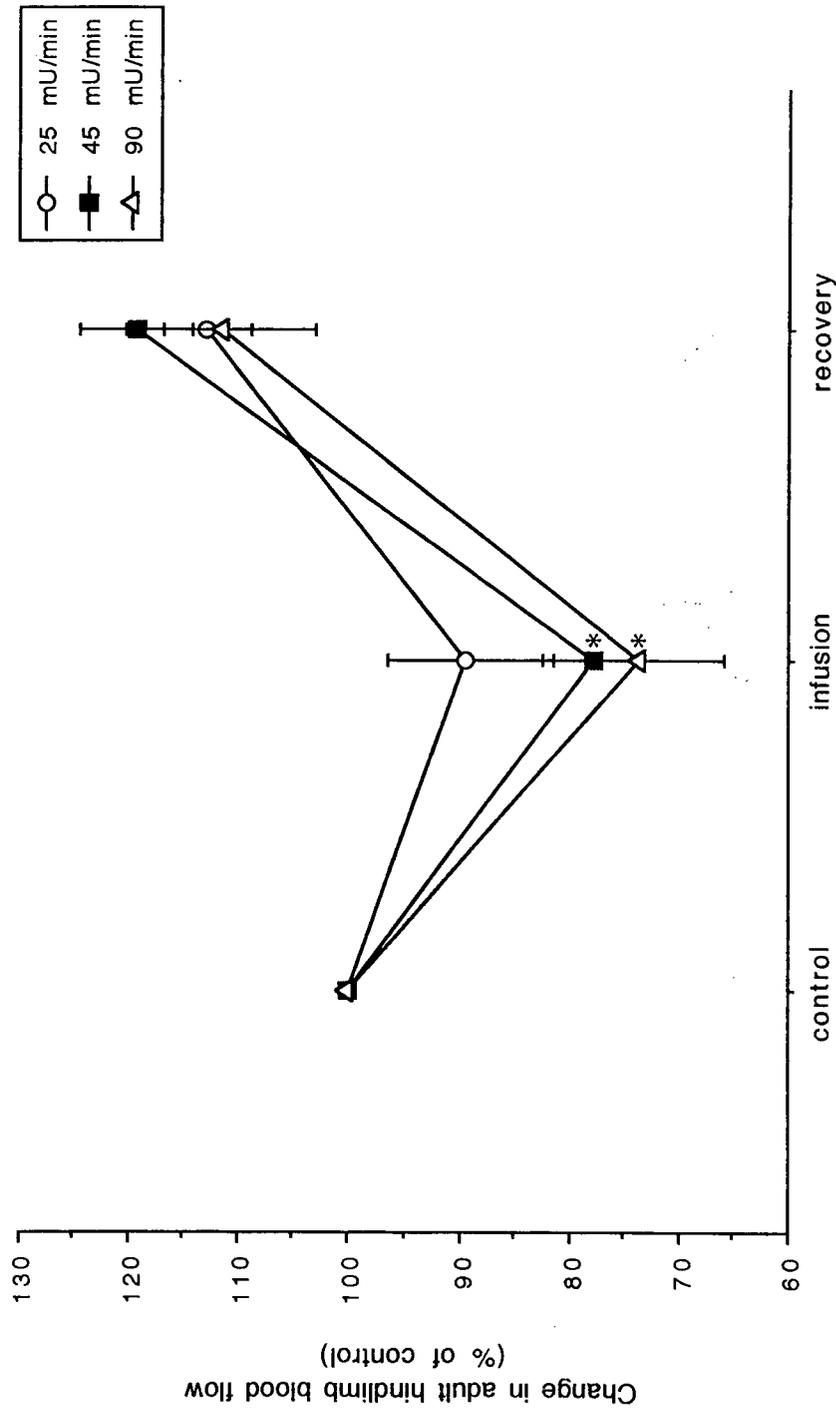
**FIGURE 39.** Change in hindlimb oxygen delivery during and after arginine vasopressin infusion to non-pregnant adult ewes. Ewes received AVP at 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments). Values are mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ).

in the fetus, the change in hindlimb oxygen delivery in the adult animals reflected the alterations in hindlimb blood flow during AVP infusion. Specifically, for the low, medium, and high arginine vasopressin doses, hindlimb blood flow during infusion was reduced to  $89.3 \pm 7.0$ ,  $77.8 \pm 4.5$ , and  $73.6 \pm 7.8\%$  of control values (Figure 40) while  $Do_2$  fell to  $80.9 \pm 6.9$ ,  $72.5 \pm 4.5$ , and  $68.6 \pm 7.6\%$  of control (Figure 39).

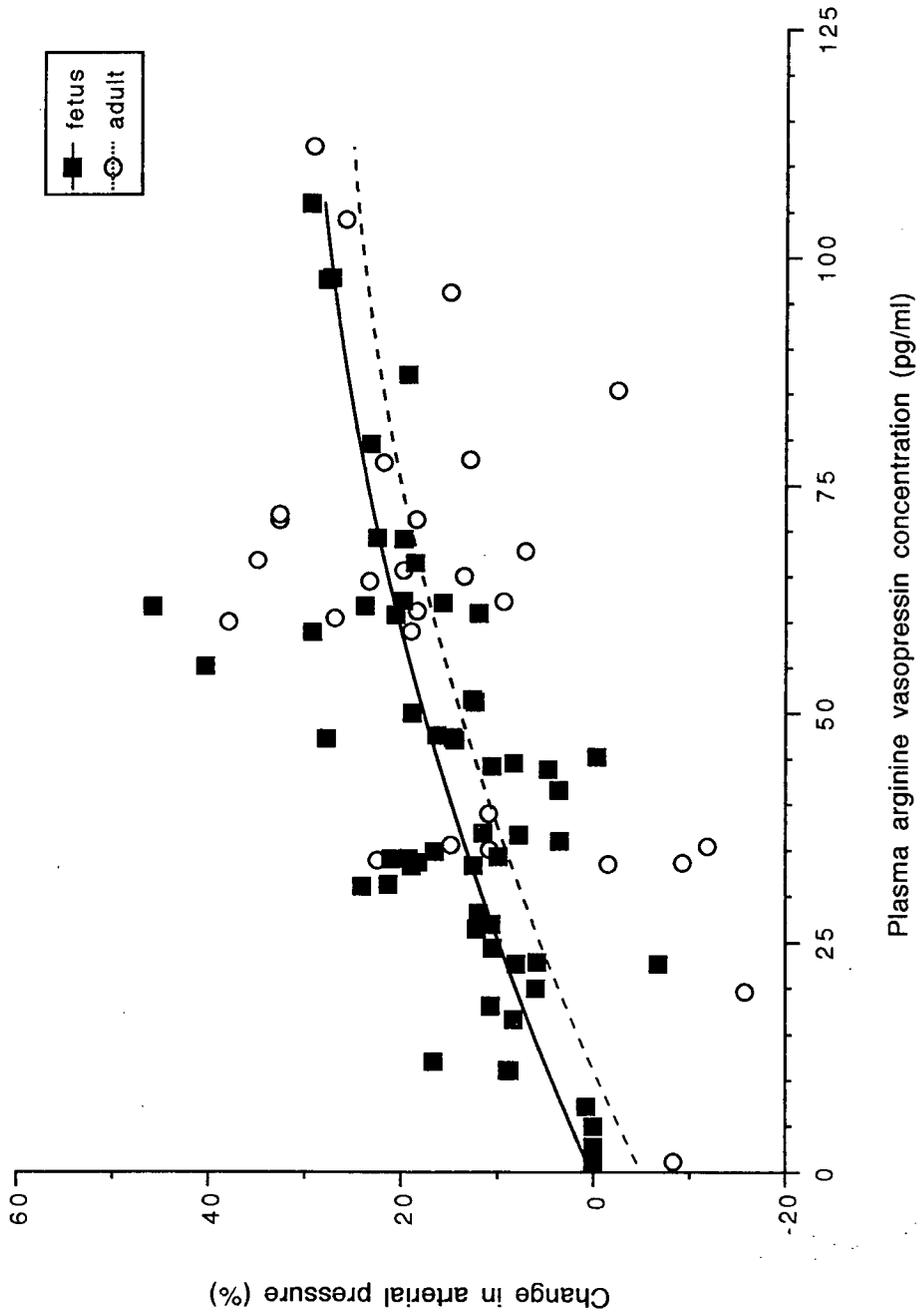
### 7.3.3 Fetal versus adult responses to arginine vasopressin concentration

In order to analyze fetal and adult responses to arginine vasopressin infusion, we wanted to compare similar levels of plasma AVP. This was achieved by combining the data obtained from the 25 and 45 mU/min (62.5 ng/min and 112.5 ng/min) doses given to the adults (5 experiments) with the fetal 0.72, 4.5 and 9.0 mU/min (1.8, 11.25, and 22.5 ng/min) doses (10 experiments). The baseline or control value for the fetuses was  $2.1 \pm 0.4$  pg/ml and  $1.4 \pm 0.2$  pg/ml in the adults. These values were not significantly different. The total AVP levels (average of control and infusion values) were also not statistically different ( $39.1 \pm 3.2$  pg/ml in the fetus;  $50.4 \pm 5.3$  pg/ml in the adult), but the average AVP concentrations during infusion were significantly higher in the adult than fetus ( $58.9 \pm 4.7$  versus  $45.0 \pm 3.0$  pg/ml). Recovery samples were not included in the comparisons so as to avoid any latent effect from the drug infusion. Thus the responses to arginine vasopressin infusion in the fetus and adult were compared over a range of approximately 1 to 112 pg/ml. The mean plasma clearance of arginine vasopressin from the fetal animals (0.72, 4.5 and 9.0 mU/min doses) was  $83.6 \pm 11.2$  ml·min<sup>-1</sup>·kg<sup>-1</sup>. In the adults, AVP clearance (25 and 45 mU/min doses) averaged  $22.5 \pm 2.5$  ml·min<sup>-1</sup>·kg<sup>-1</sup>.

Increasing plasma AVP levels were associated with a rise in arterial pressure with the hypertension for a given arginine vasopressin level tending to be greater in the fetal sheep (Figure 41). The data were best described by polynomial regression equations. However, no significant difference in the fetal and adult regression equations was found. Over the plasma arginine vasopressin concentrations studied, both fetal and adult sheep



**FIGURE 40.** Change in hindlimb blood flow during and after arginine vasopressin infusion to non-pregnant adult ewes. The ewes received AVP at a dose of 25 mU/min (2 experiments), 45 mU/min (3 experiments), or 90 mU/min (3 experiments). Blood flow was measured with a transit-time flow transducer on the external iliac artery. Values are mean  $\pm$  SEM. Asterisks indicate significant difference from control values ( $p < 0.05$ ).



**FIGURE 41.** Relationship between percentage change in arterial pressure and plasma arginine vasopressin concentration during the infusion of vasopressin to fetal (10 experiments) and non-pregnant adult (5 experiments) sheep. The regression equations are not significantly different:  
 fetus:  $y = -0.002x^2 + 0.427x + 0.195$   $r = 0.724$  ( $p < 0.0001$ )  
 adult:  $y = -0.002x^2 + 0.465x - 4.992$   $r = 0.659$  ( $p = 0.0001$ )

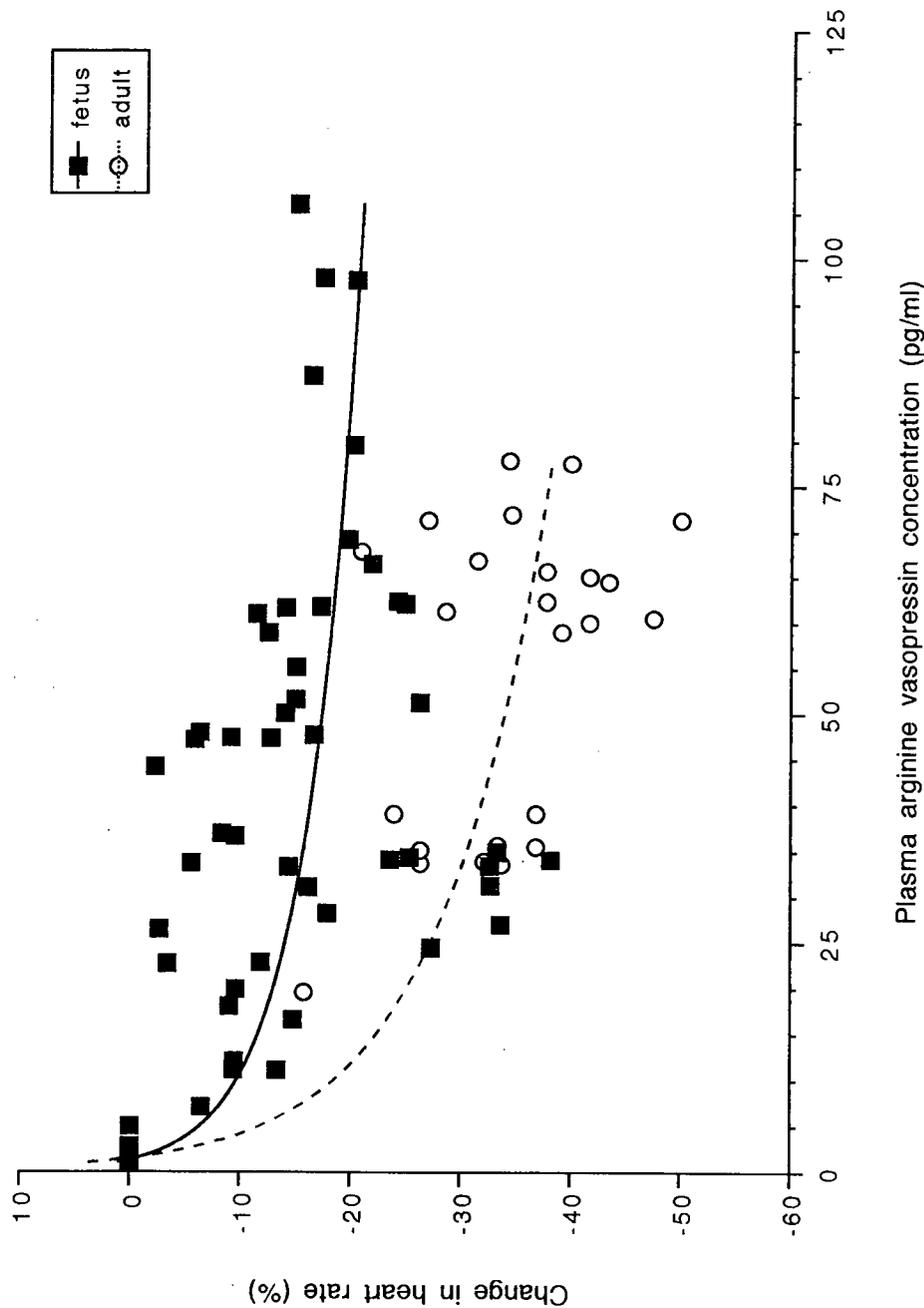
exhibited bradycardia as AVP levels increased (Figure 42). Although there was no difference between the y-intercepts, the logarithmic decline was significantly greater in the adult animals compared to the fetal results. For example, at a plasma arginine vasopressin concentration of 75 pg/ml, fetal heart rate had decreased approximately 18% from the control values compared to 36% in the adult sheep.

Figure 43 illustrates the relationship between plasma arginine vasopressin concentration and the percentage change in hindlimb blood flow in both adult and fetal sheep. As AVP levels increased, hindlimb blood flow, as measured with a transit-time flow transducer on the external iliac artery, fell dramatically. The data were best described by logarithmic regression equations. Although the change in hindlimb blood flow was greater in the fetus compared to the adult, the regression equations were not significantly different.

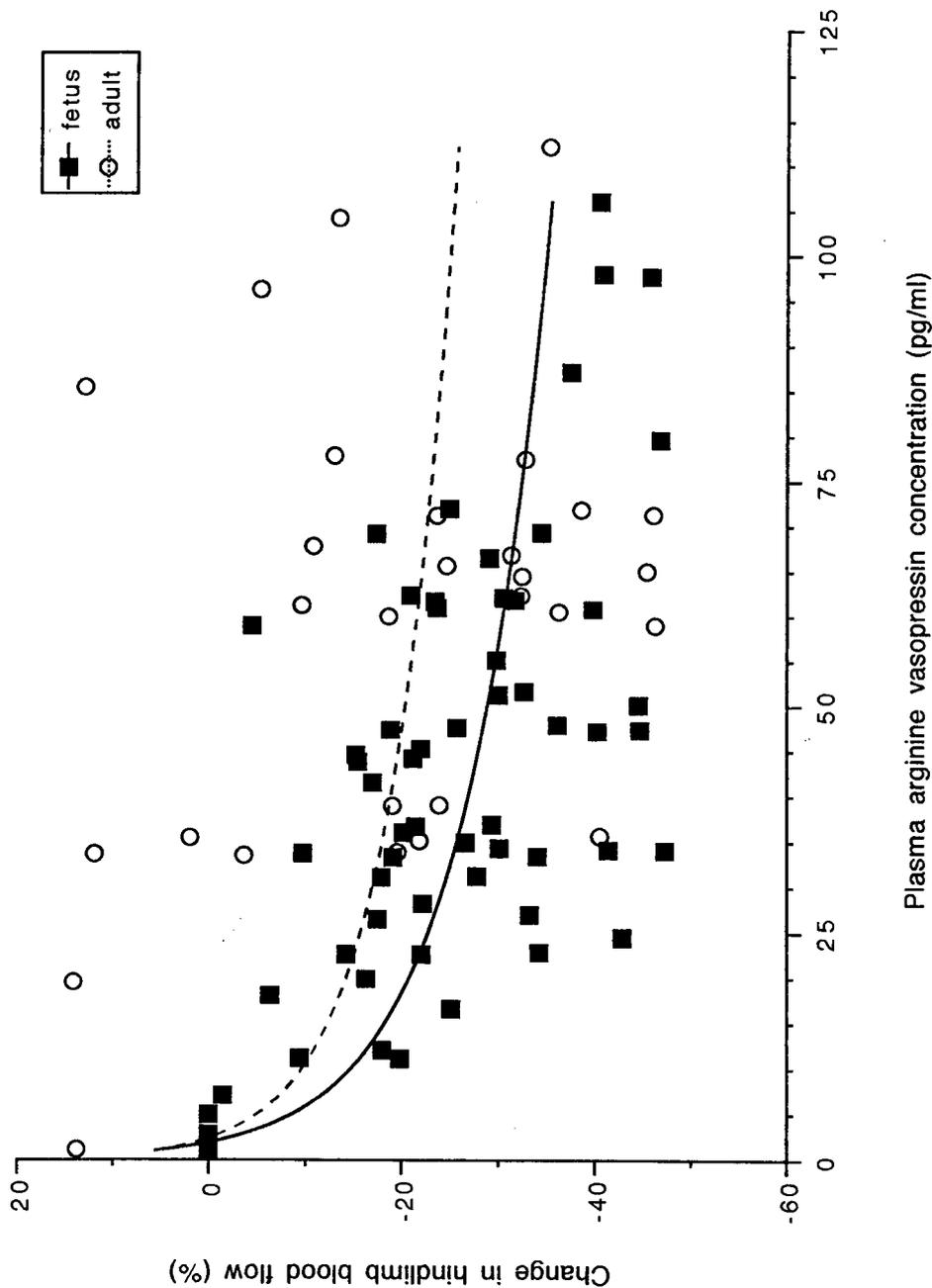
Hindlimb vascular resistance is normally calculated as follows:

$$\text{Hindlimb vascular resistance} = \frac{(\text{arterial pressure} - \text{hindlimb venous pressure})}{\text{hindlimb blood flow}}$$

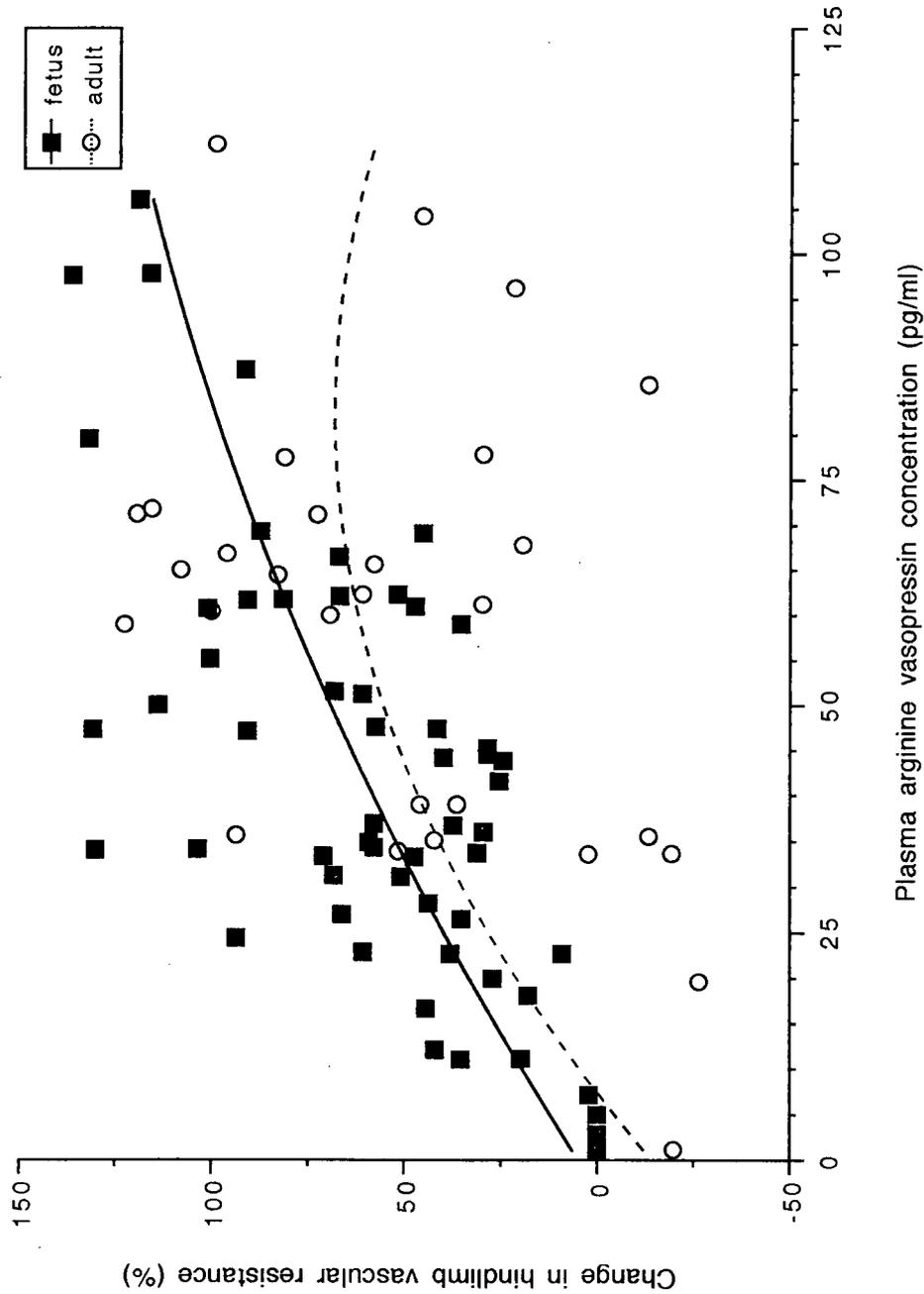
However, in the current study, venous pressures were not available. As venous pressure is generally low (~1–3 mmHg) in both adults and fetuses, we considered it to be negligible and estimated hindlimb resistance as arterial pressure over hindlimb blood flow. Thus, we likely slightly overestimated our calculation of vascular resistance in the hindlimb. Figure 44 shows the relationship between plasma arginine vasopressin levels and the percentage change in hindlimb resistance from control for fetal and non-pregnant ewes. Over the range of AVP concentrations examined (1–112 pg/ml), hindlimb resistance rose with increasing AVP levels with no difference in the regression equations seen between fetal and adult sheep.



**FIGURE 42.** Relationship between percentage change in heart rate and plasma arginine vasopressin concentration during the infusion of vasopressin to fetal (9 experiments) and non-pregnant adult (4 experiments) sheep. The slopes are significantly different ( $p < 0.01$ ): fetus:  $y = -10.979 \text{LOG}(x) + 1.282$   $r = 0.583$  ( $p < 0.0001$ ) adult:  $y = -22.165 \text{LOG}(x) + 3.687$   $r = 0.887$  ( $p < 0.0001$ )



**FIGURE 43.** Relationship between percentage change in hindlimb blood flow and plasma arginine vasopressin concentration during the infusion of vasopressin to fetal (10 experiments) and non-pregnant adult (5 experiments) sheep. Blood flow was measured with transit-time flow transducers on the external iliac arteries. The regression equations are not significantly different: fetus:  $y = -20.242\text{LOG}(x) + 5.557$   $r = 0.752$  ( $p < 0.0001$ )  
 adult:  $y = -15.357\text{LOG}(x) + 5.738$   $r = 0.543$  ( $p < 0.0001$ )



**FIGURE 44.** Relationship between percentage change in estimated hindlimb vascular resistance and plasma arginine vasopressin concentrations during the infusion of vasopressin to fetal (10 experiments) and non-pregnant adult (5 experiments) sheep. The regression equations are not significantly different:

$$\text{fetus: } y = -0.004x^2 + 1.499x + 4.889 \quad r = 0.749 \quad p < 0.0001$$

$$\text{adult: } y = -0.012x^2 + 1.991x - 14.101 \quad r = 0.624 \quad p = 0.0005$$

## 7.4 Discussion

### 7.4.1 AVP levels-normal and pathophysiological

Under normal conditions arginine vasopressin levels in the fetal and adult sheep are very low: <10 pg/ml as measured by bioassay (Rurak, 1978; Alexander *et al.*, 1974) or <5 pg/ml when determined by radioimmunoassay (Akagi and Challis, 1990b; Akagi and Challis, 1990a; Iwamoto *et al.*, 1979). In the current study, control AVP levels were  $1.8 \pm 0.2$  and  $1.3 \pm 0.2$  pg/ml in the fetal and adult animals, respectively. Exogenous AVP administration led to plasma vasopressin levels of 7–106 pg/ml in the fetus. As there were no significant changes in fetal hemoglobin concentration and the blood lost to sampling was replaced with an equal volume of maternal blood after every sample, we do not believe that changes in blood volume contributed to the rise in plasma AVP levels. The hemorrhage-induced threshold for arginine vasopressin release occurs at withdrawal of ~13% of fetal blood volume (Ross *et al.*, 1986b). In the current study <2% of fetal blood volume was removed at each sample. Furthermore, in fetuses receiving saline vehicle infusions only, the blood sampling protocol was identical to animals receiving the hormone and the saline animals did not exhibit a change in AVP concentrations.

Rurak (1978) administered exogenous arginine vasopressin to fetal lambs at doses of 2.25, 4.5, and 9.0 mU/min ( $0.5$  to  $4.1$  mU·min<sup>-1</sup>·kg<sup>-1</sup>). This resulted in fetal plasma AVP levels of 15–505 pg/ml (6–202  $\mu$ U/ml). Despite administering similar doses of exogenous arginine vasopressin (0.17, 4.5, and 9.0 mU/min), when expressed per kg fetal weight our doses were lower ( $0.2$ – $3.2$  mU·min<sup>-1</sup>·kg<sup>-1</sup>) which is reflected in our lower plasma AVP concentrations (7–106 pg/ml or 3–42  $\mu$ U/ml). Our hormone levels were more similar to those of Iwamoto (1979) who achieved plasma AVP levels of 17–91 pg/ml (6.8–36.4  $\mu$ U/ml) by administering vasopressin to fetuses at 0.9 to 2.3 mU·min<sup>-1</sup>·kg<sup>-1</sup>.

The fetal arginine vasopressin levels achieved in this study appear to be physiologically relevant. Akagi (1990b) examined the effect of graded hypoxemia, achieved

by reducing maternal inspired oxygen, on AVP levels in fetal lambs of 125 to 147 days gestation. No change in plasma AVP levels was observed when carotid arterial  $P_{O_2}$  was reduced by 4.0 to 5.3 mmHg; however, a reduction in  $P_{O_2}$  of 8.3 mmHg was associated with a significant rise in plasma arginine vasopressin levels to 16.3–24.2 pg/ml. In addition, Daniel *et al.* (1983) examined the effect of various types of hypoxemia on fetal arginine vasopressin levels. Reduced maternal inspired oxygen (10% oxygen), partial occlusion of the umbilical cord for 20 minutes (blood flow values were not given), and complete occlusion of the umbilical cord for 2 minutes resulted in plasma AVP levels of  $31.9 \pm 0.91$ ,  $50.1 \pm 11.45$ , and  $160.4 \pm 12.45$  pg/ml, respectively. In a hemorrhage study, Drummond (1980) observed that a 20% reduction in fetal blood volume resulted in plasma AVP levels of  $31.7 \pm 14.7$  pg/ml in late gestation. With more severe hemorrhage (Robillard *et al.*, 1979) or hypoxemia (Rurak, 1978) vasopressin levels rise to  $>200$  pg/ml. Plasma arginine vasopressin concentrations of  $>450$  pg/ml have been reported in fetuses during spontaneous (Stark *et al.*, 1979) and induced parturition (Stark *et al.*, 1981). No rise in vasopressin levels was seen during the preparturient period suggesting that the elevated vasopressin levels are a result of intrapartum stress and are not related to the initiation of parturition (Stark *et al.*, 1979). A recent study has demonstrated that the antepartum and intrapartum arginine vasopressin concentrations in normal and growth-restricted late gestation fetal lambs appear to be quite different (Tan, 1997). In the normal fetuses, AVP rose from  $4.2 \pm 0.8$  pg/ml pre-labour to a maximum of  $98.3 \pm 30.2$  pg/ml at delivery. In contrast, the growth-restricted fetal lambs had a pre-labour AVP concentration approximately 2.7 times higher ( $11.7 \pm 1.4$  pg/ml). At delivery, AVP levels in these fetuses averaged  $480.1 \pm 102.7$  pg/ml. Thus, the growth-restricted fetus has an elevated basal AVP level compared to appropriate-for-gestational-age fetal lambs. Furthermore, the stress of labour is associated with increased arginine vasopressin levels, with poor fetal condition, such as growth-restriction, significantly augmenting this pattern.

Rurak (1978) also administered arginine vasopressin to non-pregnant ewes at doses of 23.1, 46.3, 92.7, and 185.3 mU/min ( $0.4$  to  $2.9$   $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) resulting in plasma concentrations of 100–1170 pg/ml. With the exception of the highest dose, we administered similar amounts of AVP (25, 45, and 90 mU/min or  $0.4$  to  $1.7$   $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ). Plasma vasopressin levels between 1 and 256 pg/ml resulted. We observed a fall in hemoglobin concentration during AVP infusion to the non-pregnant ewes. Adult animals did not receive blood to replace that lost to sampling; however, only 4 ml of blood was removed during each sample for a total loss of 52 ml over the course of an experiment. Assuming an adult sheep blood volume of 53.5 ml/kg (Giles *et al.*, 1977), this represents a total blood loss of <2% which is unlikely to have contributed significantly to the rise in arginine vasopressin levels. Rurak (1978) also reported a fall in hematocrit in adult sheep given exogenous arginine vasopressin. This may be due to a rise in plasma volume, which is an AVP effect reported in adult dogs (Szczepanska-Sadowska, 1973).

Elevated plasma arginine vasopressin levels, similar to the range achieved by this study, are also seen in newborn and adult sheep in response to stress. Like the fetus, hemorrhage in adult sheep is associated with increases in plasma AVP levels (Wintour *et al.*, 1995; Hjelmqvist *et al.*, 1991; Cameron *et al.*, 1985) and lambs subjected to phlebotomy of 10 to 20% of blood volume experience 37- to 44-fold increases in AVP (Leake *et al.*, 1979). Uterine contractions, particularly at delivery, and vaginocervical stimulation increase vasopressin concentrations in the ewe (Kendrick *et al.*, 1991). Unlike fetal lambs, plasma arginine vasopressin levels do not increase significantly in adult ewes breathing 9–10% oxygen (Curran-Everett *et al.*, 1988; Stegner *et al.*, 1984b; Rurak, 1978). However, vasopressin levels of >300 pg/ml are seen with severe hypoxemia when maternal inspired air is reduced to 5% oxygen (Stark *et al.*, 1984b). Thus, hypoxemia is a potent stimulus for vasopressin release in fetal lambs, but a more profound hypoxemia is required for a similar AVP secretion in adult ewes (Stark *et al.*, 1982).

#### 7.4.2 Blood gas and metabolic responses

The blood gas and metabolic variables obtained during the control periods are within the normal range seen for fetal and adult sheep in our laboratory. The fall in pH and base excess seen in the recovery phase following the infusion of vehicle (saline) is puzzling (Table 14). When these data were calculated as a change from the individual animal's control, no significant change in these variables was found suggesting that inter-animal variation contributed to this observation.

Rurak and Gruber (1984) examined the effect of vasopressin on fetal lamb oxygenation using an AVP infusion rate similar to ours (4.5 mU/min for 60 minutes or 1.4 to 3.5 mU·min<sup>-1</sup>·kg<sup>-1</sup>). They observed a significant increase in fetal arterial  $P_{O_2}$  following 60 minutes of AVP infusion (from  $20.0 \pm 1.0$  to  $23.1 \pm 1.0$  mmHg) but not at the 30 minute sample; significant declines in pH and base excess were observed at both points. Iwamoto (1979), employing lower doses of AVP (0.9 to 2.3 mU·min<sup>-1</sup>·kg<sup>-1</sup>), also reported a rise in  $P_{O_2}$  after 30 minutes of arginine vasopressin infusion (from  $21.9 \pm 0.7$  to  $24.1 \pm 0.8$  mmHg) but no change in pH. In the current study, fetal arterial  $P_{O_2}$  rose from  $19.2 \pm 0.6$  to  $20.0 \pm 0.5$  mmHg and pH fell from  $7.316 \pm 0.011$  to  $7.311 \pm 0.007$  but the changes did not reach statistical significance (Table 14).

Rurak and Gruber (1984) attributed the rise in vascular  $P_{O_2}$  seen during vasopressin infusion to an increase in umbilical blood flow and hence increased oxygen delivery to the fetus. As oxygen consumption was unaltered, the lower extraction of oxygen from the blood led to a rise in  $P_{O_2}$ . Iwamoto (1979) did not see a significant rise in umbilical blood flow but peripheral blood flow was reduced 45%. In our study, umbilical blood flow was not measured but fetal hindlimb blood flow fell to  $71.8 \pm 1.9\%$  and  $67.7 \pm 3.2\%$  when AVP was administered at 4.5 and 9.0 mU/min, respectively. This correlated with significant decreases in hindlimb oxygen delivery to  $72.5 \pm 2.0\%$  and  $69.9 \pm 4.2\%$  of control values. Increased fractional oxygen extraction meant that hindlimb consumption was unchanged during AVP infusion. The rise in oxygen extraction led to a reduction in hindlimb venous

$P_{O_2}$  from  $14.9 \pm 0.6$  to  $14.1 \pm 0.5$  mmHg at the 4.5 mU/min dose. Although this drop was not significant, the steepness of the relationship between fetal  $P_{O_2}$  and oxygen saturation (oxyhemoglobin dissociation curve) at this value was reflected in a significant increase in hindlimb arterial-venous oxygen content difference during AVP infusion (from  $0.58 \pm 0.05$  to  $0.79 \pm 0.06$  mM). The observation that  $P_{O_2}$  in the descending aorta did not fall suggests that greater umbilical blood flow and/or reduced placental oxygen extraction occurred and were able to compensate for the changes in oxygenation seen across the hindlimb.

As with the fetus, no change in arterial  $P_{O_2}$  or pH was observed in the non-pregnant ewe. The observation that exogenous AVP does not have an effect on adult blood gases has been previously reported (Nuyt *et al.*, 1996; Harper and Rose, 1987; Rurak, 1978). Arterial lactate concentrations were elevated during AVP infusion, reaching values of  $1.10 \pm 0.43$ ,  $0.59 \pm 0.05$ , and  $0.76 \pm 0.07$  mM at the 25, 45, and 90 mU/min doses, respectively. As venous values were not available, we could not determine hindlimb lactate flux for the adults. Thus, it is unclear whether lactate release from the hindlimb contributed to the rise in circulating lactate levels or whether other peripheral tissues are responsible. Lactate values began to decline during the recovery phase but were still above control levels. This lactic acidemia likely contributed to the elevated base excess during the recovery phase, but was insufficient to alter arterial pH. Hindlimb oxygen delivery was reduced to  $80.9 \pm 6.9\%$ ,  $72.6 \pm 4.5\%$ , and  $68.6 \pm 7.6\%$  of control values during the low, medium, and high AVP doses. This reflects vasoconstriction in the hindlimb tissues as illustrated by the similar reduction in mean hindlimb blood flow ( $89.3 \pm 7.0\%$ ,  $77.8 \pm 4.5\%$ , and  $73.6 \pm 7.8\%$  of control values). Peripheral vasoconstriction has been reported in adult sheep (Vizsolyi and Perks, 1976) and dogs (Liard *et al.*, 1982; Heyndrickx *et al.*, 1976) in response to AVP administration. Adult hindlimb blood flow was quite variable over the course of the sampling (Figure 38). The variability in these data reflects the difficulty of obtaining hindlimb blood flow from semi-mobile adult animals. For example, when the animals lie

down their legs are positioned beneath the body resulting in a reduction in arterial blood flow while a switch to a standing position often causes a transient hyperemia.

#### 7.4.3 Cardiovascular responses

Since 1895 it has been recognized that vasopressin causes an increase in arterial pressure and vasoconstriction in most vascular beds (Share, 1988; Liard *et al.*, 1982; Iwamoto *et al.*, 1979). In the current study, infusion of AVP at 4.5 mU/min was associated with a reduction in hindlimb blood flow of  $28.2 \pm 1.6\%$ . Fetal arterial pressure rose  $16.5 \pm 1.6\%$ , and heart rate fell  $17.2 \pm 1.5\%$ . Previous reports in which AVP was administered at  $\sim 4$  mU/min observed fetal arterial pressure increases of 5–29% and 20–25% reductions in heart rate (Irion *et al.*, 1990; Tomita *et al.*, 1985; Wiriyathian *et al.*, 1983; Rurak, 1978). Iwamoto (1979), whose range of infusion doses ( $0.9$  to  $2.3$  mU $\cdot$ min $^{-1}\cdot$ kg $^{-1}$ ; mean =  $1.4 \pm 0.1$  mU $\cdot$ min $^{-1}\cdot$ kg $^{-1}$ ) is most similar to ours, reported a 19% rise in arterial pressures and 17% fall in heart rate in the fetus. Thus, the degree of hypertension and bradycardia noted in our fetuses in response to arginine vasopressin infusion is similar to the values observed in other studies.

Over the range of plasma AVP concentrations we achieved (1–112 pg/ml) by administering vasopressin at a maximum dose of 9.0 mU/min to the fetus, arterial pressure rose steadily (Figure 41) while heart rate fell dramatically and then appeared to plateau (Figure 42). Wiriyathian (1983) found that the rise in arterial pressure is vasopressin dose-dependent but the fall in heart rate is not. These data are in contrast to Rurak (1978) who reported that the bradycardia was AVP dose-dependent but hypertension was not. Both of these studies involved maximum vasopressin infusions of  $\sim 9$  mU/min. At infusion rates  $< 9$  mU/min, Irion's data (1990) appear to support those of Wiriyathian, i.e., arterial pressure but not heart rate changes in a dose-dependent manner. However, in fetal lambs, the change in heart rate with vasopressin dose occurs in a step-like pattern with approximately a ten-fold increase in AVP dose necessary to further alter heart rate (Irion *et al.*, 1990). For

example, fetal heart rate was reduced ~20% at doses of 2.4 and 8 mU/min, fell another 10% at AVP doses of 24 and 80 mU/min, and at 240 mU/min heart rate was 40% less than control (Irion *et al.*, 1990). In contrast, arterial pressure increases in a curvilinear fashion: rising in a dose-dependent manner then leveling off at ~30% above control values at doses beyond 24 mU/min. Thus, when considered over a wide range of arginine vasopressin administration (~0–300 mU/min), both fetal heart rate and arterial pressure initially change in a dose-dependent manner with arterial pressure then reaching a plateau (Irion *et al.*, 1990; Tomita *et al.*, 1985). This is still consistent with our observations. At the doses we employed (maximum 9.0 mU/min), arterial pressure was still rising as AVP levels were not high enough to have resulted in a plateau. With increasing vasopressin levels, fetal heart rate fell rapidly and then plateaued as would be expected at our doses. Tomita's data (1985) suggest that if we had administered higher doses of arginine vasopressin (~24 mU/min), fetal heart rate would have experienced another rapid decline followed by a plateau phase, and arterial pressure would also reach a plateau.

Adult sheep also exhibit hypertension and bradycardia during the exogenous infusion of arginine vasopressin. We administered AVP at doses of 25, 45, and 90 mU/min to non-pregnant ewes and noted rises in arterial pressure of  $10.0 \pm 5.1\%$ ,  $17.9 \pm 2.6\%$ , and  $35.9 \pm 3.8\%$  as well as reductions in heart rate of  $28.5 \pm 1.9\%$ ,  $39.9\% \pm 1.6$ , and  $38.0 \pm 3.4\%$ , at the respective doses. Rurak (1978) infused similar doses of AVP and achieved arterial pressures 9–16% above control and 20–45% reductions in heart rate. When vasopressin was given at  $3.2 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ , a 27% rise in arterial pressure and 48% decline in heart rate was seen in adult sheep (Harper and Rose, 1987). Thus, the cardiovascular responses seen in our ewes are similar to those observed in other studies with the exception of the degree of hypertension seen at the 90 mU/min dose (~36% above control) which is higher than previously reported. This is not due to differences in AVP doses as our levels were in the lower range obtained in the other studies. A possible difference is that our

infusion period lasted 90 minutes as opposed to only 30 minutes in the Harper and Rose (1987) study and 60 minutes in that of Rurak (1978).

At the three vasopressin doses used in our study, adult arterial pressure appeared to increase in a dose-dependent manner (Figure 36) but the degree of bradycardia was similar at the 45 and 90 mU/min doses (Figure 37). As was seen with the fetus, when these variables were examined over the range of plasma AVP levels achieved (1–112 pg/ml), arterial pressure rose steadily (Figure 41) while heart rate initially fell rapidly and then leveled off (Figure 42). Ling (1989) noted dose-dependent changes in both arterial pressure and heart rate in adult sheep given AVP at levels similar to ours. However, in Ling's study the highest plasma AVP concentration achieved was ~70 pg/ml which is similar to the concentration we observed ( $71.1 \pm 7.6$  pg/ml) with our 45 mU/min dose. The dose-dependent nature of vasopressin-induced bradycardia was not apparent in fetal lambs until a wider range of arginine vasopressin infusion rates was used (Irion *et al.*, 1990; Tomita *et al.*, 1985). Similar experiments employing a wider range of infusion rates will also be required in adult sheep to determine whether the vasopressin-induced bradycardia in adults is of a dose-dependent nature.

It is apparent from the present and previous studies that cardiovascular changes occur at AVP concentrations that are pathophysiologically relevant (e.g., during hypoxemia or hemorrhage) in fetal and adult sheep. The hypertension appears to be a result of marked vasopressin-induced peripheral vasoconstriction. In both the fetus and adult sheep, we noted large reductions in hindlimb blood flow during vasopressin infusion. Iwamoto (1979) also reported reductions in flow to the peripheral circulation and the gut.

The mechanism by which arginine vasopressin affects the heart, however, is not certain. The increase in blood pressure observed with vasopressin infusion could stimulate the baroreceptors resulting in bradycardia. However, in fetal and adult sheep, bradycardia was observed following intravenous AVP infusion even after the hypertensive response was blocked with nitroprusside or selective vasoconstrictor ( $V_1$ -receptor) antagonists (Ervin *et*

*al.*, 1992; Harper and Rose, 1987). No change in heart rate or arterial pressure is seen following infusion of a selective  $V_2$ -receptor agonist (desmopressin) (Ervin *et al.*, 1992). In addition, in fetal lambs given AVP, the fall in heart rate has been reported to precede or occur simultaneously with the pressor effect (Wiriyathian *et al.*, 1983; Iwamoto *et al.*, 1979). In the Iwamoto study (1979), the average time for the onset of bradycardia following AVP infusion was  $4.0 \pm 0.4$  minutes while hypertension began on average  $5.3 \pm 1.1$  minutes after the initiation of drug administration. Iwamoto (1979) also administered atropine to fetal lambs during vasopressin infusion to determine if the bradycardia was vagally mediated. Atropine failed to return heart rate to control levels. Furthermore, vasopressin administration to bilaterally vagotomized fetal lambs (Miyake *et al.*, 1991) or adult dogs (Varma *et al.*, 1969) results in persistent but smaller decreases in heart rate. These data suggest that the fall in heart rate observed during vasopressin infusion is not solely a reflex response to the increase in arterial pressure. Bradycardia may also be caused by vasopressin-induced constriction of coronary vessels (Varma *et al.*, 1969) as, in adult dogs (Liard *et al.*, 1982; Heyndrickx *et al.*, 1976) and goats (Fernandez *et al.*, 1998), vasopressin infusion reduces coronary blood flow. However, blood flow to the heart is unchanged during AVP administration to fetal lambs (Iwamoto *et al.*, 1979) negating this as the cause of myocardial depression in the fetus. In adult dogs a persistent although attenuated bradycardia is seen in response to vasopressin infusion following complete denervation of the heart (Wang *et al.*, 1987). Furthermore, vasopressin-induced bradycardia is still seen in fetal lambs following autonomic blockade with hexamethonium (Tomita *et al.*, 1985). Thus, although the mechanism is not clear, it appears that vasopressin acts, at least in part, directly on the heart to produce bradycardia in the adult and fetus.

The difficulties encountered when attempting to determine the exact role that AVP plays in cardiovascular regulation reflect the intricacies of the *in vivo* system. Given the numerous interactions involved in cardiovascular regulation, including those among the autonomic nervous system, renin-angiotensin system, and arginine vasopressin, it is difficult

to experimentally alter one variable without influencing the remaining mechanisms (Bennett and Gardiner, 1985). Elevated vasopressin levels seen under stressful conditions clearly do affect the cardiovascular system of fetal and adult sheep. The role played by AVP at basal concentrations is less apparent but likely also considerable and may involve both pressor and anti-diuretic functions (Share, 1988; Bennett and Gardiner, 1985; Cowley *et al.*, 1983).

#### 7.4.4 Fetal versus adult responses

There are very few reports in the literature in which cardiovascular responses to vasopressin infusion have been compared in fetal and adult sheep. Rurak (1978) infused AVP into fetal lambs at 0.5 to 4.1  $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  and into non-pregnant ewes at 0.4 to 2.9  $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  for 60 minutes. This resulted in plasma vasopressin concentrations of ~15–505 pg/ml in the fetuses and 100–1200 pg/ml in the adults. Hypertension and bradycardia were seen in both groups, but the pressor effect of AVP was greater in the fetal lamb. Relative to the adult, the slope of the dose-response curve for arterial pressure was increased and shifted to the left. In the fetal lambs, arterial pressure rose 27 to 43% while heart rate fell 14 to 41%. Arterial pressure was elevated 9 to 16% above control values in the adult ewes while heart rate fell 20 to 45%.

In contrast to the results of Rurak, Harper and Rose (1987) reported a similar rise in arterial pressure but a greater fall in heart rate in the ewe compared to the fetus. Exogenous vasopressin was infused to fetal and adult sheep at 3.2  $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  for 30 minutes. Fetal plasma AVP levels averaged  $113.2 \pm 40.7$  pg/ml but adult plasma concentrations were not reported. Arterial pressure rose similarly in the fetus and ewe (23.8% versus 26.5%) but fetal heart rate fell only half as much as the adult (26.2% compared to 47.6%).

The range of arginine vasopressin doses administered in the current study were slightly lower than in the above two reports. AVP was given for 90 minutes at 0.2 to 3.2  $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  (mean =  $1.6 \pm 0.3$   $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) to the fetal lambs and between 0.4 and 0.9  $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  (mean =  $0.6 \pm 0.1$   $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) to the adults. Over the range of plasma

vasopressin concentrations studied (1–112 pg/ml), arterial pressure rose an average of  $15.5 \pm 1.3\%$  in the fetuses and  $14.5 \pm 2.7\%$  in the adults. Vasopressin-induced bradycardia was twice as great in the adult compared to the fetus ( $34.2 \pm 1.7\%$  versus  $16.3 \pm 1.3\%$ ). Thus our findings are similar to Harper and Rose (1987) in that exogenous AVP administration led to similar pressor effects but a greater bradycardia in the adult relative to fetal sheep. Our data were generally in the lower range of that reported by Rurak (1978) and Harper and Rose (1987) reflecting our lower AVP doses. Neither of these authors measured hindlimb blood flow, which we found to fall to a similar degree in the fetus and adult during vasopressin infusion. Although venous pressures were not available, hypertension and vasoconstriction indicate an increase in vascular resistance. A regression analysis of estimated hindlimb resistance yielded no significant difference between the fetus and ewe. There is, therefore, no evidence for a greater sensitivity of fetal vascular smooth muscle to arginine vasopressin. In contrast, the fetus does appear to be less sensitive to the negative chronotropic effect of vasopressin. This may be a protective mechanism as, compared to the adult, the fetus has a limited ability to increase stroke volume in order to maintain cardiac output when heart rate is reduced (Heymann, 1989).

A possible explanation for the differing results in the above studies comparing AVP cardiovascular responses in the adult and fetus could be differences in the phase of the estrous cycle of the non-pregnant ewes. In adult rats, the pressor response to arginine vasopressin is related to both gender and stage of the estrous cycle with males and estrous females demonstrating a greater response than non-estrous females (Wang *et al.*, 1997; Wang *et al.*, 1996). This appears to be due to a difference in vasopressin release as metabolic clearance of AVP is not different (Crofton *et al.*, 1988). This variation with phase of the estrous cycle suggests that the gonadal steroids may play a role. In ovariectomized rats, plasma vasopressin concentration is decreased by progesterone while estrogen has been reported to have no effect on (Crofton *et al.*, 1985) or to increase AVP levels (Skowsky *et al.*, 1979). In non-pregnant adult ewes, progesterone concentrations similar to

those observed during the luteal phase of the sheep estrous cycle (with or without appropriate levels of estradiol) cause significant reductions in arterial pressure and increase plasma volume (Pecins-Thompson and Keller-Wood, 1997; Roesch and Keller-Wood, 1997). Unlike with rats, progesterone implants in sheep are associated with slightly increased levels of AVP; however, vasopressin levels are increased to a similar degree with sham implants (Pecins-Thompson and Keller-Wood, 1997).

Rurak (1978) administered vasopressin to his non-pregnant ewes at 0.4 to 2.9  $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ . As the AVP doses in the current study were on the lower end and those of Harper and Rose (1987) were on the higher end of this range, we would expect his non-pregnant ewes to demonstrate increases in arterial pressure intermediate to these two studies (i.e., between 14.5 and 26.5%). However, Rurak observed only 9–16% increases in arterial pressure. This blunted pressor response may be due to his ewes being in the luteal phase of the estrous cycle (increased progesterone concentration). This explanation cannot be ruled out as in none of these studies was the estrous cycle phase of the ewes determined.

Clearance of arginine vasopressin in our fetal lambs was 3.7 times as great as in the adult ewes ( $83.6 \pm 11.2 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  versus  $22.5 \pm 2.5 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ). These clearance values are similar to those in previous fetal and adult sheep studies (Harper and Rose, 1987; Tomita *et al.*, 1985; Stegner *et al.*, 1984a; Wiriyathian *et al.*, 1983). However, these values are higher than those reported by Rurak (1978) who calculated clearances of  $23.3 \pm 1.6 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  in the fetal lamb and  $8.1 \pm 0.5 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  in the non-pregnant ewe. This may reflect that plasma AVP concentrations were determined by radioimmunoassay in the former studies while bioassay was used by Rurak. Bioassays for arginine vasopressin are much less precise as they tend to be less specific and less reproducible (Mohring *et al.*, 1982; Johnston, 1972). An overestimate in vasopressin values would lead to an underestimate of clearance. Despite differences in the absolute values, Rurak (1978) also found fetal clearance of AVP to be ~3-fold greater than in the adult. Under basal

conditions, AVP levels in the adult and fetus are similar, thus the fetus must have a much higher rate of vasopressin secretion.

The placenta has been suggested as the major site of vasopressin clearance as, in exteriorized fetal lambs, AVP levels in the umbilical vein are substantially lower than in the umbilical artery (Jones and Rurak, 1976). However, in lambs studied *in utero*, no significant plasma AVP arteriovenous concentration gradient was found across the umbilical circulation (Wiriyathian *et al.*, 1983). Moreover, a lack of correlation between maternal and fetal paired blood samples and between twin fetal lambs indicates that arginine vasopressin does not cross the placenta (Iwamoto *et al.*, 1979; Rurak, 1978). Elevations in fetal plasma AVP concentrations due to fetal stress (hemorrhage or hypoxemia) are associated with high levels of vasopressin in the urine (Ross *et al.*, 1986a; Ross *et al.*, 1986b; Daniel *et al.*, 1984). Thus, it appears that AVP is cleared by the fetal kidney as it is in adults. This is supported by the observed increase of arginine vasopressin levels in amniotic fluid, at rates consistent with fetal urine excretion following administration of the hormone (Ross *et al.*, 1985; Stark *et al.*, 1984a; Wiriyathian *et al.*, 1983). Because the amnion and chorion of the ovine fetus are extensively vascularized, substances may be transferred between the amniotic fluid and the fetal circulation via blood vessels on the surface of these membranes (Hedriana *et al.*, 1997; Brace, 1995; Hedriana *et al.*, 1995; Brace *et al.*, 1992; Gilbert *et al.*, 1991b). For example, AVP injected intra-amniotically leads to increased vasopressin levels in fetal plasma even when the esophagus is ligated (Gilbert *et al.*, 1991b) or a glove is sewn over the fetal head (Gilbert *et al.*, 1991a). During intravenous AVP infusion, blood flow to the fetal membranes initially increases and then declines (Hedriana *et al.*, 1997). The increased blood flow could facilitate AVP clearance from plasma to the amniotic compartment while the subsequent decline in perfusion would reduce the rate of AVP resorption via the intramembranous pathway. However, although AVP appears able to cross from the amniotic cavity to the plasma via the intramembranous pathway, no evidence has been presented for the converse route (i.e., from plasma directly to amniotic fluid). Thus, AVP is

most likely cleared from the fetal circulation by the kidney while amniotic fluid may serve as a reservoir for the hormone.

In the current study, arginine vasopressin infused to fetal lambs at 4.5 mU/min resulted in a reduction in hindlimb blood flow to  $71.8 \pm 1.9\%$  of control. This is similar to the reduction in flow observed during mild ischemia ( $77.4 \pm 0.3\%$  of control) achieved by partial inflation of an occluder on the fetal external iliac artery (Section 6). There were no differences in the change in arterial  $P_{O_2}$  ( $0.8 \pm 0.3$  versus  $-1.1 \pm 0.6$  mmHg from control), oxygen saturation ( $1.4 \pm 0.9$  versus  $-1.7 \pm 1.1\%$  from control), or oxygen content ( $0.00 \pm 0.05$  versus  $-0.21 \pm 0.13$  mM below control) between the vasopressin and mild ischemia experiments. In both studies, lactate was released from the hindlimb, significantly more so with mild ischemia ( $-3.16 \pm 1.75 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) than with AVP ( $-0.64 \pm 0.53 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ). The greater efflux with mild ischemia likely reflects the small sample size ( $n=3$ ) as lactate efflux during moderate ( $0.73 \pm 1.1 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) and severe ischemia ( $-0.25 \pm 1.62 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ) were more consistent with the vasopressin results. Vasopressin administration was also associated with a greater hindlimb glucose uptake compared to mild ischemia ( $4.92 \pm 0.34$  versus  $0.73 \pm 0.57 \mu\text{mol}\cdot\text{min}^{-1}\cdot 100\text{g}^{-1}$ ). However, glucose flux was consistently low in all the ischemia animals, even at control. It should be noted that the lactate and glucose fluxes were not significantly different from their respective control values for either experiment.

No significant bradycardia or hypertension were observed with mild ischemia. In contrast, during administration of 4.5 mU/min AVP, fetal arterial pressure was significantly elevated (to  $16.5 \pm 1.6\%$  above control), and heart rate was reduced  $17.2 \pm 1.5\%$  from the control value. The degree to which hindlimb oxygen delivery was reduced was similar for the arginine vasopressin infusion ( $72.5 \pm 2.0\%$  of control) and mild ischemia ( $73.1 \pm 3.1\%$  of control) experiments. In both cases, oxygen consumption was maintained due to a rise in extraction.

In summary, we infused arginine vasopressin to fetal lambs and adult ewes to achieve plasma hormone levels which are observed during various forms of stress including hemorrhage and hypoxemia. Both the fetus and non-pregnant ewe responded similarly to the AVP administration. No changes in arterial pH or  $P_{O_2}$  were seen. Vasopressin did reduce oxygen delivery to the hindlimb by decreasing hindlimb blood flow. Over the range of plasma AVP levels studied (1–112 pg/ml), adult and fetal sheep demonstrated similar degrees of hypertension and increased vascular resistance. However, bradycardia was more profound in the adult. Thus, we did not find evidence for a greater sensitivity of fetal vascular smooth muscle to vasopressin, but the fetal heart does appear to be less sensitive to the negative chronotropic effects of this hormone.

In addition, the reductions in hindlimb blood flow and oxygen delivery observed with the 4.5 mU/min arginine vasopressin infusion were similar to those seen with mild ischemia. The fetuses responded similarly to the two perturbations: oxygen consumption was maintained by a rise in extraction and there were no differences in arterial  $P_{O_2}$ , oxygen saturation, or  $O_2$  content. Lactate efflux from the hindlimb was less during vasopressin administration, and glucose uptake was greater. However, neither of these variables were significantly different from their respective control values. No significant cardiovascular changes were seen with mild ischemia while exogenous arginine vasopressin resulted in fetal bradycardia and hypertension. Thus, for a similar reduction in hindlimb blood flow, the fetus responded similarly whether this reduction was caused by mechanical means (partial occlusion of the external iliac artery) or by exogenous administration of a hormonal vasoconstricting agent (arginine vasopressin). The exception to this was in the cardiovascular response. That the bradycardia and hypertension recorded during AVP infusion were not evident during mild ischemia very likely reflects the local nature of the latter perturbation compared to the whole body effects of vasopressin infusion.

## 8. SUMMARY AND CONCLUSIONS

The purpose of our experiments was to determine hindlimb blood flow and metabolic responses to normal and pathophysiological conditions in the late gestation fetal lamb. The hindlimb, which is composed of skin, bone, and skeletal muscle, is considered to be representative of the fetal carcass. As the carcass, or non-visceral tissues, represents the majority of fetal mass and receives a large proportion of cardiac output, metabolic changes in these tissues can profoundly affect the fetus as a whole. We examined the fetal vascular anatomy and devised a surgical protocol which allowed us to sample blood entering and leaving the hindlimb as well as to continuously measure hindlimb blood flow using a transit-time flow transducer. Normal weight and blood flow distribution patterns could then be determined for the hindlimb. Under normal conditions, hindlimb blood flow appears to fluctuate with fetal behavioural state. As the mechanisms involved are unknown, we examined this relationship further.

Despite the significant nature of the carcass, little information is available regarding its metabolic responses to abnormal situations such as reduced oxygen supply. Although, circulating lactate levels are known to rise more markedly when oxygen delivery is reduced by hypoxemia than when reduced by decreased blood flow, the role of the carcass in this response has not been examined. This prompted our study of hindlimb reactions to hypoxemia and ischemia. Arginine vasopressin (AVP) has been implicated in the cardiovascular and vasoconstrictor responses to hypoxemia in the fetus and adult. However, a direct comparison of these responses in the adult and fetus has not been previously conducted. In addition, the vasoactive properties of vasopressin presented an opportunity to compare fetal responses to vasoconstriction achieved by exogenous administration of this hormone versus that accomplished by mechanical means in the ischemia experiments.

*Hindlimb Blood Flow Measurement: Correlation of Radionuclide-Labeled Microsphere and Transit-Time Flow Transducer Techniques*

In order to study hindlimb blood flow and oxygen consumption in the late gestation fetal lamb, we needed to devise a surgical protocol for sampling blood entering and leaving the hindlimb as well as obtain continuous measurements of hindlimb blood flow. Catheters positioned in the pudendoepigastric artery and vein and advanced into the external iliac artery and vein, respectively, permitted sampling of hindlimb blood. To investigate whether a transit-time flow transducer positioned on the external iliac artery could provide accurate, continuous blood flow readings, measurements obtained by this method were compared to results achieved by injection of 15  $\mu\text{m}$  diameter radionuclide-labeled microspheres. Five fetal lambs were instrumented with tracheal and vascular catheters, a vascular occluder on the external iliac artery proximal to the circumflex iliac artery and a transit-time flow transducer distal to this vessel. Hindlimb blood flow was altered by partial inflation of the occluder. Findings were as follows:

- i). Blood flow measurements obtained by the two methods show a close correlation ( $r = 0.865$  to  $0.999$ ).
- ii). These results, in addition to evidence from vascular casting, indicate that the circumflex iliac, internal iliac, and pudendoepigastric arteries appear to be potential sources of error when measuring total hindlimb blood flow with the transit-time flow transducer. Ligation of these vessels (or catheterization of in the case of the pudendoepigastric artery) improved the correlation between the two methods.
- iii). With the incorporation of ligation of these vessels into our surgical protocol, a transit-time flow transducer on the external iliac artery can be used to provide continuous accurate determinations of hindlimb blood flow in the late gestation fetal lamb.

### *Hindlimb Blood Flow and Weight Distribution*

There is little information on the effects of occlusive-type catheterization on hindlimb blood flow and growth. In 6 late gestation fetal lambs, we compared hindlimb weight and blood flow distribution under normal conditions. Longer, larger diameter (1.19 mm outer diameter) catheters were inserted into the non-study limb compared to the study limb (0.94 mm outer diameter). Blood flow was measured with a transit-time flow transducer on the external iliac artery. We observed:

- i). In late gestation (~135 days), the hindlimb is approximately 7.34% of fetal body weight.
- ii). The study and non-study limbs did not differ statistically in terms of hindlimb weight, but blood flow was  $13.4 \pm 1.8\%$  less in the non-study limb.
- iii). This difference in blood flow between the study and non-study limbs appears to be due to differences in the surgical instrumentation (longer, larger diameter catheters used in the non-study limb) rather than to an inherent difference in blood flow between right and left hindlimbs.
- iv). Relative to the percentage of hindlimb weight that they comprise, fetal hindlimb skin and bone are more highly perfused than skeletal muscle i.e., receive a higher proportion of hindlimb blood flow. Although muscle comprised approximately 45% of hindlimb weight, it received only 22% of total hindlimb blood flow.

### *Behavioural State Modulation of Hindlimb Blood Flow*

The late gestation fetal lamb, skeletal muscle activity, in the form of breathing and body movements, are associated with specific patterns of electrocortical (ECoG) and electro-ocular (EoG) activity. In 8 experiments, ECoG and EoG activity were monitored in 7 fetal lambs between 126 and 137 days gestation. Fetal blood gas status, and metabolic and cardiovascular variables were determined during a control (pre-blockade) period and following administration of gallamine triethiodide or pancuronium bromide (post-blockade). These neuromuscular blocking agents abolish fetal breathing and body movements. Our results are as follows:

- i). Neuromuscular blockade did not alter sleep cycle length or the amount of time the fetus spent in each electrocortical state.
- ii). Following neuromuscular blockade, mean hindlimb blood flow was reduced  $8.3 \pm 3.5\%$  ( $p = 0.0510$ ) suggesting that skeletal muscle activity is associated with greater hindlimb blood flow. Blood flow tended to be higher during high voltage electrocortical activity (quiet sleep) both before and during neuromuscular blockade, but the values failed to reach statistical significance. Thus, more data is required before this relationship can be considered conclusive. The current data suggest that neuromuscular blockade did not alter the apparent behavioural state modulation of flow in the fetal lamb hindlimb. As skeletal muscle activity does not appear to be responsible for this phenomenon, this relationship may be directly mediated by the central nervous system.
- iii). The difficulty in obtaining statistically significant differences in hindlimb blood flow with behavioural state or neuromuscular blockade may reflect that the changes in blood flow being considered, when expressed in either absolute or percentage terms, are very small. The average flow difference between electrocortical states was  $\sim 1.5 \text{ ml} \cdot \text{min}^{-1}$ .

$1 \cdot 100\text{g}^{-1}$  or  $\sim 5\%$ , and neuromuscular blockade reduced flow by  $\sim 3.1 \text{ ml} \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$  or  $\sim 8\%$ . Thus, animal condition, measurement variability, and artifacts during data collection may impact the results. In addition, fetal breathing movements, which are associated with the low voltage state and are also abolished by neuromuscular blockade, may also contribute to the reduction in mean hindlimb blood flow seen following gallamine or pancuronium injections. Uterine contractures, postural changes by the ewe, and fetal arousal (awake) episodes are other potential confounding factors.

- iv). Based on the polygraph recordings, hindlimb blood flow appeared to be more variable during quiet sleep and neuromuscular blockade seemed to reduce this variability. We attributed this trend to the prevalence of limb movements during high voltage activity. Variability in mean hindlimb blood flow, expressed as a coefficient of variation, fell from  $7.2 \pm 0.4\%$  pre-blockade to  $7.0 \pm 0.5\%$  post-blockade. During high voltage ECoG activity, blood flow variability was reduced from  $7.1 \pm 0.6\%$  pre-blockade to  $6.1 \pm 0.5\%$  post-blockade. However, these differences were not significant. This lack of significance may reflect that our data acquisition system did not record data frequently enough to capture the rapid variations in blood flow associated with hindlimb activity.
- v). Neuromuscular blockade resulted in an increase in fetal oxygenation with fetal  $P_{\text{O}_2}$  increasing 15.3%, oxygen saturation 17.1%, and oxygen content 17.3%. Hindlimb oxygen consumption fell  $11.8 \pm 4.6\%$ . This suggests that inhibition of skeletal muscle activity, such as occurs during fetal hypoxemia, may be a compensatory mechanism which lowers fetal oxygen demands when faced with a reduction in oxygen supply. No difference in oxygen consumption was seen between the high and low voltage ECoG states. This may be the result of the low sample size (4 pairs of samples) or that the complexity and duration of hindlimb movements during the episodes of high voltage activity were insufficient to increase oxygen consumption.

### *Hypoxemia and Ischemia*

The objective of this study was to compare fetal metabolic and cardiovascular responses to two methods of reducing oxygen delivery to the hindlimb. Hypoxemia is, by definition, a reduction in arterial  $P_{O_2}$  while ischemia causes a reduction in perfusion while  $P_{O_2}$  is maintained. Because fetal  $P_{O_2}$  is low by postnatal standards, factors which reduce  $P_{O_2}$  even lower may affect oxygen diffusion into fetal tissues and thus lower oxygen consumption. In addition, when oxygen delivery is reduced by hypoxemia, circulating lactate levels are higher than when oxygen delivery is reduced via decreased blood flow. This led to the hypothesis that hypoxemia would be less well tolerated by the fetus than ischemia and this would be reflected in an earlier fall in oxygen consumption and/or greater release of lactate by the hindlimb which would contribute to systemic lactic acidemia. Hypoxemia studies, achieved by reducing maternal inspired oxygen levels, were conducted on 7 fetal lambs. Six fetuses were involved in the ischemia experiments, with blood flow reduced by partial inflation of a vascular occluder on the external iliac artery. The results were classified as mild, moderate, or severe based on the reduction in hindlimb oxygen delivery achieved (to ~75, 50, and 25% of the control values, respectively). Our results are as follows:

- i). Arterial and venous  $P_{O_2}$  values fell significantly with increasing severity of hypoxemia. During severe hypoxemia, arterial  $P_{O_2}$  fell from a control value of  $20.2 \pm 0.6$  mmHg to  $13.0 \pm 1.0$  mmHg and venous  $P_{O_2}$  fell to  $7.4 \pm 1.2$  mmHg from a control value of  $16.4 \pm 0.5$  mmHg. In contrast, arterial  $P_{O_2}$  was unchanged with ischemia and the reduction in venous  $P_{O_2}$  was much less (from  $17.6 \pm 0.9$  mmHg during control to  $14.3 \pm 1.2$  mmHg during severe ischemia). With severe hypoxemia, there were also significant reductions in pH, base excess, and oxygen saturation. No changes in these variables were observed with ischemia.

- ii). The fall in oxygen delivery seen with ischemia was a reflection of the reduction in hindlimb blood flow. Although no change in blood flow was seen with mild hypoxemia, the moderate and severe levels were associated with reduced hindlimb perfusion which exacerbated the reduction in oxygen delivery achieved by lowering arterial  $P_{O_2}$ .
- iii). A curvilinear relationship between hindlimb oxygen delivery and consumption was found with no difference in the curves for hypoxemia and ischemia. For both perturbations, a rise in oxygen extraction allowed oxygen consumption to be maintained until delivery was reduced by ~50%. An earlier, but not significant, reduction in consumption seen with hypoxemia may reflect inhibition of skeletal muscle activity. Our oxygen delivery versus consumption results align well with data from other researchers despite the fact that our values are based on hindlimb rather than whole body responses.
- iv). Despite greater reductions in arterial and venous (tissue)  $P_{O_2}$  values during hypoxemia compared to ischemia, the oxygen diffusion gradient (arterial-venous  $P_{O_2}$ ) was not statistically different for the two interventions. As a result, for similar reductions in oxygen delivery, hindlimb oxygen consumption was similar for both hypoxemia and ischemia.
- v). Glucose was taken up by the hindlimb during the control period. Glucose continued to be taken up during and after both hypoxemia and ischemia at rates not different from control.
- vi). Hindlimb lactate changed from net uptake during control to net release during both hypoxemia and ischemia, with the pooled mean lactate efflux values significantly greater than the controls. Lactate was released from the hindlimb during the hypoxemia intervention, whereas with ischemia, the majority of lactate output occurred

during the recovery or post-ischemia period. However, there was no difference in the overall magnitude of the lactate efflux between the two perturbations.

- vii). Arterial lactate values rose significantly with hypoxemia (to  $5.07 \pm 0.48$  mM during severe hypoxemia) but were unchanged with ischemia, which reflects the local nature of hindlimb ischemia versus the whole body effects of hypoxemia. By extrapolating hindlimb lactate efflux to the entire carcass and using published values for fetal lactate clearance during normoxia and severe hypoxemia, it was estimated that carcass lactate output could account for ~29 to 100% of systemic lactate production during hypoxemia. Thus, the carcass (non-visceral tissues) could be an important source of the elevated circulating lactate concentrations observed during this perturbation.
- viii). Hypoxemia is associated with hypertension, bradycardia, and increased vascular resistance. In contrast, no change in arterial pressure or heart rate was observed with ischemia, although hindlimb vascular resistance rose markedly. The increase in resistance with both hypoxemia and ischemia appears to be mainly a reflection of decreased hindlimb blood flow.
- ix). We conclude that, for similar reductions in oxygen delivery, hypoxemia is not associated with an earlier fall in oxygen consumption or greater hindlimb lactate efflux compared to ischemia. Thus, there is no evidence that hypoxemia is less well tolerated than hindlimb ischemia in the late gestation fetal lamb.

### *Arginine Vasopressin Infusion*

In both fetal and adult sheep, arginine vasopressin (AVP) is released from the posterior pituitary in response to stresses such as hypoxemia and hemorrhage. In addition to its antidiuretic effects, AVP acts on vascular smooth muscle  $V_1$  receptors to elicit a pressor response. In this study, we compared the effects of exogenous AVP administration in fetal and non-pregnant adult ewes. A 90 minute infusion of AVP was given to fetal lambs at doses of 0.72 (n=1), 4.5 (n=8), and 9.0 mU/min (n=1) yielding a range of 0.2 to 3.2  $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  fetal weight. Two fetuses received vehicle (saline) only. AVP was also administered to adult ewes at 25 (n=2), 45 (n=3), and 90 mU/min (n=3) yielding a range of 0.3 to 1.7  $\text{mU}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  adult weight. Plasma vasopressin levels, determined by radioimmunoassay, varied from 7–106 pg/ml in the fetuses and 1–256 pg/ml in the adults. Our observations are as follows:

- i). Basal AVP levels were not different between fetal and non-pregnant adult sheep ( $1.8 \pm 0.2$  versus  $1.3 \pm 0.2$  pg/ml).
- ii). The plasma hormone levels achieved are consistent with those seen in various forms of stress including hypoxemia and hemorrhage.
- iii). No change in circulating AVP levels, blood gases, or metabolic variables were seen in the 2 fetuses that received vehicle (saline) only.
- iv). No change in arterial blood gases or pH were seen in the fetal or adult animals during AVP administration. Arterial glucose and lactate concentrations tended to rise during vasopressin infusion in the fetus, but the fluxes was not different from control values. Likewise, circulating glucose and lactate levels increased in the adult, but flux values could not be determined.
- v). In both fetal and adult sheep, hindlimb blood flow fell significantly with arginine vasopressin infusion. This was reflected in similar reductions in oxygen delivery.

Hindlimb oxygen consumption was not determined in the adults, but, in the fetal lambs, consumption was maintained by a rise in oxygen extraction.

- vi). Fetal and adult responses to vasopressin were compared over a range of approximately 1–112 pg/ml. The dose response curves for the 2 groups demonstrated similar degrees of hypertension and increased hindlimb vascular resistance. However, the decline in heart rate was much more profound in the adult (averaging  $34.2 \pm 1.7\%$  compared to  $16.3 \pm 1.3\%$  in the fetus).
- vii). We conclude that there is no evidence for a greater sensitivity of fetal vascular smooth muscle to arginine vasopressin, but the fetal heart does appear to be less sensitive to the negative chronotropic effects of this hormone.
- viii). As has been found by other researchers, clearance of arginine vasopressin by the fetus is approximately 3 times as great as in the adult ( $83.6 \pm 11.2$  versus  $22.5 \pm 2.5$  ml·min<sup>-1</sup>·kg<sup>-1</sup>).
- ix). The reduction in fetal hindlimb blood flow and oxygen delivery seen at the 4.5 mU/min dose is similar to that achieved during mild hindlimb ischemia. The fetuses responded similarly to the two perturbations: oxygen consumption was maintained by a rise in extraction, and arterial  $P_{O_2}$ ,  $O_2$  saturation and content were similar. In neither case was lactate efflux or glucose uptake different from control values. However, bradycardia and hypertension were seen with AVP administration, but were not evident with ischemia. This reflects the local nature of ischemia compared to the whole body responses generated by vasopressin. Thus, with the exception of the cardiovascular effects, the fetus responds similarly to vasoconstriction achieved by a hormonal vasoconstricting agent (arginine vasopressin) or by mechanical means (ischemia).

### *General conclusions*

Contrary to popular belief, the fetus is very responsive to its environment. Even in normal situations, the late gestation fetus experiences cyclical changes in behavioural state, including electrocortical and electro-ocular activity, as well as fetal breathing and body movements. Blood flow, cardiovascular activity, and metabolic variables respond to these behavioural state alterations. For example, hindlimb blood flow tends to be higher and more variable during high voltage electrocortical activity, but more data are required before these relationships can be considered conclusive. However, our data suggest that neuromuscular blockade, which abolishes skeletal muscle activity, does not alter the apparent behavioural state modulation of hindlimb blood flow. Thus, this relationship may not be due to a linkage between skeletal muscle activity and ECoG state but rather may result from direct action of the central nervous system.

The fetus is also relatively adaptable to pathophysiological situations. When faced with a reduction in oxygen delivery, the fetus attempts to maintain oxygen consumption by increasing the extraction of oxygen from the blood. Blood flow may also be preferentially redistributed to the vital organs, such as the heart and brain, and away from peripheral tissues like the carcass. However, these compensatory mechanisms can be limited by the development of systemic and tissue lactic acidemia. In addition, when oxygen supply is diminished, total body oxygen demands are lowered by reducing or abolishing fetal breathing and body movements.

For similar reductions in oxygen delivery, hypoxemia and ischemia appear to be equally well tolerated: the oxygen delivery versus consumption curves are similar and the magnitude of hindlimb lactate efflux is not different for the two perturbations. However, circulating lactate concentrations are significantly greater with hypoxemia. The carcass contribution to this situation could be substantial, accounting for ~29—100% of the total systemic lactate production during this perturbation. Hypoxemia has whole body effects while ischemia is primarily of a local nature. This is evident in the cardiovascular responses

to these interventions: no significant cardiovascular changes are evident with ischemia, while hypoxemia is associated with fetal bradycardia and hypertension.

Arginine vasopressin has been implicated in the cardiovascular responses to hypoxemia observed in both the fetus and adult. In our study, over a similar range of arginine vasopressin levels, fetal and non-pregnant adult sheep exhibited similar degrees of hypertension and peripheral vasoconstriction. However, the fetus appears to be less sensitive to the negative chronotropic effects of this hormone. In addition, when similar reductions in hindlimb blood flow were compared, the fetus responded similarly whether this was achieved hormonally (arginine vasopressin) or by mechanical means (ischemia). Vasopressin infusion elicited systemic cardiovascular effects (hypertension and bradycardia) which were not evident with ischemia. Thus, both hypoxemia and arginine vasopressin generate whole body responses in the fetus while hindlimb ischemia is a local phenomenon.

In conclusion, we were able to catheterize and instrument the hindlimb of the late gestation *in utero* fetal lamb in order to monitor its responses to normal and abnormal conditions. As the hindlimb is composed of skin, skeletal muscle, and bone, it is considered to be representative of the fetal carcass (non-visceral tissues). Our studies illustrate that the hindlimb, and thus the carcass, plays a major role in the dynamic nature of the fetus under normal conditions as well as in its ability to adapt to pathophysiological situations.

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**10. APPENDIX. Unsuccessful fetal surgical preparations**

## APPENDIX. Unsuccessful fetal surgical preparations.

Ewe Number	Gestational Age (days)		Number of Fetuses	Operated Fetus Weight (kg)	Reason for Failure
	Surgery	Delivery			
E1165	124	129	3	2.152	died in utero
E1116	118	123	2	2.363	ewe died during hypoxemia experiment
E146	116	120	1	2.528	died in utero
E1213	123	123	1	2.997	fetus died during surgery
E131x	120	123	2	2.037	died in utero (operated on second fetus)
	126	128		3.386	second fetus died in utero
E172x	125	128	2	3.022	died in utero
E2206b	119	119	2	1.524	died in utero (operated on second fetus)
	125	129		1.403	second fetus died in utero
E786	125	129	2	4.339	died in utero
E3220	119	121	2	3.371	died in utero