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MONASH UNIVERSITY
THESIS ACCEPTED IN SATISFACTION OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
ON.....13 May 2003 .....
.....[redacted].....
Sec. Research Graduate School Committee

ERRATA

p vii, para 1:	"indicates" rather than "indicate"
p xvii, EGTA:	"animoethyl" rather than "aminoeethyl"
p 2, para 2:	"The inverse relationship…is continuous…and has been demonstrated…" rather than "The inverse relationship…are continuous…and have also been demonstrated…"
p 10, para 3:	"Ijzerman" rather than "IJzerman"
p 13, para 2:	"coronary artery disease" rather than "coronary heart disease"
p 15, para 2:	delete left parenthesis between "including" and "Phillips"
p 17, para 3:	change "…the feed-restriction, and also in terms of the arteries studied" and read "the feed-restriction and location of the arteries studied"
p 33, para 2:	change the sentence that begins "In contrast to persistent…" and read "In contrast to the persistent fetal hypertension during 21 days of UPE (108–129d GA) reported in one study (Murotsuki *et al.*, 1997), another study reported UPE for a similar period but at a later gestational age (120–140d GA) does not result in a persistent elevation in arterial pressure (Cock & Harding, 1997)."
p 34, para 1:	change the sentence that begins "Given UPE can…" and read "UPE during different periods of gestation can result in persistent hypertension (Murotsuki *et al.*, 1997) or relative hypotension (Cock & Harding, 1997) that persists into the postnatal period (Louey *et al.*, 2000)."
p 46, para 2, line 4:	"have" rather than "has"
p 49, para 3:	delete "typically" between "performed" and "at"
p 57, para 2:	insert "ketamine" between "and" and "45mg/kg"
p 67, last para:	"Laplace" rather than "La Place"
p 88, Table 2-7:	correct constant (k_2) for Group 2 is 106.4±3.2
p 89, last para:	"differences" rather than "difference"
p 99, para 1:	"Figure 2-25" rather than "Figure 2-24" and "Figure 2-25B" rather than "Figure 2-24B"
p 100, Figure title:	"Figure 2-25" rather than "Figure 2-24"
p 102, para 3:	insert "1" between "Group" and "and"
p 104, para 3:	insert "there remained" between "but" and "a"
p 124, para 3, line 3:	"hypotension" rather than "hypertension"
p 128, para 2, line 3:	change to read "…was inserted caudally into the vessel…"
p 136, para 4:	"15" rather than "12"
p 145, para 3, line 2:	"increases" rather than "increase"
p 145, para 3, line 2:	"changes" rather than "change"
p 149, para 1:	"Appendix 3-4C" rather than "Appendix 3-2"
p 172, para 2:	change "there being no" to read "the absence of a"
p 199, para 1:	change "is able to" to read "provides a means to"
p 214, line 7:	change "Lamb #33"… to read "Lamb #29…"
p 216, Table:	post mortem details for animals #29 and #33 are incorrect. The table should indicate that animal #29 died on 12/04/01, aged 881d and animal #33 underwent post mortem on 04/04/01, aged 876d
p 226:	change "…female IUGR sheep had significantly less lean muscle…" to read "…female IUGR sheep had significantly more lean muscle…"
p 244, Figure:	add "p(trt)<0.05" to Figure 4-33A
p 246, para 1:	change last sentence to read "In some animals, the femoral arteries of both legs had been catheterised during the course of the study to replace non-functional catheters; in these animals, segments of femoral artery were not collected for the study of arterial wall mechanical properties."
p 251, para 3:	"Figure 4-39A" rather than "Figure 4-9A"
p 270, para 2:	change "…superimposed on each other but they are not…" to read "…superimposed on each other. They are not…"

The effects of intrauterine growth restriction on postnatal growth, arterial pressure and the vasculature

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Bachelor of Science (Biomedical) (Honours)

A thesis submitted for the fulfilment of the requirements of the degree of

Doctor of Philosophy

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At birth our death is sealed, and our end is consequent on our beginning.

MARCUS MANILIUS

ASTRONOMICA, CH. 4, L. 923

1ST CENTURY A.D.

TABLE OF CONTENTS

SUMMARY

Increasing evidence from epidemiological and animal studies indicate that a sub-optimal intrauterine environment can have adverse consequences on postnatal health. In terms of arterial pressure, the majority of, although not all, human and animal studies have found that a sub-optimal *in utero* environment leads to postnatal hypertension. The mechanisms by which a gestational insult may programme postnatal cardiovascular function remain unclear, and may differ between animal models. The overall aim of this thesis was to determine the effects of a sub-optimal *in utero* environment, in late gestation, on postnatal somatic growth, arterial pressure and the passive mechanical properties of resistance arteries. These aims were investigated using two different animal models of fetal growth restriction: restriction of utero-placental blood flow in guinea pigs and placental insufficiency in sheep.

Following the ablation of several utero-placental arteries in mid-gestation guinea pigs, offspring were allowed to be born and were studied up to one year after birth. Offspring were divided into three groups according to birth weight: low birth weight (<95g), average birth weight (95–110g) and high birth weight (>110g). Although mean body weights and lengths were significantly different between the three groups at birth, catch-up growth occurred in the low birth weight group in the early postnatal period. After 4 postnatal weeks, the body weights of the low birth weight offspring did not differ from those of the average birth weight group; body weights were not different between the three groups after 9 months. In the one year old adult, arterial pressure and heart rate were not related to birth weight, nor were there significant relationships between birth weight and arterial mechanical properties at this age.

Placental insufficiency, induced by umbilico-placental embolisation (UPE) in fetal sheep from 0.8–0.95 of gestation resulted in a 37% reduction in birth weight at 140d GA (term~147d); body length and ponderal index were reduced by 7% and 8%

respectively. Although arterial pressures were not different between control and intrauterine growth restricted (IUGR) fetuses, the cerebral, mesenteric and femoral resistance arteries from IUGR fetuses were less compliant than those from controls, potentially predisposing these IUGR fetuses to later hypertension.

When UPE was continued until birth, IUGR lambs were 41% lighter and were born, on average, 4 days earlier than controls. IUGR sheep had lower arterial pressure than controls during the first postnatal year. Among young adult females (~2 years of age), there were no differences between IUGR and control sheep in terms of body weight, body composition and arterial pressure. In contrast, male IUGR sheep at this age had lower body weights, were shorter (in body length and height) and had less body fat compared with male controls; arterial pressure was also lower in the male IUGR sheep. At two years, femoral resistance arteries from IUGR sheep were less compliant than those from controls; arterial compliance of cerebral, renal and mesenteric resistance arteries did not differ between the two groups at this age.

The studies presented in this thesis show that fetal growth restriction resulting from impaired placental function in late gestation does not lead to postnatal hypertension. Fetal growth restriction is associated with decreased compliance (determined under passive conditions) of some resistance arteries from the near-term ovine fetus but these alterations do not persist to adulthood. In the postnatal guinea pig and sheep, passive arterial compliance of resistance arteries was not related to birth weight. Both IUGR guinea pigs and female IUGR sheep exhibit postnatal catch-up growth by adulthood; however, IUGR is associated with failure to catch-up in body weight and length, and with reduced adiposity in young adult male sheep.

DECLARATION

I hereby declare that, to the best of my knowledge and belief, this thesis contains neither material previously published or written by another person, or experimental data from another person's work except where due reference is made in the text. This thesis contains no material which has been submitted for the award of any degree or diploma at Monash University or equivalent institution.

[redacted]
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Special thanks to my family for their support from the beginning.

PUBLICATIONS

† data from selected animals included in these papers/abstracts have been included in this thesis (see Chapter 4).

* tissue collected from animals in this thesis were used in these studies; these data are not discussed in this thesis.

data presented in these papers/abstracts are not discussed in this thesis.

All other papers and abstracts are based on data presented in this thesis.

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LIST OF ABBREVIATIONS AND SYMBOLS

AGA	appropriate for gestational age
ANOVA	analysis of variance
BET	betamethasone
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BWt	body weight
bpm	beats per minute
Ca^{2+}	calcium
cm	centimetres
CO_2	carbon dioxide
CRL	crown-to-rump length
CSA	cross-sectional area
d	days
DBP	diastolic pressure
DEX	dexamethasone
DXA	dual emission x-ray atomoscope, dual emission x-ray absorptiometry
ECM	extracellular matrix
e.g.	*exempli gratia*, for example
EGTA	ethylene glycol-bis (β-aminoeethyl ether) -*N,N*-tetraacetic acid
EMG	electromyography
et al.	*et alii*, and others
F	cortisol
FET	fetal
FOAD	Fetal Origins of Adult Disease
G	gauge
g	grams
GA	gestational age
g/dL	grams per decilitre
GFR	glomerular filtration rate
glu	glucose
h	hours
Hct	hematocrit
HPA	hypothalamo-pituitary-adrenal
HR	heart rate
ID	inner diameter
ID/WT	inner diameter to wall thickness ratio
i.e.	*id est*, that is
i.m.	intra-muscular
i.p.	intra-peritoneal
IU	international units
IUGR	intrauterine growth restriction
i.v.	intravenous
k	rate constant
KCl	potassium chloride
kg	kilograms
KH_2PO_4	potassium dihydrogen phosphate
kPa	kilopascals
L	length

l	litre
LBW	low birth weight
LPD	low protein diet
MAP	mean arterial pressure
MAT	maternal
mg	milligrams
$MgSO_4$	magnesium sulphate
ml	millilitres
mM	millimolar
mm	millimetres
mmHg	millimetres of mercury
mmol/L	millimoles/litre
mo	months
NaCl	sodium chloride
$NaHCO_2$	sodium hydrogen carbonate
N.B.	*nota bene*, note well
NIDDM	non-insulin dependent diabetes mellitus
ng/ml	nanograms per millilitre
N_2O	nitrous oxide
NRL	nose-to-rump length
n.s.	not significant
O_2	oxygen
OD	outer diameter
P	pressure
Pa_{CO_2}	arterial partial pressure of CO_2
Pa_{O_2}	arterial partial pressure of O_2
pH	potential of hydrogen
pHa	pH of arterial blood
PHIMR	Prince Henry's Institute of Medical Research
PI	ponderal index
P-IUGR	preterm-born IUGR
PM	post mortem
PR	placental restriction, placentally restricted
PRA	plasma renin activity
PSS	physiological saline solution
R	internal radius
r	Pearson's correlation coefficient
RAS	renin-angiotensin system
rpm	rotations per minute
Sa_{O_2}	arterial oxygen saturation
SBP	systolic pressure
s.c.	subcutaneous
SD	standard deviation
SDS	standard deviation score
sec	seconds
SEM	standard error of the mean
SGA	small for gestational age
SHR	spontaneously hypertensive rat
SNK	Student-Newman-Keuls
TG	thoracic girth
tHb	total hemoglobin concentration
T-IUGR	term-born IUGR
trt	treatment
U-IUGR	unoperated (naturally occurring) IUGR
UN	undernutrition
UPE	umbilico-placental embolisation
V	luminal volume
vs	versus
v/v	volume per volume
wk	weeks
WT	wall thickness
w/v	weight per volume
yr	years

11β-HSD	11β-hydroxysteroid dehydrogenase
°C	degrees Celsius
♀	female
♂	male
µg	micrograms
µm	micrometres
∏	pi
±	plus or minus
<	less than
≤	less than or equal to
>	greater than
≥	greater than or equal to

Chapter 1

Literature Review

1.1 The Fetal Origins of Adult Disease Hypothesis

The Fetal Origins of Adult Disease (FOAD) hypothesis proposes that perturbations to the fetal environment can programme the developing fetus for disease later in life (Barker, 1995b). Programming refers to the concept that events during critical or sensitive periods of development (during embryonic, fetal or early postnatal life) can lead to permanent or long-term changes to the structure or function of organs (Lucas, 1991).

The concept that early life experience can influence postnatal physiology and health is not new (Widdowson & McCance, 1963; Ravelli *et al.*, 1976; Forsdahl, 1978) but in the last 10–15 years, many epidemiological studies, and more recently animal studies, have shown a sub-optimal intrauterine environment can have long-term consequences on the postnatal health of the individual. Investigations of relationships between early growth and later health outcomes flourished after initial epidemiological studies in this field conducted by David Barker and his colleagues at the Medical Research Council Environmental Epidemiology Unit, Southampton, UK. Follow-up of adults born in the UK (Barker & Osmond, 1986; Barker *et al.*, 1989a), in particular Preston (Barker *et al.*, 1990), Hertfordshire (Barker *et al.*, 1989b) and Sheffield (Barker *et al.*, 1993b) where detailed records were taken at birth and in the first postnatal year, indicated that poor fetal growth (as indicated by reduced size at birth) was associated with increased mortality from cardiovascular disease in adulthood. There have since been numerous epidemiological studies reporting a relationship between small size at birth (indices including low birth weight and low ponderal index at birth) and increased incidence of cardiovascular disease and metabolic dysfunction in the adult. A variety of animal

models have provided supporting evidence and have further demonstrated that sub-optimal *in utero* conditions can programme postnatal dysfunction without necessarily affecting size at birth.

1.1.1 Size at birth and later disease: Overview of epidemiological evidence supporting the fetal origins of adult disease hypothesis

Over the past 10–15 years, a large number of epidemiological studies have investigated the relationship between size at birth and postnatal health. The inverse relationship between size at birth and later risk of cardiovascular disease are continuous across a range of birth weights, and have also been demonstrated within the normal birth weight range (Barker *et al.*, 1992; Law *et al.*, 2002). In the majority of epidemiological studies, birth weight has been used as an indicator of a sub-optimal prenatal environment. In addition, postnatal pathophysiology has been associated with other markers of reduced fetal growth such as thinness, reduced body length and disproportionate head size at birth (Barker *et al.*, 1992). Given that numerous reviews (including those by Barker, 1993; Goldberg & Prentice, 1994; Law & Shiell, 1996; Huxley *et al.*, 2000; Osmond & Barker, 2000), and many epidemiological studies indicate increased risk for postnatal cardiovascular disease in low birth weight individuals, the following sections will only provide a brief outline of the epidemiological evidence for FOAD.

1.1.1a Hypertension and cardiovascular disease

In humans, low birth weight has been associated with an increased risk of cardiovascular diseases including ischaemic heart disease (Barker & Osmond, 1986; Leon *et al.*, 1998) and coronary heart disease (Martyn *et al.*, 1996; Stein *et al.*, 1996). Hypertension, a risk factor for cardiovascular disease (McCarron *et al.*, 2002), has also been proposed to have its origins *in utero* (Law *et al.*, 1993). Inverse relationships between size at birth and postnatal arterial pressure have been demonstrated in a number of age groups ranging from children to the elderly, and are apparently independent of postnatal lifestyle factors known to influence cardiovascular health (see reviews by Law & Shiell, 1996; Huxley *et al.*, 2000). The relationship between arterial pressure and birth weight appears to strengthen with age; the reported increase in systolic pressure per 1kg decrease in birth weight is 1–2mmHg in children, 2–3mmHg in middle aged adults and 3–5mmHg in adults aged 51 and older (Law *et al.*, 1993).

Although different groups of individuals were studied at each age, it was proposed from these data that the initiating events for this postnatal hypertension occurred during fetal life, and the hypertension was amplified with age (Law *et al.*, 1993).

1.1.1b Non-insulin dependent diabetes and metabolic syndrome

Reduced fetal growth (in particular reduced ponderal index, which is indicative of thinness) has been associated with impaired glucose tolerance, insulin resistance and increased risk for type 2 diabetes (i.e. NIDDM, non-insulin dependent diabetes mellitus) in adulthood (Hales *et al.*, 1991; Phillips *et al.*, 1994). When postnatal growth was also considered, the greatest risk for NIDDM and insulin resistance was in individuals whose compromised fetal growth was followed by accelerated postnatal weight gain or obesity (Phillips *et al.*, 1994; Forsen *et al.*, 2000). Given the increased risk for conditions including hypertension, NIDDM and hyperlipidemia, individuals that were small at birth may be at an increased risk for the metabolic syndrome (Syndrome X) (Barker *et al.*, 1993a). The "thrifty phenotype hypothesis", proposed by Hales and Barker (1992), suggests these adverse metabolic consequences were the result of permanent metabolic alterations that help ensure survival in the face of fetal undernutrition; when adequate levels of nutrition are restored, these alterations may become maladaptive, leading to metabolic dysfunction.

1.1.2 Small size at birth and later disease? Evidence from human studies not supporting the fetal origins of adult disease hypothesis

Despite apparently overwhelming evidence in humans that reduced fetal growth leads to adult disease, several studies have failed to find a relationship between size at birth and arterial pressure in children (Moore *et al.*, 1996; Bergel *et al.*, 2000) and adults (Falkner *et al.*, 1998; Siewert-Delle & Ljungman, 1998; Kumaran *et al.*, 2000; Leeson *et al.*, 2001). The relationship between birth weight and arterial pressure can change with age. A longitudinal study in children showed a positive relationship at one postnatal week, an inverse relationship at 3 months and a U-shaped relationship at 4 years (Launer *et al.*, 1993). Another study in 8 year old children found no significant relationship between birth weight and arterial pressure (Moore *et al.*, 1996), apparently not supporting the FOAD hypothesis. However, in the same cohort at 20 years of age, a 1kg

decrease in birth weight was associated with a 2–3mmHg increase in systolic pressure (Moore *et al.*, 1999).

In contrast to inverse relationships at older ages, a positive correlation between systolic pressure and birth weight has been shown in the first week after birth (Contis & Lind, 1963; Lee *et al.*, 1976; Versmold *et al.*, 1981; Lauer *et al.*, 1993; O'Sullivan *et al.*, 1996). In children, arterial pressure is related to body size (weight and height) (Voors *et al.*, 1977), and it is possible that the positive correlations (Contis & Lind, 1963; Lee *et al.*, 1976; Versmold *et al.*, 1981; O'Sullivan *et al.*, 1996) reported soon after birth were related to lower body weight of these IUGR newborns. In newborns whose birth weights were less than 1000g, arterial pressures in SGA and AGA infants of similar weights are not different; however, arterial pressures of SGA infants are lower than those of AGA infants with similar gestation lengths (Versmold *et al.*, 1981). Although reported in several studies in newborns, only one study in adults has reported lower systolic pressure in low birth weight individuals (Hoy *et al.*, 1999).

In adolescence, the relationship between birth weight and arterial pressure is unclear, with some studies demonstrating an inverse relationship (Nilsson *et al.*, 1997) while others report no significant relationship (Matthes *et al.*, 1994; Laor *et al.*, 1997). The validity of studies of FOAD in adolescents has been questioned (Barker & Law, 1994; Davis, 1994) as puberty and pubertal growth spurts can affect arterial pressure (Lever & Harrap, 1992); however, in these studies, the stage of puberty of the subjects is not always known or reported (Matthes *et al.*, 1994).

Typically, epidemiological studies of FOAD report a 2–3mmHg increase in arterial pressure for each 1kg decrease in birth weight (Huxley *et al.*, 2000) which is a small effect on arterial pressure for a relatively large decrease in birth weight. The size of the effect appears to be dependent on age (Law *et al.*, 1993) and the study size, with larger studies reporting weaker trends (Huxley *et al.*, 2002). Birth weight and other measures of reduced fetal growth are proxy measures of a sub-optimal intrauterine environment, and studies in animals indicate that perturbations to the *in utero* environment can modify postnatal cardiovascular function without affecting size at birth (Langley-Evans *et al.*, 1996b; Dodic *et al.*, 1998; Hawkins *et al.*, 2000a). As size at birth may be a poor indicator of the intrauterine environment, it is possible the effects of sub-optimal

intrauterine conditions have been underestimated in humans; these relationships between size at birth and later health do not consider individuals whose postnatal health may have been modified by a prenatal insult that did not alter size at birth (Hennessy, 2002).

1.2 Intrauterine growth restriction

1.2.1 Prenatal growth, body weight and proportions at birth

Low birth weight (LBW) is defined as weighing less than 2500g at birth (Wilcox, 2001). However, this definition does not distinguish between infants born preterm (and therefore expected to have low birth weights due to reduced gestation length) and infants who are small for gestational age (SGA). By definition, SGA infants have birth weights below the 10^{th} percentile of weight for a given gestational age when compared with a reference population (Wilcox, 2001). Although use of the SGA classification distinguishes between infants who were light for their gestational age and those born preterm but were an appropriate size for their gestational age (AGA), this classification does not distinguish between SGA fetuses who had slow growth throughout gestation and those whose growth was compromised for only part of gestation. Prenatal insults that lead to intrauterine growth restriction (IUGR) can alter the body proportions of the fetus, with different effects on body weight and length depending on the gestational timing of the insult. It is generally believed that constrained fetal growth throughout gestation leads to proportionate reductions in weight and length at birth whereas fetal growth restriction later in gestation leads to disproportionate (asymmetrical) body growth (Villar & Belizan, 1982; Wollmann, 1998); features of disproportionate fetal growth include reduced body weight, reduced ponderal index (indicative of thinness) and a large head circumference relative to abdominal circumference (brain or head "sparing"). It should be noted that body proportionality in IUGR infants is a continuum and there are not two distinct groups of infants that are clearly defined as symmetrically or asymmetrically growth restricted (Kramer *et al.*, 1989).

Umbilical cord blood samples indicate SGA fetuses and newborns are hypoxemic, hypercapnic, acidemic, hypoglycemic and hyperlactemic (Economides & Nicolaides, 1989; Nicolaides *et al.*, 1989; Economides *et al.*, 1991; Lackman *et al.*, 2001). Similar alterations to arterial blood gases and glucose concentrations have been shown in

several animal models of IUGR (refer to Section 1.5). In late gestation, chronic fetal hypoxemia leads to a redistribution of blood flow to favour organs vital for fetal survival (e.g. brain, heart, adrenals) while blood flow to less vital fetal organs (e.g. kidneys, mesentery, carcass) is maintained or reduced (Cohn *et al.*, 1974; Peeters *et al.*, 1979; Bocking *et al.*, 1988). This redistribution of cardiac output can lead to "brain sparing" where growth of the brain and head continues at the expense of the trunk (i.e. disproportionate fetal growth). Although asymmetrical growth restriction is the more common type of IUGR in humans (Wollmann, 1998; Regnault *et al.*, 2002), both symmetrical and asymmetrical growth restriction have been associated with increased risk for hypertension in later life (Barker *et al.*, 1993a), indicating that perturbations leading to reduced fetal growth during any period of gestation can lead to elevated postnatal arterial pressure.

1.2.2 Factors affecting fetal growth: Relationship to the fetal origins of adult disease hypothesis

A sub-optimal intrauterine environment can induce metabolic, endocrine or structural adaptations in the fetus. These adaptations may be beneficial for survival in the short term, but may also programme persistent changes to fetal tissues that lead to maladaptive changes in the long term. Although human studies demonstrate relationships between reduced fetal growth and postnatal dysfunction, evidence from animal studies indicate that some *in utero* perturbations can modify postnatal cardiovascular function without affecting fetal growth or size at birth. Recent studies also indicate the postnatal environment may amplify the adverse effects of a sub-optimal intrauterine environment on postnatal health. Figure 1-1 shows a possible pathway between a sub-optimal *in utero* environment and postnatal dysfunction and disease; the effects of some of these perturbations on somatic growth and arterial pressure in humans are outlined in the following sections.

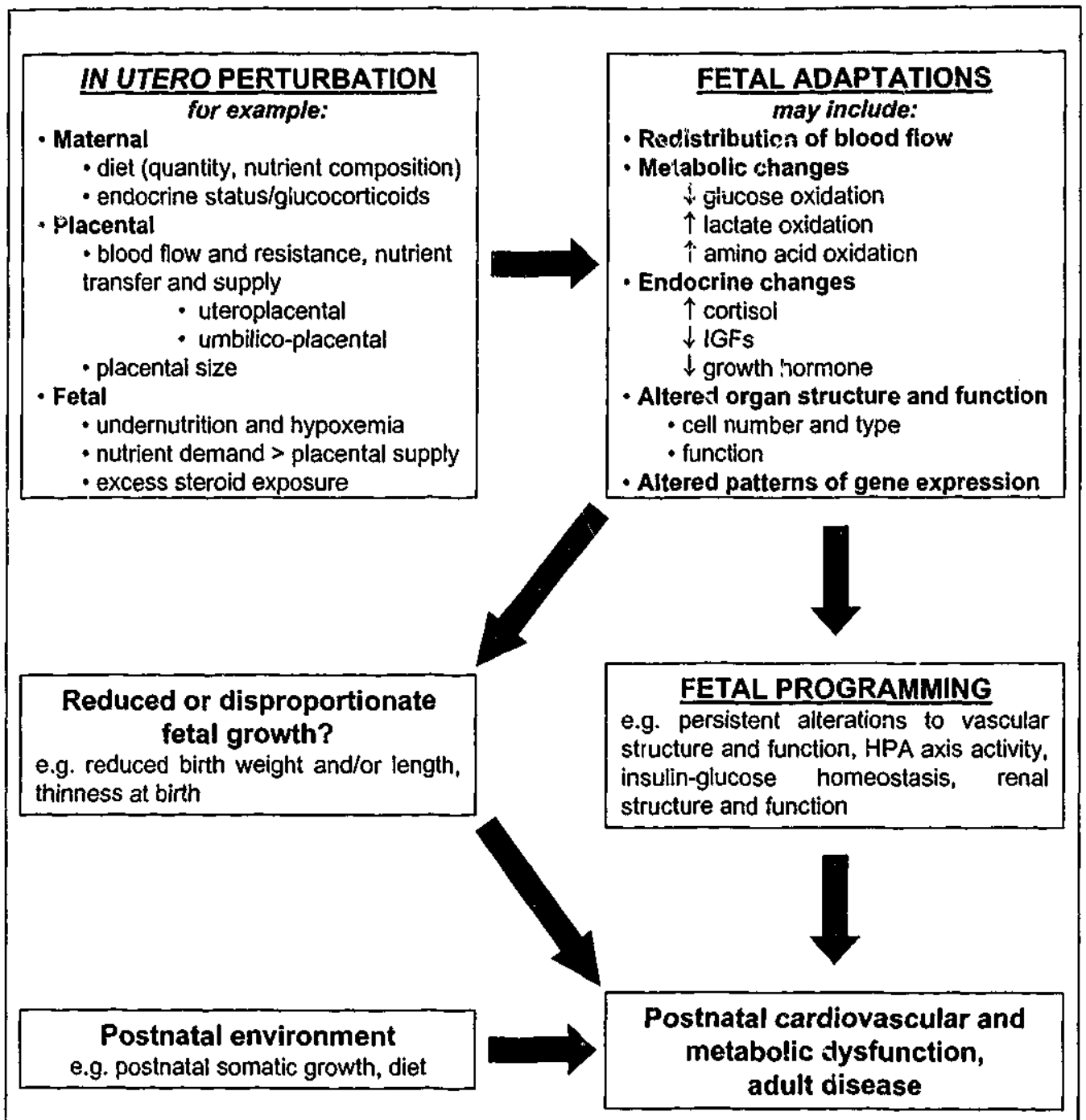


Figure 1-1. A possible pathway between a sub-optimal intrauterine environment and postnatal dysfunction. Perturbations to the *in utero* environment may induce persistent fetal adaptations that to ensure survival in the adverse conditions but become maladaptive in the postnatal period. These programmed changes can lead to cardiovascular and metabolic dysfunction that may be amplified by postnatal environmental or lifestyle factors. Adapted from figures in Goldberg & Prentice, 1994; Langley-Evans *et al.*, 1999; Barker, 2001; Harding, 2001.

1.2.2a Maternal diet

Studies in adults born during and after the time of the Dutch Winter Famine have demonstrated that severe maternal undernutrition (20–40% of pre-famine energy intake) during different periods of pregnancy can have differential effects on fetal growth and

the postnatal health of the offspring (Smith, 1947; Roseboom *et al.*, 2001a). Maternal malnutrition in early gestation (followed by adequate nutrition later in gestation when the famine ended) was associated with increased body weight and length at birth, while famine exposure at mid- and late gestation led to reduced birth weight and length (Roseboom *et al.*, 2001a). Adult arterial pressure was inversely related to birth weight but was not different from that of adults born in the year prior to, or following the famine (i.e. adults not exposed to the famine *in utero*), suggesting that maternal nutritional status was not a key factor in programming postnatal hypertension (Roseboom *et al.*, 1999). In both famine-affected and non-famine areas, low dietary protein relative to carbohydrate intake in the third trimester (rather than reduced total caloric intake) was associated with elevated adult arterial pressure in offspring (Roseboom *et al.*, 2001b). Glucose tolerance was reduced in adults exposed to famine in mid-late gestation, while an increased incidence of coronary heart disease and adult obesity was reported in those undernourished in early gestation (Ravelli *et al.*, 1976; Roseboom *et al.*, 2000; Roseboom *et al.*, 2001a). In contrast, prenatal or early postnatal exposure to famine conditions associated with the Leningrad siege did not alter adult arterial pressure, glucose tolerance, plasma insulin and lipid concentrations despite reductions in birth weight and similar famine conditions to the Dutch Winter Famine (Stanner *et al.*, 1997).

Poor maternal weight gain during pregnancy has been associated with hypertension in offspring in some (Godfrey *et al.*, 1994; Shiell *et al.*, 2001), but not all (Laor *et al.*, 1997) studies. Although birth and placental weights were not related to maternal energy, carbohydrate, protein or fat intakes in one study (Mathews *et al.*, 1999), another study reported that a low calorie maternal diet that was high in protein and low in carbohydrates led to reduced birth weight and hypertension in adulthood (Shiell *et al.*, 2001). Nutrient imbalance in the maternal diet, rather than high dietary protein intake, can lead to elevated arterial pressure in offspring; both high protein, low carbohydrate and low protein, high carbohydrate diets have been associated with hypertension in the adult (Campbell *et al.*, 1996). The composition of the maternal diet may have gender-specific effects on the arterial pressure of the offspring. In adolescent males, systolic pressure was inversely related to maternal protein intake whereas in females, systolic pressure was inversely related to maternal fat intake, despite no relationship

between total maternal energy intake and arterial pressure of the offspring (Adair *et al.*, 2001).

These studies indicate that poor maternal diet, cause by either reduced energy intake or altered dietary nutrient composition can lead to elevated arterial pressure in offspring. These dietary factors have been manipulated in animal models to investigate the underlying mechanisms for the programming of postnatal hypertension; these are discussed in Section 1.5.2.

1.2.2b Gestation length

The FOAD hypothesis proposes the relationships between birth weight and later cardiovascular and metabolic dysfunction are due to impaired fetal growth resulting from fetal undernutrition (Barker, 1995a), rather than reduced birth weight associated with preterm birth. Gestation length of subjects in epidemiological studies (including Barker *et al.*, 1990; Nilsson *et al.*, 1997) are calculated from the mother's recalled last menstrual period, obtained from hospital records. Estimation of gestation length by this method alone is often inaccurate (Savitz *et al.*, 2002), and in some cohorts gestation length is not known in a high proportion of subjects (Phillips *et al.*, 1994). Few studies have investigated the relationship between birth weight in prematurely born individuals and later cardiovascular and metabolic disease, and those who have, report conflicting findings. A study in 8 year old children born preterm (<34 weeks of gestation) reported no direct relationship between birth weight and arterial pressure but arterial pressure decreased with decreasing weight for gestational age (GA) (Morley *et al.*, 1994); that is, for a given gestational age, low birth weight was associated with reduced arterial pressure. In contrast, a study in young adults reported that low birth weight associated with preterm birth (~33 weeks GA) led to an increased risk of hypertension compared with term-born individuals; disease risk was not different between preterm AGA and preterm SGA individuals (Irving *et al.*, 2000). A study in older males (49 years of age) indicated that the relationship between birth weight and arterial pressure is dependent on gestation length (Siewert-Delle & Ljungman, 1998). In males born preterm (≤37 weeks GA), both gestation length and birth weight were independently and inversely related to arterial pressure; in term and post-term born adults, there were no associations between arterial pressure and any measures at birth (Siewert-Delle & Ljungman, 1998).

Although these studies indicate that gestation length can affect the relationship between birth weight and arterial pressure, findings are conflicting and the exact role of gestation length in FOAD remains unclear.

1.2.2c Multiple gestation

The relationship between birth weight and cardiovascular health in twins remains controversial (Doyle *et al.*, 1999; Leon, 1999; Phillips & Osmond, 1999; Ross, 1999). In twins, birth weight is usually less that than of singletons, and reduced fetal growth is evident from 28 weeks of gestation (Min *et al.*, 2000). Despite lower birth weight, studies indicate that twins do not have increased adult mortality (Christensen *et al.*, 1995), risk of ischaemic heart disease (Vagero & Leon, 1994), hypertension or altered glucose tolerance (Baird *et al.*, 2001). One study has shown the inverse relationship between birth weight and arterial pressure is stronger in twins than in singletons (Dwyer *et al.*, 1999), in contrast with another study in which reduced birth weight from twinning was associated with lower arterial pressure than in singletons (Williams & Poulton, 1999).

Studies in twin pairs with discordant birth weights show environmental influences such as reduced placental transfer of nutrients, rather than maternal nutrition, play a key role in the programming of postnatal cardiovascular function. Lower fetal plasma amino acid concentrations have been measured in the lighter twin of a discordant birth weight twin pair (Bajoria *et al.*, 2001). Plasma amino acid concentrations were not different in twin pairs with concordant birth weights, nor were they different in mothers of concordant, or discordant twins (Bajoria *et al.*, 2001). Intra-pair comparisons in twins with discordant birth weights show higher arterial pressure (Poulter *et al.*, 1999; IJzerman *et al.*, 2000) and incidence of NIDDM (Poulsen *et al.*, 1997) in the lower birth weight twin; however, this has not been shown in all studies (Loos *et al.*, 2001b). The inverse relationship between birth weight and arterial pressure has been demonstrated in both monozygotic and dizygotic twins (Christensen *et al.*, 2001). This relationship was attenuated in intra-pair comparisons of monozygotic twins (Christensen *et al.*, 2001) and is in contrast to findings in another study in which there was a significant inverse relationship between birth weight and arterial pressure in monozygotic but not in dizygotic twins (Johansson-Kark *et al.*, 2002). Metabolic dysfunction, but not

hypertension, has been demonstrated in the lighter twin of discordant monozygotic and dizygotic twin pairs (Bo *et al.*, 2000). Given that monozygotic twins are genetically identical, this finding indicates that the fetal environment plays a key role in programming of postnatal metabolic function, rather than maternal nutrition and genetic factors.

1.2.2d Placental size and function

In humans, the relationship between placental size and postnatal cardiovascular disease remains unclear. Elevated arterial pressure has been shown in LBW adults who had had low placental weights (Campbell *et al.*, 1996) or relatively large placentae (Barker *et al.*, 1990). Although birth weight is strongly correlated with placental weight (Williams *et al.*, 1997), placental weight is a crude measure that does not provide insight into placental function, which is important for nutrient supply to the fetus. Placentae from SGA infants may have lower total villous surface areas for nutrient exchange in the absence of changes to the placenta/birth weight ratio (Woods *et al.*, 1982). Animal studies demonstrate the importance of placental size and function for normal fetal growth. The effects on fetal growth, cardiovascular and endocrine parameters following experimentally induced reductions in placental size or substrate transfer are discussed in Sections 1.5.3–1.5.5.

1.2.3 Postnatal growth of the IUGR infant

1.2.3a Postnatal catch-up growth

Catch-up growth is defined as a period of rapid growth, with above average growth rates, following a period of growth restriction (Wit & Boersma, 2002). In follow-up studies of postnatal somatic growth, SGA is often defined as having a birth weight and/or body length below 2 standard deviation scores (–2SDS) of mean weight or length (Albertsson-Wikland *et al.*, 1993; Albertsson-Wikland & Karlberg, 1994; Karlberg & Albertsson-Wikland, 1995; Karlberg *et al.*, 1997) and catch-up growth is said to have occurred when weight or height increases to the normal range, i.e. within 2SDS of the mean weight or height (Karlberg *et al.*, 1997).

A high proportion, but not all of, IUGR infants exhibit catch-up in weight and height in the early postnatal period. Infants who are light and thin at birth catch up in weight and

height within the first postnatal year while those with reduced weight but normal ponderal index at birth remain lighter and shorter than infants of appropriate birth weights (Villar *et al.*, 1982). The majority of SGA infants exhibit rapid catch-up growth within the first postnatal year; 86–90% catch up in height by one year and only 6–8% fail to reach a height within the normal range by 18 years (Karlberg & Albertsson-Wikland, 1995). Of individuals who were short for gestational age at birth, 62% had low birth weights, but by five postnatal months only 16% remain short and 10% remain light compared with normally grown infants (Karlberg *et al.*, 1997). Catch-up in body weight occurs at different rates in males and females. Body weight of 85% of SGA females were within the normal range by 4 months; in contrast, weight catch-up is more rapid in males and by 3 months, 85% of SGA males were within the normal range for weight (Albertsson-Wikland *et al.*, 1993). Similarly, there were gender-specific differences in catch-up with height. In addition to having low birth weights, 54% of males and 69% of females have reduced body length at birth (Albertsson-Wikland *et al.*, 1993) but in contrast to the more rapid weight catch-up in males, height catch-up was more rapid in females. By two years, only 15% of SGA females and 22% of SGA males remained shorter than appropriately growth infants (Albertsson-Wikland *et al.*, 1993). At final height at 18 years, only 6–8% of individuals who were light or short at birth remained below –2SDS of mean height (Albertsson-Wikland & Karlberg, 1994; Karlberg & Albertsson-Wikland, 1995; Karlberg *et al.*, 1997).

1.2.3b Postnatal body composition

At birth, SGA infants have reduced body fat, lean mass and bone mineral content (BMC) compared with AGA newborns (Lapillonne *et al.*, 1997). These reductions are related to lower body weights in the SGA infants rather than altered body composition resulting from a sub-optimal intrauterine environment; body compositions are not different between SGA and AGA infants of similar weights (Lapillonne *et al.*, 1997). However, age-related changes in body composition differ between SGA and AGA infants. In addition to reduced BMC at birth, the postnatal increase in BMC in SGA infants is less than that of AGA infants (Minton *et al.*, 1983). Low birth weight adults have reduced total BMC and bone mineral density, lean mass and body fat but for any given body weight, body fat content is higher in individuals that had been light at birth

(Gale *et al.*, 2001). An inverse association between birth weight and the incidence of abdominal obesity has been demonstrated in children (Garnett *et al.*, 2001) and adults (Law *et al.*, 1992; Loos *et al.*, 2001a), although this relationship has not been consistently shown in females (Eriksson *et al.*, 2001; Gale *et al.*, 2001). In males, low birth weight and thinness at birth are associated with increased body mass index (BMI) (Eriksson *et al.*, 2001) and subcutaneous body fat (Loos *et al.*, 2001a) in adulthood.

1.2.3c Postnatal catch-up growth and the fetal origins of adult disease hypothesis

The increased risk for abdominal obesity in LBW individuals may contribute to their increased risk for cardiovascular disease (Daniels *et al.*, 1999), and recent studies indicate accelerated postnatal growth can amplify the risk for cardiovascular and metabolic dysfunction. Individuals who had low birth weights but exhibited increased growth rates in terms of weight, height or BMI are at a higher risk for hypertension (Seidman *et al.*, 1991; Law *et al.*, 2002; Zhao *et al.*, 2002) and NIDDM (Forsen *et al.*, 2000), than those who failed to catch up (Hoy *et al.*, 1999). Insulin resistance is greatest in individuals who were thin at birth but became obese as adults (Phillips *et al.*, 1994). Similarly, thinness at birth followed by catch-up growth with average or above average body weights at 7 years has been associated with increased death rates from coronary heart disease in adulthood (Eriksson *et al.*, 1999). It is also possible that childhood growth and BMI are better predictors for later hypertension than birth weight, as the relationships between hypertension and rapid postnatal growth can be evident in the absence of a significant relationship between arterial pressure and birth weight alone (Falkner *et al.*, 1998; Bergel *et al.*, 2000). The relationship between pre- and postnatal growth and cardiovascular disease may differ between females and males. One study (Osmond *et al.*, 1993) indicated that the highest death rates from cardiovascular disease were in women of low birth weight but above average body weight at one year, and in men of low weight at birth and at one year. However, these gender-specific differences may be related to different timing for catch-up growth in males and females (Albertsson-Wikland *et al.*, 1993).

1.3 Possible mechanisms for programming of postnatal hypertension and somatic growth

While the majority of human and animal studies indicate that perturbations to the intrauterine environment can modify postnatal cardiovascular and metabolic function, the mechanisms for these alterations remain unclear. Given the variety of gestational insults that can occur in humans and that may be induced in animals, and the range of functional effects between these models (refer to Section 1.5 and Tables 1-1–1-4), it is likely that the mechanisms for the altered arterial pressure may differ between different gestational insults.

1.3.1 "Thrifty phenotype hypothesis" and metabolic "thrift"

The "thrifty phenotype hypothesis" suggests that slowing of fetal metabolism and growth may provide short-term benefits for survival but the persistence of nutritional "thrift" in times of adequate nutrition may lead to later metabolic dysfunction and obesity (Hales & Barker, 1992). A study in postnatal pigs provides support for the hypothesis of persistent metabolic "thrift" following undernutrition; severe undernutrition led to reduced body fat but upon refeeding, the amount of body fat became greater than in pigs that had not been undernourished and refed (Widdowson & Shaw, 1973). It is possible that fetal undernutrition associated with a sub-optimal *in utero* environment, followed by adequate postnatal nutrition (i.e. refeeding) can lead to postnatal catch-up growth and increased risk for obesity (refer to Section 1.2.3).

In humans, reduced caloric intake (either under controlled or famine conditions), followed by a period of adequate nutrition can raise arterial pressures above than pre-feed restriction arterial pressures (Brozek *et al.*, 1948). Persistent hypertension following a cycle of undernutrition and refeeding has also been demonstrated in postnatal rats (Ernsberger *et al.*, 1996; Ernsberger *et al.*, 1998), mice (Smith-Vaniz *et al.*, 1970) and pigs (Smith *et al.*, 1964). Undernutrition, followed by adequate nutrition is associated with decreased aortic distensibility (Hembrough & Riedesel, 1970) and structural alterations including left ventricular hypertrophy, myocardial lesions, and fibrotic plaques evident in several large arteries (Smith *et al.*, 1964; Smith-Vaniz *et al.*, 1970).

1.3.2 Resetting of the hypothalamo-pituitary-adrenal axis

The role of glucocorticoids in normal fetal development, and their potential role in the programming of postnatal function, including hypertension, have been extensively reviewed (see Seckl *et al.*, 2000; Challis *et al.*, 2001; Seckl, 2001; Bertram & Hanson, 2002; Matthews, 2002), and only the key points will be outlined below.

In humans, blood cortisol concentrations are elevated in SGA fetuses (Economides *et al.*, 1991) and in LBW adults (Phillips *et al.*, 2000). Such individuals may also exhibit increased cortisol responses to stress (Reynolds *et al.*, 2001). Similarly, several animal models of a sub-optimal intrauterine environment have reported increased cortisol in the fetus (including (Phillips *et al.*, 1996; Cock *et al.*, 2001b) and altered responses to hypothalamo-pituitary-adrenal (HPA) axis challenges (Hawkins *et al.*, 2000a). However, not all perturbations to the *in utero* environment are associated with alterations to the HPA axis (Dodic *et al.*, 2002d), indicating that other neuroendocrine and/or organ systems must be involved.

1.3.3 Altered renal development

The kidney plays an essential role in the long-term regulation of arterial pressure (Folkow, 1982), and impaired renal development has been proposed to be a key mechanism in the fetal programming of arterial pressure (Brenner *et al.*, 1988; Brenner & Chertow, 1994). Nephron number declines with age, and individuals born with fewer nephrons may be at an increased risk of developing hypertension (Brenner *et al.*, 1988). Reduced nephron number, leading to a reduced surface area for filtration, can lead to hypertension, which can result in hyperfiltration and glomerulosclerosis; these can then lead to further loss of glomeruli and amplification of the hypertension (Brenner *et al.*, 1988). In humans, nephrogenesis is normally completed by 36 weeks of gestation (Hinchliffe *et al.*, 1991), and reduced nephron endowment at birth could have lasting effects on renal function and arterial pressure. However, despite reduced glomerular numbers reported in human SGA infants, glomerular volume may be not different (Hinchliffe *et al.*, 1992) or increased (Manalich *et al.*, 2000) compared with that in AGA infants.

Relative kidney weight is reduced in several animal models of asymmetric fetal growth restriction (Langley-Evans *et al.*, 1996a; Vehaskari *et al.*, 2001; Woods *et al.*, 2001) and reduced glomerular number has been reported in IUGR lambs (Bains *et al.*, 1996), piglets (Bauer *et al.*, 2002), rabbits (Bassan *et al.*, 2000) and rats (Merlet-Benichou *et al.*, 1994; Celsi *et al.*, 1998; Woods *et al.*, 2001). In some of these models, IUGR has also been associated with reduced glomerular filtration rates (GFR) and increased sodium retention (Merlet-Benichou *et al.*, 1994; Celsi *et al.*, 1998; Bauer *et al.*, 2000) which may be related to fewer glomeruli in the kidneys. However, these impairments in renal function have not been shown in all animal studies (Woods *et al.*, 2001), and it is possible that reductions in nephron number are compensated for by increased glomerular diameter or volume (Merlet-Benichou *et al.*, 1994; Lucas *et al.*, 1997; Woods *et al.*, 2001).

1.3.4 Altered vascular development

1.3.4a Arterial structure and mechanical properties

Barker *et al.* (1990) proposed that adaptations by a fetus in a sub-optimal *in utero* environment may include redistribution of cardiac output, asymmetrical growth restriction and alterations to arterial structure. Alterations to arterial structure may include impaired elastin synthesis in the aorta and other conduit arteries; reduced arterial compliance from these impairments may predispose the fetus for later hypertension (Martyn & Greenwald, 1997). Results have differed in the few human studies that have investigated the relationship between birth weight and arterial compliance (measured by pulse wave velocity). One study has reported reduced arterial compliance in LBW adults, independent of arterial pressure (Martyn *et al.*, 1995) while others have reported no association between birth weight and arterial compliance (Kumaran *et al.*, 2000; Montgomery *et al.*, 2000).

To date, few studies in animals have investigated the long-term effects of a compromised *in utero* environment on vascular development. Vascular responsiveness to pharmacological agents has been studied in offspring from feed-restricted mothers (refer to next section) but the effects of a sub-optimal intrauterine environment on the structural composition and mechanical properties of arteries has not been fully investigated. A brief (16h) period of growth inhibition in late gestation fetal rats reduces

elastin and collagen contents in the aorta; these reductions were evident at 26 postnatal weeks but not earlier (Berry & Looker, 1973). The effects of this prenatal growth inhibition and altered structural composition of the aorta on arterial pressure and compliance were not reported. Chronic hypoxia in late gestation chick embryos is associated with reduced lumen diameters and decreased lumen:wall thickness ratios in the aorta (Rouwet *et al.*, 2002). These structural alterations to the vasculature might be expected to result in elevated arterial pressure, but in this study, embryos were not hypertensive (Rouwet *et al.*, 2002). In late gestation fetal sheep, carotid artery distensibility differs between twins with discordant body weights; arterial distensibility was lower (i.e. stiffer artery) in the heavier of the twin pair (Roach, 1970) but the relationship between birth weight, arterial pressure and distensibility was not reported. These studies indicate that *in utero* perturbations, including hypoxia and growth restriction, can affect arterial structure and mechanical properties, but the functional consequences, in particular the long-term effects on arterial pressure, remain unknown.

1.3.4b Vascular responsiveness

Although few studies have investigated the effects of sub-optimal intrauterine growth and oxygen supply on arterial structure and mechanical properties, several studies in humans and animals have reported that the prenatal environment can modify endothelial function. In humans, flow-related dilation is impaired in low birth weight young adults (Goodfellow *et al.*, 1998; Leeson *et al.*, 2001) and children (Leeson *et al.*, 1997), as is endothelium-dependent vasodilation (Martin *et al.*, 2000). Endothelium-independent vasodilation does not appear to be altered in low birth weight individuals (Goodfellow *et al.*, 1998; Martin *et al.*, 2000; Leeson *et al.*, 2001).

In animals, the effects of maternal undernutrition on vascular responses to pharmacological agents can vary between studies due to differences in the gestational timing, duration and severity of the feed-restriction, and also in terms of the arteries studied. In rats, a 50% reduction in maternal feed intake throughout gestation leads to increased vasoconstrictor responses and decreased endothelium-dependent relaxation in aortic segments, with no effect on endothelium-independent relaxation (Franco *et al.*, 2002). Similarly, another study showed that endothelium-dependent relaxation was reduced in mesenteric arteries from offspring whose mothers were undernourished

(50% of *ad libitum* feed intake) in the second half of gestation. In contrast to the findings of Franco *et al.* (2002), offspring were not hypertensive, vasoconstrictor responses were not altered, and endothelium-independent vasodilation was increased (Holemans *et al.*, 1999). Although a less severe feed restriction (70% of *ad libitum* feed intake) throughout gestation did not lead to persistent alterations to vasoconstrictor or endothelium-dependent dilator responses, endothelium-independent vasodilator responses were blunted in femoral arteries of offspring from feed-restricted rat dams (Ozaki *et al.*, 2001). In sheep, mild maternal undernutrition (85% of *ad libitum* feed intake) had no effect on the vascular responsiveness of femoral resistance arteries but more severe feed restriction (50% of *ad libitum* feed intake) led to blunted endothelium-dependent and -independent vasodilation in these arteries (Ozaki *et al.*, 2000). These studies indicate that maternal feed restriction can modify vascular responsiveness in offspring, and these changes may be evident in the absence of alterations to arterial pressure. However, these effects on small artery function have only been reported following one type of gestational perturbation and the consequences of other intrauterine insults remain unknown.

1.4 Arterial wall structure and mechanical properties

1.4.1 Structural proteins in the arterial wall: Elastin and collagen

1.4.1a Elastin: Synthesis and age-related changes

Mature elastin is a relatively insoluble scleroprotein found in the extracellular matrix (ECM) of tissues including arteries, lung, skin and ligaments (Gosline, 1976; Rosenbloom, 1984). This protein plays an important role in the elastic recoil of the tissues in which it is found (Gosline, 1976; Rosenbloom, 1984). Arterial elastin is synthesised by fibroblasts and smooth muscle cells (Rucker & Tinker, 1977), and is composed of an amorphous component surrounded by smaller microfibrillar components (Rosenbloom, 1984). The properties of elastin are dependent on intact cross-links (including desmosine and isodesmosine) between lysine residues on individual elastin fibres (Rosenbloom, 1984).

Tropoelastin is the soluble precursor to elastin and differs from mature elastin in amino acid composition and by the lack of desmosine and isodesmosine cross-links (Sandberg *et al.*, 1969). Tropoelastin mRNA levels strongly correlate with elastin synthesis

(Rosenbloom, 1984) which occurs in arteries predominantly in the perinatal period, with minimal turnover of mature elastin in adulthood (Walford *et al.*, 1964; Shapiro *et al.*, 1991). In the chick embryo, tropoelastin gene expression in the aorta increases near term (Barrineau *et al.*, 1981) and in the sheep, rapid accumulation of arterial elastin in several vascular beds occurs between late gestation (140d GA) and three postnatal weeks (Bendeck & Langille, 1991; Bendeck *et al.*, 1994). In rats, there is a rapid increase in aortic elastin content and concentration in the first 12–18 weeks after birth; following this, elastin content continues to gradually increase and elastin concentration gradually decreases throughout adulthood (Looker & Berry, 1972). The relative stability of aortic elastin content throughout adulthood has been demonstrated in sheep (Wells *et al.*, 1999) and quails (Lefevre & Rucker, 1980); humans have no major loss or gain of aortic elastin after the second to third decade of life (Lansing, 1954). In contrast, elastin content in the pulmonary artery increases slightly between the third and eighth decades of life (Lansing, 1954). Age-related changes in the amino acid composition and decreased cross-linkage of elastin have been measured in human aortas (Lansing, 1954; John & Thomas, 1972) may contribute to decreased arterial elasticity with age (Yater & Birkeland, 1930). In humans, desmosine and isodesmosine cross-linkage in arterial elastic fibres decreases between birth and the ninth decade of life (John & Thomas, 1972; Hornebeck *et al.*, 1978); thus the decline in arterial elasticity with age (Yater & Birkeland, 1930) may be related to elastin cross-linkage rather than arterial elastin content.

Elastin synthesis is regulated by a number of factors including oxygenation, hormones, and growth factors (see reviews by Rucker & Tinker, 1977; Wight, 1996). Tropoelastin gene expression is upregulated by glucocorticoids; in sheep, tropoelastin mRNA increases 2–3 fold following 48-hour exposure to dexamethasone in late gestation (Bendeck *et al.*, 1994). In contrast, hypoxia downregulates tropoelastin gene expression *in vitro*; mRNA levels were proportionately decreased with increasing severity and also increasing durations of hypoxia in both neonatal calf pulmonary artery smooth muscle cells (Durmowicz *et al.*, 1991) and neonatal rat lung fibroblasts (Berk *et al.*, 1999). As a number of these factors that are shown to regulate elastin synthesis are altered in human IUGR and animal models of IUGR, it is possible to that sub-optimal intrauterine conditions could affect elastin synthesis. Given the long half-life of this protein, alterations to elastin synthesis *in utero* may have potentially life-long effects. However,

it is also possible that after birth, when conditions are no longer compromised, delayed compensatory elastin synthesis may occur. Following a period of hypoxia, during which elastin gene transcription was reduced, subsequent recovery in normoxic conditions has been shown to increase elastin gene transcription by 51% compared with controls not exposed to hypoxia (Berk *et al.*, 1999).

1.4.1b Collagen: Synthesis and age-related changes

There are several types of collagens, distributed in different tissues (see review by Berg & Kerr, 1992). Collagen is relatively inextensible and provides structural support and tensile strength to tissues (Gosline, 1976). In arteries, collagen is formed by vascular smooth muscle cells (Wight, 1996), and unlike elastin, collagen turnover is continuous throughout life (Berg & Kerr, 1992). In sheep, arterial collagen content increases during fetal (Roach, 1970), perinatal (Bendeck & Langille, 1991; Bendeck *et al.*, 1994) and postnatal life until adulthood (Wells *et al.*, 1999). In the rat, the collagen content of the aorta also increases throughout postnatal life; aortic collagen concentration increases rapidly in the early postnatal period but remains relatively stable after 12 postnatal weeks (Looker & Berry, 1972).

Several factors have been shown to alter collagen synthesis rates in a variety of tissues (see review by Wight, 1996). Hypoxia upregulates pro-α_1 collagen RNA in cultured human cardiac fibroblasts (Agocha *et al.*, 1997). In contrast, feed restriction in rats delays the age-related accumulation of collagen in organs including the kidney, liver and lung (Deyl *et al.*, 1971), with decreases in collagen production related to the severity and duration of the feed-restriction (Spanheimer *et al.*, 1991). In rats, hypertension increases collagen synthesis in a range of arteries including the aorta, mesenteric arteries and cerebral arteries (Ooshima *et al.*, 1974; Ooshima *et al.*, 1975; Iwatsuki *et al.*, 1977; Bashey *et al.*, 1989). This increased collagen synthesis occurred as a result of elevated arterial pressure; collagen synthesis was not altered in the veins of hypertensive rats (Iwatsuki *et al.*, 1977) and returned to control synthesis rates in arteries when hypertensive rats are given antihypertensive agents (Ooshima *et al.*, 1974; Ooshima *et al.*, 1975). Hypertension in rats is also associated with a more rapid turnover of arterial collagen (17d *vs* 70d in normotensive animals) (Nissen *et al.*, 1978). Higher collagen content has been measured in aortas and mesenteric arteries from

hypertensive rats (Ooshima *et al.*, 1974; Iwatsuki *et al.*, 1977), but another study found hypertensive rats had lower total aortic collagen content than normotensive rats (Bashey *et al.*, 1989); this latter study also indicated that type I collagen was increased, and type V collagen was decreased in the hypertensive rats. The lower total collagen content was not consistent with the increased arterial stiffness measured in the hypertensive rats, although the role of other structural components in the arterial wall, or the effect of altered ratios of the different types of collagen on the passive mechanical properties are not known (Bashey *et al.*, 1989).

1.4.1c Elastin and collagen in the arterial wall: Mechanical properties

The amount of elastin and collagen (absolute amounts and relative proportions) and passive mechanical properties differ between arteries from different vascular beds (Fischer & Llaurado, 1966; Cox, 1978). Although elastin and collagen are found in close association with each other, they contribute differently to the mechanical properties of the tissue. Elastin, as its name implies, provides tissues with elastic properties whereas collagen provides a rigid constraint to limit tissue elasticity while also providing tensile strength to the tissue (Gosline, 1976). The elastic modulus (Young's modulus of elasticity) provides a measurement of arterial stiffness and is calculated by deriving the slope of the stress-strain curve (Shadwick, 1999); increased arterial stiffness is associated with an increased slope of the curve (Figure 1-2). The mechanical properties of arteries at low pressures and strains are mainly determined by the amount of elastin in the artery (Roach & Burton, 1957; Gosline, 1976; Berry, 1978; Cox, 1978; Oxlund & Andreassen, 1980). In contrast, arterial mechanical properties at high pressures are determined by collagen (Roach & Burton, 1957; Gosline, 1976; Berry, 1978). However, the incremental elastic modulus at high strains is related more to the proportion of collagen fibres that contribute to wall stress (Cox, 1978), and cross-linkage of collagen fibres (Wells *et al.*, 1999), rather than total collagen content or concentration. The passive mechanical properties at (and above) physiological pressures are determined by relative contributions and interactions between elastin and collagen (Roach & Burton, 1957; Wolinsky & Glagov, 1963).

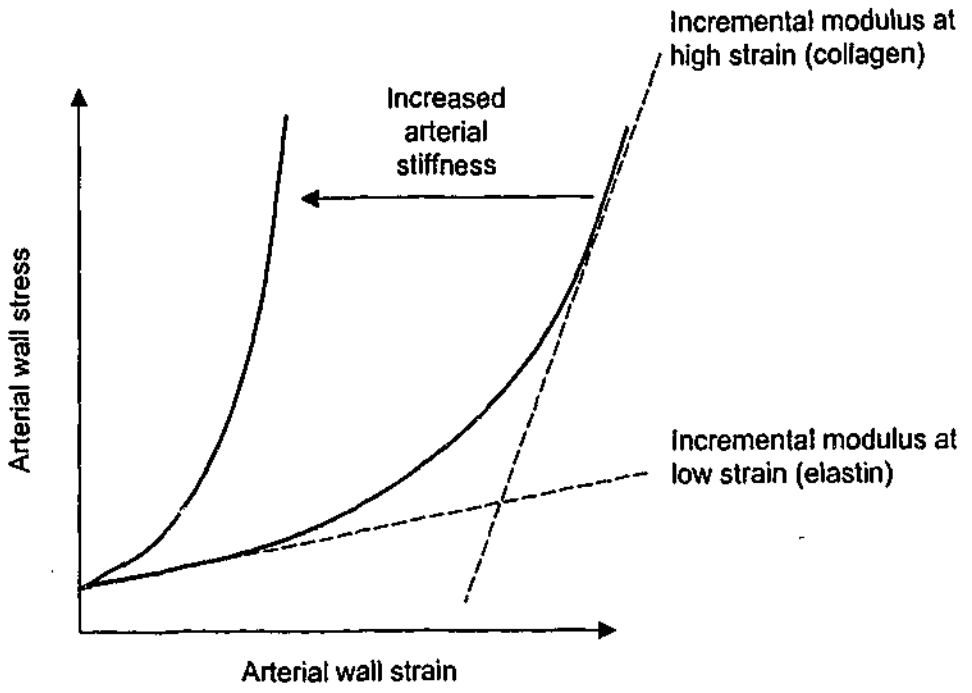


Figure 1-2. The stress-strain relationship and elastic modulus. The relationship between arterial wall stress and strain (Young's elastic modulus, solid curves) can be used as a measure of arterial stiffness. The overall slope of the curve on the left is greater than that of the curve on the right, indicating a stiffer, less compliant artery. Arterial compliance at low strains is predominantly due to elastin, and arterial stiffness at high strains is predominantly due to collagen (dashed lines). At physiological pressures, arterial mechanical properties are due to the combined mechanical properties of collagen and elastin. Adapted from figures in Dobrin, 1978; Wells *et al.*, 1999.

In humans, arterial pressure (Miall & Lovell, 1967) and arterial stiffness (Yater & Birkeland, 1930) increase with age. The elasticity of the human aorta has been shown to increase between th ages of 7 and 27 years of age, after which there is a decline in elasticity (Yater & Birkeland, 1930; Wilens, 1937). An age-related increase in arterial stiffness has also been demonstrated in animals including sheep (Roach, 1970; Wells *et al.*, 1998) and rats (Bashey *et al.*, 1989; Bruel & Oxlund, 1996). Hypertension is also associated with increased aortic stiffness and the age-related increase in arterial stiffness is accelerated in hypertensiv ats (Bashey *et al.*, 1989). An age-related increase in the collagen:elastin ratio may contribute to decreased arterial compliance with age (Cox, 1977). Although some studies indicate the collagen:elastin ratio is a useful indicator of arterial distensibility (Fischer & Llaurado, 1966; Cox, 1977), not all studies have found a correlation between this ratio and arterial mechanical properties (Cox, 1978). The age-related decrease in arterial elasticity may be related to a combination of inefficient elastic recoil following extension and increased resistance from the wall media which

also becomes thicker with age (Wilens, 1937). The decrease in arterial elasticity may also be related to age-related changes to the amino acid composition or the elastin and/or cross-linkage of individual fibres (Lansing, 1954; John & Thomas, 1972) as outlined in Section 1.4.1a. However, the decline in elasticity appears not to be due to an age-related decrease in arterial elastin content (Lansing, 1954).

1.4.2 Arterial wall structure: Geometric design and mechanical properties

In addition to the structural composition of the arterial wall, geometrical design is important in determining the mechanical properties of an artery (see reviews by Dobrin, 1978; Folkow, 1982). The elastic nature of arteries, together with active contributions from the vascular smooth muscle, can alter the lumen diameter and wall thickness of arteries in response to short-term and intermittent changes in arterial pressure to maintain blood flow to tissues (Folkow, 1982, 1991). As arterial pressure increases, lumen diameter usually increases and the arterial wall becomes thinner to maintain arterial wall tension (Wolinsky & Glagov, 1963); these changes are dependent on the artery being thin-walled and relatively compliant. Persistently elevated arterial pressure can result in arterial wall thickening which occurs at the expense of the lumen; these modifications can strengthen the arterial wall against the increased pressures, but can also result in decreased arterial compliance and lumen size (Folkow, 1978; Mulvany, 1991). Peripheral resistance is largely determined by the lumen diameter of resistance arteries, and according to the Poiseuille relationship, a small change in lumen diameter translates to a large change in resistance to blood flow; therefore, persistent reductions in lumen diameter can lead to chronically elevated arterial pressure (Folkow, 1982; Mulvany, 1999). Both active and passive properties determine the overall mechanical properties of the artery *in vivo* (Mulvany, 1999). Smooth muscle activity contributes to the active but not to passive mechanical properties of arteries (Berry *et al.*, 1975), which are mainly determined by arterial structural components such as elastin and collagen (Dobrin, 1978). It has been proposed that arterial mechanical properties should be tested under passive conditions, in the absence of smooth muscle activity, in order to determine the effects of a perturbation on vascular structure (Mulvany, 1999).

1.4.2a Changes with arterial pressure

Alterations to arterial structure and mechanical properties associated with hypertension have been extensively reviewed (see Berry, 1978; Folkow, 1978; Mulvany, 1991) therefore only key points will be outlined in this section. Hypertension is associated with structural and mechanical alterations to the arterial wall, including increased arterial wall thickness, reduced lumen diameter (or cross-sectional area), reduced lumen:wall thickness or lumen:media ratios, increased wall tensions and reduced arterial elasticity (Wolinsky, 1972; Berry & Greenwald, 1976; Berry, 1978; Folkow, 1978; Mulvany, 1993a); these modifications occur in response to the elevated arterial pressure and can further amplify the hypertension (Mulvany, 1991).

Structural alterations can either be the result of vascular growth (i.e. thickening of the arterial wall at the expense of lumen size) or vascular remodelling (Figure 1-3) (Mulvany, 1993a). Vascular remodelling was initially thought to involve the rearrangement of material in the arterial wall around a smaller lumen, with no increase in the amount of material in the vascular wall (Mulvany, 1993a). More recently, a new classification system indicates that remodelling can involve an increase (hypertrophic), decrease (hypotrophic) or no change (eutrophic) in the material in the arterial wall, and the artery can be outwardly (increase in lumen size) or inwardly (decrease in lumen size) remodelled (Mulvany, 1999).

Although alterations to arterial wall structure and mechanical properties are often secondary to the hypertension (i.e. adaptations that occur in response to the elevated pressure), they may also be apparent prior to the onset of hypertension (Lee & Smeda, 1985). Changes to arterial wall structure and mechanical properties including reduced arterial compliance, increased arterial wall thickness and cross-sectional area are apparent in a range of arteries including the aorta, renal and mesenteric arteries from spontaneously hypertensive rats (SHR) that are in the pre-hypertensive state (Lee & Smeda, 1985; van Gorp *et al.*, 2000). It is therefore possible that primary alterations to arterial structure may predispose the individual to hypertension, and secondary alterations further amplify the hypertension. Likewise, it is possible that arterial structural development is altered in a sub-optimal intrauterine environment and predisposes the fetus for later hypertension.

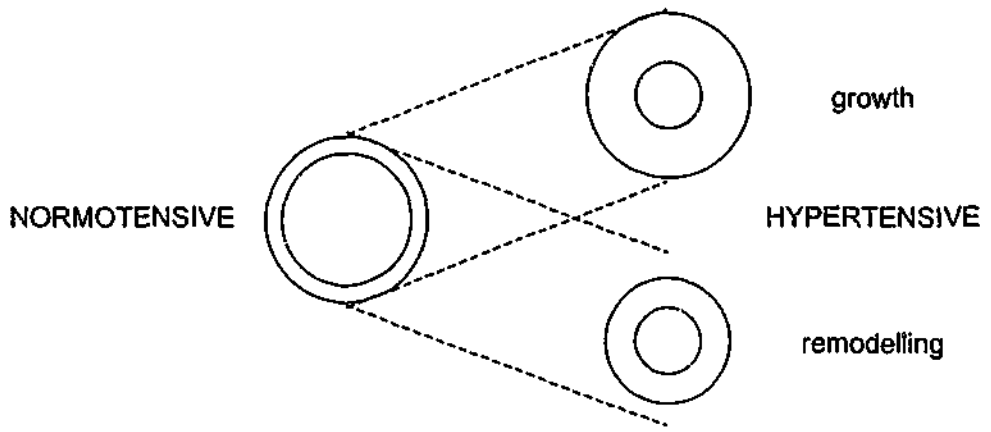


Figure 1-3. Hypothetical arterial cross-sections in normotension and hypertension. Schematic diagram of the cross-sectional areas of arteries in normotensive (left) and hypertensive (right) states. Reductions in lumen diameter and cross-sectional area associated with hypertension can occur as a result of growth of the arterial wall into the lumen (top), or vascular remodelling (bottom). Vascular remodelling involves rearrangement of material in the arterial wall with no change in the cross-sectional area of the vascular wall compared with the normotensive state. Figure adapted from Mulvany, 1993b.

Studies in humans and animals indicate that IUGR fetuses may be hypoxemic, hypoglycemic and/or hypercortisolemic (refer to Section 1.2 and 1.5) which could result in altered synthesis of the structural proteins elastin and collagen in the arterial wall (refer to Sections 1.4.1a and 1.4.1b). The perinatal period is a critical period for the synthesis of elastin, and combined with the long half-life and minimal turnover of this ECM protein in the adult, it is likely that sub-optimal perinatal conditions would have lasting effects on arterial structure and mechanical properties. It has been hypothesised that arterial elastin synthesis is impaired in IUGR fetuses, thereby predisposing these individuals to hypertension (Martyn & Greenwald, 1997), and altered arterial structure may be a key factor not only in the initiation of hypertension *in utero*, but also in the amplification of hypertension in the postnatal IUGR individual.

1.5 Investigating mechanisms underlying the fetal origins of adult disease using animal models

Intrauterine growth restriction has been studied for several decades in animal models. The effects of different gestational insults on fetal growth, blood gas and hormonal status, and cardiovascular development have been reported. While earlier studies have

investigated the short-term effects of these gestational insults on fetal development, more recent studies have allowed offspring to be born for follow-up studies in order to determine the long-term consequences of sub-optimal intrauterine conditions.

In humans, small size at birth (as indicated by a reduced birth weight, thinness at birth, and other related measures) has been used as an indicator of an adverse intrauterine environment. While the majority of evidence in humans indicates a relationship between reduced size at birth and later pathophysiology, animal studies do not consistently find the same relationship between birth size and postnatal cardiovascular function, in particular arterial pressure. Following are summaries of several animal models used to determine the effects of a sub-optimal intrauterine environment on birth size, and fetal and postnatal parameters including somatic growth and arterial pressure.

1.5.1 Naturally occurring variations in birth weight

In polytocous species, fetal weight can vary depending on fetal number and position in the uterine horn (McLaren & Michie, 1960). Individual birth weights of offspring tend to be lower in large litters than those from smaller litters; this has been shown in guinea pigs (Ibsen, 1928; Eckstein & McKeown, 1955), rats (Barr *et al.*, 1969), mice (McLaren & Michie, 1960) and pigs (Milligan *et al.*, 2002). In large litters, fetal size can also be influenced by uterine position due to unequal blood supply to different parts of the uterus, and these positional effects on birth weight can differ also between species (McLaren & Michie, 1960). In rats, the smallest fetus is usually found at the ovarian end of the uterine horns and the largest in the middle position of each uterine horn (Barr *et al.*, 1969). In contrast, the smallest fetuses in mice are found at the ovarian end and in the middle positions of the uterine horn (McLaren & Michie, 1960). There is a trend for fetal guinea pigs at the cervical end of the uterine horn to have greater body weights than those found at the ovarian end (Turner & Trudinger, 2000); these IUGR fetuses also have reduced placental weights, blood flow and nutrient transfer rates (Saintonge & Rosso, 1981; Myers *et al.*, 1982).

Studies have used naturally occurring variations in birth weight to investigate relationships between size at birth and postnatal physiological function in pigs. IUGR piglets are asymmetrically growth restricted (Bauer *et al.*, 2000) and catch up in body

weight between 3 months (juvenile) and 12 months (adult) of age (Poore & Fowden, 2002). The relationship between birth weight and arterial pressure in newborn piglets is unclear. Mean arterial pressure (MAP) has been reported to be lower (–6mmHg) in IUGR piglets (Bauer *et al.*, 2000) or not different from that of normal birth weight controls (Bauer *et al.*, 2002). Despite these conflicting findings with regard to arterial pressure, renal development is impaired in these IUGR piglets, as evidenced by a reduced GFR and glomerular number (Bauer *et al.*, 2000; Bauer *et al.*, 2002) but the long term consequences of these impairments, however, remain unknown. Although there are no significant differences in the MAP of low and high birth weight juvenile pigs, MAP is inversely correlated with birth weight at this age, but not in the adult (Poore *et al.*, 2002). Low weight and thinness at birth was associated with impaired glucose tolerance in the adult (Poore & Fowden, 2002). The mechanisms for the inverse relationship between birth weight and arterial pressure in this model remains unclear, although increased sympathetic nervous system activity and re-setting of the HPA axis and renin-angiotensin system (RAS) may be involved (Poore *et al.*, 2002).

1.5.2 Altered maternal nutrition

1.5.2a Feed restriction

Rats Severe maternal feed restriction (30% of *ad libitum* feed intake) throughout gestation produces IUGR in offspring who do not catch up in weight until 30 postnatal weeks (Woodall *et al.*, 1996). Adult offspring from feed-restricted dams are hypertensive, and an inverse relationship between birth weight and systolic pressure is apparent at 30 weeks (Woodall *et al.*, 1996). Increased food intake in the young adult offspring following severe maternal feed restriction has been reported, and a postnatal hypercaloric diet can amplify hypertension in these offspring (Vickers *et al.*, 2000). Mild maternal feed restriction (70% of *ad libitum* feed intake) throughout gestation also induces persistent hypertension in offspring with the hypertension being evident at an earlier age in male offspring compared with females (Ozaki *et al.*, 2001). This level of maternal undernutrition was also associated with reduced vasoconstrictor responses to phenylephrine and noradrenaline at 20 postnatal days; however, these blunted responses did not persist and were not present at 100 or 200 days of age, at which time the offspring were hypertensive (Ozaki *et al.*, 2001). At postnatal 100 days, endothelium-independent vasodilation was blunted in femoral resistance arteries of both

male and female offspring from undernourished dams; endothelium-dependent relaxation was not different between groups in the postnatal period (Ozaki *et al.*, 2001). In contrast, increased vasoconstrictor responses and decreased endothelium-dependent vasodilation were reported in hypertensive offspring following a 50% reduction in maternal food intake throughout gestation (Franco *et al.*, 2002). These studies indicate that maternal feed restriction in rats leads to hypertension in the offspring, however, the mechanisms for this hypertension may differ between nutritional regimes.

Guinea pigs Mild maternal undernutrition (85% of *ad libitum* feed intake) throughout gestation has gender-specific effects on the prenatal growth of offspring. At birth, male offspring were 17% lighter than controls from *ad libitum* fed mothers and asymmetrically growth restricted but in female offspring, size at birth was not reduced (Kind *et al.*, 2002). Similar gender-specific effects on birth size were reported in an earlier study using the same prenatal nutritional regime (Kind *et al.*, 1999). It is unclear why maternal feed restriction reduced fetal growth in male but not female offspring; the authors suggested low numbers of female offspring in both studies may have been a contributing factor (Kind *et al.*, 1999; Kind *et al.*, 2002). After birth, growth rates (both absolute and fractional) of offspring from *ad libitum* fed and feed-restricted mothers were not different (Kind *et al.*, 2002), and as a result the IUGR male offspring remained lighter than control males at 100 postnatal days (Kind *et al.*, 1999; Kind *et al.*, 2002). At this age, IUGR males (but not females) were hypertensive (Kind *et al.*, 2002), insulin intolerant (Kind *et al.*, 2003), and had altered cholesterol metabolism (Kind *et al.*, 1999). Although systolic pressure was inversely correlated with birth weight and birth weight/birth length ratio in the offspring from *ad libitum* fed mothers, arterial pressure in the total cohort of guinea pigs (all offspring from *ad libitum* fed and feed-restricted mothers) was not correlated with birth weight (Kind *et al.*, 2002). The underlying mechanisms for the postnatal hypertension in IUGR males are not known, nor is it clear whether hypertension in the female offspring from feed-restricted mothers develops at a later age.

Sheep The effects of maternal feed restriction in sheep on fetal and postnatal arterial pressure differ between studies due to differences in the duration, gestational timing and severity of the nutritional insult. Mild maternal undernutrition (85% of *ad libitum* feed intake) during the first 70 days of gestation did not alter fetal body weight, individual

organ weights or arterial blood gas status in late gestation (Hawkins *et al.*, 1999, 2000b; Hawkins *et al.*, 2000c). At 0.8 gestation, the arterial pressures of fetuses from feed-restricted ewes were lower than that of controls (Hawkins *et al.*, 2000c). The relative hypotension was not due to altered vasodilator or vasoconstrictor function in femoral resistance arteries (Ozaki *et al.*, 2000), but was associated with a blunted cortisol response to a HPA axis challenge (Hawkins *et al.*, 2000a). Neither the relative hypotension or reduced cortisol responses persisted into the postnatal period, and at 3 months, lambs from undernourished ewes had elevated arterial pressure and increased HPA axis activity compared with controls (Hawkins *et al.*, 2000a). Blunted vasodilator responses in resistance arteries from offspring of ewes more severely undernourished (50% of *ad libitum* feed intake) in early gestation (Ozaki *et al.*, 2000) may lead to hypertension, but fetal or postnatal arterial pressures of these offspring have not been reported.

Severe maternal feed restriction (3% of *ad libitum* feed intake) for 20 days at 0.7 of gestation led to a reduced birth weight in female offspring (Oliver *et al.*, 2002) but 10 days of feed restriction had no effect on the birth weight of lambs. Arterial pressure was not different between lambs from control and undernourished ewes at either 5 or 30 postnatal months (Oliver *et al.*, 2002). Birth weight was inversely correlated with arterial pressure at five months (–4mmHg/kg increase in birth weight), but this relationship did not persist to 30 months (Oliver *et al.*, 2002). Maternal feed restriction (50% of *ad libitum* feed intake) in the last 30 days of gestation did not reduce fetal body weight but was associated with elevated fetal arterial pressure (Edwards & McMillen, 2001). In addition, these fetuses showed increased pressor responses to angiotensin II during the first 10 days of maternal feed restriction but these increased responses were not evident in the final 10 days of the feed restriction (Edwards & McMillen, 2001).

1.5.2b Protein restriction

In rats, a maternal high protein diet throughout gestation does not affect birth weight, postnatal somatic growth, adult arterial pressure or nephron endowment (Zimanyi *et al.*, 2002). In contrast, isocaloric protein restriction diets can induce hypertension in offspring, with variable effects on birth weight (Langley-Evans *et al.*, 1996a). One week before birth, fetuses from protein-restricted rat dams have significantly greater body

weights than controls, but lower weight gain in these offspring in this final week results in birth weights that are either lower or not different to those of controls (Langley-Evans *et al.*, 1996a). At term, fetuses are disproportionately grown and have increased body lengths, reduced ponderal index and evidence of brain sparing; at weaning, body weights of offspring from protein-restricted dams are significantly greater than controls (Langley-Evans *et al.*, 1996a).

Maternal protein restriction for either a brief period or throughout gestation can induce hypertension in offspring (Langley-Evans *et al.*, 1996b), without altering maternal arterial pressure (Langley-Evans *et al.*, 1994). The hypertension in offspring is evident at 4 postnatal weeks (Langley-Evans *et al.*, 1996b), and persists to at least 9 weeks (Langley & Jackson, 1994). An inverse relationship between postnatal systolic pressure of offspring and maternal protein intake has been reported (Langley & Jackson, 1994). Brief periconceptional maternal protein restriction reduces blastocyst cell numbers and has gender-specific consequences on somatic growth and arterial pressure of offspring (Kwong *et al.*, 2000). Female offspring from protein restricted dams were growth restricted at birth, but not hypertensive in adulthood; in contrast, birth weights of male offspring were not altered, but had persistent hypertension (Kwong *et al.*, 2000).

Maternal protein restriction is also associated with reduced glomerular number and plasma renin activity (PRA) in offspring, both are which are evident prior to the onset of hypertension (Vehaskari *et al.*, 2001). Alterations to the RAS of offspring have been reported in another study. Maternal protein restriction in the final week of gestation leads to increased PRA, but normal plasma angiotensin II concentrations; in contrast, protein restriction throughout gestation or in the first or second week of gestation leads to lower circulating angiotensin II levels in offspring, with normal PRA (Langley-Evans *et al.*, 1996b). Blood glucocorticoid levels and the HPA axis in offspring are also modified by maternal protein restriction, and the postnatal hypertension in the offspring appears to be maintained by glucocorticoids; following adrenalectomy, hypertensive offspring become normotensive but the hypertension is restored after treatment with glucocorticoids (Gardner *et al.*, 1997).

1.5.3 Reduction of placental size

The surgical removal of endometrial caruncles (placental attachment sites) in ewes prior to mating (carunclectomy) leads to reduced placental mass and fetal growth restriction. Birth weight is correlated with placental mass, and is inversely related to the number of caruncles removed (Alexander, 1964). Carunclectomy leads to asymmetrical growth restriction, but the degree of growth restriction can vary greatly (from 10–48%) between studies (Robinson *et al.*, 1979; Owens *et al.*, 1987b; Owens *et al.*, 1994; Phillips *et al.*, 1996; Edwards *et al.*, 1999). In late gestation, fetuses from placentally restricted (PR) ewes are hypoxemic, hypoglycemic and hypercortisolemic compared with controls (Robinson *et al.*, 1979; Owens *et al.*, 1987a, b; Phillips *et al.*, 1996). The development of the fetal HPA axis is altered by carunclectomy (Phillips *et al.*, 1996). Despite the absence of differences in the resting arterial pressure of control and IUGR fetuses (Robinson *et al.*, 1983; Edwards *et al.*, 1999), the fetal RAS appears to play a role in the maintenance of arterial pressure in the IUGR fetuses but not in control fetuses (Edwards *et al.*, 1999). These data indicate that this gestational insult can induce structural and functional alterations to the IUGR fetus but do not adversely affect arterial pressure. At birth, offspring from PR ewes were growth restricted and hypotensive relative to controls in the early postnatal period; after two postnatal months the IUGR lambs remained smaller and were hypertensive relative to controls (Robinson *et al.*, 1998). The mechanisms for the early postnatal hypotension followed by hypertension remain unknown.

1.5.4 Reduced utero-placental blood flow

Reduced utero-placental blood flow induced by unilateral artery ligation in mid-late gestation produces asymmetrical growth restriction in fetal rats (Wigglesworth, 1964) and guinea pigs (Lafeber *et al.*, 1984). Partial ligation of the uterine artery supplying one uterine horn restricts the growth of the fetuses within that horn while an intact arterial supply to the contralateral horn permits normal growth. Thus both IUGR and control offspring are present within a litter.

Guinea pigs In guinea pigs, uterine artery ligation performed in mid-gestation (30–37d GA) is associated with reduced placental blood flow (Jansson *et al.*, 1986; Widmark *et al.*, 1990), a 40% reduction in placental weight (Carter & Detmer, 1990; Widmark *et*

al., 1990; Detmer *et al.*, 1991) and reductions of 42–67% in fetal body weight near term (Lafeber *et al.*, 1984; Jones *et al.*, 1987; Carter & Detmer, 1990; Detmer *et al.*, 1991; Persson & Jansson, 1992). Although arterial pH and blood gases do not differ between control and IUGR guinea pigs near term (Lafeber *et al.*, 1984; Widmark *et al.*, 1990), the IUGR fetuses are hypoglycemic compared to controls in the non-ligated horn (Widmark *et al.*, 1990).

Few studies have reported the effects of unilateral uterine artery ligation on arterial pressure in guinea pigs (refer to Table 1-2). Effects on fetal arterial pressure have differed between studies, with one study reporting no difference in the MAP of control and IUGR fetuses (Carter & Detmer, 1990) while a later study reported IUGR fetuses had lower MAP than controls (Detmer *et al.*, 1991). After birth, the IUGR guinea pigs from ligated horns remain smaller than those from non-ligated horns for the first 3–4 postnatal months (Lafeber *et al.*, 1984; Persson & Jansson, 1992); it is unknown whether catch-up in body size occurs in IUGR guinea pigs after this age. Only one study has reported the postnatal effects of mid-gestational uterine artery ligation on arterial pressure in the guinea pig. In the entire cohort of young adult offspring (at 3–4 months), arterial pressure was not correlated with birth weight but further analysis showed that IUGR guinea pigs had elevated arterial pressure compared with their normally grown littermates (Persson & Jansson, 1992). The authors proposed that the relationship between birth weight and arterial pressure would increase with advancing age but this relationship has not been investigated at older ages, nor have the mechanisms for this elevated arterial pressure been investigated.

Rats Unilateral uterine artery ligation in late gestation (17d GA) leads to an 18% reduction in body weight in the near-term fetal rat compared with fetuses in the non-ligated uterine horn (Wigglesworth, 1964). Placental weights are reduced and these IUGR fetuses are hypoxemic, hypercapnic, acidemic and hypoglycemic (Ogata *et al.*, 1986). In contrast to findings in guinea pigs, arterial pressure at 3–4 postnatal months is not related to birth weight in male or female offspring following uterine artery ligation (Jansson & Lambert, 1999).

1.5.5 Placental insufficiency

In sheep, daily embolisation of the umbilico-placental circulation leads to chronic placental insufficiency and fetal growth restriction. During umbilico-placental embolisation (UPE). fetuses are hypoxemic (Gagnon *et al.*, 1994; Cock & Harding, 1997; Murotsuki *et al.*, 1997; Louey *et al.*, 2000; Gagnon *et al.*, 2002); in some studies, placental embolisation has also been associated with hypoglycemia and/or hypercapnia.

The severity of the hypoxemia can vary between studies, as can the duration and gestational timing of UPE; consequently, effects on fetal growth and arterial pressure can differ between studies (refer to Table 1-3). Daily placental embolisation for 10 days (between 0.84 and 0.91 of gestation), during which fetal arterial oxygen content is reduced by 30–35%, does not alter fetal body weight or fetal arterial pressure (Gagnon *et al.*, 1994). In contrast, fetal growth restriction can result from more severe reductions in fetal arterial oxygen levels (40–50% of pre-UPE values), and arterial pressure is elevated within five days of the commencement of UPE (Cock & Harding, 1997; Murotsuki *et al.*, 1997; Gagnon *et al.*, 2002). In contrast to persistent fetal hypertension in fetuses subjected to 21 days of UPE (108–129d GA) reported in one study (Murotsuki *et al.*, 1997), another reported UPE for a similar period, but at a different gestational age (120–140d GA) did not result in a persistent elevation in fetal arterial pressure UPE (Cock & Harding, 1997). In the latter study, the arterial pressure of control fetuses progressively increased during gestation, but the UPE fetuses failed to show this gestational increase. Despite significantly higher MAP in UPE fetuses five days after the commencement of UPE, by 140d GA, the MAP of UPE fetuses was significantly lower than that of controls (Cock & Harding, 1997).

When allowed to be born, lambs subjected to UPE from 0.8 gestation until birth have reduced weight and ponderal index at birth, and remain lighter than controls during the first 8 postnatal weeks; this trend is evident in the IUGR lambs born at term (Louey *et al.*, 2000) and those born approximately one week prior to term (Cock *et al.*, 2001a). The relative hypotension reported in the late gestation fetus after 20d of UPE (Cock & Harding, 1997) was apparent at the first postnatal measurement at 4d (MAP –6mmHg) and the relative hypotension persisted for the remainder of the 8 week postnatal study period (Louey *et al.*, 2000).

The mechanisms for the altered arterial pressure following UPE are not clear, and may differ depending on the gestational timing of the embolisation. Given UPE can either lead to persistent fetal hypertension (Murotsuki *et al.*, 1997) or relative hypotension near term (Cock & Harding, 1997) that persists into the postnatal period (Louey *et al.*, 2000). Placental embolisation increases the vascular resistance of the placenta (Trudinger *et al.*, 1987; Gagnon *et al.*, 1996), which may increase the total peripheral vascular resistance of the feto-placental unit; increased vascular resistance in the fetus may in turn result in elevated arterial pressure in the UPE fetuses. While this may explain persistent (Murotsuki *et al.*, 1997) and transient (Cock & Harding, 1997) fetal hypertension, it does not explain the hypotension measured in the late gestation fetus by this latter study. The relative hypotension reported by Cock and Harding (1997) was not due to differences in renal function; GFR and kidney weight were not different between control and UPE fetuses.

Exposure of the UPE fetuses to increased circulating levels of glucocorticoids as a result of fetal hypoxemia may lead to hypertension (refer to next section). However, just as the effects of UPE on arterial pressure differ between studies, the relationship between fetal arterial pressure and plasma cortisol concentrations are also not consistent between studies. Late gestational UPE leads to increased fetal arterial pressure and cortisol levels (Murotsuki *et al.*, 1997) as well as reduced 11β-hydroxysteroid dehydrogenase (11β-HSD) activity and gene expression in the fetal kidney (Murotsuki *et al.*, 1998), suggesting that increased exposure to glucocorticoids may play a role in the persistent elevation in arterial pressure in these fetuses. However, glucocorticoid exposure does not fully explain the effects of UPE on fetal arterial pressure. Gagnon *et al.* (1994) reported no increase in MAP despite elevated cortisol concentrations in UPE fetuses, and Cock and Harding (1997) reported elevated arterial pressure in UPE fetuses at an age when plasma cortisol concentrations were not different from that of controls (Cock *et al.*, 2001b). Elevated plasma cortisol has been measured at 130d GA, 20 days after the commencement of UPE (Murotsuki *et al.*, 1996), and also at 135d and 140d GA (15 and 20 days after the commencement of UPE) in another study (Cock *et al.*, 2001b). It is unclear from these studies whether the increase in cortisol was a consequence of UPE or was related to the earlier pre-partum cortisol surge in these animals. The pre- and postnatal cortisol profiles of term-born UPE lambs do not differ from controls (Louey *et al.*, 2000). UPE fetuses that are born approximately one week

before term exhibit an earlier pre-partum rise in cortisol but to similar levels as controls (Cock *et al.*, 2001a) and the postnatal cortisol concentrations were not different between these growth restricted lambs and controls (*unpublished data*). As there is no difference in the prenatal or postnatal cortisol concentrations in UPE lambs compared with controls, it is unlikely that the postnatal hypotension following UPE was due to differences in circulating cortisol levels. The relative postnatal hypotension could also not be explained by altered PRA which was not different between the two groups during the 8 postnatal weeks except at one postnatal week when it was significantly higher in the IUGR lambs (Louey *et al.*, 2000).

In children, arterial pressure is correlated with current body weight (Voors *et al.*, 1977) and it is possible that the lower arterial pressure in the IUGR lambs was due to smaller body size during this early postnatal period (Louey *et al.*, 2000). Indeed, covariance analysis indicated that the relative hypotension could be explained by reduced body size in the IUGR lambs (Louey *et al.*, 2000). It was unclear from this study whether the IUGR lambs would remain hypotensive relative to controls after 8 postnatal weeks, nor was it known when, or if, postnatal catch-up in body weight would occur, and what the effect of postnatal catch-up would be on arterial pressure. It is possible that UPE programmes hypotension rather than hypertension but it is also possible that the lower arterial pressure would not persist. After 8 postnatal weeks, IUGR lambs may become normotensive (as reported by Moss *et al.*, 2001, following *in utero* glucocorticoid exposure; refer to next section) or hypertensive (as reported by Robinson *et al.*, 1998, following carunclectomy; refer to Section 1.5.3).

1.5.6 *In utero* glucocorticoid exposure

Elevated plasma cortisol concentrations have been measured in human SGA fetuses (Economides *et al.*, 1991). Animal studies indicate that exogenous glucocorticoid exposure during gestation can retard fetal growth (Edwards *et al.*, 1993; Jobe *et al.*, 1998) and models of induced IUGR can lead to increase cortisol concentrations (Phillips *et al.*, 1996; Cock *et al.*, 2001b). Ordinarily, placental 11β-HSD converts maternal cortisol to cortisone, thereby preventing exposure of the fetus to excessive maternal cortisol (Edwards *et al.*, 1993). In rats, low activity of placental 11β-HSD has been reported in low birth weight rats with disproportionately large placentae indicating

these animals may be exposed to increased maternal glucocorticoids, which in turn may programme increased postnatal arterial pressure (Benediktsson *et al.*, 1993).

Rats In rats, exposure to high levels of glucocorticoids *in utero* can cause fetal growth restriction and postnatal hypertension in offspring. Daily treatment of pregnant rats with dexamethasone throughout gestation significantly reduces the birth weight of the pups, and at 140–150 postnatal days, systolic pressure is elevated in male and female offspring; diastolic pressures were not different between dexamethasone treated and control offspring at this age (Benediktsson *et al.*, 1993). The gestational timing of excess glucocorticoid exposure appears to be important in fetal growth and postnatal arterial pressure. Maternal dexamethasone treatment at a similar dose in the first or second week of gestation does not affect birth weight, or glucose tolerance at 6 months in offspring (Nyirenda *et al.*, 1998). In contrast, this same treatment in the third (final) week of gestation led to a 9% reduction in birth weight and was associated with glucose intolerance at 6 months of age (Nyirenda *et al.*, 1998). The arterial pressure of these animals at this age is not known. In a similar study, maternal dexamethasone treatment in the final week of gestation reduced birth weight by 9%, and led to hypertension in male and female offspring evident at 3 postnatal months and persisted to one year of age (Sugden *et al.*, 2001).

Sheep Systolic and diastolic pressures are elevated in immature fetuses (103–120d GA) following a 24-hour infusion of cortisol, but arterial pressure in older fetuses (130–137d GA) is not altered by this treatment (Tangalakis *et al.*, 1992). In another study, a 48-hour infusion of dexamethasone in 125d fetal sheep led to elevated arterial pressure that persisted after the dexamethasone infusion ended (Derks *et al.*, 1997). A similar infusion of betamethasone also increased arterial pressure but this infusion induced labour and whether hypertension persisted after the infusion was stopped is not known (Derks *et al.*, 1997).

Brief exposure to glucocorticoids early in ovine gestation can programme persistent postnatal hypertension, but does not affect size at birth. Gestational timing appears to be important in the programming of postnatal hypertension. A 48-hour maternal infusion of dexamethasone (0.48mg/h) between 22d and 29d GA leads to elevated arterial pressure in female offspring, but the same infusion between 59d and 66d GA does not

alter postnatal arterial pressure (Dodic *et al.*, 1998). A 48-hour maternal cortisol infusion (5mg/h) commencing at 22d GA can also programme postnatal hypertension in both male and female offspring (Dodic *et al.*, 2002b). The dose and duration of the glucocorticoid exposure is also important in the programming of arterial pressure. A 20 day low dose infusion of dexamethasone (1mg/day) does not result in hypertensive offspring (Moritz *et al.*, 2002), despite the infusion commencing at a time previously shown to programme postnatal hypertension (Dodic *et al.*, 1998). Hypertension in the dexamethasone-exposed group (22–29d GA) was evident at 4 postnatal months (+6mmHg) (Dodic *et al.*, 1998) and was amplified with age; at 40 months, this difference was 12mmHg (Dodic *et al.*, 1999). Follow-up studies of the hypertensive offspring from the dexamethasone treated ewes indicate they have an increased cardiac output (due to an increased stroke volume) (Dodic *et al.*, 1999). This prenatal insult was not associated with alterations to the HPA or RAS systems of offspring in terms of basal hormone concentrations or responses to physiological stressors such as hemorrhage (Dodic *et al.*, 1998; Peers *et al.*, 2001; Dodic *et al.*, 2002d) or alterations to glucose tolerance in the adult (Gatford *et al.*, 2002). More recent studies following this early gestational corticosteroid challenge indicate that altered gene expression of components of the RAS in the kidney and brain observed in the late gestation fetus and adult offspring may play key roles in the persistent elevation in postnatal arterial pressure (Dodic *et al.*, 2002a).

In contrast to the postnatal hypertensive effects of a brief, early gestational exposure to exogenous glucocorticoids, exposure later in gestation may reduce fetal growth but does not programme postnatal hypertension. Betamethasone reduces fetal body weight without affecting body length when administered as a single intramuscular injection to the ewe at 104d GA (–11% and –14% birth weight at 125d and 145d GA respectively) or when administered as three weekly doses commencing at 104d GA (–25% and –19% birth weight at 125d and 145d GA; Jobe *et al.*, 1998). In contrast, not all studies have found a significant reduction in birth weight following a single maternal injection of betamethasone at 104d GA; arterial pressures of these lambs during the first year after birth were not different to controls (Moss *et al.*, 2001; Moss *et al.*, 2002). Similarly, direct administration of either a single (0.5mg/kg) or repeated (4 doses at weekly intervals) doses of betamethasone intramuscularly to the fetus did not alter birth weight or arterial pressure (Moss *et al.*, 2001). In contrast, maternal injections of

betamethasone every 3 weeks from 104d GA reduced birth weight (–19%), body weight and arterial pressure during the first postnatal month (Moss *et al.*, 2002). Following 4 weekly maternal injections, birth weight was also reduced and lambs remained 33% lighter and were hypotensive relative to controls at 3 months of age (Moss *et al.*, 2001). This relative hypotension did not persist and by 6 months, arterial pressures and body weights were not different to those of controls (Moss *et al.*, 2001).

At present, evidence from studies in sheep indicate that the gestational timing, dose and duration of glucocorticoid exposure are important in programming postnatal hypertension. Brief exposure (48h) to exogenous glucocorticoids early in gestation does not reduce fetal growth but can lead to persistent hypertension in the postnatal sheep. Conversely, single or repeated exposure to glucocorticoids later in gestation do not lead to postnatal hypertension.

1.5.7 Intrauterine perturbations in animals: A summary of effects on size at birth and arterial pressure

Studies in animals have demonstrated that perturbations to the *in utero* environment can induce functional changes in the postnatal offspring without necessarily affecting size at birth. The effects of these insults on postnatal body growth and arterial pressure differ between models due to differences in the duration, severity and gestational timing of the insults. Tables 1-1–1-4 summarise the effects of different intrauterine perturbations on birth weight, arterial pressure and postnatal body weight in rats, guinea pigs and sheep, as described in the previous sections.

Table 1-1. Effects of perturbations to the *in utero* environment on body weight and arterial pressure in rats. Models of sub-optimal intrauterine conditions include unilateral uterine artery ligation (LIG), maternal undernutrition (UN), maternal low protein diets (LPD)* and glucocorticoid (dexamethasone, DEX) exposure. Effects of the *in utero* perturbation on birth weight, resting arterial pressure (systolic pressure, SBP, unless otherwise stated; MAP, mean arterial pressure; DBP, diastolic pressure) and postnatal body weight are indicated below, and the age at which measurements were made are indicated in parentheses.

mean numerical values not stated in paper; * diets of control dams contained ~18% protein; an increase, decrease, or no difference in the parameter compared with controls are indicated by ↑, ↓, and ↔, respectively.

Study	Perturbation	Birth weight	Arterial pressure	Postnatal body weight
Jansson & Lambert (1999)	LIG at 18d GA	↓ #	↔ (3–4mo)	–
Langley-Evans *et al.* (1994)	LPD 9%, -14–22d GA	↓ 10%	♀ ↑ 30mmHg, ♂ ↑ 28mmHg (4wk)	–
Langley-Evans *et al.* (1996b)	LPD 9% 0–22d GA	↔	♀ ↑ 28mmHg, ♂ ↑ 26mmHg (4wk)	♀ ↔, ♂ ↑ 17% (4wk)
	0–7d GA	↔	♀ ↔, ♂ ↑ 15mmHg (4wk)	♀ ↔, ♂ ↑ 39% (4wk)
	8–14d GA	↑ 6%	♀ ↑ 15mmHg, ♂ ↑ 9mmHg (4wk)	♀ ↔, ♂ ↑ 26% (4wk)
	14–22d GA	↔	♀ ↑ 20mmHg, ♂ ↑ 16mmHg (4wk)	♀ ↔, ♂ ↔ (4wk)
Kwong *et al.* (2000)	LPD 9%, 0–4.25d GA	♀ ↓ 6% ♂ ↔	♀ ↔ (4wk, 11wk) ♂ ↑ 12mmHg (4wk), ↑ 9mmHg (11wk)	♀ ↑ 14% (4wk), ↔ (11wk) ♂ ↑ 10% (4wk), ↔ (7wk)
Woods *et al.* (2001)	LPD 8.5%, 0d–birth	↓ 13%	♂ MAP ↑ 9mmHg (21wk)	♂ ↔ (3wk, 21wk)
Woodall *et al.* (1996)	UN 30% *ad libitum*, 1–23d GA	↓ 27%	↑ 7mmHg (30wk), ↑ 5mmHg (56wk)	↓ 14% (3wk), ↔ (30wk)
Holemans *et al.* (1999)	UN 50% *ad libitum*, 11–23d GA	Not given	♀ ↔ (100d)	♀ ↓ # (3–13wk)
Ozaki *et al.* (2001)	UN 70% *ad libitum*, 0–18d	↓ 16%	MAP ♀ ↔, ♂ ↑ 12mmHg (60d) MAP ♀ ↑ 13mmHg, ♂ ↑ 15mmHg (100d) MAP ♀ ↑ 17mmHg, ♂ ↑ 16mmHg (200d)	↔ (3wk)
Benediktsson *et al.* (1993)	DEX 100µg/kg daily throughout pregnancy	↓ 15%	♀ SBP ↑ 9mmHg, DBP ↔ (21wk) ♂ SBP ↑ 13mmHg, DBP ↔ (21wk)	–
Celsi *et al.* (1998)	DEX 100µg/kg daily throughout pregnancy	↓ 29%	MAP ↑ 23mmHg (60d)	↔ (3wk)

Table 1-2. Effects of perturbations to the *in utero* environment on body weight and arterial pressure in guinea pigs. Models of sub-optimal intrauterine conditions include unilateral uterine artery ligation (LIG) and maternal undernutrition (UN). Effects of the *in utero* perturbation on birth weight, resting arterial pressure (MAP, mean arterial pressure; SBP, systolic pressure) and postnatal body weight are indicated below, and the age at which measurements were made are indicated in parentheses.

Increase, decrease, or no difference in the parameter compared with controls are indicated by ↑, ↓, and ↔, respectively.

Study	Perturbation	Birth weight	Arterial pressure	Postnatal body weight
Kind *et al.* (2002)	UN 85% *ad libitum* 4–6wk before mating–term	♀ ↔ ♂ ↓ 17%	♀ ↔ ♂ SBP ↑ 7mmHg (100d)	♀ ↓ 8% (100d) ♂ ↓ 11% (100d)
Carter & Detmer (1990)	LIG at 30–33d GA	↓ 50%	↔ (60–64d GA)	–
Detmer *et al.* (1991)	LIG at 30–32d GA	↓ 42%	MAP ↓ 3mmHg (60–64d GA)	–
Persson & Jansson (1992)	LIG at 32–37d GA	↓ 46%	MAP ↑ 7mmHg compared with littermates (3–4mo)	↓ 17% (3–4 mo)

Table 1-3. Effects of perturbations to the *in utero* environment on body weight and arterial pressure in fetal sheep. Models of sub-optimal intrauterine conditions include maternal undernutrition (UN), placental restriction by carunclectomy (PR), umbilico-placental embolisation (UPE) and excess glucocorticoid exposure by either cortisol, dexamethasone (DEX) or betamethasone (BET) administered to the ewe (MAT) or fetus (FET). Effects of the *in utero* perturbation on fetal arterial blood parameters (O_2, either Pa_{O_2} or Sa_{O_2} as reported; glu, glucose concentration; F, plasma cortisol concentration), body weight and resting arterial pressure (mean arterial pressure, MAP, unless otherwise stated; SBP, systolic pressure, DBP, diastolic pressure) are indicated below, and the age at which measurements were made are indicated in parentheses.

mean numerical values not stated in paper; increase, decrease, or no difference in the parameter compared with controls are indicated by ↑, ↓, and ↔, respectively.

Study	Perturbation	Blood parameters	Body weight	Arterial pressure
Hawkins *et al.* (2000a, 2000c)	UN 85% *ad libitum* 0–70d GA	↔O_2, ↔glu, ↔F	↔ (131d GA)	↓ (114–126d GA)#
Edwards & McMillen (2001)	UN 50% *ad libitum* 115–145d GA	↔O_2, ↓glu, ↔F	↔ (145d GA)	↑ 5–7mmHg
Edwards *et al.* (1999)	PR	↓O_2	↓ 47% (147d GA)	↔ (115–145d GA)
Murotsuki *et al.* (1997)	UPE 108–129d GA	↓O_2	↓ 28% (129d GA)	↑ 7mmHg
Gagnon *et al.* (2002)	UPE 124–129d GA	↓O_2	↓ 18% (129d GA)	↑ 5mmHg
Gagnon *et al.* (1994)	UPE 125–135d GA	↓O_2	↔ (135d GA)	↔
Cock & Harding (1997)	UPE 120–140d GA	↓O_2, ↓glu	↓ 20% (140d GA)	↑ 5mmHg (125d GA) ↓ 7mmHg (140d GA)
Louey *et al.* (2000)	UPE 120–146d GA	↓O_2, ↓glu, ↔F	↓ 33% (birth)	↑ 7mmHg (121d GA) ↔ (125–140d GA)
Tangalakis *et al.* (1992)	FET Cortisol 100µg/hr, 24h Immature (103–120d GA) Mature (130–137d GA)	 ↔O_2, ↔glu, ↑F ↔O_2, ↔glu, ↑F		 SBP ↑ 8mmHg, DBP ↑ 3mmHg ↔
Derks *et al.* (1997)	FET BET 10µg/hr, 48h from 125d GA FET DEX 10µg/hr, 48h from 125d GA	↔ ↔		↑ 9mmHg ↑ 7–10mmHg
Forhead *et al.* (2000)	FET Cortisol 2–3mg/kg/day, 5 days from 129d GA	↔O_2		↑ 8mmHg

Table 1-4. Effects of perturbations to the *in utero* environment on body weight and postnatal arterial pressure in sheep. Models of sub-optimal intrauterine conditions include global maternal undernutrition (UN), placental restriction by carunclectomy (PR), umbilico-placental embolisation (UPE) and excess glucocorticoid exposure by either cortisol, dexamethasone (DEX) or betamethasone (BET) administered to the ewe (MAT) or fetus (FET). Effects of the *in utero* perturbation on birth weight, resting arterial pressure and postnatal body weight are indicated below, and the age at which measurements were made are indicated in parentheses.

mean numerical values not stated in paper; increase, decrease, or no difference in the parameter compared with controls are indicated by ↑, ↓, and ↔, respectively.

Study	Perturbation	Birth weight	Mean arterial pressure	Postnatal body weight
Hawkins *et al.* (2000a)	UN 85% *ad libitum* 0–70d GA	↔	↑ (3mo) #	↔
Oliver *et al.* (2002)	UN 3% *ad libitum*			
	105–115d GA	↔	↔ (5mo and 30mo)	↔ (3mo)
	105–125d GA	♀ ↓ 14%; ♂ ↔	↔ (5mo and 30mo)	↔ (3 mo)
Robinson *et al.* (1998)	PR	↓ #	↓ (birth–60d) # ↑ (after 60d) #	↓ #
Louey *et al.* (2000)	UPE 120d–146d GA	↓ 33%	↓ 4mmHg (birth–8wk)	↓ 20% (8wk)
Dodic *et al.* (1998) and later studies by the same authors	MAT DEX 0.48mg/h, 48h			
	Between 22–29d GA	↔	↑ 6–12mmHg (4mo–5yr)	↔
	Between 59–66d GA	↔	↔	↔
Moritz *et al.* (2002)	MAT DEX 1mg/d, 25–45d GA	♂ (↓) 7%	♂ ↔ (2mo)	♂ (↓) 13% (2mo)
Dodic *et al.* (2002b)	MAT Cortisol 5mg/h, 48h from 22d GA	↔	♀ ↑ 8mmHg (18mo) ♂ ↑ 11mmHg (18 mo)	↔
Moss *et al.* (2001)	BET 0.5mg/kg			
	MAT 104d GA	↔	↔	↔
	MAT 104d, 111d, 118d, 125d GA	↓ 34%	↓ 8mmHg (3mo), ↔ (6mo)	↓ 33% (3mo), ↔ (6mo)
	FET 104d GA	↔	↔	↔
	FET 104d, 111d, 118d, 125d GA	↔	↔	↔

1.6 Aims

The overall aim of this thesis was to investigate the relationships between birth weight and somatic growth, arterial pressure and arterial wall mechanical properties. Two models of late gestational fetal growth restriction were used in the studies described in this thesis: restriction of utero-placental blood flow in guinea pigs and chronic placental insufficiency in sheep. These two models are clinically relevant in terms of gestational timing (Wollmann, 1998), and the placental nature of the insult (Regnault *et al.*, 2002); late gestational IUGR associated with placental insufficiency is the most common type of IUGR in humans. In addition, epidemiological studies disproportionate fetal growth from a late gestational insult (Villar & Belizan, 1982) has associated with postnatal hypertension (Barker *et al.*, 1992).

In the first experimental study (Chapter 2), IUGR was induced in pregnant guinea pigs by placental restriction at 0.5 of gestation and offspring were studied up to one year of postnatal age, an age at which the effects of the prenatal environment on somatic growth and arterial pressure has not be previously reported. The use of a small animal species allows follow-up studies from mid-gestation to adulthood to be conducted within a reasonable period of time. Rats have been used extensively to investigate the mechanisms underlying fetal programming, but there are several advantages in the use of guinea pigs rather than rats for these studies, including a relatively long gestation, similar placental structure to humans, and similar renal maturity at birth to humans.

Placental insufficiency was induced in fetal sheep from 0.8 of gestation by umbilico-placental embolisation (as described in Chapters 3 and 4). Fetuses were studied until 0.95 gestation (Chapter 3) to investigate the short-term effects of placental insufficiency on arterial pressure and mechanics. Although previous studies from our laboratory have reported the effects of UPE on fetal arterial pressure (Cock & Harding, 1997; Louey *et al.*, 2000), the passive mechanical properties of resistance arteries in the near-term UPE fetus are not known. Altered mechanical properties of these arteries may explain the relative hypotension measured in the early postnatal period (Louey *et al.*, 2000) or potentially predispose the IUGR fetus for later hypertension.

A separate group of fetuses were allowed to be born, in order to determine the long-term effects of late gestational UPE on postnatal growth and arterial pressure

between birth and two years. I have previously shown that IUGR lambs remain lighter and have lower arterial pressure compared with controls during the first two postnatal months (Louey *et al.*, 2000) but somatic growth and arterial pressure after this age was not known. Based on previous studies in the sheep, the IUGR lambs may have become hypertensive (Robinson *et al.*, 1998) or normotensive (Moss *et al.*, 2001) relative to controls. The effects of UPE on passive arterial mechanics in the young adult sheep have also not previously been reported.

1.6.1 Specific aims of studies in this thesis

Study 1: The relationship between birth weight and postnatal growth, arterial pressure and arterial wall mechanical properties in adult guinea pigs

1. To determine the postnatal growth profile of guinea pigs of differing birth weights during the first postnatal year.
2. To determine the effects of birth weight on arterial pressure at one year in guinea pigs.
3. To determine the effects of birth weight on passive arterial wall mechanical properties at one year in guinea pigs.

Study 2: The effects of intrauterine growth restriction on arterial pressure and arterial wall mechanical properties in near-term fetal sheep

1. To determine the effects of placental insufficiency on arterial pressure in late gestation fetal sheep.
2. To determine the effects of late gestational placental insufficiency on passive arterial wall mechanical properties in the near-term fetal sheep.

Study 3: The effects of intrauterine growth restriction on postnatal growth, arterial pressure and arterial wall mechanical properties in adult sheep

1. To determine the postnatal growth profile of sheep following late gestational placental insufficiency.
2. To determine the effects of birth weight on postnatal arterial pressure in sheep during the first two postnatal years.
3. To determine the effects of birth weight on passive arterial wall mechanical properties in two year old sheep.

Chapter 2

The relationship between birth weight and postnatal growth, arterial pressure and arterial wall mechanical properties in adult guinea pigs

2.1 Introduction

The effects of fetal programming on postnatal cardiovascular function have been extensively studied using small animal models. An advantage of using small animals in studies of this nature is that many of the effects of an adverse fetal environment do not become evident until adulthood; small animals reach adulthood in shorter times relative to larger animals, thus allowing follow-up studies to be performed in shorter periods. Manipulations including maternal undernutrition and uterine artery ligation have been performed in small animals such as guinea pigs and rats, with the majority of these studies being conducted in rats. However, there are several distinct advantages of using guinea pigs rather than rats in developmental studies, in particular, studies involving the fetal programming on arterial pressure.

Guinea pigs have similar placentae to humans, and therefore have been used in a number of studies investigating placental function (Myers *et al.*, 1982; Carter & Detmer, 1990; Jansson, 1990; Dwyer *et al.*, 1992). While both humans and guinea pigs have discoid hemochorial placentae, the exchange surface is of a villous nature in the human placenta (as it also is in ruminants), but is labyrinthine in the guinea pig placenta (Enders, 1965). Another advantage of using guinea pigs in developmental studies is that they have a relatively long gestation (58–75d of gestation, depending on the strain; average 68d) compared with other small animals such as the mouse and rat (both 21–23d). This relatively long gestation allows surgical manipulations of fetal growth to be performed, and the effects on the fetus to be studied.

Another advantage of guinea pigs is that the newborn is more mature than the newborn rat. When investigating the effects of fetal growth on postnatal arterial pressure, it is important to consider the development of the kidney, which plays a major role in the regulation of arterial pressure (Folkow, 1982). Like the human (Hinchliffe *et al.*, 1991), nephrogenesis in the guinea pig is complete before birth (Merlet-Benichou *et al.*, 1981), whereas nephrogenesis in the rat is not complete until a week after birth (Larsson, 1975). Therefore at birth, the guinea pig pup is more like a human newborn with respect to renal development.

2.1.1 Methods for the induction of IUGR in guinea pigs

2.1.1a Maternal undernutrition

Maternal undernutrition throughout pregnancy has been shown to reduce birth weight in guinea pigs; moderate undernutrition (85% of *ad libitum* feed intake) can lead to a 13% reduction in birth weight (Kind *et al.*, 1999) while more severe undernutrition (60% of *ad libitum* feed intake) results in a more severe reduction (39%) in fetal body weight (Dwyer *et al.*, 1992). These effects on fetal growth, and also postnatal growth, appear to gender-specific. Female offspring from feed-restricted mothers are not growth restricted at birth, nor are body weights different at 3 postnatal months; in contrast, male offspring from feed-restricted mothers have reduced birth weights and remain lighter at 3 months compared with male offspring from *ad libitum* fed mothers (Kind *et al.*, 1999; Kind *et al.*, 2002). With regard to postnatal arterial pressure, moderate maternal undernutrition led to elevated systolic pressure (+7mmHg) in male offspring at 3 months of age (Kind *et al.*, 2002).

2.1.1b Uterine artery ligation

A widely used technique for inducing intrauterine growth restriction (IUGR) in small animals such as guinea pigs and rats is unilateral uterine artery ligation. In the guinea pig, this procedure is typically performed typically at mid-gestation and involves ligating the uterine artery supplying one pregnant uterine horn, while leaving the uterine artery supplying the other uterine horn unligated. This procedure is thought to result in reduced oxygen and nutrient supply to the fetuses in the ligated horn, and causes a 40–67% reduction in body weight compared with pups from the contralateral, control horns (Lafeber *et al.*, 1984; Jones *et al.*, 1987; Carter & Detmer, 1990; Detmer *et al.*,

1991; Persson & Jansson, 1992). The effects of unilateral uterine artery ligation on fetuses can be variable. Of 372 pregnant guinea pigs that underwent surgery for unilateral uterine artery ligation, Lafeber *et al.* (1984) reported that 29% delivered litters in which all fetuses had birth weights in the normal birth weight range. In the same cohort, 30% delivered litters in which some fetuses were growth restricted (birth weights below 60% of normal mean birth weight), and 31% delivered litters with growth restricted fetuses, but these fetuses were dead or resorbed. When the fetuses were allowed to be born, the IUGR guinea pigs remain smaller than controls up to at least 3–4 months after birth (Lafeber *et al.*, 1984; Persson & Jansson, 1992). At this age, the IUGR offspring also have higher arterial pressure than their appropriately grown littermates (Persson & Jansson, 1992). However, this relationship between birth weight and arterial pressure was only found when the degree of birth weight reduction (% difference in birth weight between IUGR and normal birth weight littermates) was compared with the difference in mean arterial pressure (MAP) between the littermate pairs. Without these data transformations, MAP was not correlated with birth weight or current weight. While Persson and Jansson (1992) suggested that the relationship between birth weight and arterial pressure might become stronger at later age, this has not been confirmed.

2.1.1c Uterine artery ablation

A variation to the unilateral uterine artery technique was recently been developed (A. Turner, MSc Thesis, 1997). This new technique involved the ablation (via diathermy) of two branches of the uterine artery supplying the placenta of each fetus, and had effects on fetal growth similar to those of unilateral uterine artery ligation (Turner, 1997). Following unilateral artery ligation at 30–35days gestational age (GA), 82% (n=41) of the fetuses from ligated horns were found to be dead within 10 days of surgery. The remaining fetuses (n=9) from the ligated horn survived until the end of the study at 59–69d GA and were 16% smaller than fetuses (n=29) from the non-ligated uterine horn (72±7g *vs* 85±18g; data are mean±SD). Using the ablation technique (also performed at 30–35d GA), 47% (n=32) of the treated fetuses died within 10 days of surgery, however, the remaining fetuses (n=17) survived until the end of the study. These surviving fetuses were growth restricted to a similar degree (–20%) to those fetuses growth restricted following the ligation technique.

Given the higher success rate of the diathermy technique used by Turner (1997), I chose to use this method to induce growth restriction in guinea pigs. While Turner (1997) described the characteristics of guinea pig offspring during the fetal period, the postnatal growth characteristics have not previously been described. The postnatal growth profiles of guinea pigs of a range of birth weights will be described in this chapter. Arterial pressures were measured in these animals at an older age (1 year) than the guinea pigs studied by Persson and Jansson (1992) to determine the relationship between birth weight and adult arterial pressure.

2.1.2 Arterial wall mechanical properties

Based on epidemiological evidence, it has been proposed that some forms of hypertension may be initiated *in utero* and amplified with age (Law *et al.*, 1993). While Persson and Jansson (1992) reported IUGR guinea pigs were hypertensive compared with their normally grown littermates, they also suggested that the relationship between birth weight and arterial pressure might be stronger at later age. The strengthening of the relationship between birth weight and arterial pressure with age would lend support to the hypothesis of *in utero* initiation of hypertension and postnatal amplification.

Arterial wall structure and mechanics are important in both the maintenance and amplification of elevated arterial pressure (Folkow, 1982) and it has been suggested that alterations to the structure of the arterial wall *in utero* may play a role in the initiation of postnatal hypertension (Martyn & Greenwald, 1997; Martyn & Greenwald, 2001). The relative proportions of arterial wall structural components such as elastin and collagen, both of which are important in the determination of arterial wall mechanical properties, may be affected by a sub-optimal *in utero* environment such as that experienced during IUGR. These components can be affected by hypoxia and nutritional deficits (Durmowicz *et al.*, 1991; Spanheimer *et al.*, 1991) and alterations to their deposition could have lasting effects on arterial mechanics (Martyn & Greenwald, 1997). It is possible that the alterations to the arterial wall that could potentially occur *in utero* may not be reflected in arterial pressure until adulthood, as changes to arterial wall structure and mechanical properties have been shown to precede alterations to arterial pressure (van Gorp *et al.*, 2000). While recent studies have investigated the effects of birth weight on small artery function (Holemans *et al.*, 1999; Ozaki *et al.*, 2000; Ozaki *et al.*,

2001), there have been no animal studies to date that have described the effects of birth weight on passive arterial wall mechanical properties in the adult.

2.2 Aims

1. To determine the postnatal growth profile of guinea pigs of differing birth weights during the first postnatal year.
2. To determine the effects of birth weight on arterial pressure at one year in guinea pigs.
3. To determine the effects of birth weight on passive arterial wall mechanical properties at one year in guinea pigs.

2.3 Methods

2.3.1 Overview of study

Date mated guinea pigs underwent surgery at mid-gestation to produce growth restricted offspring by ablation of selected blood vessels supplying placentae. In normal guinea pig gestation, fetal position in the uterine horns affects fetal weight (Turner & Trudinger, 2000), such that fetuses at the cervical end of the uterine horn are heavier than those located towards the tip of the horn. The utero-placental artery ablation technique was used with the aim to increase the incidence and degree of IUGR within each litter.

All guinea pigs were allowed to deliver their offspring spontaneously. Since offspring were born spontaneously, it was not possible to differentiate between fetuses that had had utero-placental vessels ablated and those which were small due to their uterine position alone. It was possible however, to conclude that the low birth weight offspring used in this study were likely to be growth restricted rather than preterm, since exact gestational age was known at the time of delivery. Measurements of body weight and body dimensions were made at regular intervals after birth. At one year of age, offspring were catheterised, and arterial pressure was recorded. Following this, the offspring were killed and resistance arteries from selected vascular beds were collected to determine their mechanical properties.

2.3.2 Experimental animals

Date mated outbred tri-colour guinea pigs were supplied by Monash Animal Services (Gippsland, VIC). Day one of gestation was defined as the day after the vaginal plug was found, following mating. The pregnant guinea pigs were received in the Department of Physiology (Monash University) animal house at an average gestational age of 25 days (term~68d GA). They were housed in plastic cages lined with straw and wood shavings, with a maximum of 4 guinea pigs in each box. Standard guinea pig food pellets and water were provided *ad libitum*.

2.3.3 Placental restriction surgery

Pregnant guinea pigs underwent surgery at approximately mid-gestation (average 32d GA, range 28–40d GA) for the ablation of utero-placental vessels to cause growth restriction in a number of fetuses, following the method described by Turner (1997). All procedures involving animals were approved by the Monash University Animal Ethics Committee (Physiology).

2.3.3a Preparation of the pregnant guinea pig for surgery

Pregnant guinea pigs were anaesthetised with a combined i.p. injection of 5mg/kg xylazine (Xylazil 20, 20mg/ml, Troy Laboratories Pty Ltd, Australia) and 45mg/kg ketamine (100mg/ml, Parnell Laboratories Pty Ltd, Australia). This dose of anaesthetic lasted for approximately 45 minutes, and a second, smaller dose was given during the surgery if required. When adequately anaesthetised (as determined by the absence of corneal reflexes and muscle tone), the abdomen of the guinea pig was shaved and cleaned thoroughly with surgical scrub (Betadine, 7.5% w/v Povidone-iodine, Faulding Pharmaceuticals, Australia), before the application of antiseptic solution (Betadine, 10% w/v Povidone-iodine, Faulding Pharmaceuticals, Australia).

All drapes and surgical instruments were sterilised by autoclaving and aseptic conditions were maintained throughout the surgical operation. Surgical caps and facemasks were worn and the surgeon's hands were washed using an antiseptic skin cleaner (Hibiclens, 4% w/v chorhexidine gluconate and 4% w/v isopropyl alcohol, ICI, Australia) before drying with a sterile handtowel. Sterile latex gloves (Gammex, Ansell, Australia) were worn and sterile drapes placed around the surgical incision site.

2.3.3b Surgical procedure

The abdomen was palpated to determine the approximate position of fetuses so the skin incision was made close to where they lay. A small amount of local anaesthetic (lignocaine hydrochloride 20mg/ml, Troy Laboratories Pty Ltd, Australia) was injected subcutaneously into the intended incision site on the skin in a line that was adjacent to, and approximately 45° from the midline. The incision was made at this angle so that it approximated the angle at which the uterine horns lay, and minimal retraction of the skin was required to expose the uterine horns. A 3cm incision was made in the skin and the muscle layer was blunt dissected to expose the abdominal contents. Gauze swabs were used to retract the intestines and provide a clear view of the uterus and adjacent fat pad (Figure 2-1). Radial arteries (branches of the uterine artery supplying individual placentae) were identified and ~50% were ablated by diathermy (approximately two per fetus). Only vessels that were supplying placentae of fetuses towards the tips of the uterine horns were ablated and those supplying placentae of fetuses at the base of the horn were left intact so that these guinea pigs could act as controls. The gauze swabs were then removed and 5ml of sterile saline was instilled in the abdominal cavity. The abdominal muscles were sutured closed in layers (monofilament polypropylene 6/0, 0.7 metric) and the skin was then sutured closed with non-absorbable suture (Vetafil Bengen, 0.30mm, Clements Stansen Medical, Australia). At the completion of the surgery, the guinea pig was returned to its cage for recovery.

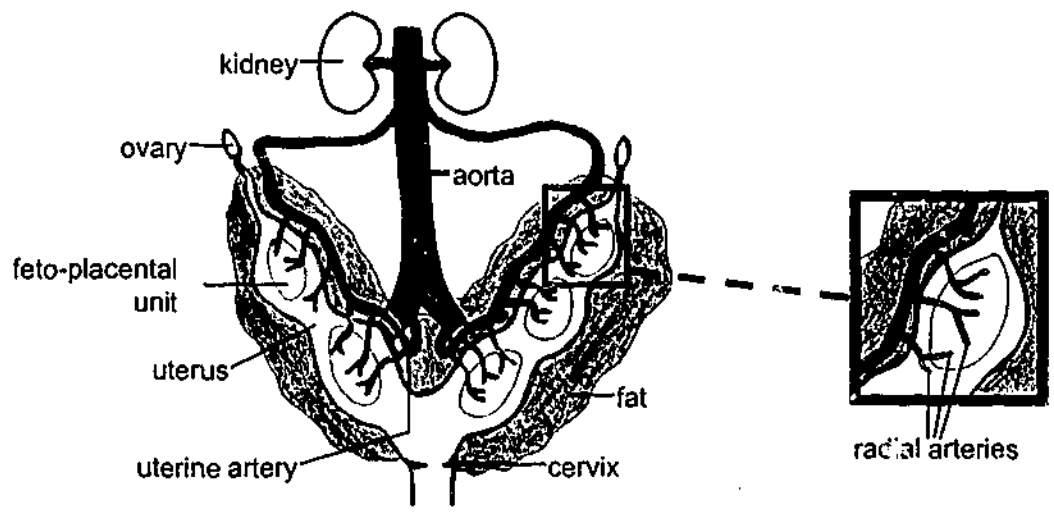


Figure 2-1. Location of arteries ablated during the placental restriction surgery. A schematic diagram of the guinea pig uterus, its arterial supply and the location of the radial arteries that were ablated by diathermy in order to induce fetal growth restriction.

2.3.4 Delivery of offspring

Following surgery, guinea pigs were checked daily for adequate wound healing. One week prior to term, the number of animals in each cage was reduced from four to two per cage. Each guinea pig was allowed to deliver spontaneously. At birth, each pup was weighed and body dimensions measured. Offspring were weaned 3 weeks after birth and separated into different cages according to gender, with a maximum of 4 guinea pigs per cage.

After the offspring were weaned, the sows were heavily sedated with a 2ml i.p. injection of pentobarbitone sodium (Nembutal, 60mg/ml, Rhône Mérieux Pty Ltd, Australia) and euthanased by a 5ml intracardiac injection of pentobarbitone sodium (Lethabarb, 325mg/ml, Virbac Pty Ltd, Australia).

2.3.5 Measurement of postnatal body dimensions

The offspring were weighed daily from birth up to two weeks of age. After this, they were weighed every second day until 4 weeks of age, when they were weighed twice per week. After 6 months of age, the offspring were weighed weekly.

Body dimensions (nose-to-rump length, thoracic girth, abdominal girth and hip circumference) were measured at birth and at weekly intervals. Nose-to-rump length (NRL) was measured as a substitute for the standard crown-to-rump length, since there was difficulty in reliably locating body landmarks for crown-to-rump length. Bony landmarks were identified and used as reference points (Figure 2-2). Each measurement (in mm) was made in duplicate. Ponderal index was calculated from the weight and NRL measurements:

$$ponderal\ index\ (g/mm^3) = body\ weight \div nose\text{-}to\text{-}rump\ length^3$$

A low ponderal index is indicative of an animal that is light for their body length, and is often used as a measure of thinness.

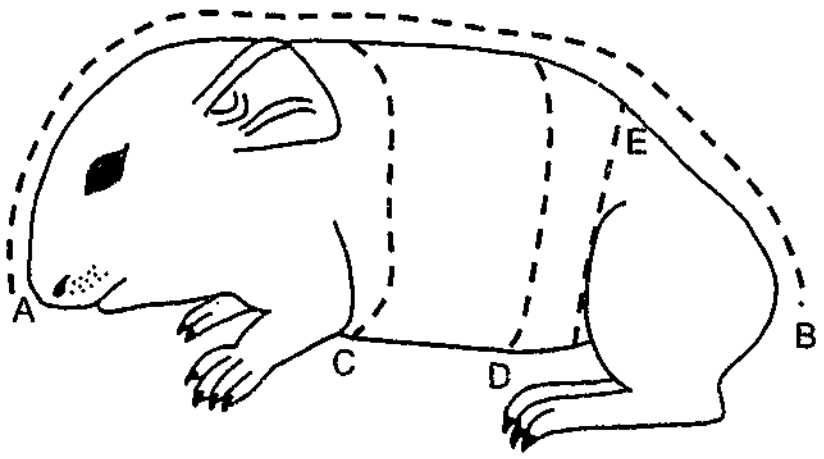


Figure 2-2. Measurement of body dimensions of the postnatal guinea pigs. A schematic diagram of the sites of measurement of body length and girths in the postnatal guinea pig offspring. Nose-to-rump length was measured from the tip of the nose (A), along the length of the spine to the coccyx (B). Thoracic girth was the chest circumference, measured at the base of the sternum (C). Abdominal girth was measured at the level of the navel (D). Hip circumference was taken at the level of the iliac crest (E).

2.3.6 Calculation of postnatal growth rates

Postnatal growth rates for weight and each body dimension were calculated as follows:

$$increase/day = (x_2 - x_1) \div (age_2 - age_1)$$

$$increase/day(\%) = (x_2 \div x_1) \div (age_2 - age_1) \times 100$$

where: x_1 = measurement (e.g. weight, NRL) at age_1

x_2 = measurement (e.g. weight, NRL) at age_2

age_1 = first age

age_2 = second age

e.g. to calculate the growth rate between 2 and 4 weeks of age, age_1 = 2 weeks, age_2 = 4 weeks.

2.3.7 Catheterisation of offspring

At approximately one year of age, offspring were catheterised for the measurement of arterial pressure. Following catheterisation, it was difficult to maintain the patency of the arterial catheter (inserted into the femoral artery) such that valid arterial pressure recordings could be made. Even though the catheters were flushed with heparinised saline (50IU/ml 0.9% sodium chloride) and filled with heparin (5000IU/ml) when not in use, catheters remained patent for only 1–2 days. It was possible to catheterise a carotid

artery rather than a femoral artery, but since catheterisation required obstruction of the artery, catheterisation of a carotid artery may have affected the functioning of carotid baroreceptors, and thus affected arterial pressure. If a carotid artery was to be catheterised, a longer recovery time between surgery and arterial pressure measurement would be required, during which, the patency of the catheters may have been lost.

2.3.7a Preparation of the one year old guinea pig offspring for surgery

Guinea pigs were anaesthetised with a combined i.p. injection of 5mg/kg xylazine and 45mg/kg. Once adequately anaesthetised, the groin area and the ventral and dorsal sides of the neck of the guinea pig were shaved, and cleaned as previously described in Section 2.3.3a.

2.3.7b Surgical procedure

A femoral artery was located by palpation of the inner aspect of a hind leg for the presence of a pulse; a skin incision was made over this site to expose the femoral artery. The artery was freed from surrounding tissue and a polyvinyl catheter (Dural Plastics, Australia, Cat. No. SV35, Medical Grade, ID 0.5mm, OD 0.9mm) was inserted approximately 3cm, such that its tip was located in the descending aorta. The catheter was tracked subcutaneously to the dorsal aspect of the neck for exteriorisation through a small incision (1–2cm) and sutured securely to the skin. A loop of the catheter was left subcutaneously in the hind limb to allow for movement. An incision was then made in the anterior aspect of the neck. A jugular vein was isolated and catheterised (Dural Plastics, Australia, Cat. No. SV45, Medical Grade, ID 0.58mm, OD 0.96mm). The catheter was subcutaneously tracked to the dorsal aspect of the neck. All incision sites were sutured closed with non-absorbable suture. Both the arterial and venous catheters were flushed with heparinised saline (50IU/ml 0.9% sodium chloride). The dead space of the arterial and venous catheters were filled with heparin (5000IU/ml) before the exteriorised end of the catheters was blocked with a pin. Prior to blocking the end of the catheters, they were cut short, such that a minimal length (<5mm) protruded at the dorsal aspect of the neck; this minimised the risk of the guinea pig damaging the catheters.

2.3.8 Measurement of arterial pressure at one year of age

Arterial pressure was measured in the catheterised guinea pig offspring at least 24 hours following the catheterisation surgery. To ensure the guinea pigs were adequately recovered from the effects of the anaesthetic and surgery, all animals were observed in the morning following the surgery (~20h after surgery), making note of their general condition and behaviour. They were checked again in the early afternoon (~24h after surgery), prior to the measurement of arterial pressure.

Guinea pigs were placed in a box (30×17×10cm) in a quiet room 10–15 minutes prior to the measurement of arterial pressure. The external end of the arterial catheter was fitted with an extension catheter (ID 0.5mm, OD 0.9mm, 60cm in length), which was connected to a pressure transducer (TFN-R Disposable Transducers, Viggo-Spectramed, USA) placed at a level approximating the heart. The transducer was connected to a pressure amplifier to measure arterial pressure. Heart rate was derived from the arterial pressure signal, via a ratemeter. Output signals from the pressure amplifier and ratemeter were logged by a digital data recording system (MacLab 8, ADInstruments Pty Ltd, Australia) which was connected to a computer using the program Chart for MacLab (Version 3.6, ADInstruments Pty Ltd, Australia) (Figure 2-3). The data recording system was configured to record at a speed of 40 samples/sec up to a range of 10V; the time scale was set to 2.5sec/division.

2.3.8a Analysis of arterial pressure recording

The arterial pressure of each conscious guinea pig was recorded for 30–40 minutes. At the conclusion of the recording session, the data were digitally analysed in 1 minute blocks to obtain average diastolic pressure, average systolic pressure and average heart rate. Data from periods when the guinea pig was moving excessively in the box were excluded from the analysis. For the majority of the recordings, all guinea pigs remained stationary in the box. Mean arterial pressure for each minute was calculated from the corresponding systolic and diastolic pressure readings using the formula:

$$MAP\ (mmHg) = diastolic + \tfrac{1}{3}\ (systolic - diastolic)$$

All valid data from the recording period were then averaged to obtain one value for diastolic, systolic and mean arterial pressure, and heart rate.

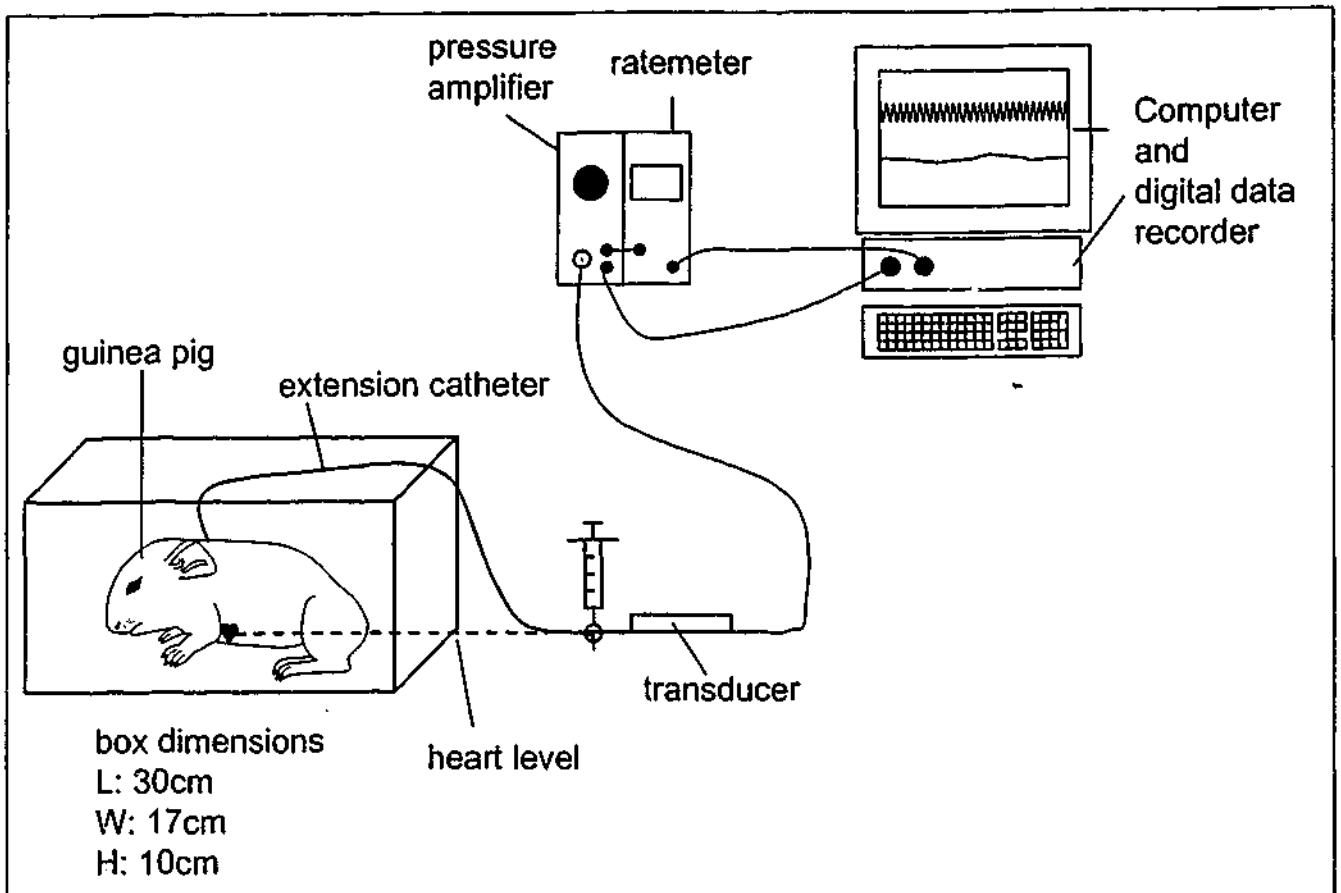


Figure 2-3. Measurement of arterial pressure in guinea pig offspring. A schematic diagram of the arrangement or recording equipment during arterial pressure measurements. The pressure transducer was positioned at a level corresponding to the heart. Data were logged using a digital data recorder (MacLab, ADInstruments, Australia).

2.3.9 Post mortem and tissue collection

Following the measurement of arterial pressure, the guinea pigs were euthanased and tissue collected for either measurements of arterial wall mechanics, or for histological analysis of arteries (data not presented in this thesis). Prior to euthanasia, all guinea pigs received an i.v. injection of heparin (1ml, 5000IU/ml) to prevent blood clotting in the arteries selected for study.

2.3.9a Collection of tissue for histology

A group of guinea pigs was killed for the collection of tissue for histological analysis of arterial wall structure. These guinea pigs received a 0.5ml i.v. injection of papaverine (papaverine hydrochloride, 120mg in 10ml, David Bull Laboratories, Australia) to maximally dilate blood vessels. The guinea pig was heavily sedated with a 2ml i.v. injection of pentobarbitone sodium (Nembutal 60mg/ml, Rhône Mérieux Pty Ltd,

Australia). Saline, followed by 4% paraformaldehyde, was infused into the left ventricle of the heart, perfusing the entire body at 50mmHg (~MAP). Once the perfusion was complete, major organs were weighed, and the kidney, femoral muscle (from the non-catheterised leg), and a segment of mesentery were collected for later dissection and study of arteries contained within (data not presented in this thesis).

2.3.9b Collection of tissue for studies of arterial wall mechanical properties

A separate group of guinea pigs was killed for the collection of arteries for *in vitro* determination of the passive mechanical properties. These guinea pigs were killed by rapid cervical dislocation and exsanguination. Organs from which arteries were collected were placed in oxygenated, calcium-free physiological saline solution (PSS) (120mM NaCl, 5mM KCl, 25mM $NaHCO_3$, 11mM glucose, 1mM KH_2PO_4, 1.4mM $MgSO_4$, 0mM Ca^{2+}) until they could be dissected.

2.3.10 Measurement of passive arterial wall mechanical properties

2.3.10a Selection of arteries

Arteries were selected from four vascular beds to investigate whether there were regional differences in the mechanical properties of arterial walls. Figure 2-4 illustrates the four vascular beds and sites from which the segments of artery were collected. Arteries were taken from a similar area of each bed; there was a degree of variation in the initial outer diameter (OD) of the vessels but there were no significant differences between the initial OD of each group for each vessel.

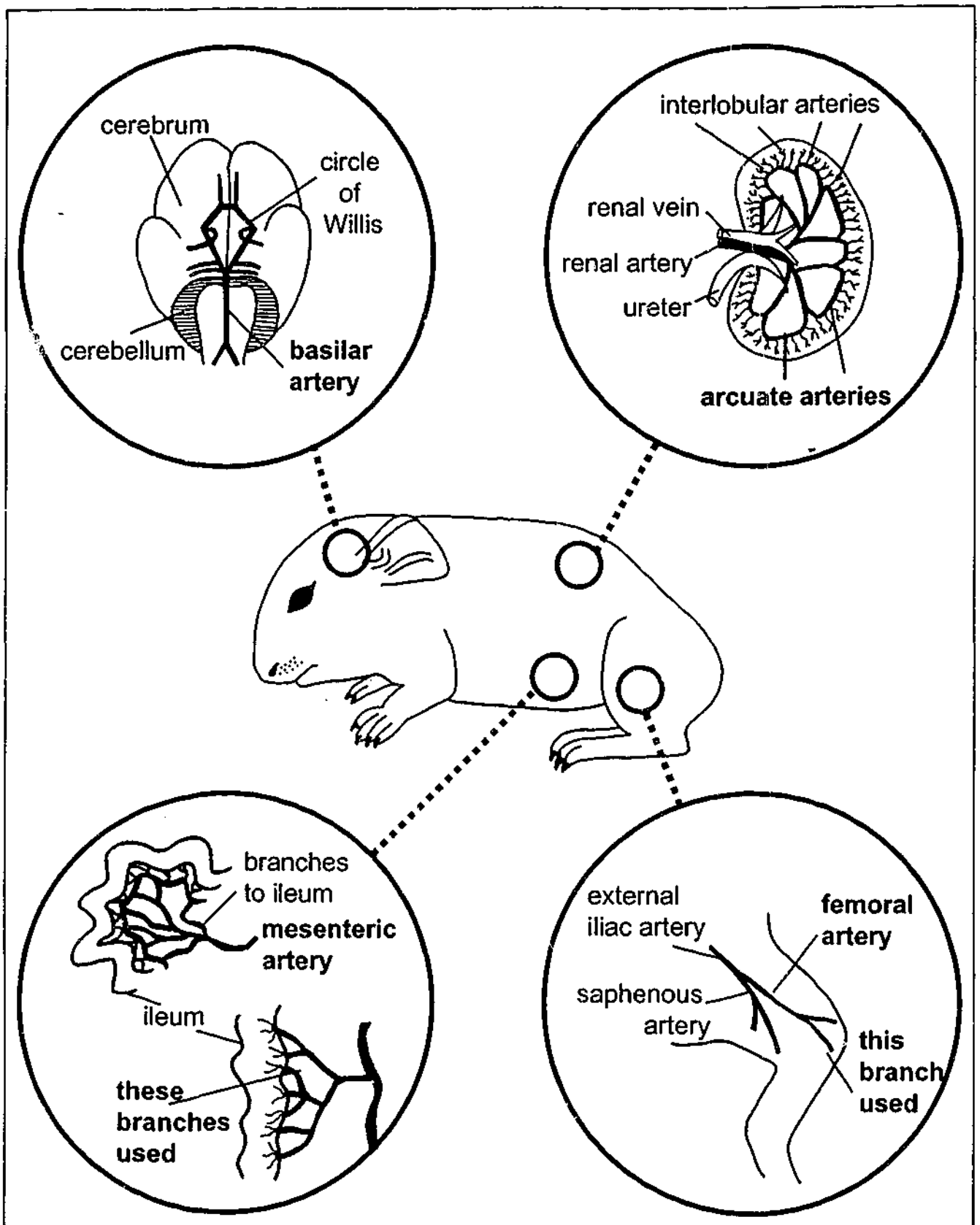


Figure 2-4. Sites of arteries collected for the determination of passive arterial wall mechanics. Schematic diagrams showing the sites from which segments of artery were collected for the determination of passive arterial wall mechanics. Segments of artery were taken from four different vascular beds: the basilar artery from the brain, the arcuate artery from the kidney, a branch of the mesenteric artery, and a branch of the femoral artery.

An artery from the brain A segment of the basilar artery (located at the base of the brain) was selected to measure vessel dimensions and determine vessel wall mechanics. One segment of the artery was dissected from each animal; the average initial OD (pressurised at 10mmHg) was 567±24μm.

An artery from skeletal muscle The femoral artery arises from a branch of the external iliac artery and runs along the inner aspect of the hind leg. The femoral artery branches in the popliteal region; one of these branches was collected for the measurement of vessel dimensions and calculation of vessel wall mechanics. One segment of the artery was dissected from each animal and was collected from the non-catheterised hind limb; the average initial OD (pressurised at 10mmHg) was 620±44μm.

An artery from the mesentery The mesenteric artery sends branches to the ileum, where they further divide before entering the gastrointestinal tract; the artery that was selected for study was the penultimate branch before entry into the ileum. One segment of artery was collected from this region; the average initial OD (pressurised at 10mmHg) was 378±25μm.

An artery from the kidney The arcuate arteries of the kidney can be found at the lower border of the renal cortex, and run in a plane parallel to the cortical border. One segment of arcuate artery was collected; the average initial OD (pressurised at 10mmHg) was 482±28μm.

2.3.10b Dissection of arteries

At post mortem, organs were collected and placed in calcium-free PSS aerated with carbogen (95% O_2, 5% CO_2). All dissections were performed under a microscope. Once a length of the artery (~1600μm) was cleaned of the surrounding connective tissue, it was mounted on a pressure myograph (Living Systems Instrumentation, VT, USA) in a warmed (35°C) bath of Ca^{2+}-free PSS containing 1mM EGTA. EGTA is a calcium chelator, therefore all measurements were made in the absence of extracellular calcium (i.e. passive mechanical properties were determined). The artery was tied to the glass cannula with a nylon monofilament, and any blood was gently flushed from the artery before the distal end of the artery was ligated to make a closed tube.

The arterial segment was slowly pressurised to 200mmHg (maximum) to ensure there were no major leaks in the vessel. The pressure-servo control system was capable of compensating for small leaks in the artery and maintaining intraluminal pressure at the nominated pressure. Where possible, branches that could potentially cause leakage from the main segment of artery were ligated using nylon monofilaments. Inflating and maintaining the artery at a high pressure for several minutes also served to stretch the artery and reduce the impact of slow dilatation over time (Bergel, 1961) that might affect the OD measurements. Following this initial pressurisation, intraluminal pressure was returned to 10mmHg.

2.3.10c The pressure myograph

A schematic diagram of the pressure myograph is shown in Figure 2-5. Once the artery was secured to the glass cannula, the pressure myograph was placed on the stage of an inverted microscope. A solution of Ca^{2+}-free PSS containing 1mM EGTA was pumped through the cannula to pressurise the artery, with the pressure transducer placed at the level of the myograph. The inlet tubing allowed inflow of this same solution so that the vessel was bathed in a warm (35°C, heated by the heating coil located at the base of the myograph) solution while measurements were made; solution was removed via outlet tubing connected to a water pump. A video camera positioned under the stage of the microscope relayed real-time images of the artery to a video monitor. Measurements of arterial dimensions were made from the monitor. This is described in more detail in Section 2.3.10d.

Pressurisation of each artery was controlled by a pressure-servo control system (PS/200/Q Pressure Servo System, Living Systems Instrumentation, VT, USA). This system comprised of a pressure-servo control unit, a pressure adjustment potentiometer, and a peristaltic pump. The artery in the myograph was pressurised by increasing the intraluminal volume. This was achieved by turning the pressure adjustment dial until the desired pressure was shown on the pressure display panel of the pressure control unit. The peristaltic pump fed solution to the lumen of the vessel to create the desired intraluminal pressure. The pressure adjust potentiometer controlled the speed of the pump to maintain the pressure set by the control unit. The system was able to compensate for small leaks in the arteries, as the pressure adjust potentiometer

compared the desired pressure with the pressure detected at the level of the transducer and adjusted the peristaltic pump flow accordingly to compensate for the leak (and associated decrease in pressure). The accuracy of the system was verified at the commencement of the study using an in-line mercury manometer.

2.3.10d Measurements of arterial dimensions

After the initial pressurisation of the arterial segment to 200mmHg, the intraluminal pressure was returned to 10mmHg. Step-wise increases in lumen pressure were made between 10–120mmHg, with measurements of arterial dimensions made at each 10mmHg increment. Segment length (L) and outer diameter (OD) were made from the projected image of the vessel on the video monitor using a standard ruler. For these measurements, the ×1.6 objective lens was used in the microscope (Figure 2-6). The length of the segment of artery was measured (in cm) as the distance between the nylon ties at each end of the vessel. Outer diameter was measured (in mm) as the distance between the external borders of the artery. Once all the L and OD measurements were made, the intraluminal pressure was returned to 10mmHg and the ×10 objective lens was used for the measurement of arterial wall thickness (WT) at each 10mmHg pressure increment. Wall thickness was measured (in mm) as the distance between the external and internal border of the arterial wall (Figure 2-6). Once all of the L, OD and WT measurements had been made on the segment of artery from one vascular bed, another artery from a different vascular bed was dissected, mounted and pressurised.

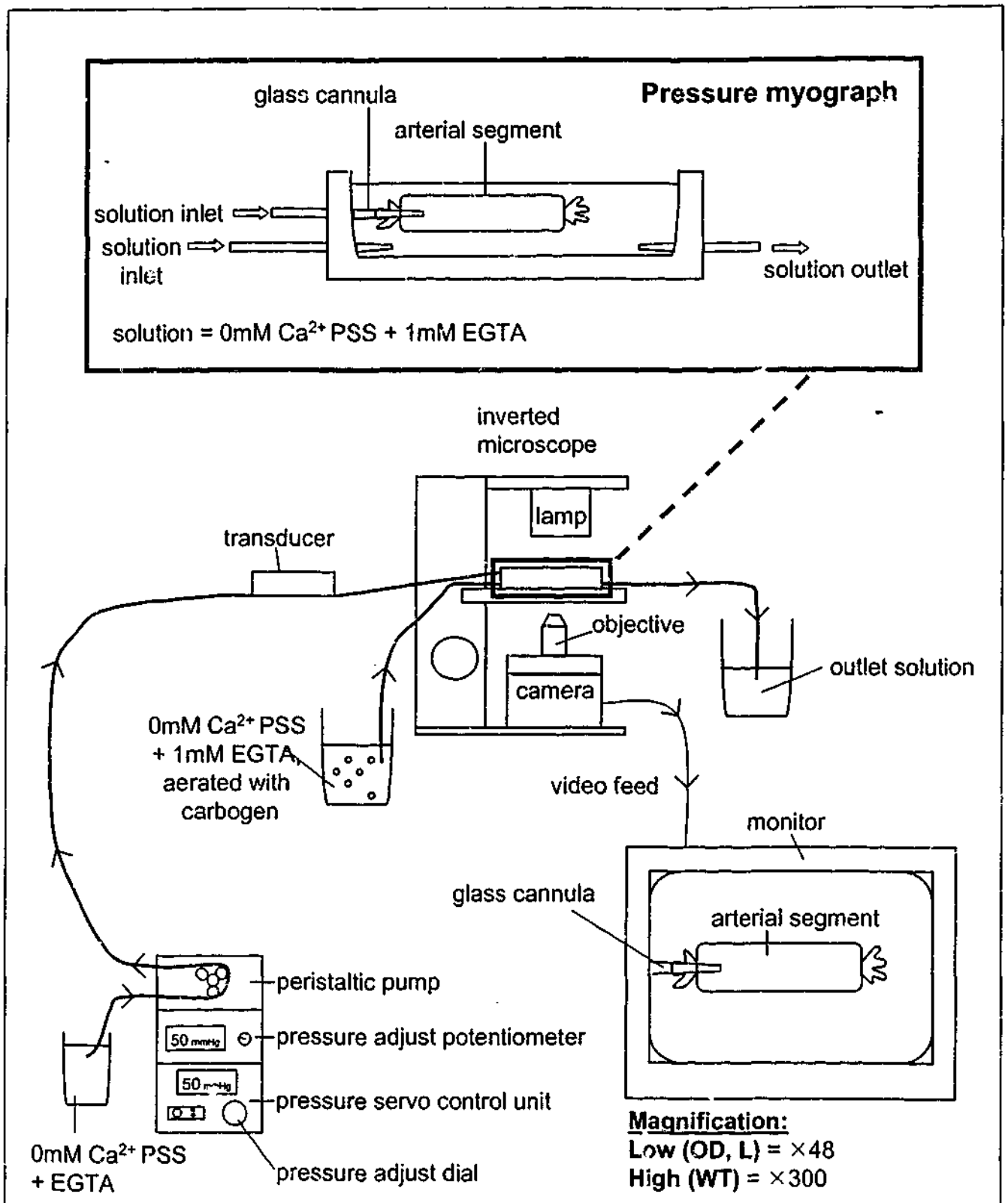


Figure 2-5. Arrangement of equipment used for the measurement of arterial wall mechanics. A schematic diagram of the pressure myograph, pressure-servo control units and monitor from which vessel dimensions were made. A detailed description of this set-up can be found in Section 2.3.10c.

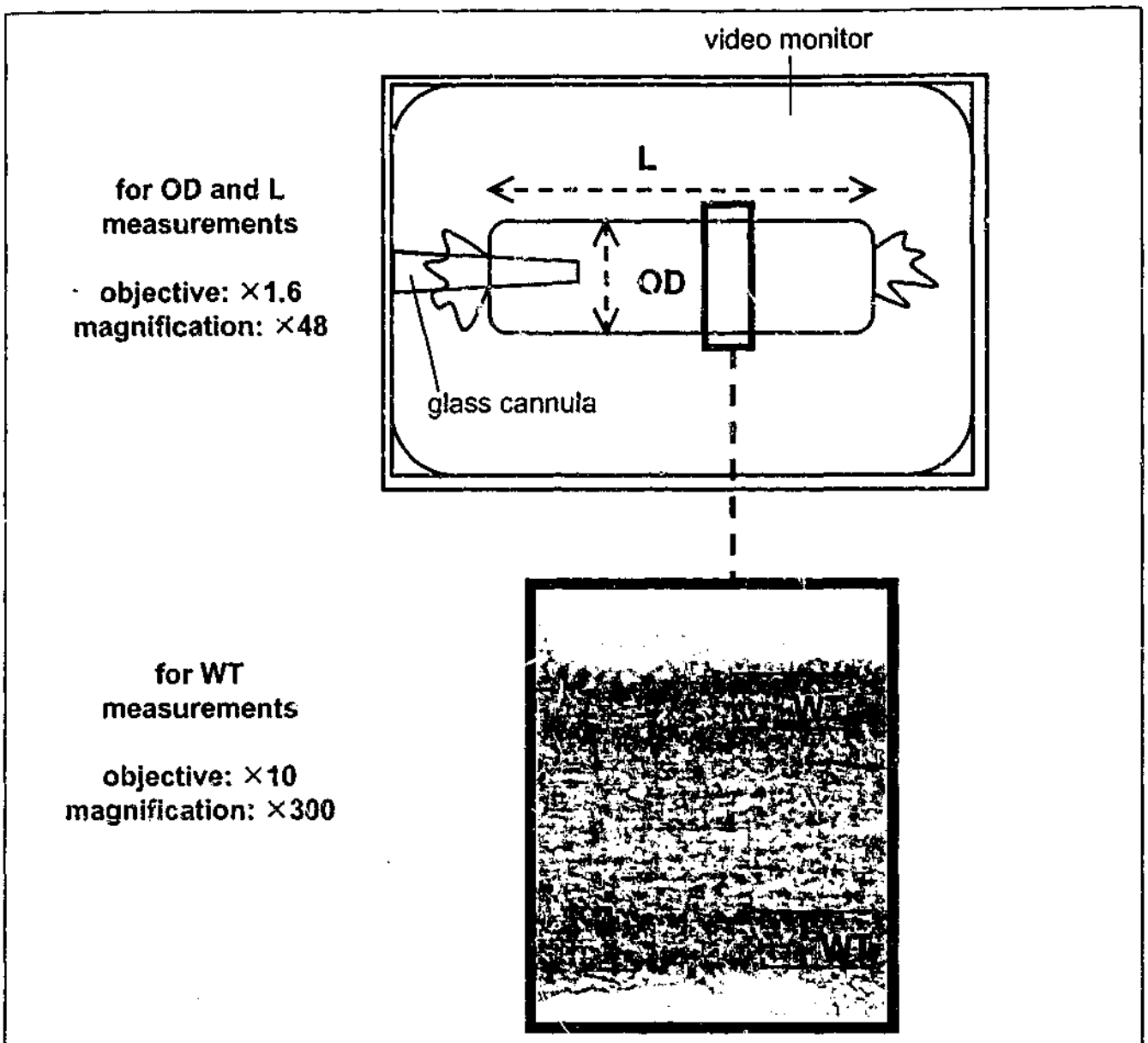


Figure 2-6. Measurement of arterial dimensions during pressurisation. Images of the artery on the myograph were projected onto a video monitor, from which measurements of segment length (L) and outer diameter (OD) were made (×48 magnification) during pressurisation. Wall thickness (WT) was measured at a higher magnification (×300). A more detailed description of these measurements can be found in Section 2.3.10d.

2.3.10e Calculations

Arterial segment dimensions All measures were converted from the value measured from the video monitor (in cm or mm) to the actual size (in μm). The conversion factors were calculated by placing a graticule slide on the stage of the microscope, projecting an image of the graticule onto the video monitor and measuring the known lengths on the graticule.

The percentage change from the initial measurement at 10mmHg intraluminal pressure has been calculated for each of the measured dimensions (OD, L, WT), and also internal (lumen) diameter (ID) which was calculated:

$$Inner\ diameter\ (\mu m) = Outer\ diameter - (2 \times Wall\ thickness)$$

$$or:\ ID = OD - 2 \times WT$$

The cross-sectional area (CSA) of the arterial wall was calculated using the formula (Pourageaud & De Mey, 1997):

$$Arterial\ wall\ cross\text{-}sectional\ area\ (\mu m^2) = artery\ CSA - lumen\ CSA$$

$$or:\ Arterial\ wall\ CSA = \Pi \times (OD \div 2)^2 - \Pi \times (ID \div 2)^2 = \Pi \times (OD^2 - ID^2) \div 4$$

Changes to the length of the arterial segment during pressurisation gave an indication of the longitudinal elastic properties of the artery while changes to the outer and inner diameters of the arterial segment, in conjunction with changes in WT, gave an indication of the circumferential elastic properties. To accommodate increasing intraluminal pressures, an elastic artery might be expected to lengthen and dilate by increasing either OD, ID or both. The wall thickness might also be expected to decrease with increasing pressures. Thus as the artery dilates, wall thickness would be expected to decrease and luminal diameter would be expected to increase. A less elastic (i.e. stiffer) artery would not be expected to lengthen or dilate to the same degree as a more elastic artery.

Arterial wall properties Chronically elevated arterial pressure can lead to vascular remodelling, so that arteries can accommodate the higher pressures. This remodelling can lead to a reduced lumen:wall thickness (arising from a decreased lumen, an increased wall thickness or a combination of both) which has also been associated with elevated arterial pressure (Lee & Smeda, 1985; Mulvany, 1993b, 1996). This vascular remodelling also has the negative effect of making the artery less compliant, which in turn can constrain vascular relaxation and result in a further increase in arterial pressure.

$$Lumen{:}wall\ thickness\ (ID/WT) = Internal\ diameter \div Wall\ thickness$$

The tension of the arterial wall can be calculated using the Law of La Place (*tension=radius ×pressure*). However, this formula assumes the vessel is thin walled,

and does not account for a wall that is not infinitely thin walled. The modified equation of circumferential wall stress allows WT to be taken into consideration by its inclusion in the formula (Hill & Ege, 1994; Crijns *et al.*, 1999):

$$Wall\ stress\ (kPa) = (Radius \times Pressure) \div (2 \times Wall\ thickness)$$

$$or:\ Wall\ stress = (R \times P) \div (2 \times WT)$$

where: R = internal radius of the vessel (i.e. ID ÷ 2)

P = pressure in Pa

<u>*Arterial compliance and distensibility*</u> Luminal volume was calculated as:

$$Lumen\ volume\ (\mu m^3) = \Pi \times (ID \div 2)^2 \times L$$

and was then expressed as the percentage change from the initial volume at 10mmHg. A less compliant artery would be expected to have a smaller change in lumen volume per change in pressure. Arterial compliance and distensibility were calculated by two methods, the first being based on the cross-sectional area (CSA) of the artery wall (Crijns *et al.*, 1999) and the second based on the luminal volume to include vessel lengthening.

$$Cross\text{-}sectional\ compliance\ (mm^2/kPa) = \Delta CSA/\Delta P = (CSA_2 - CSA_1) \div (P_2 - P_1)$$

$$Cross\text{-}sectional\ distensibility\ (kPa^{-1}) = \Delta CSA/CSA_i/\Delta P$$

$$Volume\ compliance\ (mm^3/kPa) = \Delta V/\Delta P = (V_2 - V_1) \div (P_2 - P_1)$$

$$Volume\ distensibility\ (kPa^{-1}) = \Delta V/V_i/\Delta P$$

where: P_1 = lower intraluminal pressure

P_2 = higher intraluminal pressure

CSA_1 and V_1 = wall cross-sectional area and volume at P_1

CSA_2 and V_2 = wall cross-sectional area and volume at P_2

CSA_i = initial wall cross-sectional area at 10mmHg intraluminal pressure

V_i = initial lumen volume at intraluminal pressure of 10mmHg

For each of these equations, the measurement at the lower pressure (P_1) in each 10mmHg increment pair (e.g. CSA at 30mmHg in the calculation of compliance or distensibility between 30mmHg and 40mmHg) is used as the "baseline" to provide an estimate of the compliance and distensibility over the 10mmHg increment.

Stress-strain relationship Wall strain was calculated (Hill & Ege, 1994):

$$Wall\ strain = (ID_x - ID_i) \div ID_i$$

where: ID_x = internal diameter at intraluminal pressure x

ID_i = initial internal diameter at 10mmHg intraluminal pressure

The stress-strain relationship (Young's modulus of elasticity) uses measures at 0mmHg or 10mmHg as reference measures (for the studies presented in this thesis, 10mmHg was used as the reference); this relationship, providing a measure of arterial stiffness may represent a more generalised "steady state" situation and is more commonly used in the literature concerning arterial mechanical properties (Hill & Ege, 1994; Muller-Delp *et al.*, 2002).

The stress-strain relationship was analysed using Origin (Version 7.0, Microcal Software Inc., CA, USA) to calculate the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment (Hill & Ege, 1994). This exponential function was used to describe the overall stress-strain relationship and the data fit this equation with a $r^2>0.93$. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low strain (elastic) range of the arteries and be^{k_2x} describes the high strain (collagenous) range of the arteries. The derived rate constants (slopes) were compared using GraphPad Instat (Version 3.05, GraphPad Software Inc., CA, USA), and a higher rate constant was consistent with a stiffer, less compliant artery as for a given strain, the stress on the arterial wall was greater.

2.3.11 Statistical analysis

One-way analysis of variance (ANOVA) were used to compare body weights and body dimensions at birth and one year of age, arterial pressure and heart rate at one year, and organ weights at post mortem. Two-way repeated measures ANOVA were used to compare body weight and growth rates throughout the postnatal period (birth–1 year); factors were treatment and age. The postnatal growth data were further analysed by one-way ANOVA at each age to determine differences in body dimensions and the growth rates between each age. Pearsons correlation coefficients were calculated for comparisons between arterial pressure body weights and postnatal growth. Arterial wall

data were analysed by two-way repeated measures ANOVA, with factors being treatment and pressure. These data were further analysed by one-way ANOVA to determine differences between groups at each pressure.

Data are presented as mean±SEM unless otherwise indicated. Statistical analyses were performed using SPSS for Windows (Version 10.0.5) with the level of significance taken at $p<0.05$, unless otherwise stated. All significant differences indicated by ANOVA were subjected to the Student-Newman-Keuls (SNK) post-hoc test to test for significant differences between individual means.

2.4 Results

2.4.1 Outcomes of the placental restriction surgery

Of the 83 guinea pigs obtained from Monash Animal Services, 31 animals delivered viable offspring that were used for postnatal studies. The remaining 52 animals that did not successfully deliver viable offspring included one that was not pregnant, three that died during surgery, 4 that died within a week following surgery, and one that died during delivery. Nine aborted their fetuses, 7 delivered their offspring that were dead or died soon after birth. Twenty-eight guinea pigs resorbed all fetuses during gestation and failed to deliver any offspring. The abdomen of these animals was palpated one week after term for the presence of any fetuses. In every case, no fetuses could be palpated, and the mothers were heavily sedated with a 2ml i.p. injection of pentobarbitone sodium (60mg/ml) before being killed with an intracardiac injection of 325mg/ml pentobarbitone sodium. Following euthanasia, the abdomen was opened for visual inspection for confirmation that all fetuses had been resorbed between the time of surgery and the time of euthanasia. The outcomes of the pregnancies are summarised in Table 2-1. The outcome of the pregnancy, with regards to the delivery of viable offspring was not related to maternal age at mating, the maternal age or body weight at the time of the placental restriction surgery, or the gestational age of the fetuses at the time of the surgery.

Details of the cohort of viable offspring are summarised in Table 2-2. The average body weight and gestational age at birth were 104.7±2.9g and 69±1d GA, respectively. The distribution and range of birth weights and gestational ages at birth are shown in Figure

2-7. The birth weights of all viable offspring were ranked in ascending order and allocated to one of three groups according to birth weight. These groups were formed such that there were similar number of pups in each group:

Group 1	Low birth weight; birth weight < 95g, n=11
Group 2	Average birth weight; birth weight between 95–110g, n=16
Group 3	High birth weight; birth weight > 110g, n=17

As the offspring were born spontaneously and it was not possible to mark them *in utero* for later identification, it was not possible to deduce which of the low birth weight offspring were growth restricted as a result of the placental restriction procedure, and which were growth restricted as a result of their *in utero* position during gestation.

Table 2-1. Summary of pregnant guinea pigs that underwent the placental restriction surgery, and the outcome of the pregnancy. A total of 83 guinea pigs were obtained from Monash Animal Services; one of which was not pregnant. Data are shown as mean±SEM, with the range of the data in parentheses.

	n	Maternal age at mating (d)	Maternal age at surgery (d)	Gestational age at surgery (d)	Maternal weight at surgery (g)
All pregnant guinea pigs	82	107±4 (62–205)	139±4 (92–235)	32±1 (28–40)	777±9 (600–1000)
Sows that failed to deliver or carry to term	44	117±6 (62–205)	148±6 (92–235)	31±1 (28–36)	780±12 (600–900)
Died during, or within a week of surgery	7	112±15 (74–191)	144±16 (105–227)	31±1 (31–36)	763±61 (620–900)
All fetuses resorbed	28	125±8 (67–205)	155±8 (102–235)	31±1 (28–36)	789±16 (600–900)
Fetuses aborted	9	97±9 (62–140)	129±8 (92–168)	32±1 (28–35)	766±17 (700–865)
Sows that carried fetuses to term	38	97±4 (67–156)	129±4 (99–189)	33±1 (29–40)	773±15 (600–1000)
Sows that delivered dead offspring at term	7	118±12 (75–156)	151±12 (110–189)	32±1 (30–35)	779±52 (600–930)
Sows that delivered live offspring at term	31	92±4 (67–152)	124±4 (99–181)	33±1 (29–40)	771±15 (600–1000)

Table 2-2. The history of guinea pig offspring used in the studies described in this chapter. This table summarises the identification numbers, gender, birth weight group, gestational age (GA) at the time of the placental restriction surgery, GA and weight at birth, and the fate of each pup. "+" refers to arterial pressure being successfully measured at one year of age. "M" refers to offspring whose arteries were collected for the determination of arterial wall mechanical properties, "H" refers to offspring whose arteries were collected for histological analysis (not in this thesis). "DS1" refers to offspring that died during the catheterisation surgery at one year, "DS2" refers to offspring that were found dead on the day following the catheterisation surgery, "DS3" refers to offspring that were found dead on the day following the arterial pressure measurement, the measurement was excluded from analysis, "FD" refers to offspring found dead at other times (age at death in parentheses), "CB" refers to a blocked, non-functional catheter via which arterial pressure could not be measured.

ID	Sex	Group	GA at fetal surgery	Birth		Arterial pressure measured	Fate	Comment
				GA (d)	Weight (g)			
1	♂	1	40	57	68		–	DS1
2	♀	2	40	64	100		M	CB
3	♂	1	32	67	87	+	H	
4	♀	2	32	67	102	+	–	DS3
5	♂	1	31	68	79		–	DS1
6	♀	2	29	70	103		M	CB
7	♀	1	29	70	93	+	H	
8	♂	2	29	70	95	+	M	
9	♀	2	29	70	103		–	DS2
10	♀	–	29	69	85		–	FD (4d)
11	♂	2	29	69	95		–	FD (280d)
12	♂	2	29	69	95		–	DS2
13	♀	3	30	71	125		M	CB
14	♂	2	32	71	100	+	H	
15	♂	3	32	71	119	+	H	
16	♀	3	32	73	144	+	M	
17	♂	2	35	70	105	+	H	
18	♀	2	35	70	106		M	CB
19	♀	2	34	69	101	+	M	
20	♂	3	34	69	113		–	DS2
21	♂	2	34	69	107	+	H	
22	♂	1	34	69	88	+	H	
23	♀	3	33	70	129	+	H	
24	♀	3	34	72	117	+	H	
25	♂	3	32	70	117		–	DS2
26	♂	3	32	70	142	+	H	
27	♂	1	32	70	90	+	–	DS3
28	♀	3	32	70	126	+	H	
29	♀	3	32	70	117		H	
30	♀	3	31	69	151	+	H	
31	♀	2	35	67	107	+	M	
32	♂	3	35	67	111	+	H	
33	♂	1	34	67	87	+	M	
34	♀	3	31	67	116	+	M	
35	♀	3	32	69	118	+	M	
36	♂	3	32	69	115	+	H	
37	♂	1	34	68	77	+	H	
38	♀	1	34	68	73	+	M	
39	♂	2	34	71	96		–	DS1
40	♀	3	33	70	127	+	M	
41	♀	3	31	70	125	+	M	
42	♀	1	31	70	63	+	M	
43	♂	2	31	70	104	+	M	
44	♀	1	31	70	93	+	M	
45	♀	2	31	70	99	+	H	
Mean±SEM			32±1	69±1	104.7±2.9			

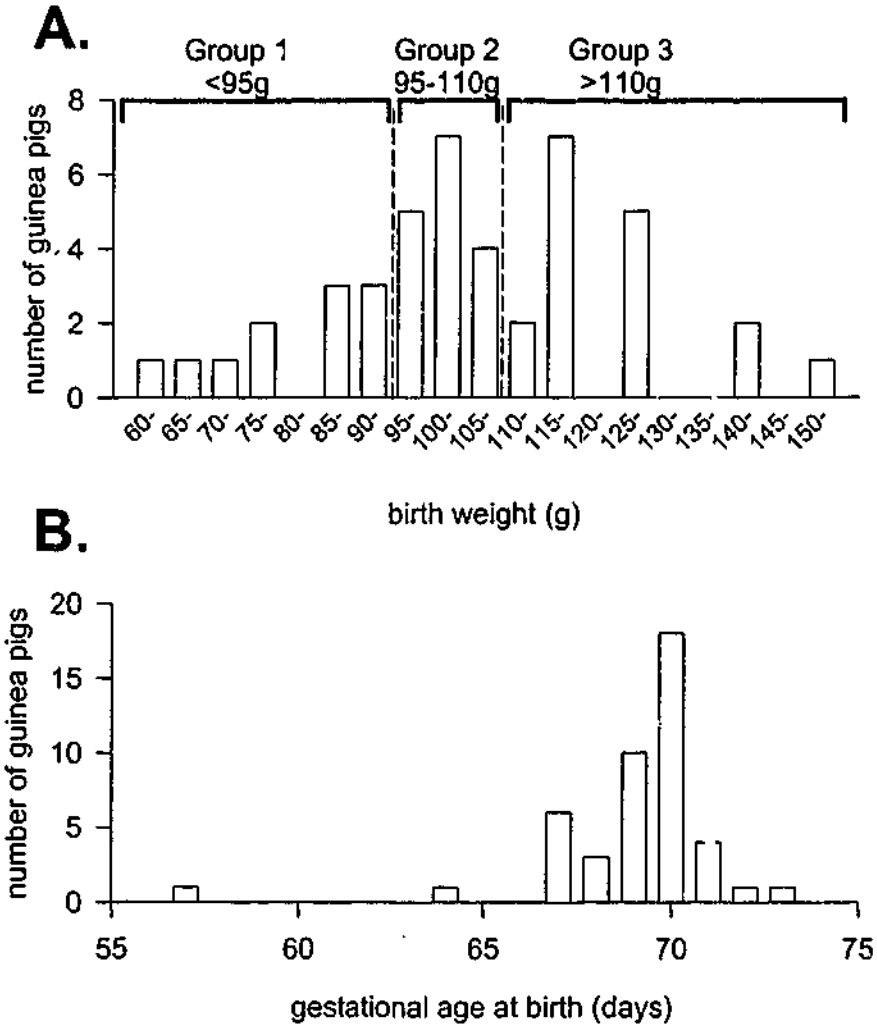


Figure 2-7. Distribution of weights and gestational ages at birth. (A) Birth weights and (B) gestational age at birth of the cohort of viable offspring. These offspring were allocated into one of three groups according to birth weight (as indicated by the bars in graph A).

2.4.2 Birth weight and body dimensions

2.4.2a Gestational age

The average gestational age at birth for Group 1 (67±1d GA) was significantly less than that of Groups 2 and 3 (Group 2: 69±1d GA, Group 3: 70±1d GA), however, this was due to one pup (ID:#1, Table 2-1) who born at 57d GA (Figure 2-7B). When this animal was excluded from the analysis, there were no differences in the gestational age at birth between the three groups (Group 1: 69±1d GA, Group 2: 69±1d GA, Group 3: 70±1d GA). Therefore, the animals in the low birth weight group were not small due to preterm birth.

2.4.2b Birth weight

Mean birth weights of the three groups of animals were significantly different from each other. Group 1 had lower birth weights than both Groups 2 and 3, and Group 3 had higher birth weights than both Groups 1 and 2 (Figure 2-8A, Table 2-3). When the preterm animal (ID:#1, Table 2-2) was excluded, the average birth weight of animals in Group 1 was 82.2±3.3g; the average birth weight of Group 1 remained less than that of animals in both Groups 2 and 3. Given the differences in birth weight were not affected by the inclusion or the exclusion of the preterm pup, it has been included in all subsequent analyses. The mean birth weights of female offspring in the three groups were significantly different from each other (Group 1: 81.5±7.2g, n=4; Group 2: 102.9±1.2g, n=8; Group 3: 126.8±3.4g, n=11). These differences in birth weight between the three groups was also evident in the male offspring (Group 1: 80.3±3.4g, n=7; Group 2: 100.0±2.2g, n=8; Group 3: 120.8±5.5g, n=6)

2.4.2c Body dimensions at birth

<u>Body length</u> Like birth weight, mean nose-to-rump length at birth was significantly different between the three groups. The average nose-to-rump length was lowest in Group 1 (163±3mm); this was significantly lower than the average nose-to-rump length of animals in Group 2 (171±2mm) or Group 3 (180±2mm). The average nose-to-rump length was significantly higher in Group 3 compared with Group 2 (Figure 2-8B).

<u>Girths and circumferences</u> There were no significant differences in the thoracic girth between the three groups at birth, although there was a trend for the animals in Group 1 to have smaller thoracic girths and the animals in Group 3 to have larger thoracic girths (Group 1: 98±4mm, Group 2: 104±2mm, Group 3: 106±2mm). Like thoracic girth, there were no significant differences in the abdominal girths of animals in the three groups at birth (Group 1: 105±4mm, Group 2: 110±2mm, Group 3: 113±3mm). The average hip circumference of animals in Group 1 (93±3mm) was significantly lower than the average hip circumference of animals in Groups 2 (103±1mm) or 3 (105±2mm). There were no differences between Groups 2 and 3, with respect to hip circumference at birth.

Ponderal index Group 1 had a lower ponderal index at birth compared with Group 3, indicating these animals in Group 1 were light for their body length compared with the those in Group 3. The mean ponderal index at birth for the Group 2 animals was not different to the mean ponderal indexes of the other two groups (Figure 2-8C).

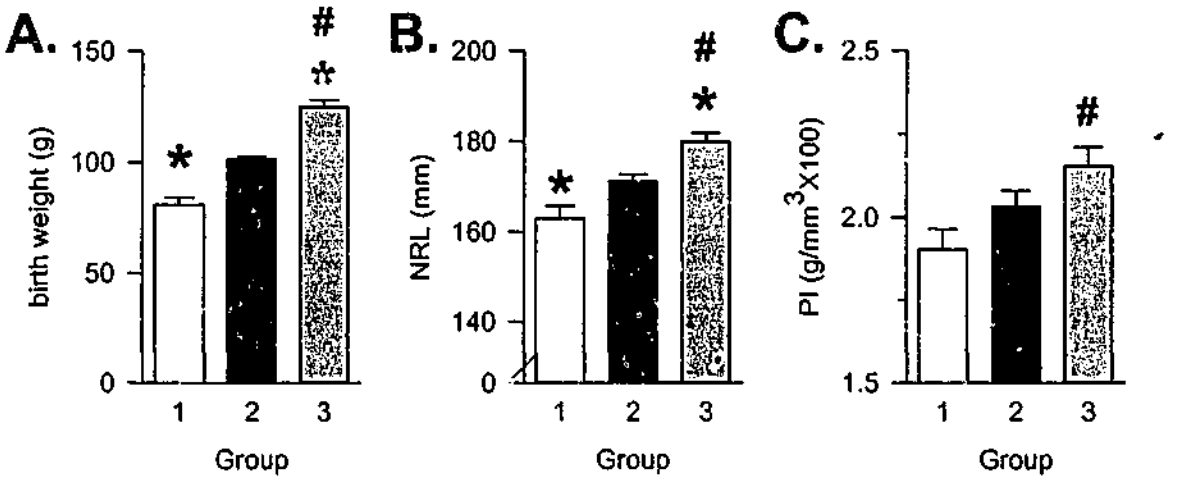


Figure 2-8. Body size at birth. Mean (A) birth weight, (B) nose-to-rump length (NRL) and (C) ponderal index (PI) of the guinea pigs in Group 1 (n=11), Group 2 (n=16) and Group 3 (n=17) at birth. * indicates a significant difference compared with Group 2; # indicates a significant difference between Groups 1 and 3.

2.4.3 Postnatal growth

2.4.3a Body weight

The body weights of the guinea pigs during the first postnatal year is shown in Table 2-3. The body weights of all three groups remained significantly different from each other between birth and two weeks of age. By 4 weeks, animals in Group 1 had caught up in body weight to those in Group 2; however, the animals in Group 3 remained heavier than those in Groups 1 and 2. After 8 weeks, there were no significant differences in the body weights of animals in Groups 2 and 3, and after 9 months, there were no significant differences in the body weights of the three groups.

Table 2-3. Body weight (in grams) from birth to one year of age. At each age, an asterisk (*) indicates a significant difference ($p<0.05$) compared with Group 2, # indicates a significant difference ($p<0.05$) compared with Group 3.

Age	Group 1	Group 2	Group 3
Birth	80.8±3.3*#	101.3±1.4	124.9±2.9*
1 week	126.1±5.0*#	148.6±4.6	186.4±6.1*
2 weeks	187.4±5.9*#	210.2±7.1	256.8±8.3*
4 weeks	268.6±12.2#	302.4±10.4	368.3±13.0*
8 weeks	482.9±15.1#	525.0±17.6	581.9±21.4
12 weeks	674.0±23.4	701.2±23.1	750.2±22.4
6 months	872.5±28.5#	920.4±23.7	968.8±21.6
9 months	1003.1±38.2	1020.1±25.6	1107.6±30.7
12 months	1014.3±37.6	1065.8±33.5	1122.63±41.9

The absolute growth rates (g/day) for the three groups of guinea pigs are shown in Figure 2-9A. After an initial increase in absolute growth rate early in the postnatal period, there was a decrease in absolute growth rate in all three groups after two weeks of age. Animals in Group 3 had significantly higher growth rates (g/day) than the other two groups only for the period between birth and one week. Two-way ANOVA revealed there was a significant effect of birth weight group on the absolute growth rates, with animals in Group 3 having a higher growth rate compared with the other two groups throughout the postnatal study period.

Higher absolute growth rates in Group 3 indicated that these offspring gained more weight (in grams) than the animals in Groups 1 and 2. While heavier animals might gain more weight than lighter animals, the relative weight increase may not have differed between heavier and lighter animals. To account for differences in the absolute weights of the groups, the growth rates of the three groups of guinea pigs were expressed as a percentage of the previous weight (i.e. relative weight increase/day) were calculated. Throughout the postnatal period, there was an effect of birth weight group on the relative weight increase, with animals in Group 1 having a higher relative growth rate compared with the other two groups. This indicates that despite being lighter at birth than the other two groups, an higher relative weight gain of the Group 1 animal led to

catch-up growth such that there were no significant differences in the body weights of Groups 1 and 2 after 4 weeks of age, and Groups 1 and 3 by 12 postnatal weeks, confirming the data in Table 2-4.

There was no significant difference in the relative growth rates of the animals in Group 2 and Group 3 during the study period. Although the body weights of these two groups remained significantly different from each other for the first 4 postnatal weeks, marginally higher (although not significant) relative growth rates in the Group 2 animals compared with Group 3 would account for the catch-up in body weights of the Group 2 animals compared with Group 3 during this time.

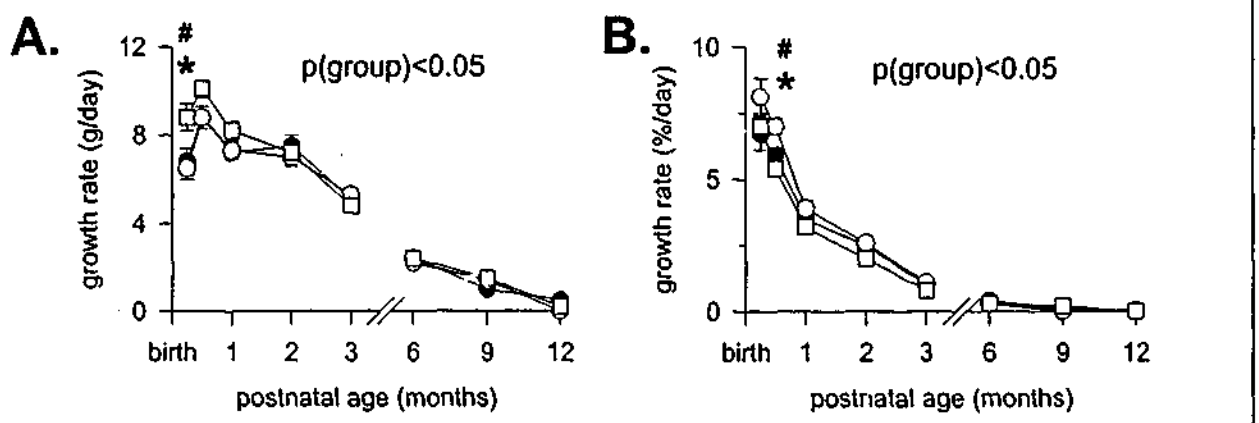


Figure 2-9. Postnatal growth rates from birth to one year of age. (A) Absolute growth rates (g/day) and (B) relative weight gain (% increase/day) in Group 1 (○, n=11), Group 2 (●, n=16) and Group 3 (□, n=17) animals. p(group)<0.05 indicates a significant effect of birth weight group throughout the study period (analysis by two-way ANOVA). * indicates a significant difference compared with Group 2 at the individual age; # indicates a significant difference between Groups 1 and 3 at the individual age (analyses by one-way ANOVA).

2.4.3b Body dimensions at one year

Body weight There was no significant difference in the body weights of the three groups at one year (Table 2-3, Figure 2-10A). The body weights of the three groups were also not different from each other when data was analysed for the female offspring alone (Group 1: 957.0±53.0g, n=4; Group 2: 1043.7±53.6g, n=7; Group 3:

1066.1±39.1g, n=11) or the males alone (Group 1: 1060.2±47.2g, n=6; Group 2: 1081.3±44.8g, n=8; Group 3: 1247.0±83.6g, n=5).

Body length Although significantly different between the three groups at birth, nose-to-rump length was not significantly different between the three groups of guinea pigs at one year of age (Figure 2-10B). By one postnatal week, the mean nose-to-rump length of Group 1 was not different from Group 2, however, they remained shorter in body length than Group 3 animals until 8 weeks of age. The nose-to-rump lengths of Groups 2 and 3 were was also only different at birth; by one postnatal week, there was no difference in the body length of these two groups.

Ponderal index Although the ponderal indexes of Group 1 and Group 3 were different at birth, there was no difference in the ponderal indexes of the three groups at one year of age (Figure 2-10C).

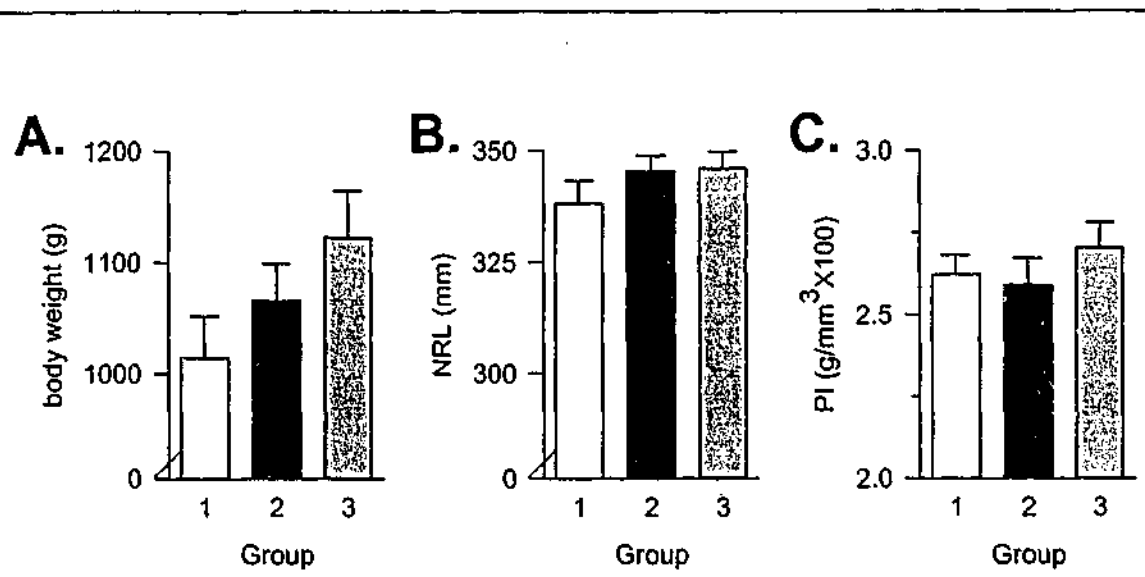


Figure 2-10. Body size at one year. Mean (A) body weight, (B) nose-to-rump length and (C) ponderal index of the guinea pigs in Group 1 (n=9), Group 2 (n=16) and Group 3 (n=16) at one year of age.

Girths and circumferences There were no significant differences in the mean thoracic girths of the three groups at any period during the first postnatal year, or at one year of age (Group 1: 193±5mm, Group 2: 193±2m,Group 3:192±2mm). While not different at birth, the mean abdominal girth of Group 1 at one year, and also at 9 months, was significantly less than that of Group 3 (265±6mm *vs* 284±5mm, $p<0.05$). The mean

abdominal girth of Group 2 (277±5mm) was not different from the other two groups at one year. Despite Group 1 having a smaller hip circumference at birth compared with Groups 2 and 3, after birth, hip circumferences did not differ between the three groups (at one year of age: Group 1: 208±3mm, Group 2: 208±2mm, Group 3: 208±3mm).

2.4.4 Arterial pressure and heart rate

Due to technical problems associated with maintaining the patency of catheters for the recording of arterial pressure, arterial pressure and heart rate were not recorded in all animals. The data from two animals (one from Group 1, the other from Group 2) were excluded from analysis, as these animals were found dead in their cages on the day following arterial pressure measurement. Although the animals appeared to have adequately recovered from the catheterisation surgery, it is not clear as to whether the arterial pressure measurements would have been compromised by the same factor that led to their premature death. Therefore the arterial pressure and heart rate measurements included for analysis arose from eight animals from Group 1, nine animals from Group 2 and 12 animals from Group 3. Arterial pressure and heart rate were recorded for 31.7±0.7 minutes, with an average of 29.5±1.0 minutes (92.6±1.7%) of each recording included in the analysis.

2.4.4a Arterial pressure

There were no significant differences in the mean arterial pressure of the three groups at one year (Figure 2-11A), although there appeared to be a trend for the animals in Group 1 to have lower mean arterial pressure (52.0±2.6mmHg) compared with the other two groups (Group 2: 56.9±2.9mmHg; Group 3: 55.5±2.9mmHg). There were also no differences in the systolic (Figure 2-11C) and diastolic pressures (Figure 2-11D) of the three groups. Mean arterial pressure in the female offspring was not different between the three groups (Group 1: 49.6±3.8mmHg, n=4; Group 2: 51.3±5.8mmHg, n=5; Group 3: 55.6±3.2mmHg, n=8), nor was it different in the male guinea pigs (Group 1: 54.4±2.7mmHg, n=4; Group 2: 53.3±5.5mmHg, n=6; Group 3: 55.4±4.5mmHg, n=4).

2.4.4b Heart rate

There was no significant difference in the heart rates (Figure 2-11B) of the three groups (Group 1: 226.4±4.1bpm; Group 2: 243.0±5.6bpm; Group 3: 240.5±5.4bpm).

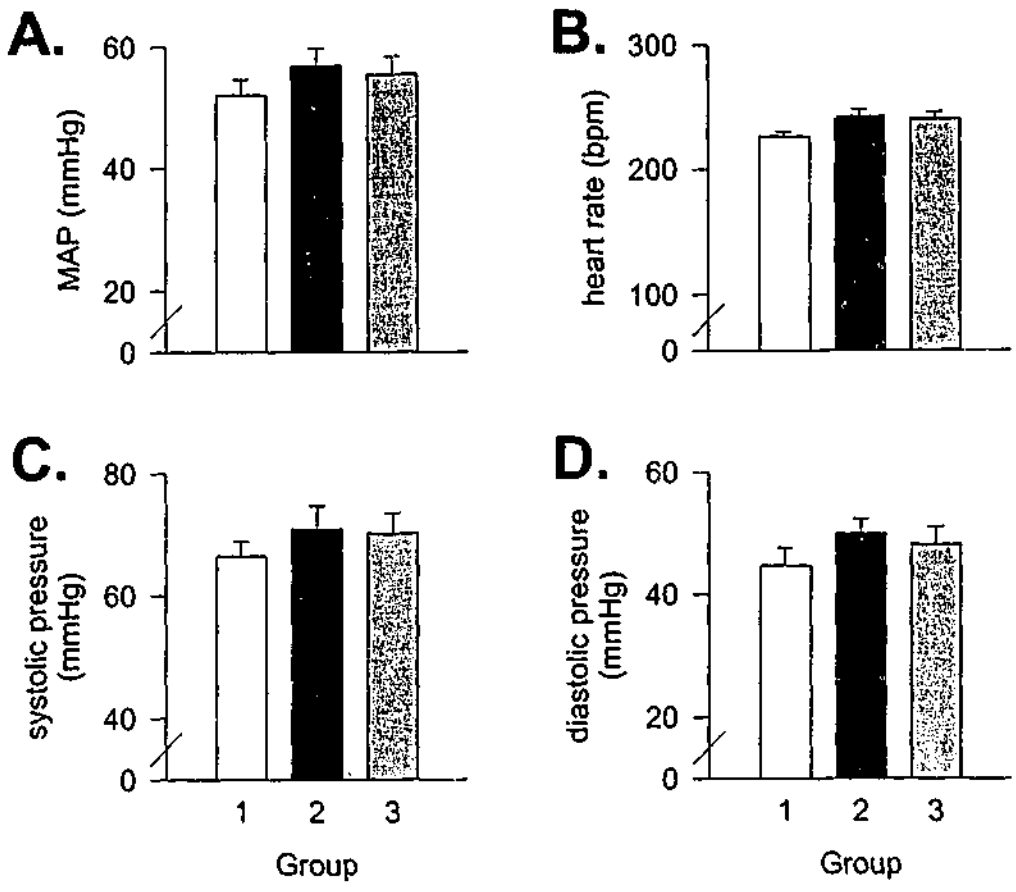


Figure 2-11. Arterial pressure and heart rate at one year of age. (A) Mean arterial pressure (MAP), (B) heart rate, (C) systolic pressure and (D) diastolic pressure in Group 1 (n=8), Group 2 (n=9) and Group 3 (n=12) guinea pigs at one year of age.

2.4.5 Correlations

There was no correlation between birth weight and mean arterial pressure at one year of age ($r=0.22$, $p=0.25$; Figure 2-12A). MAP at one year was also not correlated to body weight at this age ($r=0.21$, $p=0.27$) or relative growth rate ($r=-0.17$, $p=0.38$; Figure 2-12B). Despite no significant differences in the thoracic girths of the three groups of guinea pigs at birth, there was a significant correlation between thoracic girth at birth and MAP at one year ($r=0.44$, $p<0.05$). There was no relationship between MAP at one year and body length at birth ($r=0.29$, $p=0.12$), ponderal index at birth ($r=-0.04$, $p=0.82$) or gestational age at birth ($r=-0.08$, $p=0.67$).

Although there were no differences in the body weights of the three groups of guinea pigs at one year of age, there was a significant correlation between body weight at birth and at one year ($r=0.43$, $p<0.01$; Figure 2-13A). Birth weight was also correlated with postnatal growth rates ($r=-0.70$, $p<0.01$; Figure 2-13B), with the lower birth weight

animals having greater growth rates that led to catch-up growth during the first postnatal year. Similar correlations were found between size at birth and at one year of age with respect to body length ($r=0.34$, $p<0.05$), thoracic girth ($r=0.56$, $p<0.01$), abdominal girth ($r=0.28$, $p=0.08$), hip circumference ($r=0.41$, $p<0.01$) and ponderal index ($r=0.28$, $p=0.07$).

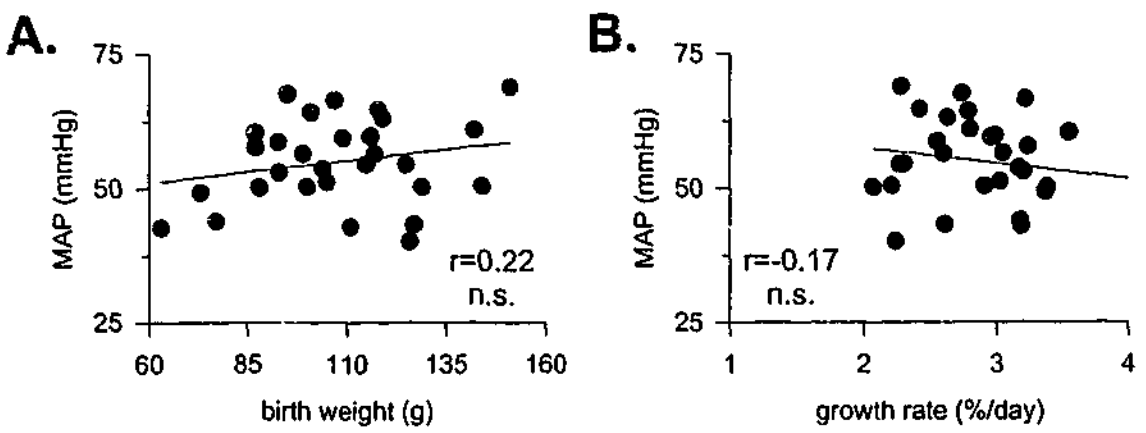


Figure 2-12. Mean arterial pressure at one year: Relationship to birth weight and postnatal growth rate. The relationship between MAP at one year of age and (A) birth weight and (B) overall growth rate between birth and one year (% weight gain/day). r value refers to Pearsons correlation coefficient for the data from the total cohort of animals; n.s. indicates correlation was not statistically significant.

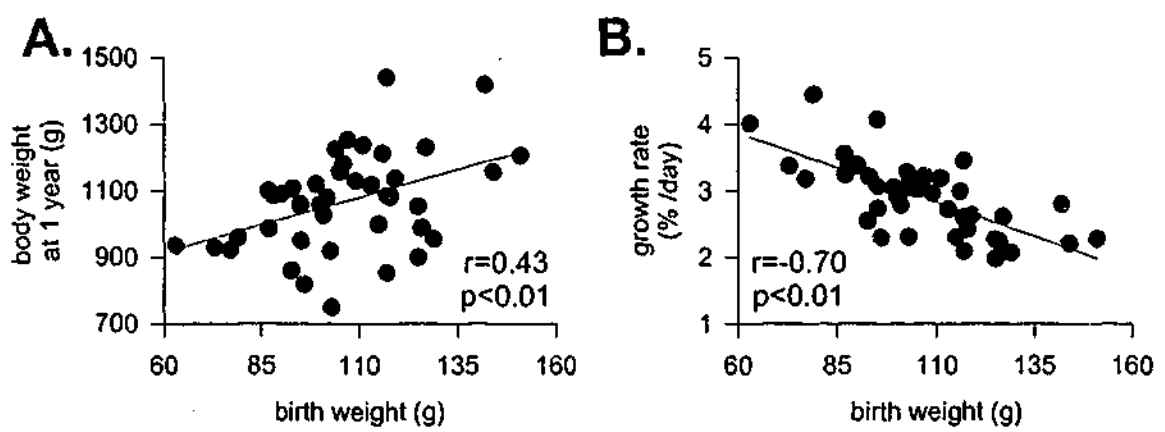


Figure 2-13. Relationship between birth weight and postnatal growth. The relationship between birth weight and (A) body weight at one year, and (B) overall growth rate between birth and one year (% weight gain/day). r value refers to Pearsons correlation coefficient for the data from the total cohort of animals; p values indicates the level of statistical significance.

2.4.6 Organ weights

Prior to post mortem, the animals were randomly divided into two groups – those that had tissues perfusion fixed for histological analysis of blood vessels (data not presented in this thesis) and those that were killed for the collection of arteries for the determination of arterial wall mechanics. The organ weights have been analysed separately due to possible effects of fixation on tissue weights.

The weights of the major organs from the animals from which tissue were collected for histological analysis are shown in Table 2-4. The organ weights of the three groups were not different from each other, with the exception of kidney weights. The combined kidney weight (left+right) of animals in Group 1 was significantly lower than that of Group 2, however, when the kidney weights were adjusted for body weight, there was no difference in the kidney weights of the three groups.

Table 2-4. Organ weights from animals from which blood vessels were collected for histological analysis. At post mortem, organs were perfusion fixed with 4% paraformaldehyde before the major organs were excised and weighed. Data shown are absolute organ weights (g) and organ weights adjusted for body weight (g/kg BWt). Asterisk (*) indicates a significant difference compared with Group 2. † includes gut contents; # combined weight of left and right kidneys.

		Group 1 n=4	Group 2 n=5	Group 3 n=8
Heart	(g)	3.5±0.4	3.3±0.1	3.4±0.2
	(g/kg BWt)	3.6±0.3	3.0±0.2	3.4±0.2
Lung	(g)	7.7±1.0	7.5±1.1	8.7±1.2
	(g/kg BWt)	8.0±1.0	6.8±1.0	8.5±1.0
Liver	(g)	38.4±3.0	39.7±2.1	41.6±3.0
	(g/kg BWt)	40.2±3.7	36.0±1.2	41.7±3.2
Gut †	(g)	133.3±11.8	143.5±19.8	166.3±10.8
	(g/kg BWt)	139.0±12.1	129.4±14.8	166.9±11.5
Kidneys #	(g)	6.3±0.4*	7.5±0.4	7.1±0.3
	(g/kg BWt)	6.6±0.5	6.8±0.3	7.1±0.4
Brain	(g)	2.8±0.2	3.3±0.2	3.6±0.2
	(g/kg BWt)	2.9±0.3	3.0±0.2	2.6±0.2

The weights of the major organs from the animals from which arteries were collected for the determination of arterial wall mechanics are shown in Table 2-5. The organs (not fixed) were collected immediately after cervical dislocation and exsanguination. In this cohort of animals, neither the absolute organ weights nor the weights adjusted for body weight differed between the three groups.

When the organ weights of all the guinea pigs was combined (data not shown), irrespective of the post mortem treatment of their tissues (i.e. fixed and unfixed), there were no significant differences in the organ weights of the three groups, either expressed as absolute organ weight, or adjusted for body weight with the exception of gut weight (adjusted for body weight) which was higher in Group 3 (163.8±7.6g/kg BWt) compared with Group 2 (135.9±6.8g/kg BWt; Group 1: 146.6±8.2g/kg BWt).

Table 2-5. Organ weights from animals from which blood vessels were collected for determination of passive arterial wall mechanical properties. At post mortem, major organs were excised and weighed (unfixed). Data shown are absolute organ weights (g) and organ weights adjusted for body weight (g/kg BWt). † includes gut contents; # combined weight of left and right kidneys.

		Group 1 n=4	**Group 2 n=8**	**Group 3 n=5**
Heart	(g)	3.2±0.2	3.9±0.3	3.8±0.3
	(g/kg BWt)	3.5±0.1	3.7±0.3	3.7±0.4
Lung	(g)	6.3±0.8	3.1±0.3	6.1±0.4
	(g/kg BWt)	6.8±0.6	5.8±0.2	6.0±0.6
Liver	(g)	30.4±0.9	35.0±2.6	33.0±2.0
	(g/kg BWt)	33.3±2.3	33.4±1.7	32.2±2.4
Gut †	(g)	146.8±9.3	145.7±6.4	163.9±11.3
	(g/kg BWt)	159.3±3.9	140.2±6.4	159.0±7.9
Kidneys #	(g)	5.5±0.5	5.8±0.1	6.1±0.2
	(g/kg BWt)	6.0±0.5	5.6±0.2	5.9±0.1
Brain	(g)	3.9±0.3	3.8±0.2	4.1±0.2
	(g/kg BWt)	4.2±0.4	3.6±0.3	4.0±0.3

2.4.7 Passive mechanical properties of the arterial wall

2.4.7a Basilar artery

Arterial dimensions (μm) during pressurisation There were no significant differences in the initial OD (pressurised to 10mmHg) of the three groups of guinea pigs (Group 1: 639±62μm; Group 2: 604±26μm; Group 3: 569±43μm). At 50mmHg, there were no significant differences in the cross-sectional areas of the lumen or the arterial wall of the segments from the three groups (Table 2-6). During pressurisation, there was a significant effect of birth weight group on both OD and ID; the diameters of the basilar arteries from Group 1 animals were larger than those from Groups 2 and 3, and the diameters of the arteries from Group 3 animals were smaller than those from the other two groups (Appendix 2-1). While the wall thickness of the arterial segments decreased significantly with increasing intraluminal pressure in all three groups, there were no significant differences in the WT of the three groups during pressurisation (Appendix 2-1).

Table 2-6. Cross-sectional area (CSA) of the basilar artery at 50mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 50mmHg intraluminal pressure (~MAP) for the three groups of guinea pigs.

	Group 1 n=4	**Group 2 n=8**	**Group 3 n=5**
Lumen CSA (mm^2)	0.36±0.10	0.30±0.03	0.25±0.03
Arterial wall CSA (mm^2)	0.09±0.01	0.07±0.01	0.07±0.02

Relative changes in arterial dimensions during pressurisation The arterial dimensions all changed significantly with increasing pressures; OD, ID and segment length all increased significantly from their initial size at 10mmHg and WT significantly decreased with increasing intraluminal pressures. While the relative increase in ID and L did not differ significantly between the three groups during pressurisation, there was a significant effect of birth weight group on the relative change in OD and WT. The relative increase in OD of the arterial segments from Group 1 animals was greater than that in arterial segments from Group 2 and Group 3 animals (Figure 2-14A). During

pressurisation, the relative wall thickness of the arteries from Group 1 animals was also greater than that of Group 2 and 3 animals (Figure 2-14B).

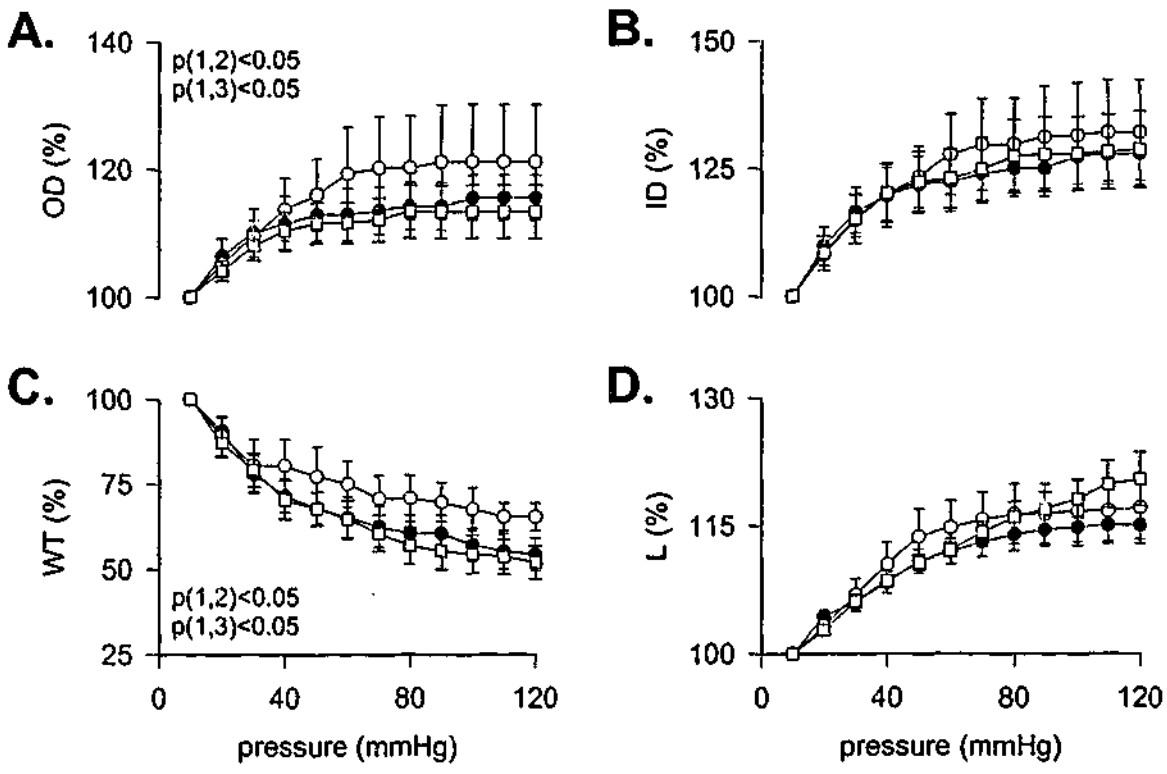


Figure 2-14. Relative dimension changes in the basilar artery during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of basilar artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5). p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.

Arterial wall properties during pressurisation There was a significant effect of intraluminal pressure on the lumen to wall thickness ratio (consistent with increasing lumen size and decreasing wall thickness with increasing pressures), however, ID/WT ratios did not differ between the three groups during pressurisation (Figure 2-15A). Similarly, there was a significant effect of intraluminal pressure on circumferential wall stress; however, there were no differences in the arterial wall stress between the three groups (Figure 2-15B).

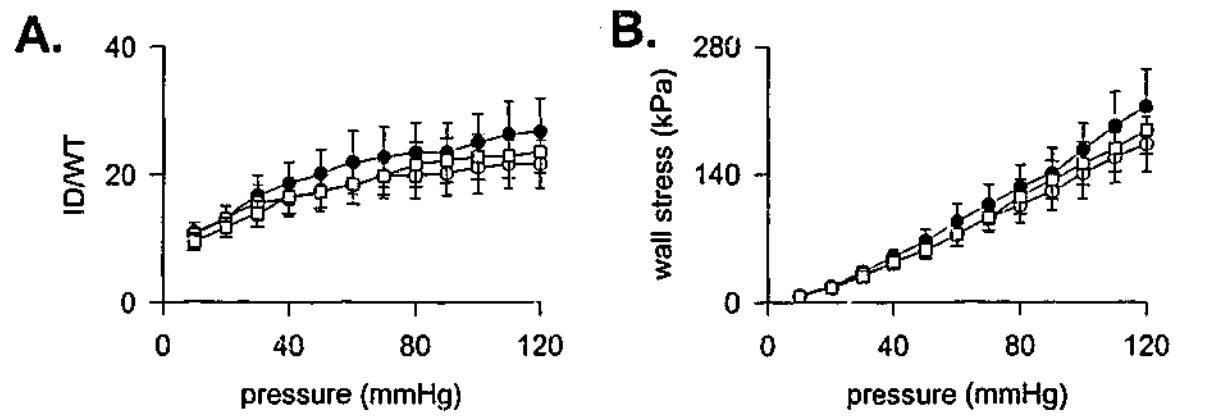


Figure 2-15. Arterial wall properties in the basilar artery during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) circumferential wall stress during pressurisation of the segments of basilar artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5).

Compliance and distensibility Luminal volume increased significantly with increasing intraluminal pressures but there were no differences in the relative volume increases between groups (Figure 2-16A). Cross-sectional compliance and distensibility significantly decreased with increasing pressures, but there were no differences between the three groups in these measures (Appendix 2-2). To include the effects of vessel lengthening during pressurisation, volume compliance and distensibility were also calculated. There was a significant effect of birth weight group on volume compliance, with the lowest birth weight group (Group 1) having highest compliance compared with Groups 2 and 3 (Figure 2-16B). There were no differences in the volume distensibility of the arteries from the three groups (Figure 2-16C).

Stress-strain relationship The stress-strain relationship for the segments of basilar artery are shown in Appendix 2-3. When these curves were fitted to the function $y=ae^{kx}$, there were no differences in the rate constants (Table 2-7), indicating that the overall stress-strain relationships in the three groups were not different. There were also no differences between the three groups when the curves were fitted by the exponential $y=ae^{k_1x}+be^{k_2x}$ (Table 2-7), indicating that the stress-strain properties of the arteries did not differ in either the elastic or collagenous range.

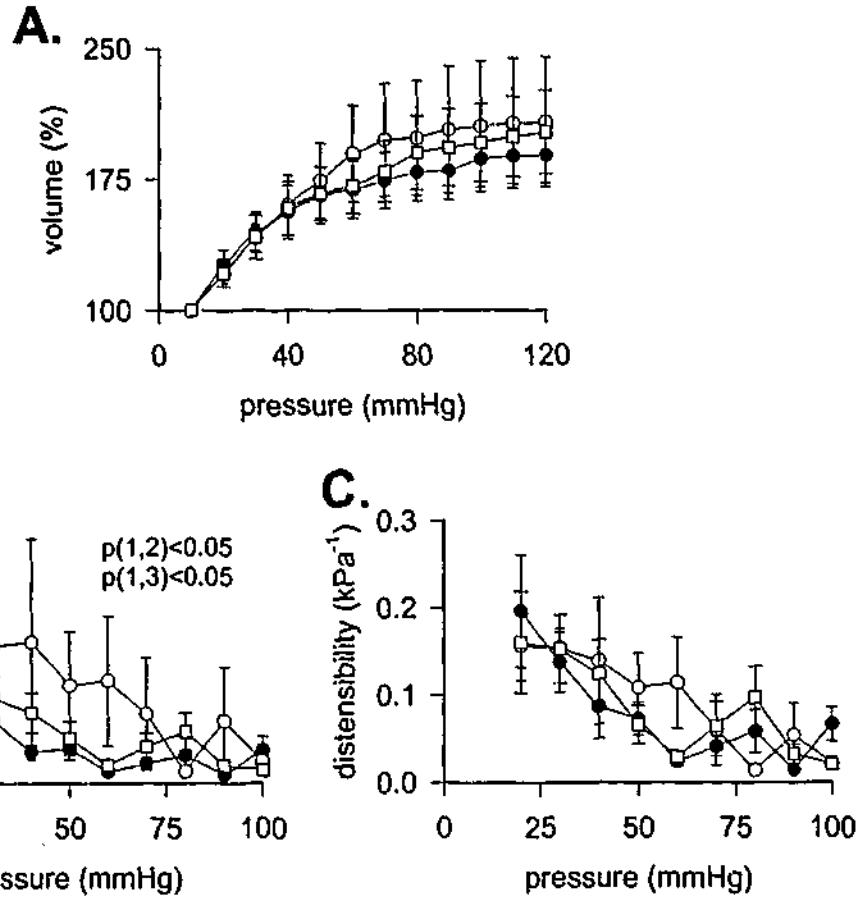


Figure 2-16. Volume compliance and distensibility of the basilar artery. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of basilar artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5). p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.

Table 2-7. Incremental elastic modulus of the basilar artery. The stress-strain relationship for the three groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries.

		Group 1 (n=4)	Group 2 (n=5)	Group 3 (n=8)
$y=ae^{kx}$	k	12.5±2.1	16.1±1.1	16.9±2.0
$y=ae^{k_1x}+be^{k_2x}$	k_1	7.3±1.0	7.3±45.0	11.0±2.6
	k_2	108.7±37.5	16.4±3.2	114.4±50

2.4.7b *Branch of the femoral artery*

Arterial dimensions (μm) during pressurisation Initial OD (pressurised to 10mmHg) was not significantly different between the three groups of guinea pigs (Group 1: 611±25μm; Group 2: 598±89μm; Group 3: 649±64μm). There were also no significant differences between the three groups with respect to lumen and arterial wall cross-sectional areas at 50mmHg (Table 2-8). Although outer and lumen diameters of the femoral segments increased significantly with increasing pressures, there were no differences in diameters between groups (Appendix 2-4). Wall thickness decreased in all groups with increasing intraluminal pressures and there was a significant effect of birth weight group on the WT of the arterial segments; arterial wall thickness was greatest in the segments from Group 1 animals and thinnest in segments from Group 3 (Appendix 2-4).

Table 2-8 Cross-sectional area (CSA) of the femoral artery branch at 50mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 50mmHg intraluminal pressure (~MAP) for the three groups of guinea pigs.

	Group 1 n=4	**Group 2 n=8**	**Group 3 n=5**
Lumen CSA (mm^2)	0.29±0.03	0.32±0.11	0.38±0.06
Arterial wall CSA (mm^2)	0.11±0.01	0.11±0.03	0.09±0.01

Relative changes in arterial dimensions during pressurisation There was a significant effect of intraluminal pressure on the relative change in all of the arterial dimensions measured. Although the relative change in OD in arterial segments of the animals from Group 2 animals was less than that in the other two groups (Figure 2-17A), there were no difference is the relative increase in ID between the three groups (Figure 2-17B). The relative change in WT was different between all three birth weight groups; arterial segments from Group 1 animals showed the least change and the segments from Group 3 animals showed the greatest change in WT during pressurisation (Figure 2-17C). There were no differences in the relative change in segment length between the three groups during pressurisation (Figure 2-17D).

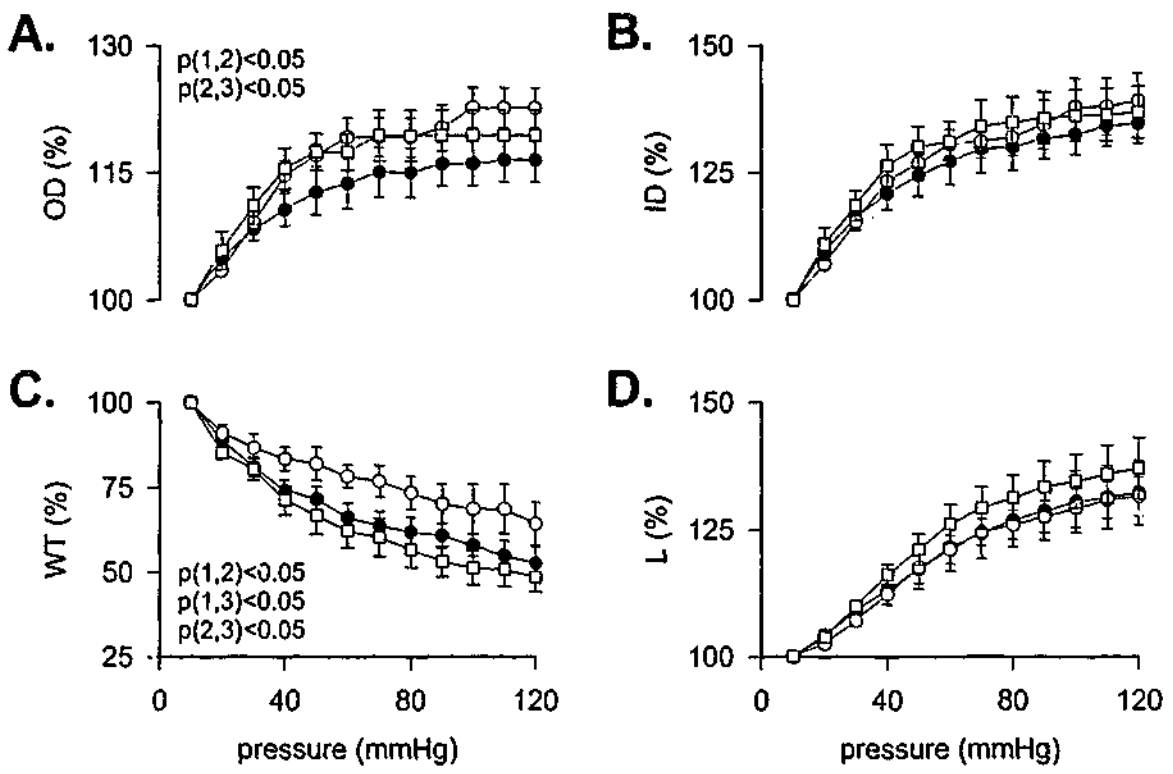


Figure 2-17. Relative dimension changes in the femoral artery branch during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of femoral artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5). p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.

Arterial wall properties during pressurisation There was a significant effect of birth weight group on the lumen to wall thickness ratio, with Group 1 animals having the lowest ID/WT and Group 3 animals having the highest ID/WT (Figure 2-18A). This alteration to the ratio was consistent with the difference in absolute WT between the three groups (Appendix 2-4). When analysed at each increment of pressure, the ID/WT was found to be significantly higher in the vessels from Group 3 compared with those from Groups 1 and 2 at all pressures except 10 and 30mmHg; there were no significant differences between Groups 1 and 2 at any pressure.

There were significant effects of pressure and birth weight group on circumferential wall stress (Figure 2-18B). Wall stress differed between the three groups throughout pressurisation and analysis at each pressure increment found that for the majority of

pressures, wall stress was greater in the arterial segments from Group 3 animals compared with the other two groups.

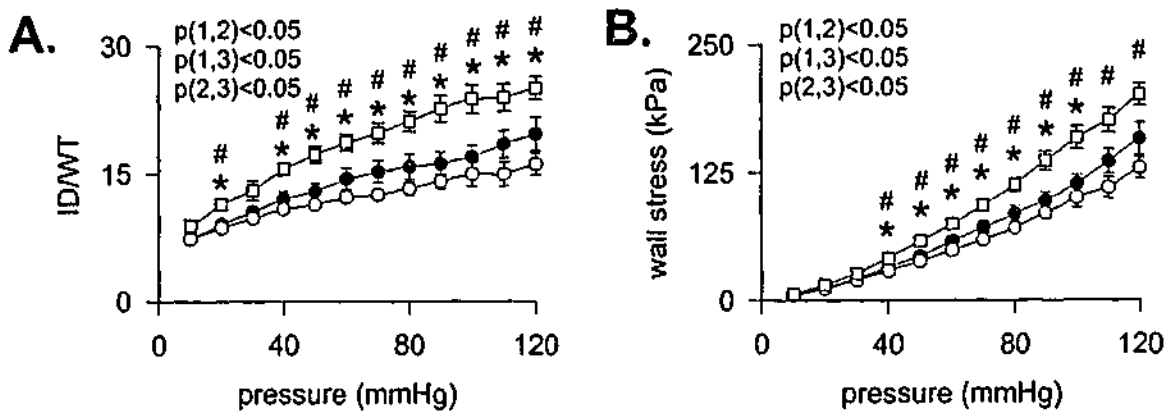


Figure 2-18. Arterial wall properties in the femoral artery branch during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) circumferential wall stress during pressurisation of the segments of femoral artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5). p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other. * indicates a significant difference compared with Group 2 at the individual pressure; # indicates a significant difference between Groups 1 and 3 at the individual pressure (analysis by one-way ANOVA).

Compliance and distensibility There was a significant effect of intraluminal pressure in the change in luminal volume (Figure 2-19A), cross-sectional compliance and distensibility (Appendix 2-5), and volume compliance and distensibility (Figure 2-19). There were no differences between birth weight groups in any of these parameters except volume compliance; arterial segments from Group 1 animals were more compliant than those from Groups 2 and 3 (Figure 2-19B), however, after standardisation for the initial luminal volume (i.e. calculation of distensibility), there were no differences between the three groups (Figure 2-19C).

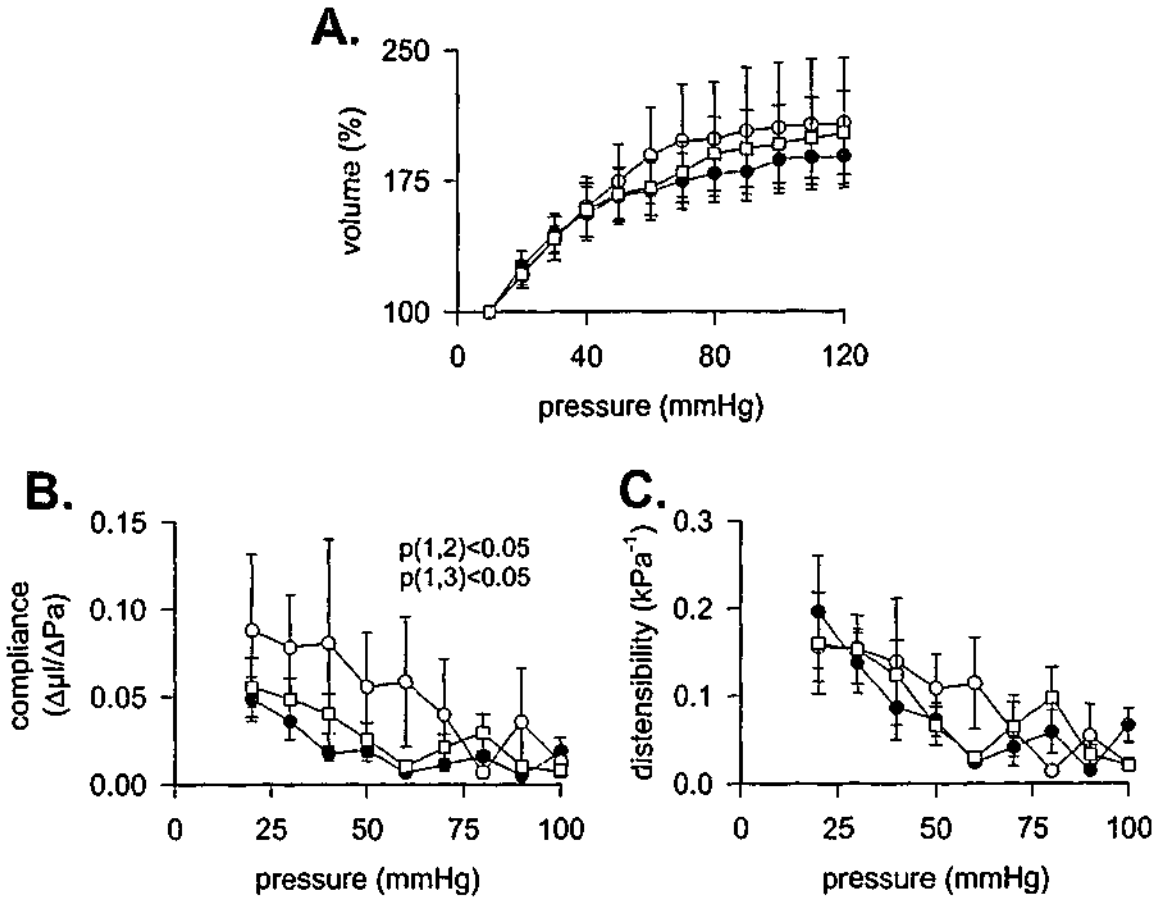


Figure 2-19. Volume compliance and distensibility of the femoral artery branch. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of femoral artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5).

Stress-strain relationship The stress-strain relationship for the femoral arteries from the three groups is shown in Appendix 2-6. The derived rate constant was significantly greater in Group 3 compared with Group 1 (Table 2-9), indicating that the arteries from Group 3 animals had greater wall stress for a given strain (i.e. the arteries were stiffer) compared with Group 1. Further analysis indicated that the difference in the overall slope of the curve was likely due to differences at high strain range at which the passive mechanical properties are mostly due to collagen (Table 2-9).

Table 2-9. Incremental elastic modulus of the femoral artery branch. The stress-strain relationship for the three groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. # indicates a significant difference between Groups 1 and 3 ($p<0.05$).

		Group 1 (n=4)	Group 2 (n=5)	Group 3 (n=8)
$y=ae^{kx}$	k	8.9±0.6	12.8±0.7	17.8±2.3#
$y=ae^{k_1x}+be^{k_2x}$	k_1	2.0±25	8.6±1.6	7.4±1.6
	k_2	10.1±5.6	33.6±13.7	60.6±13.8#

2.4.7c Branch of the mesenteric artery

Arterial dimensions (μm) during pressurisation The starting OD (pressurised to 10mmHg) was not different between the three groups of guinea pigs (Group 1: 354±32μm; Group 2: 402±49μm; Group 3: 361±33μm). When pressurised to 50mmHg, there were no differences in the cross-sectional area of the lumen or arterial wall between the three groups (Table 2-10).

There was a significant effect of birth weight group on OD and ID, with arterial segments from Group 2 animals having larger diameters compared with Groups 1 and 3 throughout pressurisation. While arterial wall thickness decreased in all groups during pressurisation, the WT of arterial segments from Group 1 animals remained greater than those from Groups 2 and 3 (Appendix 2-7).

Relative changes in arterial dimensions during pressurisation The dimensions of the arterial segments changed significantly with increasing intraluminal pressures. Although the relative increases in ID and segment length during pressurisation were not different between the three birth weight groups, the relative increase in OD was significantly less in Group 3 compared with Groups 1 and 2 (Figure 2-20A). The relative decrease in WT was less in the arterial segments from Group 1 animals compared with those from the higher birth weight groups (Figure 2-20C).

Table 2-10. Cross-sectional area (CSA) of the mesenteric artery branch at 50mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 50mmHg intraluminal pressure (~MAP) for the three groups of guinea pigs.

	Group 1 n=4	**Group 2 n=8**	**Group 3 n=5**
Lumen CSA (mm^2)	0.11±0.03	0.16±0.04	0.11±0.02
Arterial wall CSA (mm^2)	0.03±0.01	0.03±0.01	0.02±0.01

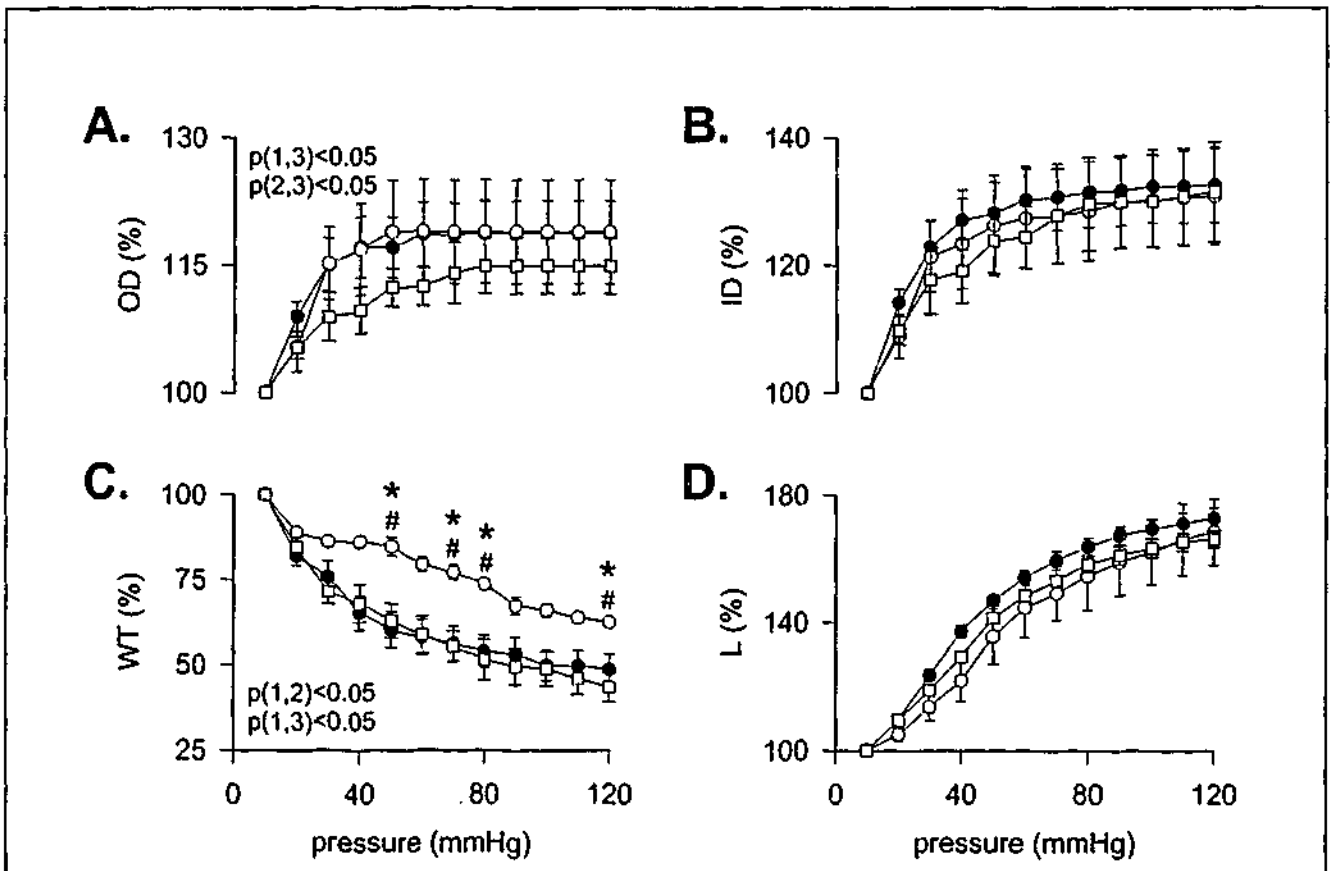


Figure 2-20. Relative dimension changes in the mesenteric artery branch during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of mesenteric artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5). p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other. * indicates a significant difference compared with Group 2 at the individual pressure; # indicates a significant difference between Groups 1 and 3 at the individual pressure (analysis by one-way ANOVA).

Arterial wall properties during pressurisation Arterial segments from Group 1 animals had significantly lower ID/WT and lower arterial wall stress compared with Groups 2 and 3 throughout pressurisation (Figure 2-21). In all three groups, ID/WT and arterial wall stress increased significantly with increasing intraluminal pressures.

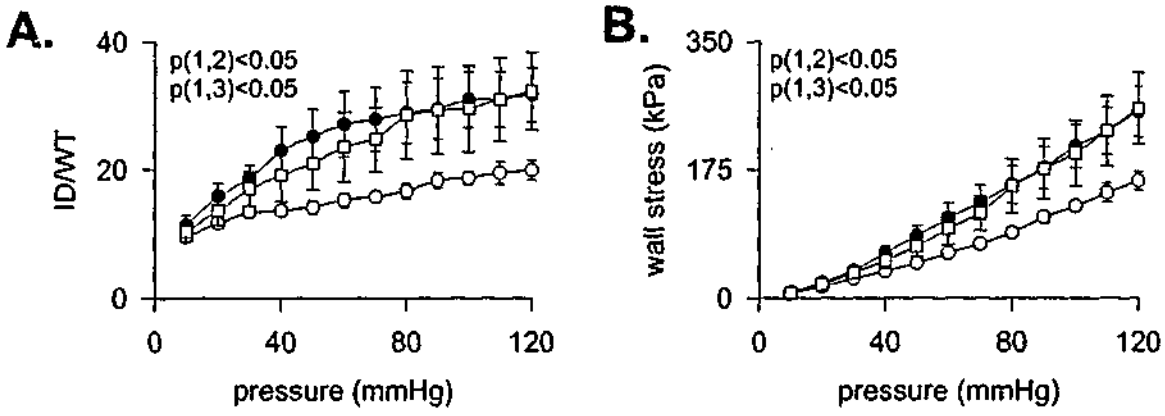


Figure 2-21. Arterial wall properties in the mesenteric artery branch during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) circumferential wall stress during pressurisation of the segments of mesenteric artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5). p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.

Compliance and distensibility There were no differences between the three birth weight groups in the relative change in luminal volume, cross-sectional compliance, cross-sectional distensibility, volume compliance or distensibility during pressurisation (Figure 2-22, Appendix 2-8). In all groups, all of these parameters changed significantly with increasing intraluminal pressures.

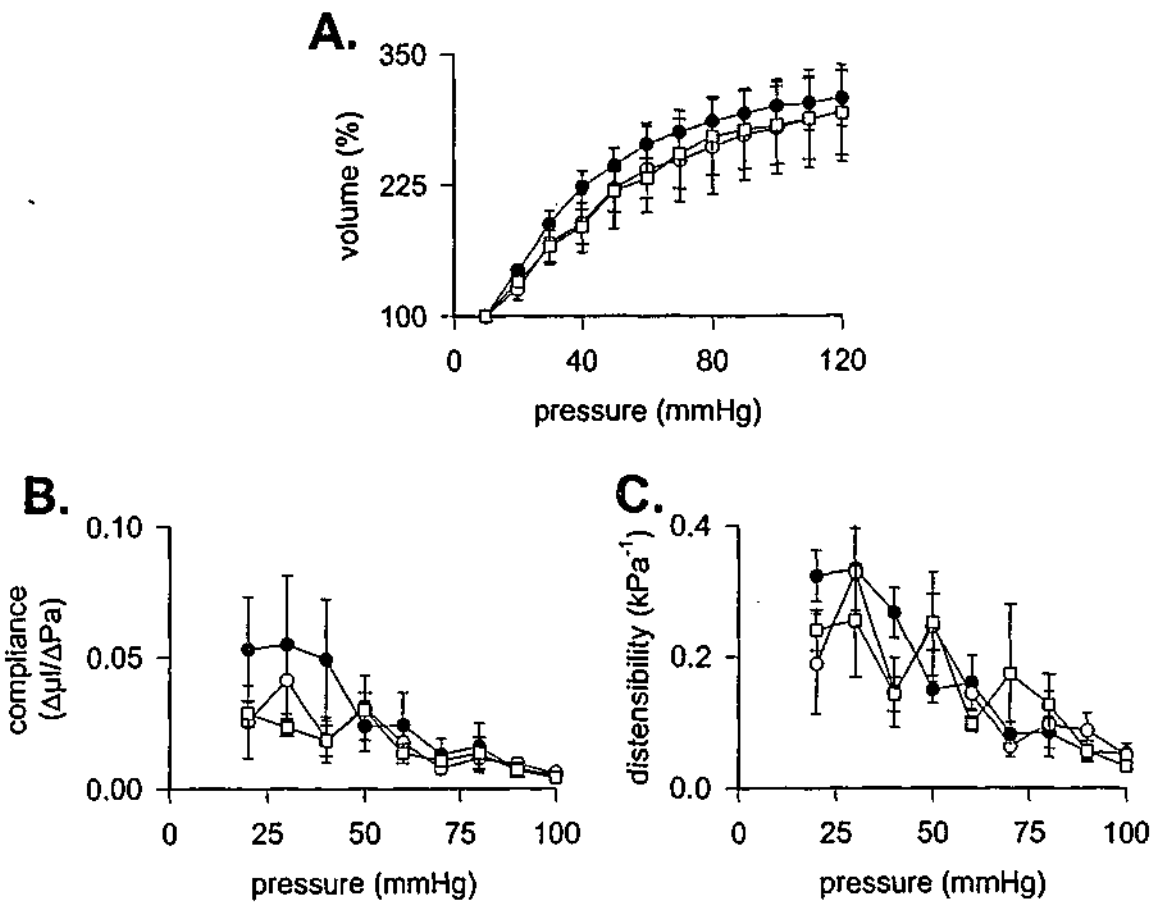


Figure 2-22. Volume compliance and distensibility of the mesenteric artery branch. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of mesenteric artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5).

Stress-strain relationship The stress-strain relationship for the segments of mesenteric artery are shown in Appendix 2-9. The rate constant for the stress-strain curve of Group 2 was significantly greater (indicating increased stiffness) than that of Group 3 (Table 2-11). While there were no significant differences between the three groups when the stress-strain relationships were analysed at the low and high strain ranges, the difference in the overall elastic modulus of Group 2 and 3 was most likely have been due to differences at the high strain (collagenous) range (Table 2-11).

Table 2-11. Incremental elastic modulus of the mesenteric artery branch. The stress-strain relationship for the three groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. * indicates a significant difference between Groups 2 and 3 ($p<0.05$).

		Group 1 (n=4)	Group 2 (n=5)	Group 3 (n=8)
$y=ae^{kx}$	k	22.5±1.8	27.4±3.4	15.5±1.6*
$y=ae^{k_1x}+be^{k_2x}$	k_1	10.1±2.4	10.4±2.6	5.8±2.3
	k_2	66.3±30.1	87.6±20.5	39.9±7.6

2.4.7d Arcuate artery

Arterial dimensions (μm) during pressurisation While the initial OD (pressurised at 10mmHg) of the three groups was not significantly different (Group 1: 514±18μm; Group 2: 408±36μm; Group 3: 528±49μm), there was a significant effect of birth weight group on OD and ID. Throughout pressurisation, the arterial segments from Group 2 animals were smaller in diameter than segments from animals in Groups 1 and 3 (Appendix 2-10). Wall thickness of the arcuate arteries decreased significantly in all groups during pressurisation and the segments from Group 2 animals had thinner arterial walls compared with the other two groups throughout pressurisation (Appendix 2-10C). There were no significant differences in the lumen and arterial wall cross-sectional areas at 50mmHg (Table 2-12).

Relative changes in arterial dimensions during pressurisation While OD increased and WT decreased significantly as intraluminal pressure increased, these relative changes during pressurisation were not different between the three groups (Figures 2-23A and 2-23C). Lumen diameter increased significantly during pressurisation; the relative increase in lumen diameter of segments from Group 1 was significantly less than the relative ID increase of segments from Group 2 (Figure 2-23B). Segment length also increased significantly with increasing intraluminal pressure; the relative increase in length was greatest in segments from Group 1 and the least in segments from Group 2 (Figure 2-23D).

Table 2-12. Cross-sectional area (CSA) of the arcuate artery at 50mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 50mmHg intraluminal pressure (~MAP) for the three groups of guinea pigs.

	Group 1 n=4	Group 2 n=8	Group 3 n=5
Lumen CSA (mm^2)	0.22±0.03	0.14±0.02	0.24±0.04
Arterial wall CSA (mm^2)	0.05±0.01	0.04±0.01	0.05±0.01

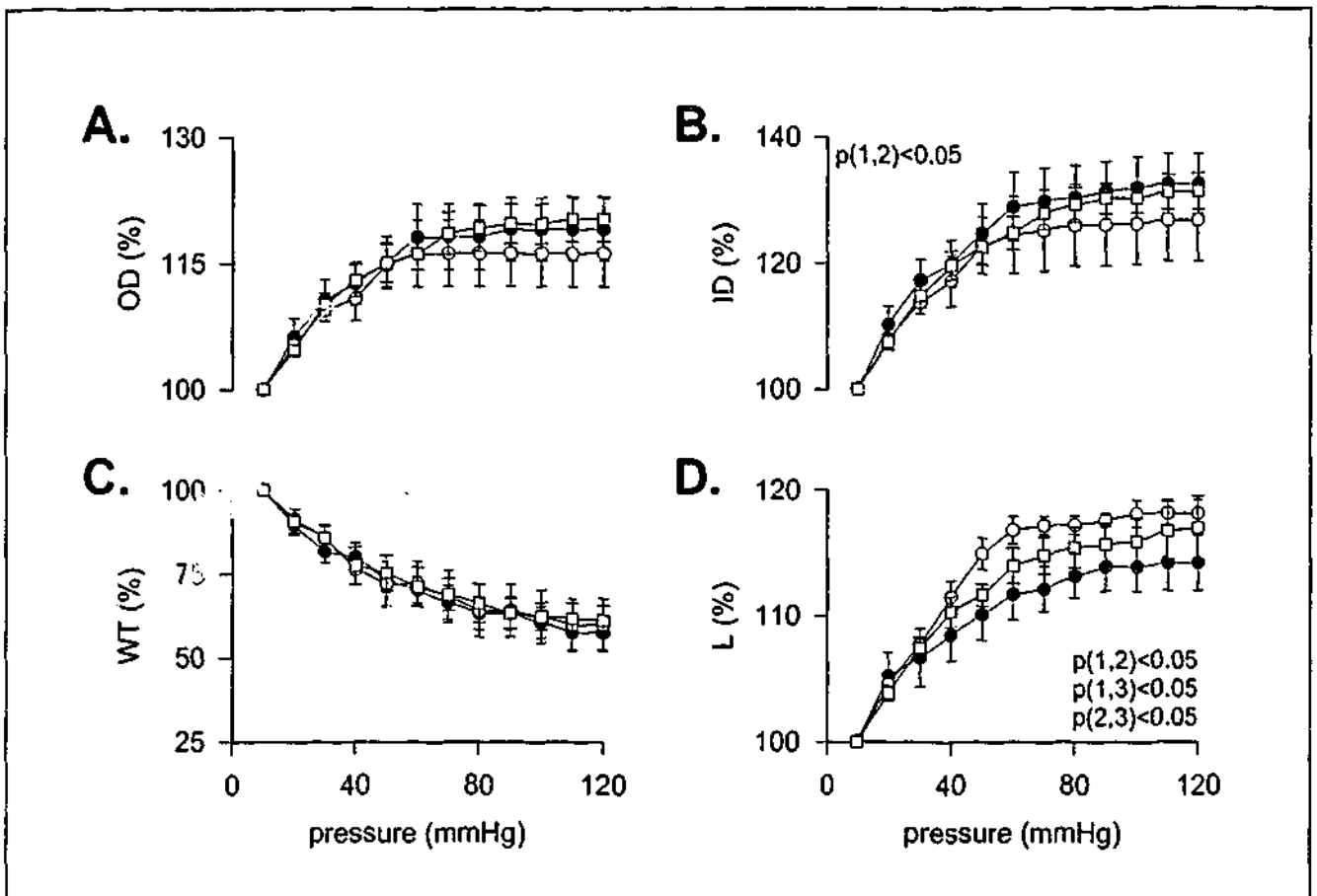


Figure 2-23. Relative dimension changes in the arcuate artery during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of arcuate artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5). p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.

Arterial wall properties during pressurisation ID/WT ratio and circumferential wall stress both increased with increasing intraluminal pressures; there were no differences in these parameters between birth weight groups (Figure 2-24).

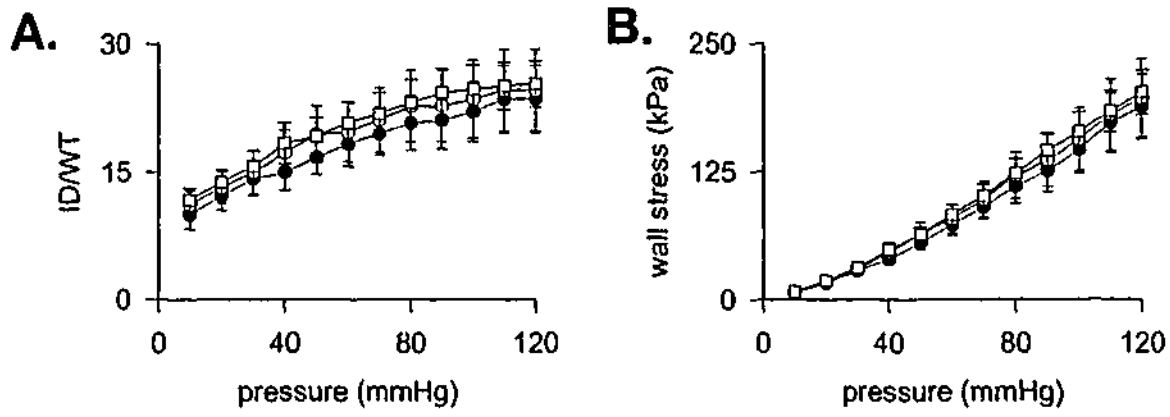


Figure 2-24. Arterial wall properties in the arcuate artery during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) circumferential wall stress during pressurisation of the segments of arcuate artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5).

Compliance and distensibility There was a significant effect of intraluminal pressure on the volume change, cross-sectional compliance and distensibility, and volume compliance and distensibility (Figure 2-24, Appendix 2-11). The relative change in lumen volume, and cross-sectional compliance and distensibility were not significantly different between the three groups. Volume compliance was higher in the segments of arcuate artery from Group 3 animals compared with segments from Group 2 animals (Figures 2-24B); there were no differences in volume distensibility between these two groups.

Stress-strain relationship Appendix 2-12 shows the stress-strain relationships for the segments of arcuate artery from the three groups of guinea pigs. Although the rate constant for the stress-strain curve of arteries from Group 1 was significantly greater than that of Group 3, suggesting increased arterial stiffness of segments from Group 1, there were no significant differences in the slopes of the curves for the three groups at either the low or high strain ranges when analysed separately (Table 2-13).

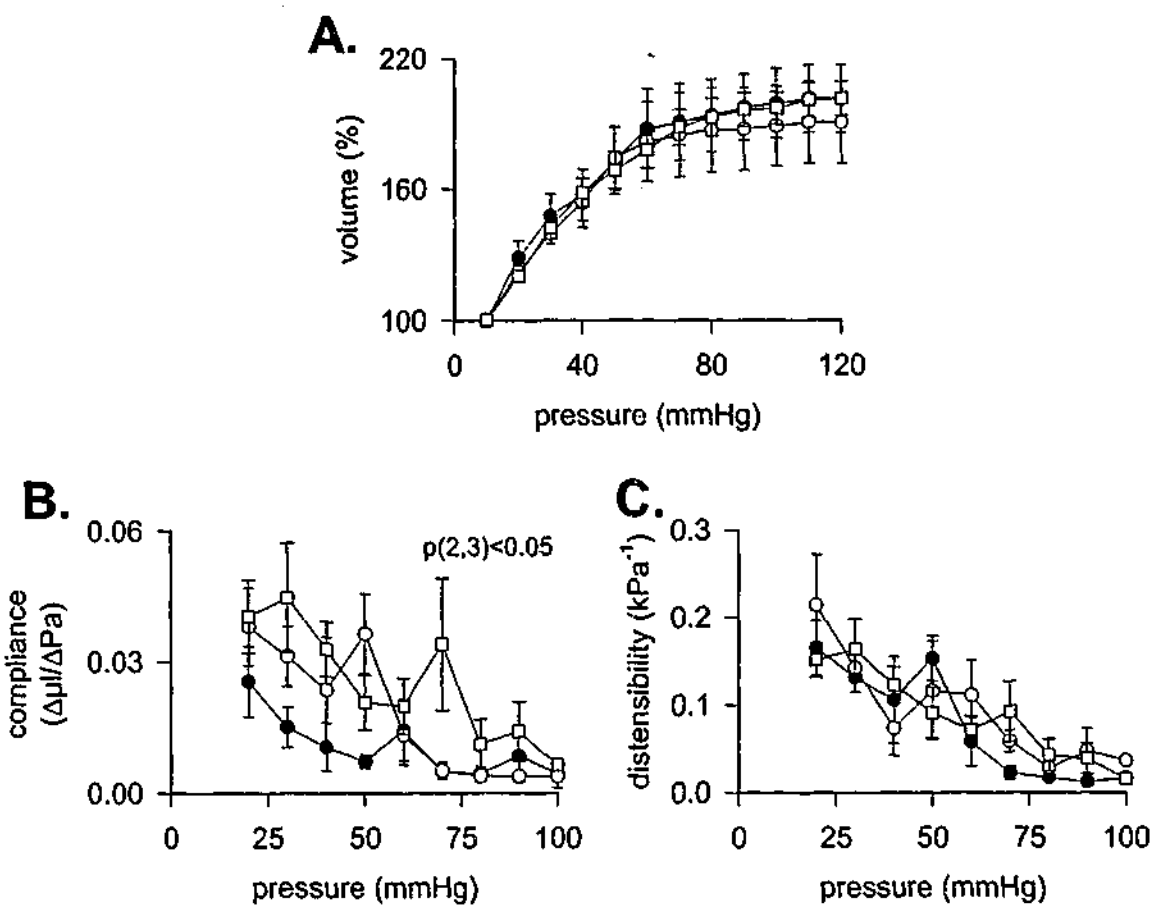


Figure 2-24. Volume compliance and distensibility of the arcuate artery. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of arcuate artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5).

Table 2-13. Incremental elastic modulus of the arcuate artery. The stress-strain relationship for the three groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. # indicates a significant difference between Groups 1 and 3 ($p<0.05$).

		Group 1 (n=4)	Group 2 (n=5)	Group 3 (n=8)
$y=ae^{kx}$	k	23.4±3.8	17.3±2.6	12.5±1.2#
$y=ae^{k_1x}+be^{k_2x}$	k_1	7.8±2.2	7.6±1.8	8.5±1.3
	k_2	68.7±19.7	64.1±22	64.9±26.5

2.4.7e Summary

Relative changes in arterial segment dimensions between vascular beds There was a significant effect of pressure on the relative change in each of OD, ID, WT and segment length from the initial dimensions at 10mmHg. The relative increase in each of the dimensions was also similar across the four vascular beds. In all arteries studied, irrespective of the vascular bed from which they were collected, the relative increase in OD was approximately 15–20% above the initial OD, the relative increase in ID was approximately 25–30% above the initial ID and the relative decrease in WT was approximately 35–40%. The arterial segments of the basilar and arcuate arteries lengthened to a similar degree, increasing by approximately 15% from their starting length, however, relative lengthening was greater in the femoral (approximately 25%) and mesenteric (approximately 50%) artery segments.

Effects of birth weight group on passive arterial wall mechanical properties Table 2-14 summarises the effects of birth weight group on arterial dimensions and mechanical properties. While there were a number of differences between the birth weight groups with respect to arterial dimensions and changes to these dimensions during pressurisation, there were few differences in arterial compliance between the three groups.

The segments of basilar artery from Group 1 animals had larger diameters, both outer and inner, and had a greater relative increase in outer diameter compared with the segments from Group 2 and 3, suggesting increased compliance during pressurisation for the arteries from Group 1. These arteries from Group 1 guinea pigs also had greater volume compliance compared with the other two groups, however, this may have been related to the larger absolute diameters, given that when initial diameter was accounted for (i.e. calculation of distensibility), there were no differences between the three groups.

There were no differences in the diameters of the segments of femoral artery from the three groups, however, arterial wall thickness (both absolute thickness, and relative changes in) differed between the three groups. Absolute, and relative thickness of the arterial walls were greatest in the segments from Group 1, and the least in the segments

from Group 3; these differences in wall thickness led to the ID/WT ratio being significantly different between the three groups of guinea pigs. Based on the decrease in ID/WT ratio it might be expected that the arteries from Group 1 were less compliant, however, there were no differences in the compliance or distensibility of arterial segments between the three groups.

Segments of mesenteric artery from guinea pigs in Group 2 had larger outer and lumen diameters compared with the other two groups. The segments from Group 1 had the greatest wall thickness (both absolute and relative). These differences in dimensions resulted in a reduced ID/WT ratio in the arteries from Group 1. However, there were no differences in the volume compliance or distensibility of the arteries from the three groups.

Although the arcuate arteries from guinea pigs in Group 2 had smaller outer and lumen diameters and thinner arterial walls compared with those from guinea pigs in Group and 3, these differences were proportional such that there were no differences in the ID/WT ratio between the three groups. The arteries from Group 3 were more compliant than those from Group 2 animals but there were no differences in volume distensibility between the three groups.

Table 2-14. Summary of the effects of birth weight group on arterial dimensions and passive arterial wall mechanical properties in the four selected vessels. ↔ indicates no difference between the three groups of guinea pigs. > indicates significant effects of birth weight group on the measured parameter, e.g. femoral artery: WT (μm) 1>2>3 indicates the arterial wall thickness was different between all three groups and was thickest in Group 1 and thinnest in Group 3; OD (%) 1,3>2 indicates a greater change in OD during pressurisation in Groups 1 and 3 compared with Group 2, there were no differences between Groups 1 and 3. # N.B. a greater change in the WT (%) indicates a relative greater decrease in WT during pressurisation, e.g. 3>2>1 indicates that Group 3 had the greatest change and Group 1 had the smallest change in WT, therefore, relative to the initial WT at 10mmHg intraluminal pressure, arterial wall thickness was greatest in Group 1 and smallest in Group 3.

	Basilar	**Femoral**	**Mesenteric**	**Arcuate**
OD (μm)	1>2>3	↔	2>1,3	1,3>2
ID (μm)	1>2>3	↔	2>1,3	1,3>2
WT (μm)	↔	1>2>3	1>2,3	1,3>2
OD (%)	1>2,3	1,3>2	1,2>3	↔
ID (%)	↔	↔	↔	2>1
WT (%) #	2,3>1	3>2>1	2,3>1	↔
L (%)	↔	↔	↔	1>3>2
ID/WT ratio	↔	3>2>1	2,3>1	↔
Wall stress	↔	3>2>1	2,3>1	↔
Volume (%)	↔	↔	↔	↔
Vol. Compliance	1>2,3	↔	↔	3>2
Vol. Distensibility	↔	↔	↔	↔
Stress-strain slope	↔	3>1	2>3	1>3
Stress-strain $slope_{low}$	↔	↔	↔	↔
Stress-strain $slope_{high}$	↔	3>1	↔	↔

2.5 Discussion

The aims of this study were to investigate the relationship between birth weight and postnatal growth, arterial pressure and passive arterial wall mechanical properties at one year in guinea pigs. At birth, guinea pigs were classified into low, average or high birth weight groups and offspring that were small at birth exhibited catch-up growth during the first postnatal year such that there were no differences in the body sizes of the three groups at adulthood. Arterial pressure in the adult guinea pig was not found to be different between the three birth weight groups, nor was it correlated with size at birth (body weight, length or ponderal index at birth). There were no major effects of birth weight group on passive mechanical properties of the arteries studied.

2.5.1 Placental restriction surgery and birth weight

A previous study by Turner (1997) found that inducing IUGR in guinea pigs by the ablation of blood vessels supplying the placentae of fetal guinea pigs was more successful in terms of fetal survival (53%), than inducing IUGR by unilateral uterine artery ligation (18%). For this reason, the diathermy technique was used to induce IUGR for studies presented in this chapter. However, of the 82 pregnant guinea pigs that underwent the placental restriction surgery for the studies described in this chapter, only 31 successfully delivered viable offspring for postnatal study.

The outcome of the pregnancy could not be predicted by the maternal age at mating, the maternal age or weight at the time of the placental restriction surgery, or the gestational age of the fetuses at the time of the surgery. The duration of the surgery (time from anaesthesia to the initial stages of recovery) ranged from 30–60 minutes, depending on the ease of location of the appropriate vessels. Although surgical time decreased with greater experience with the technique, the outcome of the pregnancy was not related to the duration of the surgery, or the total dose of the anaesthetic (for longer duration surgeries, a second smaller dose was administered if required). The success rate did improve slightly as the length of the surgery decreased, but a high rate of fetal death and resorption, including fetuses that had not had any utero-placental vessels ablated. Within litters in which viable offspring were delivered, there was a high degree of fetal death, such that within each litter only 1–2 offspring were born, despite more fetuses being present, as determined by palpation and/or visualisation at the time of surgery

(~mid-gestation). Fetal death and resorption occurred in 34% of the pregnant guinea pigs; these animals failed to deliver any offspring at all.

It is possible that at least some of the low birth weight guinea pigs in this study were low birth weight due to factors other than the surgical intervention. In the study by Turner (1997), guinea pigs and their fetuses were killed at 59–69 days of gestation, and the body dimensions of the fetuses described at post mortem. There were no postnatal studies of the offspring. Since fetuses were *in situ* and *in utero* at the time of post mortem examination, it was possible to determine exactly which fetuses had utero-placental vessels ablated, and which fetuses were low birth weight due to non-surgical factors (Turner, 1997). In my study, the litters delivered spontaneously, thus it was not possible to confirm if the low birth weight was the result of the ablation of utero-placental vessels, or merely due to natural factors such as position in the uterine horn or genetic predisposition. Fetuses were not marked at the time of surgery to allow identification of those that had utero-placental vessels ablated and those that had not. I could have injected dyes into the amniotic sacs of each surgically treated fetus to mark it but this may have adversely affected the fetal outcome; it is also likely that the dyes may not have lasted for the 30–35 days between surgery and delivery. It may have also been possible to deliver the fetuses by caesarean section, however, this may have compromised the guinea pig mothers by subjecting them to a second surgery, and subsequently they may not have recovered adequately to immediately nurse their young.

In the cohort of guinea pigs studied by Turner (1997), the mean fetal weight at post mortem near term (59–69d GA) was 90.6±19.9g (mean±SD, n=19) in sham-operated controls, and 72.9±17.0g (n=14) in IUGR fetuses. In the cohort of animals described in this chapter, the mean birth weight (±SD) for Group 2 was 101.3±5.8g (n=16), while the mean birth weight for Group 1 (low birth weight) was 80.8±10.5g (n=11). While the animals in my study were heavier than those described by Turner (1997), the variation within each group was lower. The cohort of guinea pigs described in this chapter includes a third group of animals that had high birth weights (124.9±11.6g, n=17). While it is unclear why the birth weights of Group 2 and 3 were greater than the birth weights of controls studied by Turner (1997), it may be related to smaller litter sizes of guinea pigs in my study. Variations in the mean birth weights of control guinea pigs may be also be related to the breed of guinea pig used by different research groups;

mean (±SEM) birth weights reported for control guinea pigs include 98.9±2.5g (IMVS coloured; Kind *et al.*, 1999), 101.3±1.4g (outbred tri-colour; this chapter) and 111.9±2.0g (Dunkin-Hartley; Persson and Jansson, 1992).

The degree of growth restriction in the studies described in this chapter, and also by Turner (1997), was less than that described by previous studies using the unilateral uterine artery ligation (Lafeber *et al.*, 1984; Jones *et al.*, 1987; Carter & Detmer, 1990; Detmer *et al.*, 1991; Persson & Jansson, 1992). Those studies reported a 40–67% reduction in the body weights of offspring from the ligated horn compared with offspring from non-ligated horns. Turner (1997) reported an 18% reduction in birth weight following unilateral uterine artery ligation. Using the diathermy technique, Turner (1997) reported a 20% reduction in the body weights of fetuses that had utero-placental vessels ablated. In the present study, the low birth weight animals (Group 1) were also 20% lighter than the Group 2 animals at birth. Mild maternal undernutrition can also cause fetal growth restriction but the degree of growth restriction is also lower than that achieved by uterine artery ligation; pups from mildly undernourished mothers were 13% lighter at birth than those from *ad libitum* fed mothers (Kind *et al.*, 1999).

The average birth weight for the total cohort of offspring described in this chapter was 104.7±2.9g. The offspring were arbitrarily divided into three groups based on birth weight so there were approximately equal numbers in each group (Figure 2-7A, Figure 2-26A). The cohort could have been divided into two groups rather than 3, with the division criteria of birth weight<105g (n=23) and birth weight>105g (n=21) (Figure 2-26B). However, based on the birth weight distribution, there were many guinea pigs with birth weights clustered around 105g, and therefore it would not have been logical to divide the cohort at this weight. Using the naturally occurring divisions in the birth weight distribution it might have been logical to divide the offspring into the following groups – Group 1: birth weight <85g (n=5), Group 2: birth weight 85–130g (n=36), Group 3: birth weight>130g (n=3) (Figure 2-26C). These divisions would, however, have resulted in very uneven group sizes, making statistical analysis difficult.

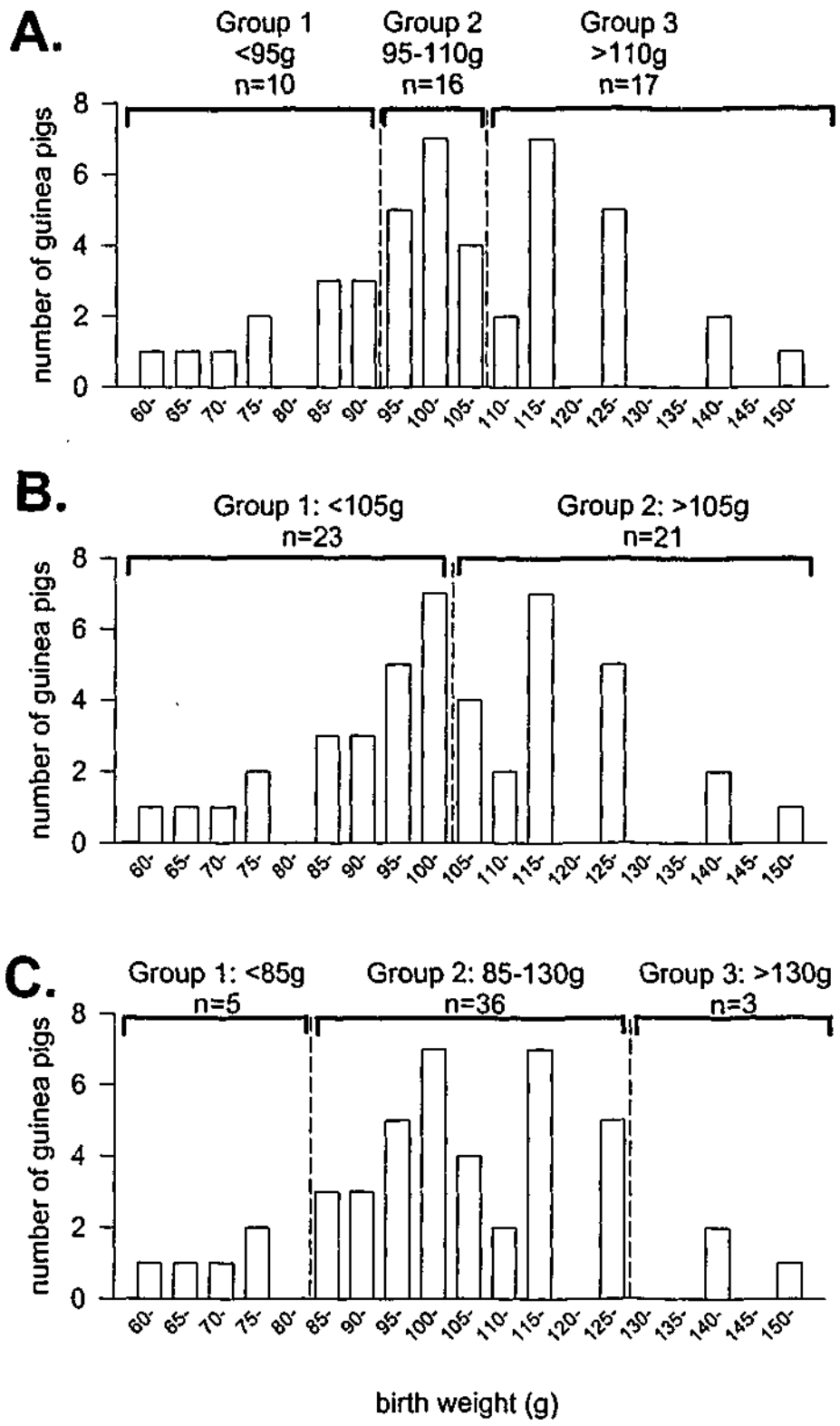


Figure 2-26. Allocation of offspring into birth weight groups based on the birth weight distribution. (A) The criteria for group allocation used for analyses in this chapter; these criteria allowed for approximately equal numbers in the three groups. Alternative criteria to divide the cohort could have been to (B) divide the guinea pigs into those with birth weights above and below the average birth weight (105g), or (C) divide at naturally occurring "breaks" in the frequency distribution.

2.5.2 Postnatal growth

Based on previous studies, it was expected that the low birth weight guinea pigs would remain smaller than normal birth weight offspring for at least 3–4 months after birth (Lafeber *et al.*, 1984; Persson & Jansson, 1992). It was also expected that by one year, the low birth weight guinea pigs would have exhibited catch-up in body weight, such that there was no difference in the body weights between the three groups.

The lowest birth weight offspring (Group 1) remained lighter than those in Group 2 for the first two postnatal weeks and remained lighter than the animals in Group 3 for the first 8 postnatal weeks; offspring in Group 2 were lighter than those in Group 3 for the first 4 postnatal weeks. In contrast with previous studies (Lafeber *et al.*, 1984; Persson & Jansson, 1992) there was no significant difference in the body weights of the three groups of guinea pigs at 12 weeks (~3 months), indicating that the offspring exhibited catch-up growth earlier than those previously reported.

Kind *et al.* (1999) found that mild undernutrition throughout pregnancy reduced birth weight significantly in male guinea pigs (–14%) but not in females (–11%). At 3 months of age (young adult), male offspring from undernourished mothers remained lighter than those of male offspring from *ad libitum* fed mothers (848.4±17.4g *vs* 922.0±20.8g); body weights of female offspring from undernourished mothers (695.3±26.0g) did not differ from female offspring from *ad libitum* fed mothers (712.5±12.2g) at this age (Kind *et al.*, 1999). Following the same undernutrition protocol, a more recent paper by the same research group (Kind *et al.*, 2002) reported similar gender-specific effects on birth weight (males: –17%, females –4%); however, in this second cohort of guinea pigs, both male and female offspring from undernourished mothers were lighter at 3 months of age compared those from mothers that were adequately nourished during pregnancy (males: 722±29g *vs* 808±13g; females: 600±29 *vs* 652±10). For the guinea pigs described in this chapter, there were no gender-specific differences in birth weight between the three groups. At birth, female guinea pigs in Group 1 were 21% and 36% lighter than females in Group 2 and 3, respectively; similarly, birth weights of males in Group 1 were 20% and 34% less than the birth weights of males in Group 2 and 3 respectively. In contrast with the studies by Kind *et al.* (1999 and 2002), body weights of male offspring in the three groups were

not significantly different from each other at 3 months (Group 1: 720.8±12.3g, n=6; Group 2: 766.4±23.9g, n=8; Group 3: 808.6±50.9g, n=5); this catch-up in growth occurred in the period between 2 and 3 months of age. Females in Group 1 (603.8±31.3g, n=4) and 2 (620.8±17.0g, n=8) were significantly lighter than the females in Group 3 (723.6±19.9g, n=11) at 3 months and remained lighter until 9 months, after which there were no significant differences in the body weights of females from the three groups. Therefore, while there were no overall differences in body weight between the three groups after 8 postnatal weeks, there were gender-specific differences that remained for up to 9 months.

Increased weight gain in the lower birth weight groups resulted in them catching up to Group 3 early in the postnatal period. Catch-up growth was also exhibited with respect to other measures of body dimensions. The differences between the three groups in terms of nose-to-rump length and hip circumference at birth were eliminated within the first postnatal year. The catch-up growth with respect to weight and length suggest that the low birth weight guinea pigs described in this chapter were growth restricted rather than genetically small. In humans, several studies have investigated the relationship between birth weight and postnatal size and have found that a high proportion of individuals born small-for-gestational age (SGA) catch up to appropriately grown infants. A longitudinal study of SGA infants found that within the first 4 years of life, 85% of these SGA babies had exhibited catch-up growth in body weight, and more than 90% had caught up in body length (i.e. height) (Albertsson-Wikland *et al.*, 1993). This evidence for catch-up was further supported by other studies reporting 85% of SGA infant catch up in height within the first two years of life (Hokken-Koelega *et al.*, 1995) and 92–94% of SGA individuals have final heights within the normal range (Albertsson-Wikland & Karlberg, 1994; Karlberg & Albertsson-Wikland, 1995; Karlberg *et al.*, 1997).

2.5.3 Arterial pressure and heart rate

Based on the previous findings of Persson and Jansson (1992), it was expected that the arterial pressure of the low birth weight guinea pigs would be higher than that of the average birth weight guinea pigs. Persson and Jansson (1992) found that MAP was not correlated with birth weight, however, there was a negative correlation between the

degree of IUGR and the difference in MAP between littermates. Persson and Jansson (1992) proposed that the relationship between birth weight and arterial pressure may become stronger at a later age; this would be consistent with the hypothesis that alterations to arterial pressure can be initiated prenatally and these changes become amplified with age (Law *et al.*, 1993).

In the study described in this chapter, arterial pressure at one year was not related to birth weight. This result differs from previous studies that have reported that IUGR guinea pigs have elevated arterial pressure (Persson & Jansson, 1992; Kind *et al.*, 2002); in the latter study, hypertension was reported in male IUGR offspring only. Kind *et al.* (2002) found no difference in the arterial pressure of female offspring from undernourished guinea pigs and those from *ad libitum* fed mothers but birth weights did not differ between these two groups of females. For the guinea pigs described in this chapter, there were no differences in the arterial pressure of the three groups of guinea pigs when analysed as males alone, females alone, or males and females combined.

It is possible that there was no significant relationship between birth weight and arterial pressure because the prenatal insult of placental restriction was not severe enough to modify postnatal cardiovascular function. At birth, animals in Group 1 were 20% lighter than those in Group 2 and 35% lighter than those in Group 3. In the study by Persson and Jansson (1992), IUGR guinea pigs were 46% lighter than controls and a significant relationship was only found after a transformation of the data; that is, they did not find a simple relationship between birth weight and arterial pressure at 3–4 months of age. For the study presented in this chapter, it was not possible to transform the data in the manner of Persson and Jansson (1992), as the majority of litters used for my study produced only one live offspring making it impossible to calculate both the degree of growth restriction or the littermate difference in MAP. However, despite only a mild reduction in birth weight (–17%) in the male offspring from undernourished mothers, these offspring were found to be hypertensive at 3 months of age compared with male offspring from *ad libitum* fed mothers. It is possible that the effects for different models of IUGR in guinea pigs on later arterial pressure depends on the gestational timing or the duration of the insult. A sub-optimal *in utero* environment throughout gestation, as induced by maternal undernutrition (Kind *et al.*, 2002), leads to elevated arterial pressure in young male adults but perturbations in the latter half of gestation (this

chapter and Persson & Jansson, 1992) does not appear to greatly affect later arterial pressure.

Previous studies have shown negative correlations between birth weight (and other measures of size at birth) and arterial pressure, such that arterial pressure increased with decreasing birth weight (Law & Shiell, 1996). For the guinea pigs described in this chapter, birth weight was not correlated with arterial pressure at one year, nor was any other descriptor of small size at birth (e.g. reduced body length, ponderal index) correlated with MAP at one year. The only measurement at birth that was correlated with adult arterial pressure was thoracic girth (r=0.44); however, thoracic girth did not differ between the three groups of guinea pigs at birth, or at any other age during the first postnatal year. Therefore these data do not support the hypothesis that small size at birth is related to later arterial pressure.

Epidemiological studies indicate that arterial pressure is related to postnatal growth, with individuals who exhibit catch-up growth (i.e. have increased postnatal growth rates) having elevated arterial pressure (Huxley *et al.*, 2000). Birth weight was strongly correlated with body weight at one year; despite catch-up in body weight and no difference in body weight between the three groups at one year, guinea pigs that were born small remained at the lower end of the weight range in adulthood (Figure 2-13). These guinea pigs however, did exhibit greater postnatal growth rates to allow for catch-up growth and as a result, postnatal growth rate was inversely correlated with birth weight. In contrast to human studies, growth rates during the first postnatal year were not correlated with MAP at one year in the guinea pigs, therefore my findings do not support the hypothesis that an increased postnatal growth rate in low birth weight individuals leads to later hypertension.

2.5.4 Passive arterial wall mechanics

It was initially expected that the low birth weight guinea pigs would have less compliant arteries, consistent with both having increased arterial pressure, and also with altered vascular wall composition resulting from a sub-optimal *in utero* environment. Given there was no effect of birth weight on arterial pressure in the adult guinea pig, it might be expected that the arterial wall mechanical properties would not be different between

the three birth weight groups. Conversely, it was also possible that differences in the mechanical properties of the arteries existed in the absence of measured differences in arterial pressure (van Gorp *et al.*, 2000).

There were no consistent alterations in the passive mechanical properties of the arteries from the three groups that suggested low birth weight group (Group 1) had the least compliant arteries and the high birth weight group (Group 3) had the most compliant arteries, or vice versa. While there were some differences between the birth weight groups with respect to absolute dimensions of the arterial segments studied, in all segments, there were no differences in the cross-sectional areas of the arterial wall or lumen at 50mmHg. Hence, it is unlikely that the resistance of these arteries *in vivo* (at pressures similar to MAP) will have differed due to physical differences in arterial lumen size. In response to increasing luminal pressures, outer and lumen diameters increased and arterial wall thickness decreased. With respect to the relative changes in these dimensions during pressurisation, the basilar, femoral and mesenteric arterial segments from Group 1 animals had greater relative arterial wall thicknesses compared with Groups 2 and 3. To accommodate the greater relative wall thickness, and in the absence of any differences in the relative ID between the three groups during pressurisation, there was a greater relative increase in OD in Group 1. This greater relative increase in OD might suggest a degree of outward remodelling (Mulvany, 1999) of these arteries from Group 1, but any arterial remodelling (if present) did not greatly impact upon the passive mechanics of the three groups, as there were no differences in the relative change in lumen diameter during pressurisation, or distensibility of the arteries from any of the vascular beds studied.

It is possible that, in the absence of differences in arterial pressure, alterations to the arterial wall mechanics may have preceded the alteration in arterial pressure (van Gorp *et al.*, 2000), however, this was not found. Alterations to the *in utero* environment that led to lower birth weights may have affected the synthesis and deposition of extracellular proteins responsible for passive mechanical properties in the arteries. The placental restriction surgery was performed to reduce the oxygen and nutrient supply to selected guinea pig fetuses and a reduced supply of these substrates may have adversely affected the synthesis and deposition of elastin and/or collagen in arteries (Durmowicz *et al.*, 1991; Spanheimer *et al.*, 1991), leading to altered relative proportions of these

proteins, and in turn affecting arterial wall mechanical properties (Roach & Burton, 1957). Given the possibility that the low birth weight guinea pigs may have been small as a result of naturally occurring differences in placental function and *in utero* position, it is possible that the fetuses were not compromised to such a degree to have an effect on arterial pressure or mechanics. Although born small, the fetuses may not have been sufficiently hypoxemic and hypoglycemic *in utero* to adversely affect the deposition of the structural components important for the determination of the arterial wall mechanical properties (i.e. elastin and collagen).

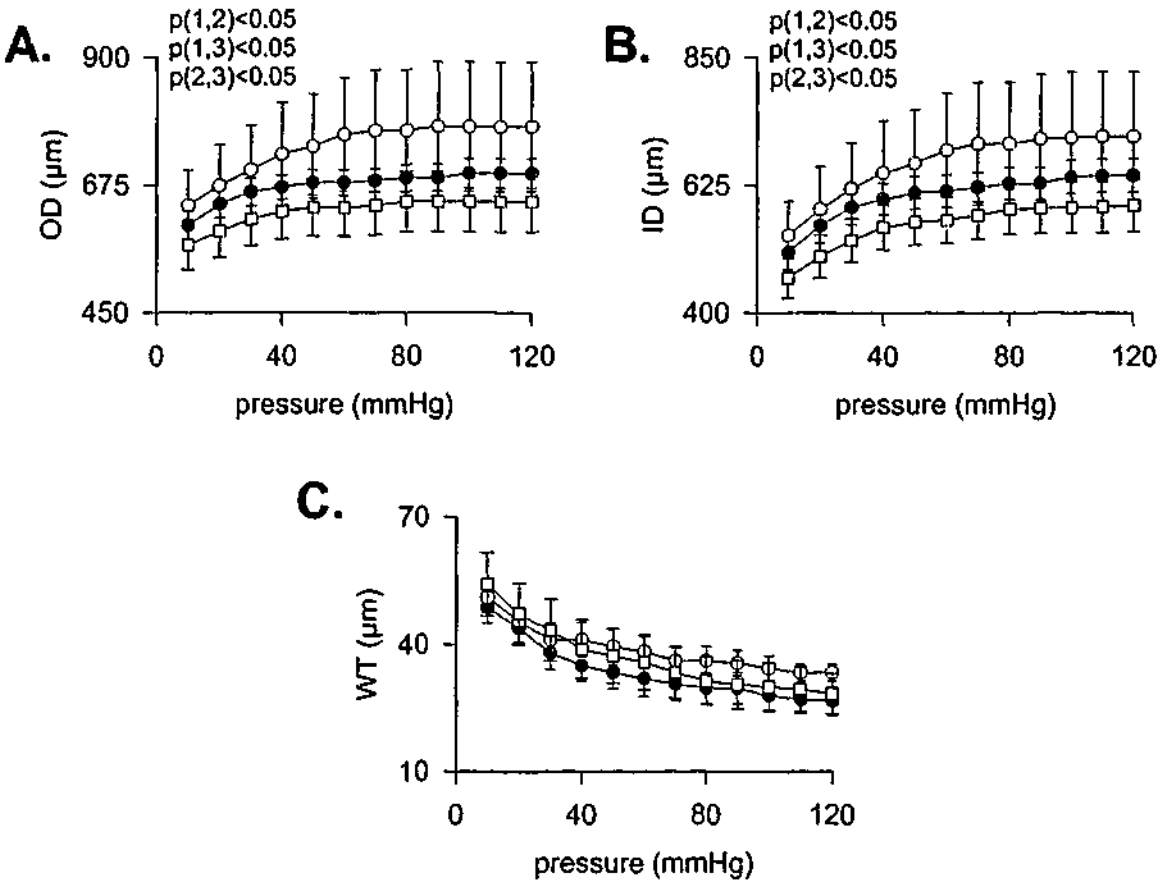
The rate constants in the low strain range of the stress-strain relationship that corresponds to the elastic range of the arteries (Roach & Burton, 1957; Wolinsky & Glagov, 1963) did not differ between the three groups for any of the four arteries studied, suggesting there were no differences in the elastic properties of arteries from the three groups. In the high strain range of the stress-strain relationship, at which collagen plays the dominant role in determining mechanical properties (Roach & Burton, 1957; Wolinsky & Glagov, 1963), differences were evident between Group 3 and Group 1 only in the segment of femoral artery; offspring from Group 3 had stiffer femoral arteries which possibly contained more cross-linked collagen than the femoral arteries from Group 1. The derived rate constant for the overall stress-strain curves was lower for mesenteric arteries from Group 3 than those from Group 2. Similarly, the overall slope of the stress-strain curve of arcuate arteries from Group 3 was less than those from Group 1. The stress-strain relationships for these two arteries did not differ in the either low and high strain ranges between the three groups, indicating the mechanical properties due to elastin alone or collagen alone were not different between groups. However, alterations to the combined properties (relative proportions) of elastin and collagen may have led to the overall differences in the stress-strain curves. While the relative proportions of these proteins can affect vascular mechanics, the arrangement of elastin and collagen in the arterial wall also plays a role in the determination of arterial wall mechanics (Cox, 1978). Further studies investigating the relative amounts of collagen and elastin, and the arrangement of these proteins in the vascular wall (via biochemical or histological analyses), could determine if these alterations in the stress-strain relationship were the result of differences in the absolute or relative amounts of elastin and collagen, the differences in the arrangement of these in the vascular wall, or a combination of both.

The arterial wall mechanical properties described in this chapter were determined in the absence of extracellular Ca^{2+} and smooth muscle activity (i.e. passive mechanics). *In vivo* arterial wall mechanics may differ from those described in this chapter due to effects of smooth muscle activity in the arterial wall (i.e. active mechanics). Recent studies in rats have investigated the effects of an altered *in utero* environment on arterial responsiveness (Holemans *et al.*, 1999; Ozaki *et al.*, 2001; Franco *et al.*, 2002), but these studies did not investigate the passive mechanical properties of these small arteries, nor was vascular responsiveness measured in arteries from guinea pigs described in this chapter. Maternal undernutrition may be able to modify vascular responsiveness in offspring, however, responses to various pharmacological agents have differed between studies (Holemans *et al.*, 1999; Ozaki *et al.*, 2001; Franco *et al.*, 2002). Vascular responsiveness in offspring may be altered in the absence of elevated arterial pressure (Holemans *et al.*, 1999), and although not measured in my study, vascular responsiveness may have differed between the three groups of guinea pigs described in this chapter.

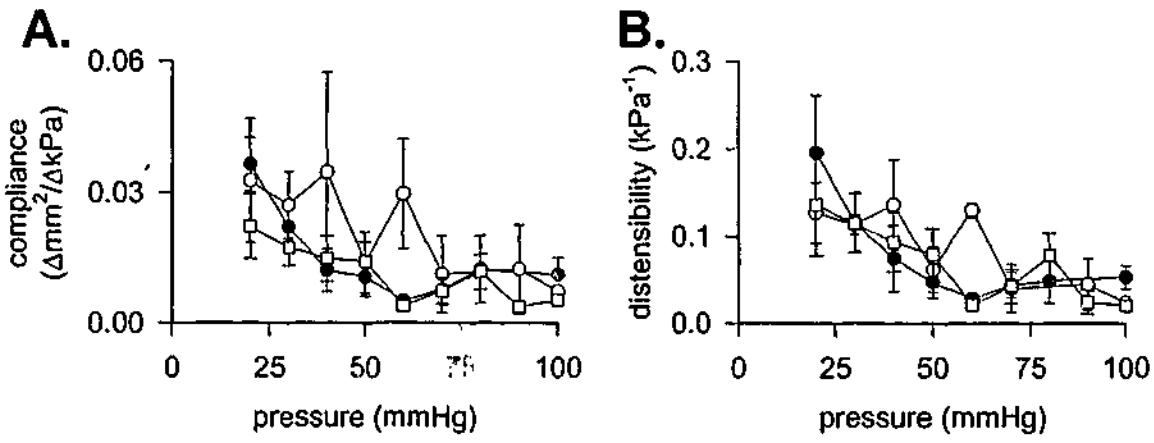
2.6 Conclusions

Guinea pigs that are born with low birth weights were able to catch up in body size to guinea pigs of average and high birth weight within the first postnatal year. However, the timing of this catch-up is gender-specific. Arterial pressure in the 1 year old adult guinea pig was not related to birth weight. While there were regional differences in the passive arterial wall mechanical properties, there were few differences in arterial mechanics between birth weight groups; this finding is consistent with no association between birth weight and arterial pressure in the adult guinea pig.

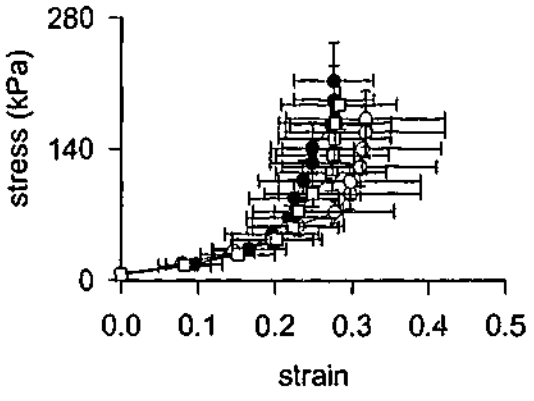
2.7 Appendices



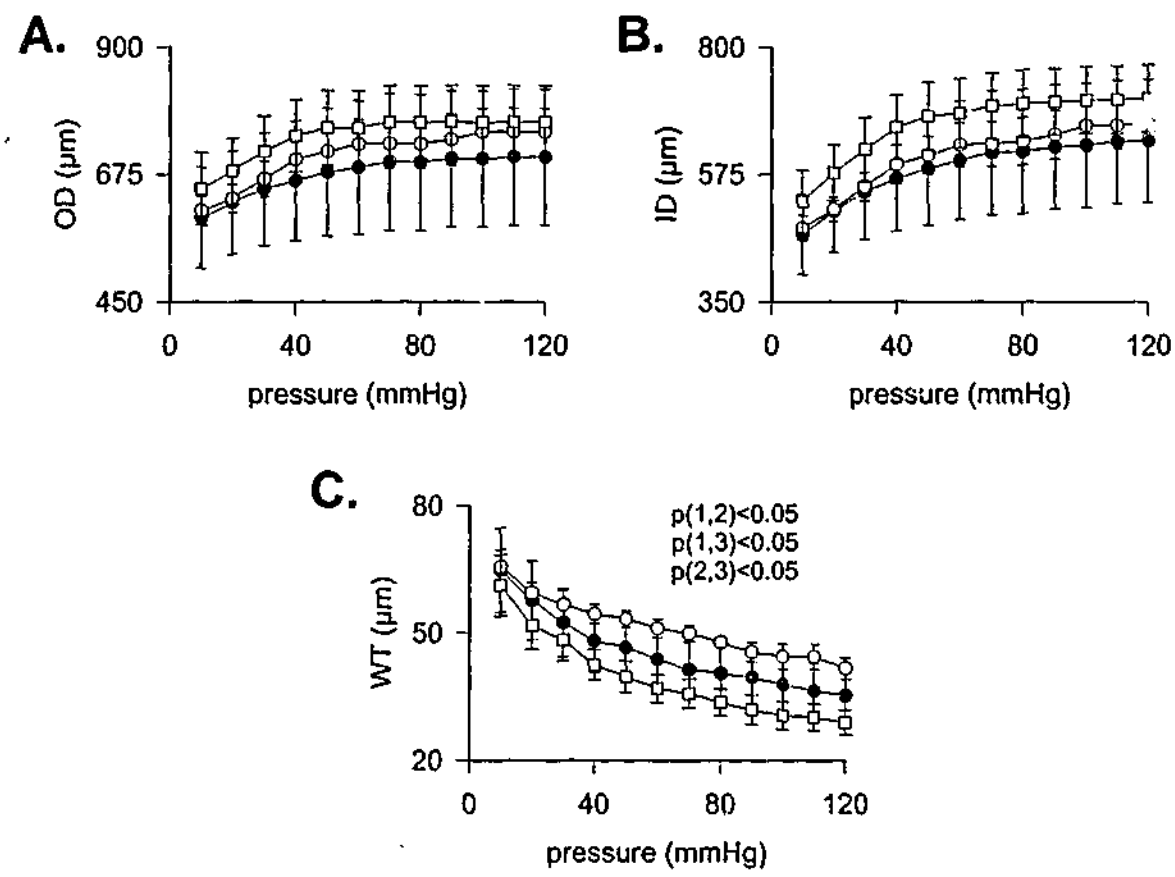
Appendix 2-1. Dimensions (µm) of the basilar artery during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of basilar artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs. p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.



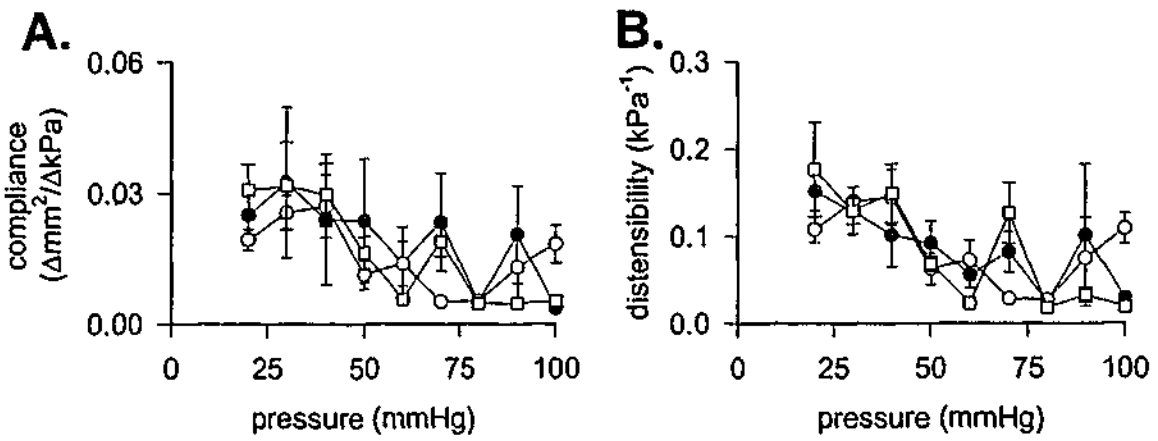
Appendix 2-2. Cross-sectional compliance and distensibility of the basilar artery. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of basilar artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs.



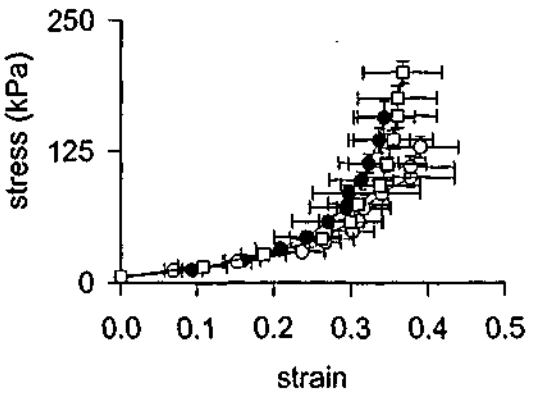
Appendix 2-3. Stress-strain relationships for the basilar artery. Stress-strain relationships for the segments of basilar artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs.



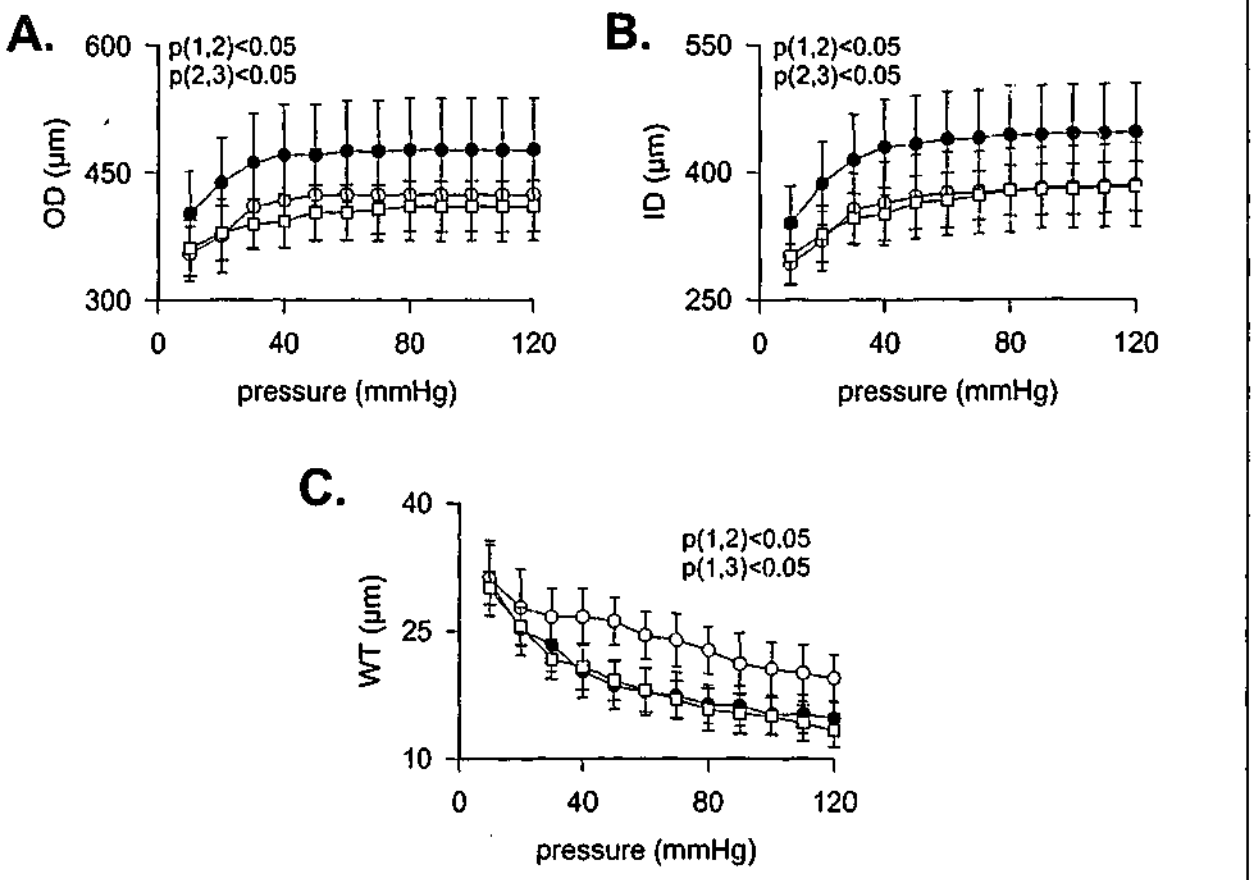
Appendix 2-4. Dimensions (µm) of the femoral artery branch during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the femoral artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs. p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.



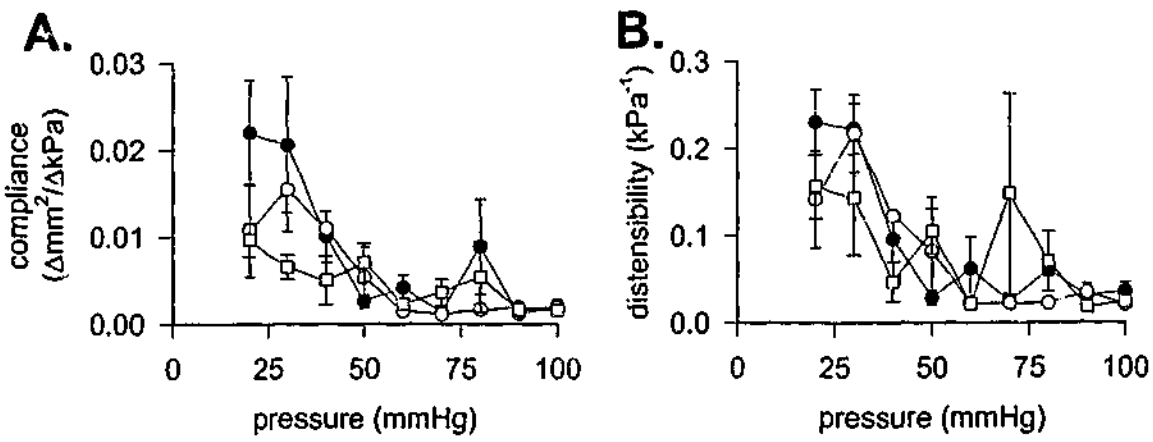
Appendix 2-5. Cross-sectional compliance and distensibility of the femoral artery branch. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of femoral artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs.



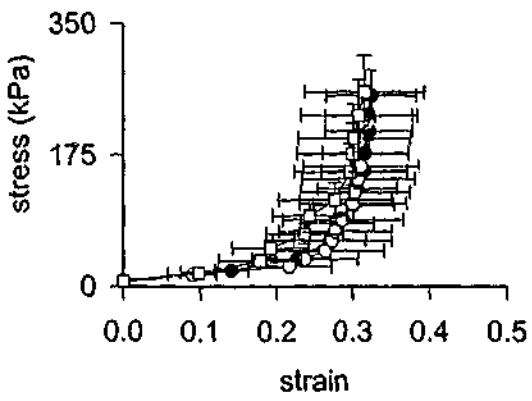
Appendix 2-6. Stress-strain relationships for the femoral artery. Stress-strain relationships for the segments of femoral artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs.



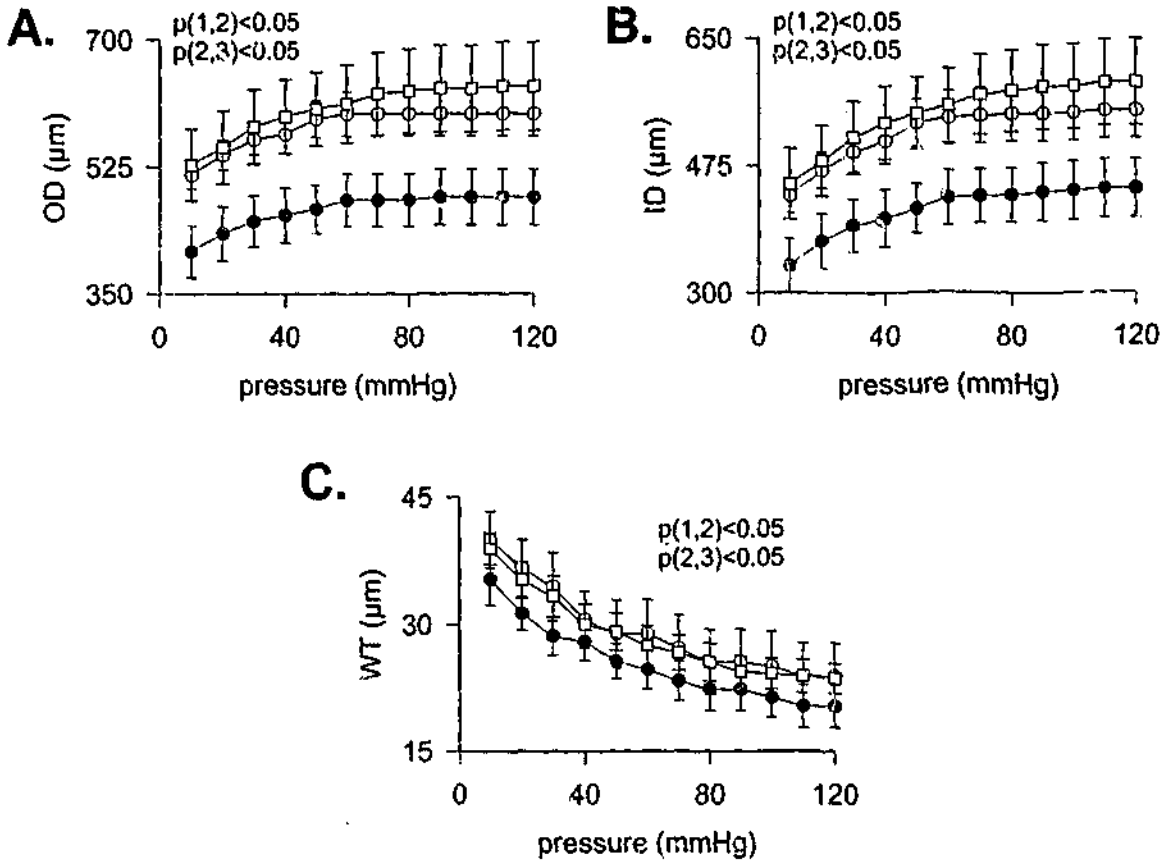
Appendix 2-7. Dimensions (µm) of the mesenteric artery branch during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of mesenteric artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs. p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.



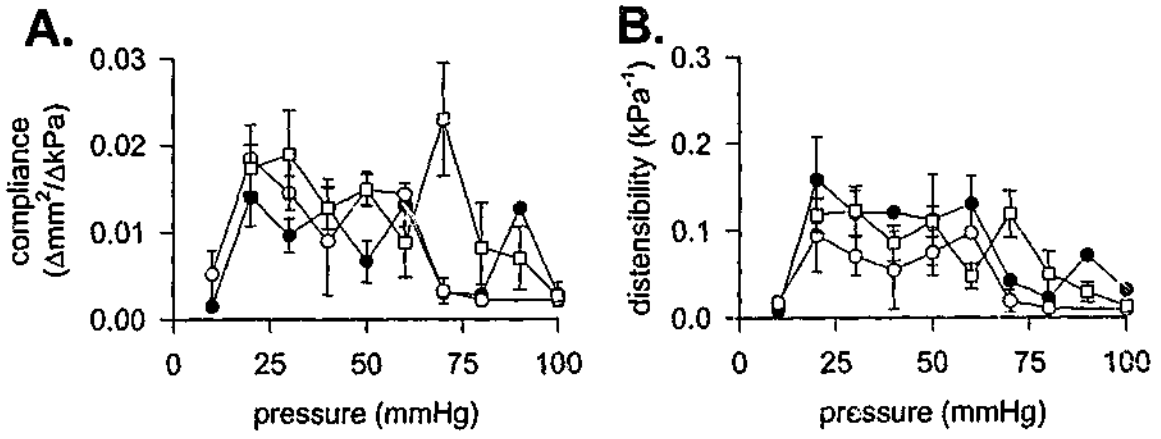
Appendix 2-8. Cross-sectional compliance and distensibility of the mesenteric artery branch. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of mesenteric artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs.



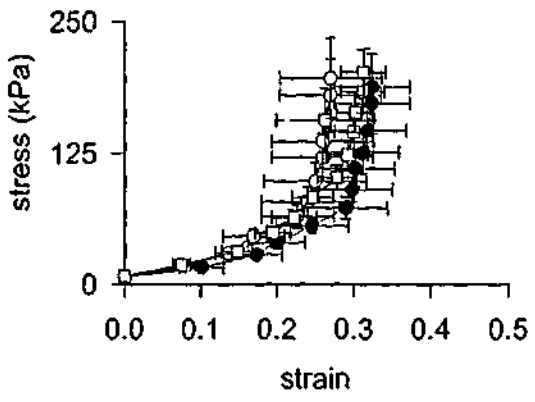
Appendix 2-9. Stress-strain relationships for the mesenteric artery. Stress-strain relationships for the segments of mesenteric artery branch from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs.



Appendix 2-10. Dimensions (µm) of the arcuate artery during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of arcuate artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs. p(group)<0.05 indicates a significant effect of group as found by two-way ANOVA; numbers in parentheses refer to the birth weight groups that differ from each other.



Appendix 2-11. Cross-sectional compliance and distensibility of the arcuate artery. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of arcuate artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs.



Appendix 2-12. Stress-strain relationships for the arcuate artery. Stress-strain relationships for the segments of arcuate artery from Group 1 (○, n=4), Group 2 (●, n=8) and Group 3 (□, n=5) guinea pigs.

Chapter 3

The effects of intrauterine growth restriction on arterial pressure and arterial wall mechanical properties in near-term fetal sheep

3.1 Introduction

Epidemiological studies indicate that a sub-optimal intrauterine environment resulting in reduced fetal growth can predispose the individual to adult diseases, including hypertension (Law & Shiell, 1996; Huxley *et al.*, 2000). It has been proposed that hypertension in the postnatal period may be initiated *in utero* and amplified with age (Law *et al.*, 1993) although the mechanisms for the initiation and amplification of hypertension in these individuals remain unknown.

In the sheep, different perturbations to the intrauterine environment can lead to altered arterial pressure in the postnatal period (Dodic *et al.*, 1998; Robinson *et al.*, 1998; Hawkins *et al.*, 2000c; Louey *et al.*, 2000; Moss *et al.*, 2001); however, the direction in which the arterial pressure is shifted differs between models (refer to Table 1-4). Some of these alterations to postnatal arterial pressure have their origins *in utero* but postnatal hypertension is not necessarily preceded by fetal hypertension. Intrauterine growth restriction (IUGR) induced by a reduced placental mass (resulting from pre-pregnancy caruncleotomy) is not associated with altered fetal arterial pressure in late gestation (Robinson *et al.*, 1983; Edwards *et al.*, 1999). Postnatally, these IUGR offspring become hypertensive relative to controls, but this hypertension is preceded by a period of hypotension soon after birth (Robinson *et al.*, 1998). Early gestational maternal undernutrition in sheep leads to postnatal hypertension in offspring (Hawkins *et al.*, 2000a); this hypertension is also preceded by a period of hypotension but in contrast to

the effects of IUGR induced by caruncleclectomy, this relative hypotension is evident in late gestation (Hawkins *et al.*, 2000c).

Late gestational placental insufficiency induced by umbilico-placental embolisation (UPE) leads to IUGR and postnatal hypotension; this relative hypotension appears to be initiated *in utero* (Louey *et al.*, 2000). However, the effects of UPE on fetal arterial pressure can differ between studies, and is dependent on the gestational timing, duration and severity of the embolisation. When fetal arterial oxygen content or saturation is reduced by 40–50% of pre-UPE values at 120d GA, fetuses become hypertensive soon after the onset of UPE (Cock & Harding, 1997; Louey *et al.*, 2000; Gagnon *et al.*, 2002). If UPE is continued for 20–26 days, the fetuses become hypotensive relative to controls (Cock & Harding, 1997; Louey *et al.*, 2000). In contrast, less severe UPE for 10 days (30–35% reduction in fetal arterial oxygen content) commencing in the same gestational period does not alter fetal arterial pressure (Gagnon *et al.*, 1994). If UPE is performed earlier in gestation (starting at 108–110d GA) and fetal oxygen content is reduced by 40–50% of pre-embolisation values, the fetuses become hypertensive for 21 days (Murotsuki *et al.*, 1997). The fetuses in these latter studies (Gagnon *et al.*, 1994; Murotsuki *et al.*, 1997), however, were not born and thus the postnatal arterial pressure of these animals is not known.

In sheep, UPE from 120d GA until term birth (~147d GA) leads to reduced fetal mean arterial pressure that persists into the postnatal period (Louey *et al.*, 2000), although the mechanisms are unclear; the postnatal hypertension is not due to altered circulating cortisol or plasma renin activity. One possible mechanism for the induction of persistent alterations to arterial pressure that is initiated *in utero* is altered development of structural components of arterial walls. Elastin and collagen are the two main structural proteins in the arterial wall that determine the passive mechanical properties of the artery (Roach & Burton, 1957; Wolinsky & Glagov, 1963; Dobrin, 1978). Both are synthesised *in utero*, and the perinatal period is a critical period for elastin synthesis (Berdeck & Langille, 1991; Wells *et al.*, 1999). There is little elastin synthesis and almost no arterial elastin turnover in adult life (Lansing, 1954; Walford *et al.*, 1964; Shapiro *et al.*, 1991). In a sub-optimal fetal environment in which a growth restricted fetus may be hypoxemic and/or hypoglycemic (Economides & Nicolaides, 1989), elastin and collagen synthesis and deposition may be decreased, as these proteins are

sensitive to changes in oxygen and nutrient supply (Durmowicz *et al.*, 1991; Spanheimer *et al.*, 1991). The amount and relative proportions of elastin and collagen are important determinants of arterial mechanical properties (Roach & Burton, 1957; Wolinsky & Glagov, 1963) and it is possible that reduced synthesis and deposition of elastin and/or collagen (which in turn alters the collagen:elastin ratio in the arterial wall) in a sub-optimal intrauterine environment could have lasting effects on the arterial pressure of the individual.

Although impaired elastin synthesis and decreased arterial compliance have been proposed as a potential mechanism underlying the fetal programming of hypertension (Martyn & Greenwald, 1997), few studies have investigated the relationship between the fetal environment and mechanical properties or the structural composition of the arterial wall. In rats, a brief period of fetal growth restriction in late gestation results in reduced aortic elastin and collagen contents in the adult (Berry & Looker, 1973). However, the reduction in these structural proteins was not apparent in the early postnatal period, and the functional consequences on arterial pressure and arterial mechanical properties are not known. In fetal sheep (mid-late gestation), there are age-related and body weight-related decreases in the distensibility of the carotid artery. Arterial distensibility decreases with advancing gestational age, and with increasing body weight (Roach, 1970), but given that fetal weight increases with gestational age, it is unclear whether the weight-related decrease in arterial distensibility was directly associated with gestational age. Further investigation of the relationship between arterial distensibility and fetal weight in twin fetuses with discordant body weights indicated that the weight-related decrease in arterial distensibility is independent of gestational age; arterial distensibility is lower in the heavier twin of the pair (Roach, 1970). These studies indicate that fetal growth restriction can be associated with reduced amounts of arterial wall structural proteins, and reduced arterial compliance, but the relationship between the fetal environment and the short- and long-term effects on arterial pressure, mechanical properties and structural composition of arteries remains unknown.

It is apparent that alterations to arterial structure and mechanical properties induced by a chronically hypoxic and hypoglycemic fetal environment could lead to alterations to the fetal arterial pressure that persist into the postnatal period. Based on previous studies

that have investigated the effect of late gestational UPE on arterial pressure of offspring, it might be expected that the arteries from UPE fetuses are more compliant than those from controls, consistent with lower arterial pressure in near-term fetuses (Cock & Harding, 1997) and early postnatal lambs (Louey *et al.*, 2000) after 20–26 days of UPE. Conversely, the opposite might also be expected, and impaired elastin synthesis in artery walls may lead to decreased arterial compliance in the fetus (Martyn & Greenwald, 1997), predisposing the offspring for later hypertension.

3.2 Aims

1. To determine the effects of placental insufficiency on arterial pressure in late gestation fetal sheep.
2. To determine the effects of late gestational placental insufficiency on passive arterial wall mechanical properties in the near-term fetal sheep.

3.3 Methods

3.3.1 Experimental animals

Studies were conducted on the fetuses of Border Leicester × Merino ewes of known mating date. Prior to surgery (116–117 days of gestation), ewes (n=18) were brought into the Department of Physiology (Monash University) animal house for acclimatisation. Before and after surgery, the ewes were housed in individual metabolic cages. They were exposed to 12 hour light and dark cycles and room temperatures of 18–20°C. They were provided daily with fresh lucerne chaff and water.

Of the ewes that underwent surgery, 15 were carrying singleton fetuses and the remaining three ewes were carrying twins. All twin fetuses were allocated to the UPE group; twin fetuses were expected to be smaller than singletons but were also subjected to late gestational UPE to further restrict their growth and maximise the degree of growth restriction. In these twin pregnancies, only one fetus was catheterised for experimental procedures; the other twin remained uncatheterised and was not used for any studies described in this chapter. All procedures involving animals were approved by the Monash University Animal Ethics Committee (Physiology).

3.3.2 Fetal surgery

3.3.2a Preparation of the ewe for surgery

Ewes were fasted for 12–24 hours prior to surgery, with only water available in this time. General anaesthesia of the ewe and fetus was induced by the intravenous injection of 1g of sodium thiopentone into the ewe's jugular vein. The ewe was then intubated with a cuffed endotracheal tube (Portex Ltd, England) and connected to an anaesthetic machine and ventilator. Anaesthesia was maintained with halothane (1.5–2%) in oxygen for the remainder of the surgery.

The ewe's neck, abdomen and right upper flank were shorn and these areas were then cleaned with Savlon (ICI, Australia). Following this, the skin was washed twice with surgical scrub (Betadine, 7.5% w/v Povidone-iodine, Faulding Pharmaceuticals, Australia), before the application of antiseptic solution (Betadine, 10% w/v Povidone-iodine, Faulding Pharmaceuticals, Australia). After the ewe was transferred onto the surgical table, a mixture of antiseptic disinfectant (Hibitane Concentrate, 5% w/v chlorhexidine gluconate and 4% w/v isopropyl alcohol, ICI, Pty Ltd, Australia) and ethanol was sprayed over the surgical incision area.

All towels, gowns, drapes and surgical instruments were sterilised by autoclaving and strict aseptic conditions were maintained throughout the surgical operation. All catheters were sterilised by either ethylene oxide gas or gamma radiation. Surgical caps and facemasks were worn, and the surgeon's hands were washed and scrubbed with antiseptic skin cleaner (Hibiclens, 4% w/v chorhexidine gluconate and 4% w/v isopropyl alcohol, ICI, Australia). Gowns and sterile latex gloves (Gammex, Ansell, Australia) were worn and drapes placed around the surgical incision site.

3.3.2b Surgical procedure

A 15cm midline incision was made in the skin and peritoneum of the lower abdomen of the ewe, running from the level of the umbilicus to the upper margin of the mammary glands, followed by a 15cm midline incision of the *linea alba*. The uterus was exposed and palpated to determine the orientation of the fetus. A 10–15cm uterine incision was made to expose the fetus, taking care not to damage any large blood vessels or placental

cotyledons. The fetal legs and rump were exposed to the level of the umbilical cord and the uterus and fetal membranes were clamped to the fetal skin to minimise loss of fluid.

An incision was made medially on the fetal upper hind limb to expose the fetal femoral artery. The vessel was freed from surrounding tissue and a catheter (Dural Plastics, Australia, Cat. No. SV65, Medical Grade, ID 0.85mm, OD 1.52mm) was inserted into the vessel caudally 7–10cm so that the tip of the catheter was located in the descending aorta at a level above the umbilical arteries but below the renal arteries (Cock & Harding, 1997); this catheter was used for blood sampling, measurement of fetal arterial pressure and UPE. The incision site was then sutured closed and the catheter was secured to the fetal skin. A catheter was sutured to the rump of the fetus (Dural Plastics, Australia, Cat. No. SV116, Medical Grade, ID 1.5mm, OD 2.7mm) to measure amniotic fluid pressure. A 2ml injection of antibiotics (Ilium Penstrep, procaine penicillin 250mg/ml, dihydrostreptomycin as sulphate 250mg/ml, procaine hydrochloride 20mg/ml, Troy Laboratories Pty Ltd, Australia) was administered intramuscularly to the fetal rump before its return to the uterus. The uterus was sutured closed using a locking stitch, with the added strength of a second overlying suture line to ensure fetal membranes were sealed and fluid loss minimised.

The free ends of the catheters were collected and exteriorised through a small incision (1–2cm) on the ewe's right upper flank. The catheters were sutured to the skin of the ewe just above this incision, flushed with sterile, heparinised saline, and fitted with sterile three-way taps. Both incision sites (midline and flank) in the ewe were then sutured closed with non-absorbable suture (Vetafil Bengen, 0.30mm, Clements Stansen Medical, Australia).

A catheter (Dural Plastics, Australia, Cat. No. SV116, Medical Grade, ID 1.5mm, OD 2.7mm) was inserted into the maternal jugular vein via an incision in the ewe's neck; this catheter was used for euthanasia of the ewe and fetus at the end of the study. The incision was then sutured closed and a three-way tap was attached to the catheter. The exteriorised fetal catheters, and maternal catheter, were kept inside plastic self-seal bags secured to elasticised netting (Setonet, Seton Products Ltd, England) around the ewe's trunk. At the completion of the surgery, the halothane administration was stopped.

When the ewe was able to breathe spontaneously and the swallow reflex returned, the endotracheal tube was deflated and removed.

3.3.2c Post operative care

Following surgery, ewes were returned to individual metabolic cages. Three to four days of recovery were allowed before experimentation commenced. Starting the day after surgery, one arterial blood sample was taken from each fetus for daily assessment of fetal health by analysis of fetal blood gases, and glucose and lactate concentrations. Fetal arterial catheters were flushed daily with sterile heparinised saline (50IU/ml 0.9% sodium chloride).

3.3.3 Blood gas tensions, glucose and lactate concentrations

Each day, arterial blood samples (0.5ml) were collected from the femoral arterial catheter into heparinised 1ml syringes. Air bubbles were expelled from the syringe, which was capped and the blood analysed immediately. Arterial blood gas tensions (pHa, Pa_{CO_2}, Pa_{O_2}, Sa_{O_2}) from whole blood were analysed with the Radiometer ABL 510 blood gas analyser (Radiometer, Denmark) and adjusted for fetal body temperature of 39°C. The remainder of the sample was analysed for blood glucose and lactate concentrations with the YSI 2300 STAT Glucose and L-Lactate analyser, (Yellow Springs Instruments, USA).

3.3.4 Umbilico-placental embolisation

The umbilico-placental circulation of fetuses in the growth restricted group was embolised from 120 days until 140 days GA (term~147d GA). UPE was performed using a 1% w/v suspension of non-radioactive, non-soluble 40–70μm spheres (Sephadex Superfine G-25, Pharmacia LKB, Sweden) in heparinised saline and 0.02% Tween 80, so that 1ml of the solution contained approximately 1 million spheres. This suspension was sterilised by autoclaving, and after vigorous mixing immediately prior to use, small volumes were injected daily into the fetal arterial catheter to decrease the fetal arterial oxygen saturation (Sa_{O_2}) to between 25%–35%, i.e. a reduction to ~50% of pre-UPE values. A fetal arterial blood sample was taken prior to embolisation, and if necessary, a small volume of microspheres was injected via the arterial catheter and

flushed into the umbilico-placental circulation with a small volume of heparinised saline. The required number of microspheres injected was titrated according to the fetal Sa_{O_2} of the most recent blood sample. Approximately 10–15 minutes after the injection of the microspheres, another arterial blood sample was taken to determine the effect of the microspheres on Sa_{O_2}. If the Sa_{O_2} was reduced to the desired level, embolisation ceased for that day. If the Sa_{O_2} had not adequately been reduced, another injection of microspheres was administered and this procedure was repeated until Sa_{O_2} had fallen to the desired level. While Sa_{O_2} and Pa_{O_2} were the main indicators of the level of embolisation, Pa_{CO_2} and pHa were also monitored. If an increase in Pa_{CO_2} or a decrease in pHa were observed, embolisation ceased for that day, regardless of whether Sa_{O_2} had reached the desired level.

In this chapter, fetuses that underwent umbilico-placental embolisation will be referred to as UPE fetuses. Fetuses that did not undergo UPE, and received saline injections only will be referred to as control fetuses.

3.3.5 Fetal arterial pressure recordings

Recordings of fetal arterial pressure were made every five days from 120d until 140d GA. Recordings were made for one hour for the control fetuses, and for one hour prior to, during and the two hours immediately following embolisation in the growth restricted fetuses. Disposable pressure transducers (TFN-R Disposable Transducers, Viggo-Spectramed, Oxnard, CA, USA) were connected to the arterial and amniotic catheters. Fetal arterial pressure (after the subtraction of amniotic sac pressure) was recorded from pressure amplifiers and fetal heart rate was derived from the arterial pressure signal, via a ratemeter. Output signals from the pressure amplifier and ratemeter were logged by a digital data recording system (PowerLab 800, ADInstruments Pty Ltd, Australia) connected to a computer using the software Chart for Windows (version 4.01, ADInstruments Pty Ltd, Australia). The data acquisition system was configured to record at a rate of 40 samples/sec up to a range of 10V; the time scale was set to 10 sec/division.

3.3.5a Analysis of arterial pressure recording

At the completion of the recording period, the data were digitally analysed in 1 minute blocks to obtain average diastolic, systolic and heart rate values for each minute. Mean arterial pressure (MAP) was calculated from the corresponding systolic and diastolic pressure readings using the formula:

$$MAP\ (mmHg) = diastolic + \tfrac{1}{3}\ (systolic - diastolic)$$

Data from the control fetuses were averaged to obtain one mean value for each of diastolic pressure, systolic pressure, MAP and heart rate for the entire recording period on each day. Data from the UPE fetuses were averaged to obtain mean values for each of diastolic pressure, systolic pressure, MAP and heart rate for each of the periods pre-UPE, during UPE, and the first and second hours following UPE on each recording day.

3.3.6 Fetal plasma cortisol concentrations

Plasma samples were collected between 120d and 140d GA for the measurement of circulating cortisol levels. One sample was collected from each control fetus every five days and two samples from each UPE fetus was collected on each of these days – one sample prior to embolisation and a second sample two hours after the completion of embolisation. Only one plasma sample was collected from the UPE fetuses at 140d GA, prior to the post mortem.

A 2ml sample of whole blood was collected into a fluoride heparin tube and centrifuged (Model J-6B, Beckman, USA) at a temperature of 4°C for 15 minutes at 3000 rpm. The plasma was stored at –20°C for later analysis by radioimmunoassay (performed by Mrs. Jan Loose), using the method described in Bocking *et al.* (1986).

3.3.7 Post mortem

At 140d GA, ewes and their fetuses were humanely killed and sections of arteries were collected for the *in vitro* determination of arterial wall mechanical properties. A number of organs were also collected for various biochemical and histological analyses (not presented in this thesis).

Five minutes before the sheep were killed, the fetuses were given a 0.5ml intravenous injection of vasodilator (papaverine hydrochloride, 120mg in 10ml, David Bull Laboratories, Australia) to maximally dilate blood vessels, followed by 5ml of heparin (Mulitiparin Heparin, 5000IU/ml, Fisons Pty Ltd, Australia) to minimise clotting in the blood vessels. The ewes were then killed with a 20ml intravenous injection of sodium pentobarbitone (Lethabarb, sodium pentobarbitone 325mg/ml, Virbac, Australia).

At post mortem, the fetus was removed from the uterus, weighed and crown-to-rump length and thoracic girth were measured. Amniotic fluid volume was measured, and the cotyledons in the placenta were counted and weighed. The major fetal organs were weighed and collected for later analysis (not presented in this thesis). In addition to the major organs, peri-renal and abdominal fat was collected and weighed. The left kidney, brain, a section of the ileum and a section of femoral muscle were collected and placed in oxygenated calcium-free physiological saline solution (PSS) until they could be dissected at the conclusion of the post mortem; segments of arteries were collected from these organs to determine the passive mechanical properties.

A number of conducting vessels were also collected for histological and biochemical analysis. Sections of thoracic aorta, inferior vena cava and carotid artery were removed and perfusion fixed at 50mmHg with 4% paraformaldehyde. Sections of aorta and carotid artery were also frozen in liquid nitrogen and stored at –70°C for later biochemical analysis of elastin and collagen content. These results are not reported in this thesis.

3.3.8 Measurement of passive arterial wall mechanical properties

3.3.8a Selection of arteries

Figure 3-1 illustrates the sites from which these arterial segments were collected. Arteries were taken from a similar area of each vascular bed and the outer diameter (OD) of the selected branches were similar between animals (~400μm).

Arteries from the brain A branch of the basilar artery (located at the base of the brain) and also an artery from the surface of the cerebrum were collected to measure their changes in dimensions during pressurisation so as to characterise the mechanical

properties of the arterial wall. One segment of the artery was dissected from each animal. The average initial OD (pressurised at 10mmHg) of the basilar artery branch was 338±20μm; the average initial OD (pressurised at 10mmHg) of the cerebral surface artery was 544±43μm.

An artery from skeletal muscle A branch of the femoral artery from the non-catheterised hind leg was collected for the determination of the mechanical properties of its walls; the selected branch was one entering the muscle. One segment of the artery was dissected from each animal; the average initial OD (pressurised at 10mmHg) was 434±36μm.

An artery from the mesentery The mechanical properties of an arterial segment of the mesenteric artery was determined; the arterial segments studied were branches of the mesenteric artery that entered the ileum. One segment of artery was collected from each fetus; the average initial OD (pressurised at 10mmHg) was 354±29μm.

An artery from the kidney A segment of the arcuate artery in the kidney was used for the study of arterial wall mechanics. This artery is located in the renal cortex and runs parallel to the cortical border. One segment of the arcuate artery was studied from each fetus; the average initial OD (pressurised at 10mmHg) was 365±25μm.

3.3.8b Dissection of arteries

Dissection of the arteries from the surrounding tissue has been described in Section 2.3.10b. In brief, organs were collected at post mortem and placed in oxygenated calcium-free PSS solution. After removal of connective tissue, the segment was tied to the glass cannula of the pressure myograph. Any blood within the arterial segment was gently flushed out before the free end of the segment was ligated. After initial inflation to the maximum pressure (200mmHg) to ensure there were no major leaks in the artery, the intraluminal pressure was returned to 10mmHg. Potential leaks in the artery were sealed by ligatures, however, the pressure-servo control system was able to compensate for minor leaks. A more detailed description of the pressure myograph and pressure-servo control circuit can be found in Section 2.3.10c. During the entire study

period, the artery was superfused with a warmed (35°C) and oxygenated solution of calcium-free EGTA-containing (1mM) PSS.

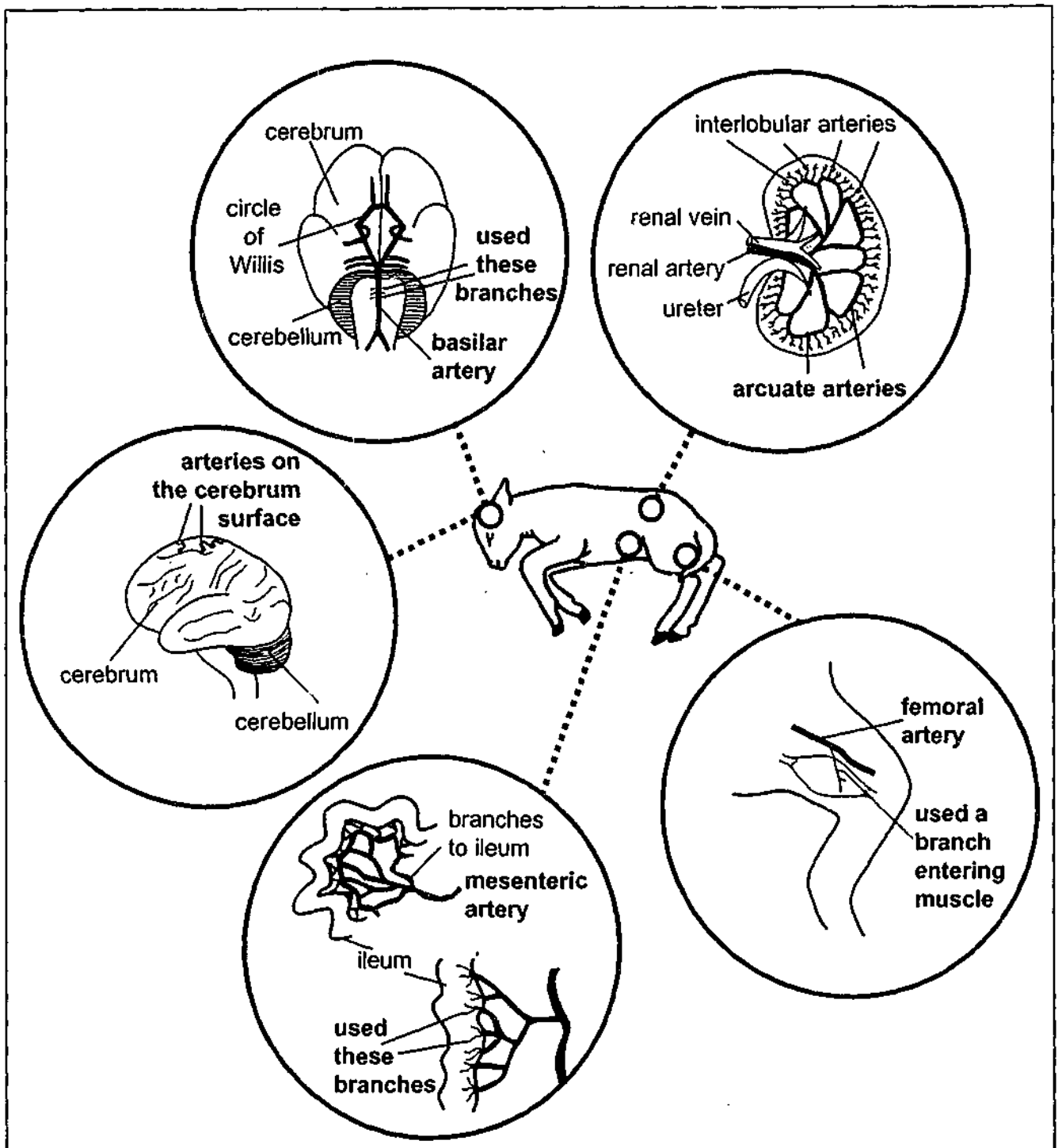


Figure 3-1. Fetal arteries collected for the determination of passive arterial wall mechanics. Schematic diagrams showing the sites from which arterial segments were collected for the determination of passive arterial wall mechanics. Segments were taken from four different vascular beds: a cerebral surface artery and a branch of the basilar artery from the brain, the arcuate artery from the kidney, a branch of the femoral artery from the skeletal muscle, and a branch of the mesenteric artery.

3.3.8c Measurements of arterial dimensions

Measurements (from a video monitor) of segment length (L) and OD were made using low magnification (×48), before wall thickness (WT) measurements were made at a higher magnification (×300); a more detailed description of these measurements can be found in Section 2.3.10d. Following the measurements at 10mmHg, the intraluminal pressure was increased by increments of 10mmHg up to 120mmHg, with measurements of L, OD and WT made at each pressure.

3.3.8d Calculations

All measurements were converted from values measured from the video monitor (in cm or mm) to the actual size (in μm). The calculations of arterial wall areas, wall stress and compliance (Hill & Ege, 1994; Pourageaud & De Mey, 1997; Crijns *et al.*, 1999) are described in more detail in Section 2.3.10e. In brief, the relative change from the initial measurement at 10mmHg intraluminal pressure has been calculated for each of:

outer diameter (OD)
inner diameter ($ID=OD-2WT$)
segment length (L)
wall thickness (WT)
and luminal volume ($V=\Pi(ID \div 2)^2 \times L$).

The following parameters were calculated from the measured OD, L and WT:

cross-sectional area of the lumen (μm^3) = $\Pi \times ID^2 \div 4$
cross-sectional area of the arterial wall (μm^3) = $\Pi(OD^2-ID^2) \div 4$
circumferential wall stress (kPa) = $(ID \div 2 \times P) \div (2 \times WT)$
cross-sectional compliance (mm^2/kPa) = $\Delta CSA/\Delta P$
cross-sectional distensibility (kPa^{-1}) = $\Delta CSA/CSA_i/\Delta P$
volume compliance (mm^3/kPa) = $\Delta V/\Delta P$
volume distensibility (kPa^{-1}) = $\Delta V/V_i/\Delta P$
wall strain = $(ID_x-ID_i) \div ID_i$.

The stress-strain relationship was analysed using Origin (Version 7.0, Microcal Software Inc., CA, USA) to calculate the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment (Hill & Ege, 1994). This exponential function was used to describe the overall

stress-strain relationship and the data fit this equation with a $r^2>0.94$. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries, and be^{k_2x} describes the high stress (collagenous) range of the arteries. The derived rate constants were compared using GraphPad Instat (Version 3.05, GraphPad Software Inc., CA, USA).

3.3.9 Statistical analysis

T-tests for independent samples were used to compare the body weights, organ weights and other measurements at post mortem in the control and UPE fetuses. Two-way repeated measures analysis of variance (ANOVA) were used to analyse the measurements in fetal blood (pHa, blood gases, glucose and lactate concentrations, plasma cortisol concentrations), arterial pressure and heart rate measurements; factors were treatment and age. Two-way repeated measures ANOVA were also used to analyse the data on arterial wall mechanics; factors were treatment and pressure.

Data are presented as mean±SEM unless otherwise indicated. Statistical analyses were performed using SPSS for Windows (Version 10.0.5) with the level of significance taken at $p<0.05$, unless otherwise stated. All significant differences indicated by ANOVA were subjected to the Student-Newman-Keuls (SNK) post-hoc test to test for significant differences between individual means.

3.4 Results

3.4.1 Outcomes of umbilico-placental embolisation

The details of the animals used in the studies described in this chapter are summarised in Table 3-1. Fetal surgery was performed on 18 pregnant ewes, 12 of which were carrying singleton fetuses and 3 carrying twins. Experiments were completed in a total of 6 control fetuses (all singletons) and 6 UPE fetuses (5 singletons, one twin). Experiments were commenced on the fetuses from 6 other ewes but were not completed in these animals due to unexpected fetal death (usually from an accidental overdose of microspheres during UPE) or maternal death. Data from fetuses whose studies were incomplete are not included in this chapter.

Table 3-1. The histories of ewes and fetuses described in this chapter. This table summarises the identification numbers of ewes and fetuses, gender of the fetus, treatment (Trt) group, the date and gestational age (GA) at surgery and at post mortem (PM). "S" refers to singleton fetuses and "T" refers to twin fetuses. "C" refers to control fetuses and "UPE" refers to fetuses that underwent umbilico-placental embolisation. "FD" refers to fetal death, the cause was usually from an accidental overdose of microspheres during UPE, "A" refers to an aborted fetus and "MD" refers to a maternal death.

Ewe ID	Fetal ID	Sex	Single/ Twin	Trt	Surgery date	GA at surgery	PM date	GA at PM	Comment
78	78.1	♂	T	UPE	12/06/00	116	17/06/00	121	FD
	78.2	♀	T	UPE	12/06/00	116	17/06/00	121	FD
82	82	♀	S	C	20/06/00	117	13/07/00	140	
132	132	♂	S	C	14/08/00	116	07/09/00	140	
133	133	♀	S	C	14/08/00	116	08/09/00	141	
134	134	♀	S	UPE	15/08/00	117	08/09/00	141	
135	135	♂	S	UPE	15/08/00	117	07/09/00	140	
158	158	♂	S	UPE	04/09/00	117	28/09/00	141	
173	173	♀	S	UPE	18/09/00	116	24/09/00	122	FD
187	187	♂	S	C	02/10/00	116	26/10/00	140	
203	203	♀	S	C	23/10/00	116	16/11/00	140	
204	204	♂	S	C	23/10/00	116	16/11/00	140	
209	209		S	UPE	30/10/00	116	08/11/00	125	FD
210	210		S	UPE	30/10/00	116	05/11/00	123	A
225	225		S	UPE	13/11/00	116	27/11/00	130	MD
226	226.1		T	UPE	13/11/00	116	27/11/00	130	FD
	226.2		T		Unoperated		27/11/00	130	
1032	1032.1	♂	T	UPE	07/05/01	116	31/05/01	140	
	1032.2	♂	T		Unoperated		31/05/01	140	
1038	1038	♂	S	UPE	14/05/01	116	07/06/01	140	
1044	1044	♂	S	UPE	28/05/01	116	21/06/01	140	

3.4.2 Fetal arterial blood parameters

3.4.2a Arterial blood gas and pH status

Figure 3-2 shows the arterial blood gas and pH values of the control and UPE fetuses prior to and during the period of UPE (120–140d GA). For the UPE animals, the data shown during the period of UPE are those obtained at the completion of the daily embolisation period.

Prior to UPE, Sa_{O_2} and Pa_{O_2} did not differ between the two groups of fetuses. However, UPE reduced fetal Sa_{O_2} to approximately 50% of the pre-embolisation values. After the first day of embolisation, the UPE fetuses had significantly lower Sa_{O_2} compared with controls; this hypoxemia was maintained for the remainder of the study period (Figure 3-2A). A similar effect was evident with Pa_{O_2}; compared with controls, UPE fetuses had significantly lower Pa_{O_2} levels from 125d GA until the end of the study (Figure 3-2B).

Throughout the period of UPE, there was a significant effect of treatment on Pa_{CO_2}; UPE fetuses were hypercapnic compared with controls (Figure 3-2C). Arterial pH did not differ between the two groups of fetuses prior to UPE however, the UPE fetuses became acidemic on the first day of UPE. The arterial pH of these fetuses remained lower than that of control fetuses for the remainder of the UPE period (Figure 3-2D).

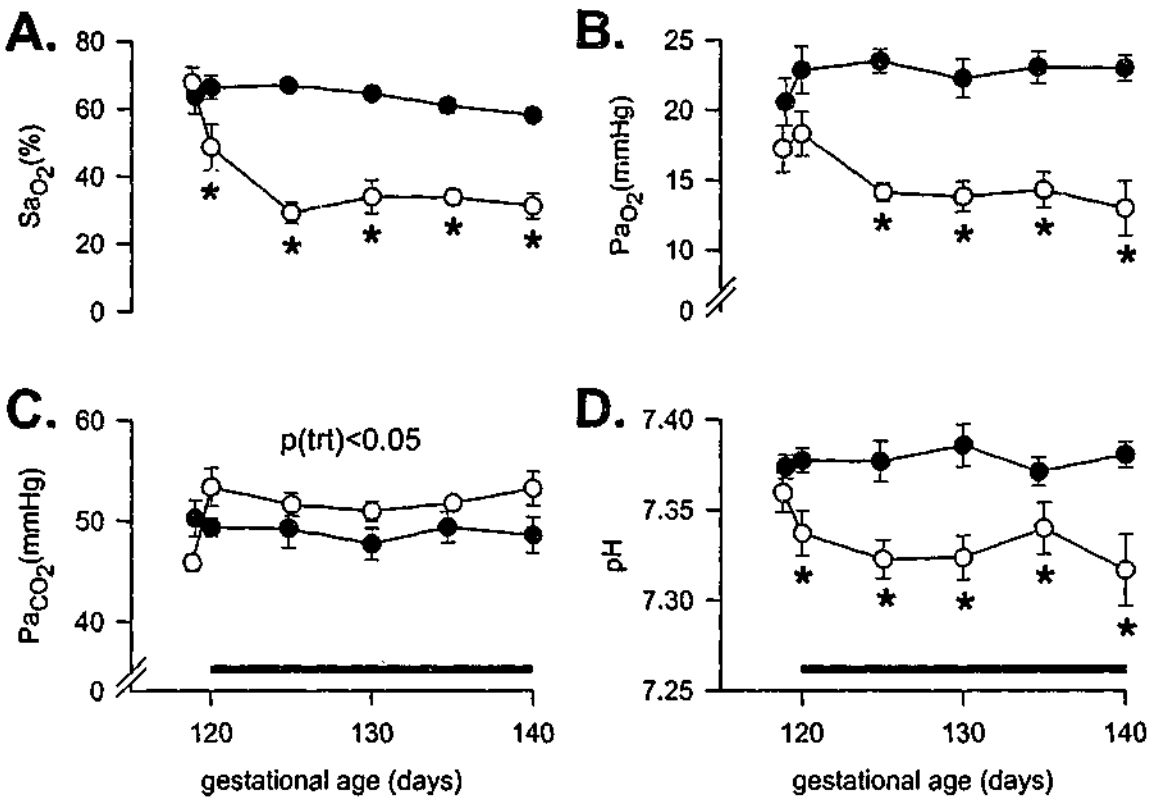


Figure 3-2. Arterial blood gas and pH status between 118–140d GA. (A) Arterial oxygen saturation (Sa_{O_2}), partial pressures of (B) oxygen (Pa_{O_2}) and (C) carbon dioxide (Pa_{CO_2}), and (D) pH in control (●, n=6) and UPE (○, n=6) fetuses in the pre-embolisation (118–119d GA) and the umbilico-placental embolisation (120–140d GA, indicated by the bar) periods. p(trt)<0.05 indicates a significant effect of treatment as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual ages (p<0.05).

3.4.2b Hemoglobin concentration and hematocrit

Hemoglobin concentrations were not different between the control and UPE fetuses at the onset of embolisation. After 10 days of embolisation, hemoglobin concentrations became significantly higher in the UPE fetuses and remained 2–3g/dL higher than those of controls until 140d GA (Figure 3-3A). As with hemoglobin concentration, hematocrit did not differ between the two groups of fetuses until 130d GA; after this age, the UPE fetuses had significantly higher hematocrits compared with controls (Figure 3-3B).

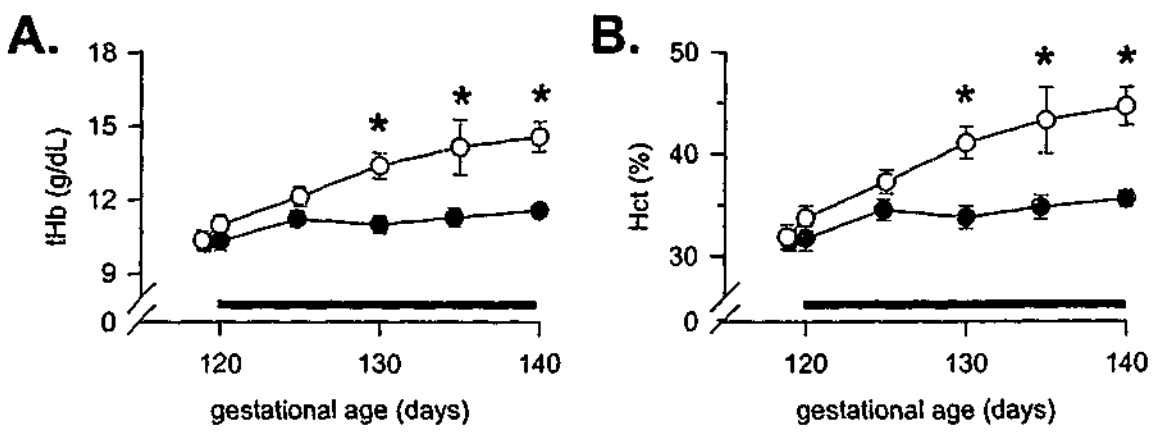


Figure 3-3. Hemoglobin concentrations and hematocrit during umbilico-placental embolisation. (A) Hemoglobin concentration (tHb) and (B) hematocrit (Hct) in control (•, n=6) and UPE (○, n=6) fetuses in the pre-embolisation (118–119d GA) and the umbilico-placental embolisation (120–140d GA, indicated by the bar) periods. Asterisks (*) indicate values that differ between groups at individual ages ($p<0.05$).

3.4.2c Blood glucose and lactate concentrations

There was no difference in the blood glucose concentrations of the control and UPE fetuses at 120d GA. By 125d GA, the UPE fetuses had lower blood glucose concentrations than controls. The UPE fetuses remained hypoglycemic relative to controls for the remainder of the study period (Figure 3-4A). There were no significant differences in the blood lactate concentrations between the two groups of fetuses during the embolisation period (Figure 3-4B).

3.4.2d Plasma cortisol concentrations

There was a significant effect of treatment group on the plasma circulating cortisol concentrations, with UPE fetuses having higher cortisol concentrations compared with controls throughout the study period (Figure 3-5A). While the UPE fetuses appeared to have higher mean plasma cortisol concentrations compared with controls at 140d GA, this difference was not significant due to a large variation in values in the UPE fetuses (control: 13.0±1.2ng/ml plasma, UPE: 39.0±15.4ng/ml plasma, $p=0.18$). The cortisol concentrations in the UPE fetuses prior to daily embolisation were not different to the cortisol concentrations two hours after the daily completion of UPE (Figure 3-5B).

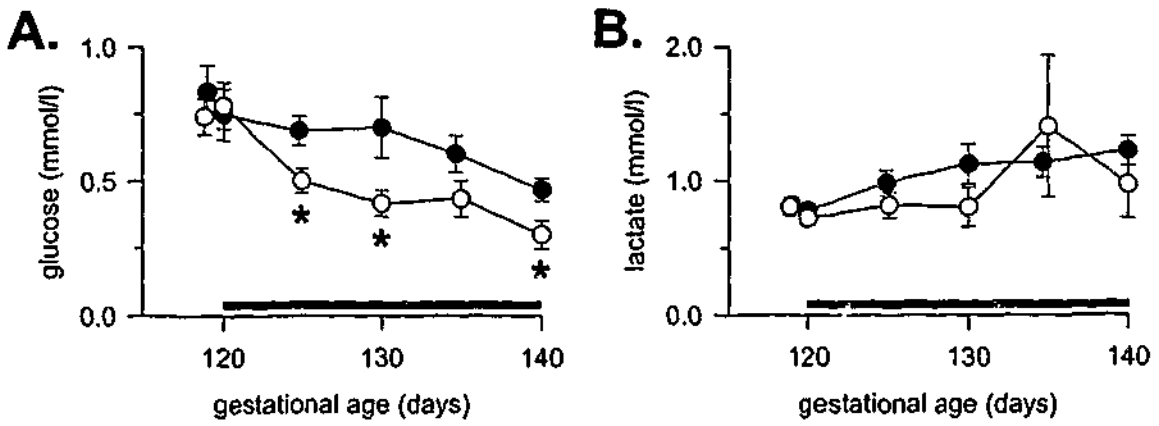


Figure 3-4. Blood glucose and lactate concentrations during umbilico-placental embolisation. (A) Blood glucose and (B) blood lactate concentrations in control (●, n=6) and UPE (○, n=6) fetuses in the pre-embolisation (118–119d GA) and the umbilico-placental embolisation (120–140d GA, indicated by the bar) periods. Asterisks (*) indicate values that differ between groups at individual ages ($p<0.05$).

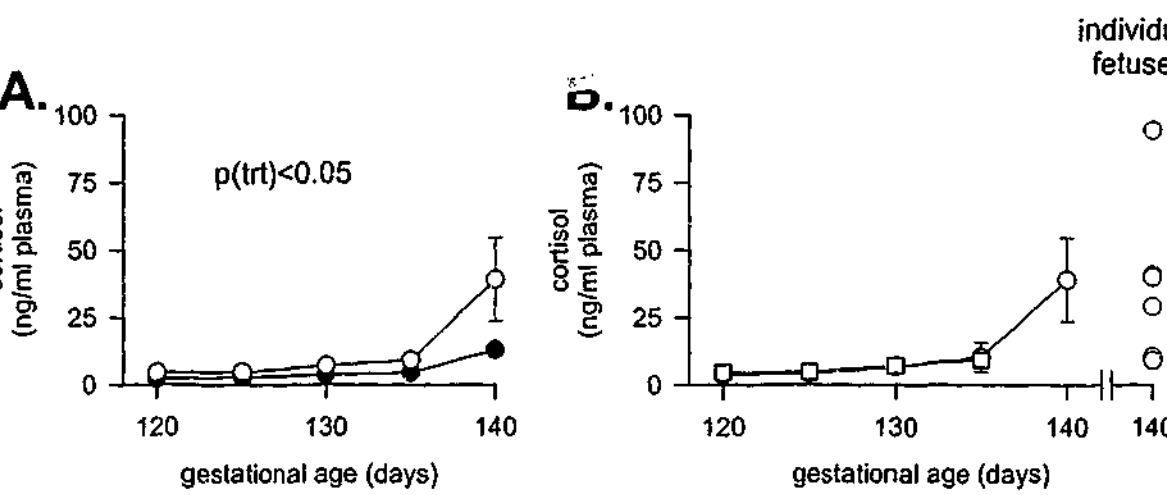


Figure 3-5. Plasma cortisol concentrations. (A) Plasma cortisol concentrations in control (●, n=6) and UPE (○, n=6) fetuses during the period of umbilico-placental embolisation (120–140d GA). Samples from UPE fetuses were collected two hours after the completion of daily UPE except at 140d GA; UPE was not performed at 140d GA and plasma samples were collected immediately prior to post mortem in the morning. (B) Plasma cortisol concentrations in UPE fetuses before (□) and two hours after (○) UPE. The cortisol concentrations for individual UPE fetuses at 140d GA are plotted on the right of graph B. $p(trt)<0.05$ indicates a significant effect of treatment as found by two-way ANOVA.

3.4.3 Fetal arterial pressure and heart rate

While there were no overall differences in the arterial pressures of the control and UPE fetuses, MAP tended (p=0.1) to be higher in the UPE fetuses at 125d GA compared with controls at this age (Figure 3-6A). The MAP of the UPE fetuses also tended (p=0.1) to be higher at 125d GA compared with pre-embolisation values at 120d GA. At 140d GA, the MAP was not significantly different between controls (42.4±2.4mmHg) and UPE fetuses (37.5±3.6mmHg). Similarly, there were no significant differences between the systolic and diastolic pressures of control and UPE fetuses during the period of placental embolisation (Figure 3-6C and 3-6D). Fetal heart rate significantly decreased with age in both groups; there were no significant differences in heart rate between the two groups (Figure 3-6B).

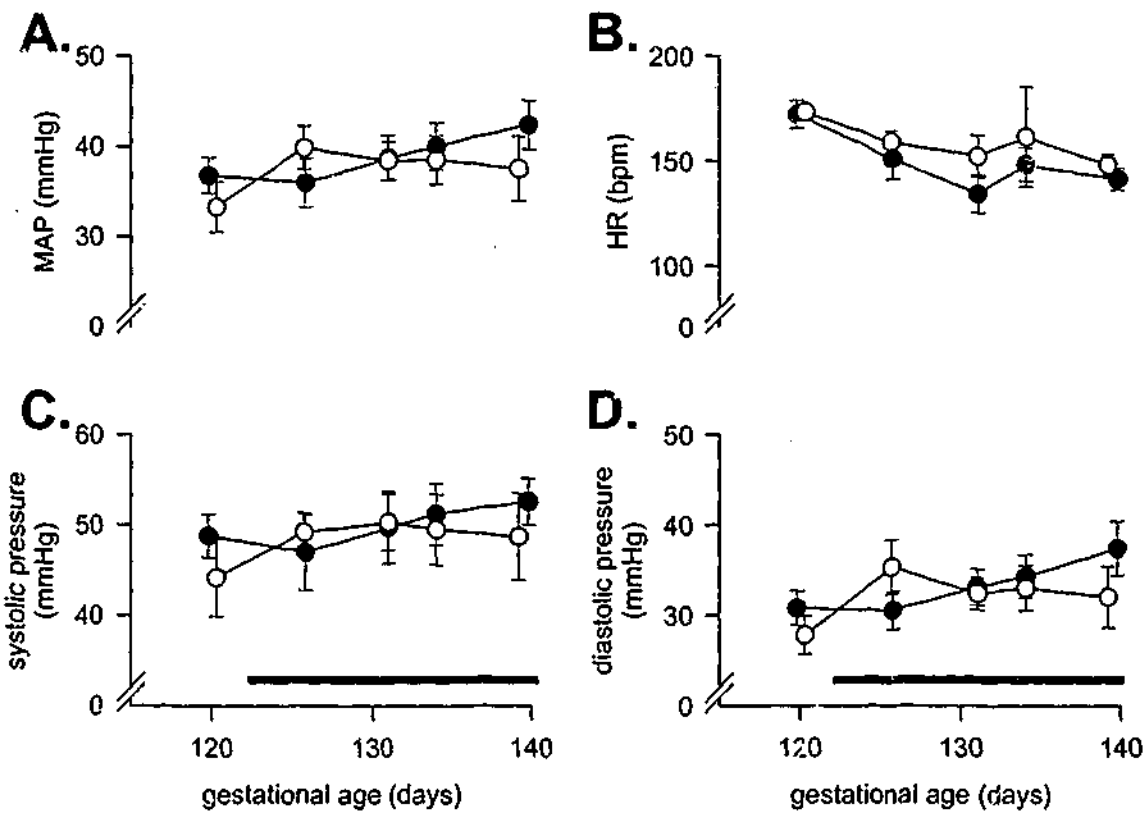


Figure 3-6. Arterial pressure and heart rate during umbilico-placental embolisation. (A) Mean arterial pressure, (B) heart rate, (C) systolic pressure and (D) diastolic pressure in control (●, n=6) and UPE (○, n=6) fetuses during the period of umbilico-placental embolisation (120–140d GA, indicated by the bar). Data from the UPE fetuses at 120d GA were taken pre-UPE; other data points taken two hours were post-embolisation.

3.4.4 Fetal body size at post mortem

Figure 3-7 shows the body weight, crown-to-rump length and ponderal index of the control and UPE fetuses at 140d GA. At post mortem, UPE fetuses were 37% lighter than controls (3.3±0.2kg *vs* 5.2±0.2kg) and the crown-to-rump length of UPE fetuses was 7% shorter than controls (53.5±1.5cm *vs* 57.4±0.7cm). The mean ponderal index of the UPE fetuses ($2.2e^{-5}$±$2.2e^{-6}$kg/cm^3) was also significantly less that that of the controls ($2.8e^{-5}$±$8.4e^{-7}$kg/cm^3), indicating the UPE fetuses were thinner than the control fetuses.

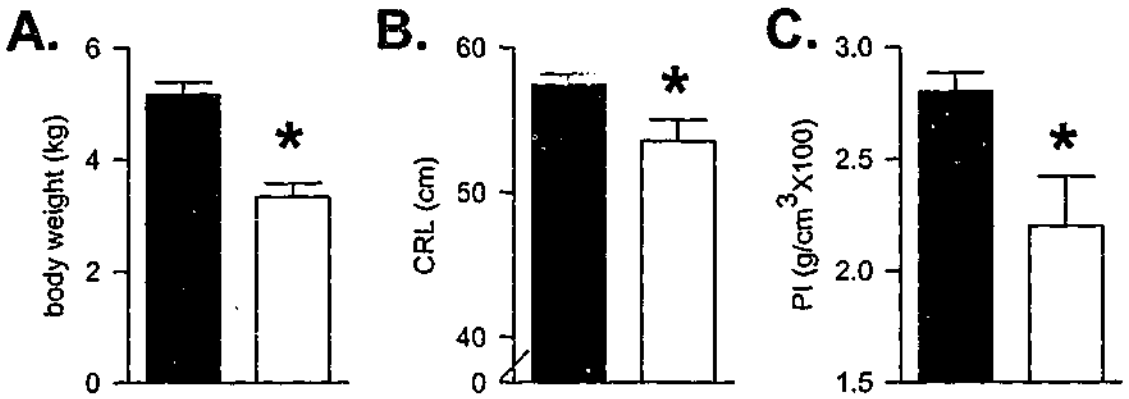


Figure 3-7. Body size of control and UPE fetuses at 140d GA. Mean (A) body weights, (B) crown-to-rump length (CRL) and (C) ponderal index (PI) of control (■, n=6) and UPE (□, n=6) fetuses at post mortem at 140d GA. Asterisks (*) indicate significant differences when compared with controls ($p<0.05$)

3.4.5 Organ weights

At 140d GA, there were no differences in the number of cotyledons or the placental weights of the UPE and control fetuses (Table 3-2). The amniotic fluid volumes collected at post mortem were also not significantly different between the groups (control: 561.5±156.2ml *vs* UPE: 309.7±88.4ml).

Heart, lung, liver, gut and kidney weights were all significantly lower in the UPE fetuses; however, when adjusted for body weight, the weights of these organs were not different to those of control fetuses (Table 3-2). Adrenal weights and the weight of abdominal fat were not different between the control and UPE fetuses in absolute terms, but when adjusted for body weight, the UPE fetuses had heavier adrenals and more

abdominal fat than controls (Table 3-2). The UPE fetuses showed evidence of brain sparing, having greater brain weight (g/kg body weight) (Table 3-2) and higher brain:liver weight ratios compared with controls (0.76±0.06 *vs* 0.45±0.03, $p<0.05$).

Table 3-2. Organ weights at 140d GA in control and UPE fetuses. Data shown are absolute organ weights (g) and organ weights adjusted for body weight (g/kg BWt). Asterisks (*) indicate values that differ between groups ($p<0.05$). # combined left and right organ weight.

		Control (n=6)	UPE (n=6)
Placenta	Weight (g)	413.8±36.9	353.8±46.5
	number of cotyledons	75±12	80±9
Heart	(g)	33.6±2.7	21.0±1.2*
	(g/kg BWt)	6.5±0.3	6.3±0.2
Lung	(g)	153.8±8.3	114.2±9.0*
	(g/kg BWt)	30.0±1.9	34.5±2.6
Liver	(g)	120.8±8.1	69.5±6.5*
	(g/kg BWt)	23.3±1.0	20.9±1.4
Gut	(g)	286.0±22.4	203.1±20.1*
	(g/kg BWt)	56.0±5.3	60.3±3.4
Kidneys#	(g)	31.2±1.3	20.5±1.8*
	(g/kg BWt)	6.1±0.2	6.1±0.3
Adrenals#	(g)	0.42±0.04	0.47±0.04
	(g/kg BWt)	0.08±0.01	0.15±0.02*
Abdom. Fat	(g)	26.3±1.9	27.9±1.6
	(g/kg BWt)	5.1±0.4	8.4±0.4*
Brain	(g)	52.8±2.2	50.6±1.9
	(g/kg BWt)	10.3±0.5	15.4±0.8*

3.4.6 Passive mechanical properties of the arterial wall

3.4.6a Branch of the basilar artery

Arterial dimensions (μm) during pressurisation There were no significant differences in the initial OD (pressurised to 10mmHg intraluminal pressure) of arteries from control (349±35μm) and UPE (327±23μm) fetuses. There were significant effects of treatment on the outer and lumen diameters, with arteries from control fetuses having greater diameters during pressurisation compared with those from UPE fetuses. Arterial wall thickness decreased significantly with increasing pressures; however, there was no difference in the WT of the two groups during pressurisation (Appendix 3-1). There were also no differences between the cross-sectional areas of the lumen or arterial wall (at 50mmHg) of arteries from control and UPE fetuses (Table 3-3).

Table 3-3. Cross-sectional area (CSA) of the basilar artery branch at 40mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 40mmHg intraluminal pressure (~fetal MAP) for the two groups of fetuses.

	Control (n=6)	UPE (n=6)
Lumen CSA (mm^2)	0.120±0.023	0.097±0.016
Arterial wall CSA (mm^2)	0.017±0.002	0.015±0.002

Relative changes in arterial dimensions during pressurisation In arteries from UPE fetuses, the relative increase in outer and lumen diameter with during pressurisation were less than the relative change in segments from control fetuses (Figure 3-8A, 3-8B). Similarly, the relative change with respect to arterial segment length was lower in segments from UPE fetuses (Figure 3-8D). Relative to initial arterial wall thickness (at 10mmHg intraluminal pressure), the segments of the basilar artery branch from UPE fetuses had thicker arterial walls (Figure 3-8C). All parameters changed significantly with increasing pressures.

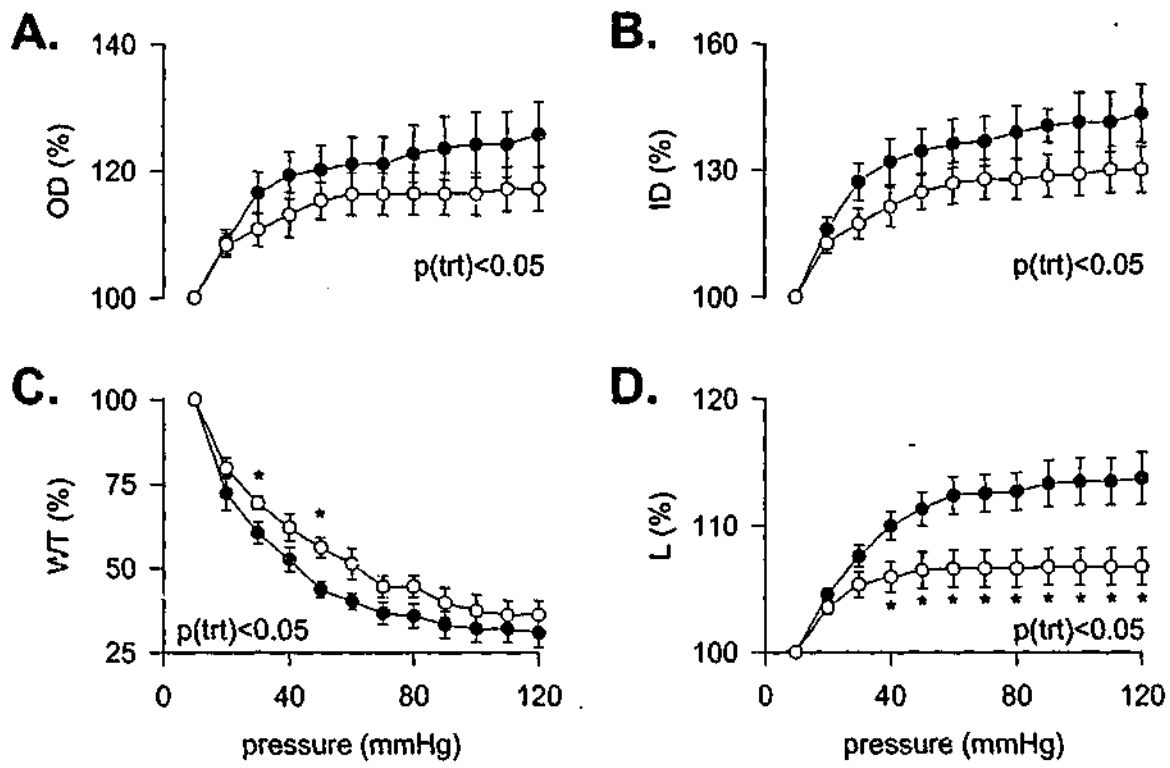


Figure 3-8. Relative dimension changes in the basilar artery branch during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of basilar artery branch from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Arterial wall properties during pressurisation There was a significant effect of intraluminal pressure on the lumen:wall thickness ratio and circumferential wall stress (Figure 3-9); however, there were no significant differences between control and UPE fetuses with respect to these parameters.

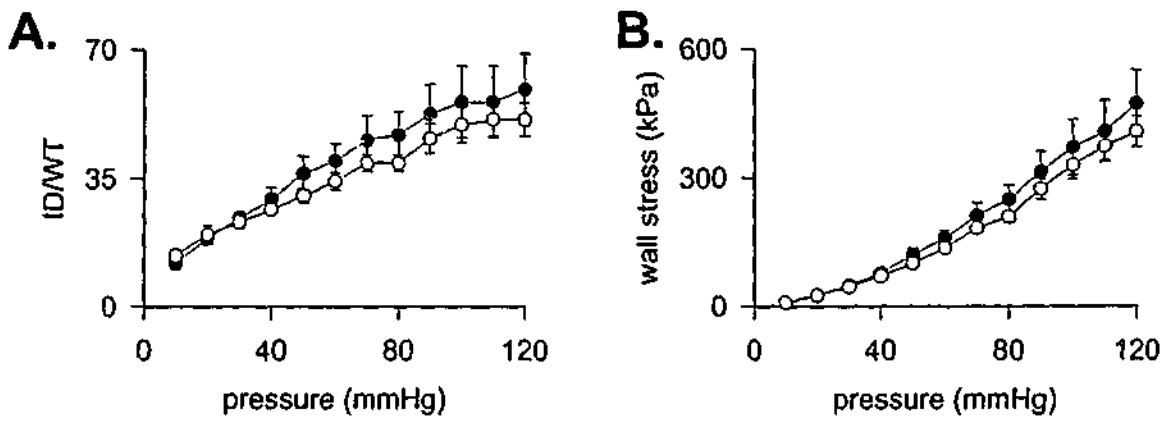


Figure 3-9. Arterial wall properties in the basilar artery branch during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of basilar artery branch from control (●, n=6) and UPE (○, n=6) fetuses.

Compliance and distensibility Luminal volume increased significantly in both groups during pressurisation. Consistent with lower relative increases in lumen diameter and segment length during pressurisation of basilar arteries from UPE fetuses, the relative change in luminal volume was also lower in the UPE fetuses compared with controls (Figure 3-10A). Volume compliance and distensibility both decreased significantly with increasing luminal pressures. However, there were no differences between the compliance or distensibility of the arterial segments from control and UPE fetuses (Figure 3-10B, 3-10C).

Stress-strain relationship The stress-strain relationships of the branches of basilar artery from control and UPE fetuses are shown in Appendix 3-3. The stress-strain curve for the UPE fetuses has been left-shifted, indicating higher circumferential wall stresses for a given strain compared with controls; the rate constant for the UPE curve was also significantly higher than that for the arteries from controls (Table 3-4). Despite a higher overall rate constant for the arteries from UPE fetuses, when the low and high strain ranges of the stress-strain curves were analysed separately, there were no significant differences in the rate constants for the two groups.

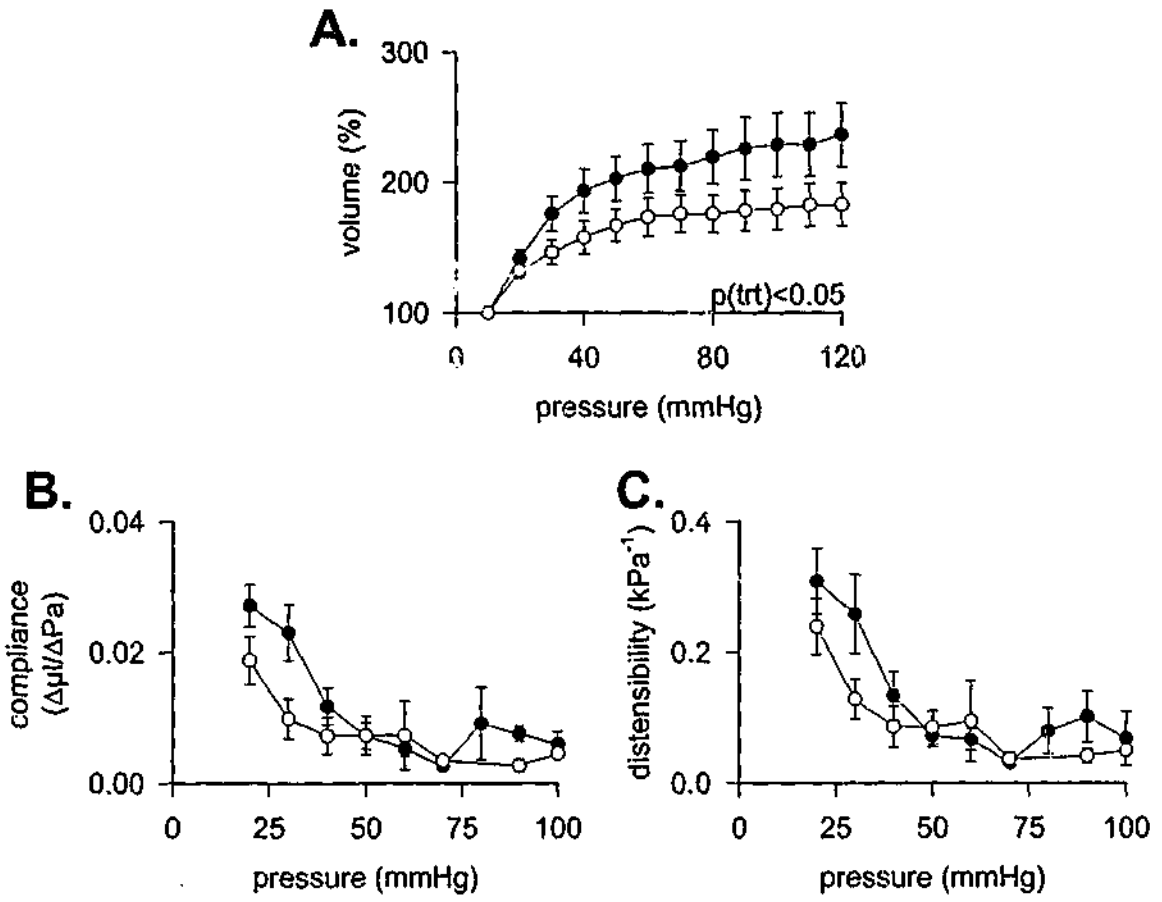


Figure 3-10. Compliance and distensibility of the basilar artery branch. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of basilar artery branch from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

Table 3-4. Incremental elastic modulus of the basilar artery branch. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. Asterisks (*) indicate a significant difference between the control and IUGR groups ($p<0.05$).

		Control (n=6)	UPE (n=6)
$y=ae^{kx}$	k	14.7±0.9	26.5±2.9*
$y=ae^{k_1x}+be^{k_2x}$	k_1	6.3±14.6	5.0±9.0
	k_2	23.9±18.6	35.9±11.9

3.4.6b Cerebral surface artery

Arterial dimensions (μm) during pressurisation There was no significant difference in the initial OD of the segments of cerebral surface artery from control (512±59μm) and UPE (576±65μm) fetuses at 10mmHg intraluminal pressure, nor was OD different between the two groups during pressurisation (Appendix 3-4). Similarly, lumen diameter was not different between the two groups during pressurisation. Arterial wall thickness decreased significantly with increasing intraluminal pressure, throughout pressurisation, the arteries from UPE fetuses had thinner arterial walls than those from controls (Appendix 3-2). Cross-sectional areas of the lumen and the arterial wall at 50mmHg were not significantly different between the control and UPE arteries (Table 3-5).

Table 3-5. Cross-sectional area (CSA) of the cerebral surface artery at 40mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 40mmHg intraluminal pressure (~fetal MAP) for the two groups of fetuses.

	Control (n=6)	**UPE (n=6)**
Lumen CSA (mm^2)	0.282±0.054	0.322±0.072
Arterial wall CSA (mm^2)	0.046±0.010	0.043±0.012

Relative changes in arterial dimensions during pressurisation Despite there being no differences in the absolute outer and lumen diameters of the cerebral surface arteries from the control and UPE fetuses, the relative changes to these diameters during pressurisation differed between the two groups; for both outer and lumen diameter, the arteries from UPE fetuses showed significantly smaller relative increases in diameter compared with controls (Figure 3-11A, 3-11B). There were no differences in the relative decrease in WT (Figure 3-11C), or the relative increase in segment lengthening (Figure 3-11D) between the two groups.

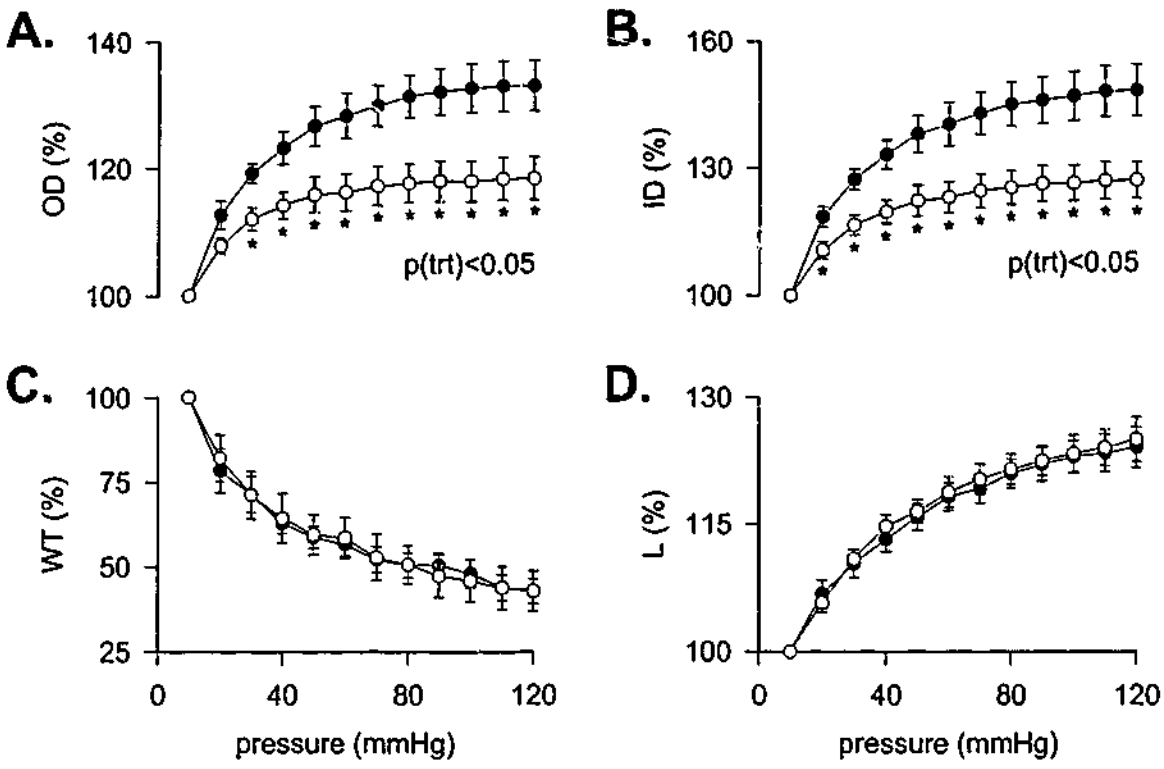


Figure 3-11. Relative dimension changes in the cerebral surface artery during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of cerebral surface artery from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Arterial wall properties during pressurisation The ID/WT ratio was significantly higher in the cerebral surface arteries from UPE fetuses throughout pressurisation (Figure 3-12A); this was consistent with a lower WT (and no difference in ID) in UPE fetuses compared with controls (Appendix 3-4). Circumferential wall stress was also significantly higher in the arterial segments from UPE fetuses compared with controls (Figure 3-12B). Both the ID/WT ratio and circumferential wall stress increased significantly with increasing intraluminal pressures.

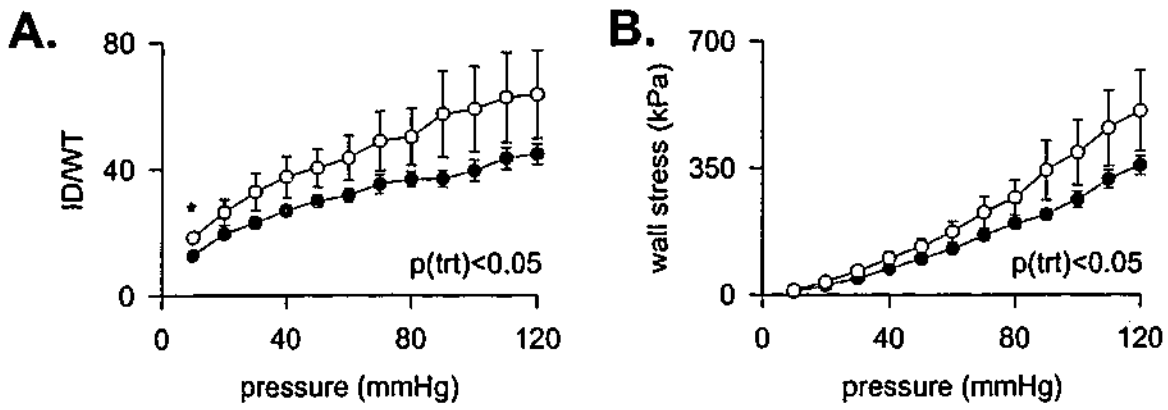


Figure 3-12. Arterial wall properties in the cerebral surface artery during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of cerebral surface artery from control (•, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Compliance and distensibility Arterial segments from UPE fetuses had smaller relative changes in luminal volume compared with those from control fetuses (Figure 3-13A), consistent with these vessels also showing lower relative changes in lumen diameter. Cross-sectional and volume compliance decreased significantly with increasing pressures; however, there were no significant differences between the two groups with respect to arterial compliance (Appendix 3-5A, Figure 3-13B). Cross-sectional distensibility was reduced in the arteries from UPE fetuses (Appendix 3-15B), and given no difference in the lengthening profiles of the two groups, volume distensibility was also lower in the UPE fetuses compared with controls (Figure 3-13C).

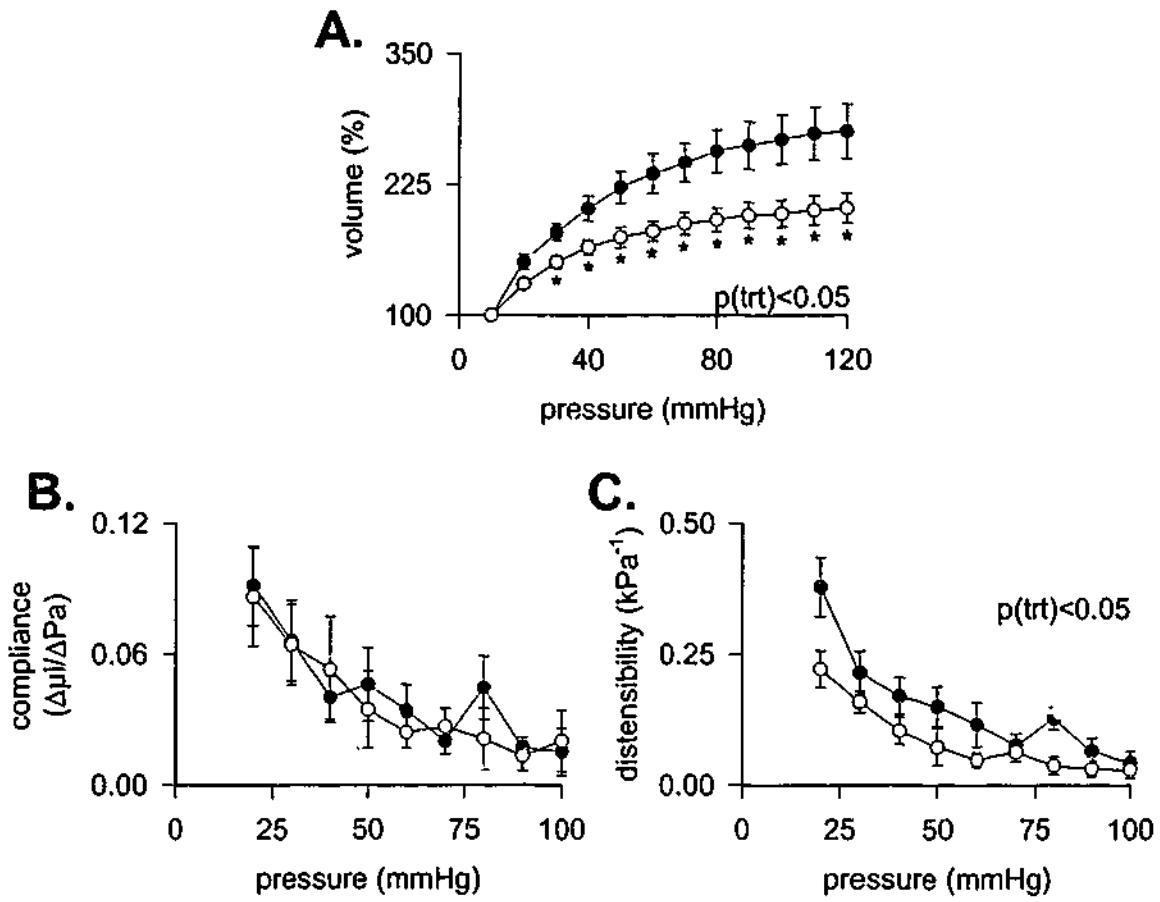


Figure 3-13. Compliance and distensibility of the cerebral surface artery. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of cerebral surface artery from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Stress-strain relationship In addition to a leftward shift of the stress-strain curve, the overall slope of the stress-strain curve of the cerebral surface artery from UPE fetuses was significantly greater than that of controls (Appendix 3-6, Table 3-6); this was consistent with increased stiffness of arteries from UPE fetuses, decreased relative changes in OD, ID, luminal volume, and decreased distensibility of these arteries. Although not significantly different, the rate constant for the low strain range of the stress-strain curve (at which mechanics are predominantly due to elastin) tended (p=0.09) to be greater in the UPE fetuses than controls, suggesting a lower elastin content in these arteries, making them stiffer in this low strain range (Table 3-6).

Table 3-6. Incremental elastic modulus of the cerebral surface artery. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. Asterisks (*) indicate a significant difference between the control and IUGR groups ($p<0.05$).

		Control (n=6)	UPE (n=6)
$y=ae^{kx}$	k	12.0±1.0	27.4±2.6*
$y=ae^{k_1x}+be^{k_2x}$	k_1	7.8±1.6	12.6±2.0
	k_2	56.3±15.3	81.7±14.8

3.4.6c Branch of the femoral artery

Arterial dimensions (μm) during pressurisation Initial OD (10mmHg intraluminal pressure) was not significantly different between controls (415±45μm) and UPE (453±59μm) fetuses; however, throughout pressurisation there was a significant effect of treatment on OD and ID (Appendix 3-7) with larger diameters in arteries from UPE fetuses. Arterial wall thickness decreased in both groups during pressurisation, but there was no difference between the groups (Appendix 3-7C). There were also no differences in the lumen and arterial wall cross-sectional areas between the two groups (Table 3-7).

Table 3-7. Cross-sectional area (CSA) of the femoral artery branch at 40mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 40mmHg intraluminal pressure (~fetal MAP) for the two groups of fetuses.

	Control (n=6)	UPE (n=6)
Lumen CSA (mm^2)	0.103±0.017	0.141±0.033
Arterial wall CSA (mm^2)	0.061±0.013	0.062±0.016

Relative changes in arterial dimensions during pressurisation The relative increase in OD in arteries from UPE fetuses was not significantly different to the relative increase in OD from control fetuses (Figure 3-14A), nor were the relative decreases in WT during pressurisation different between the two groups of fetuses (Figure 3-14C). The segments of femoral artery branch from UPE fetuses did not show the same degree of relative lengthening as the segments of artery from control fetuses (Figure 3-14D).

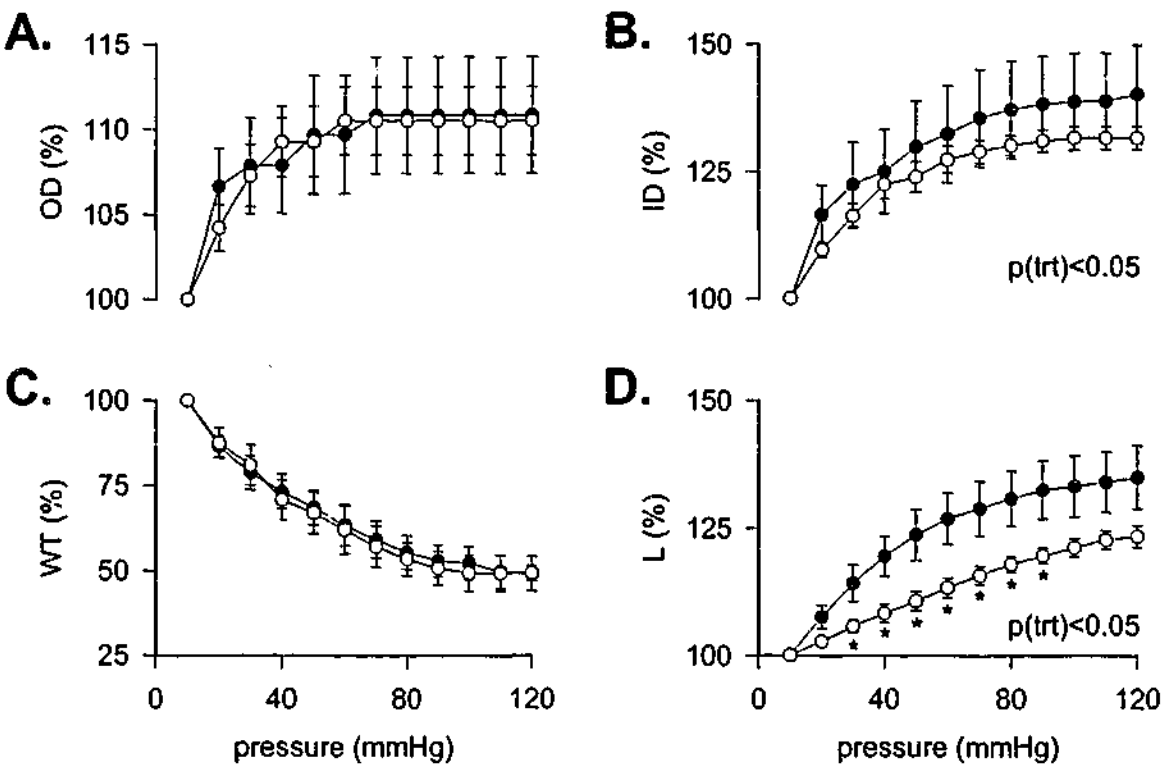


Figure 3-14. Relative dimension changes in the femoral artery branch during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of femoral artery branch from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Arterial wall properties during pressurisation The ID/WT ratio increased significantly during pressurisation (Figure 3-15A), and was higher in arteries from UPE fetuses compared with controls. This finding was consistent with greater ID in the arteries from UPE fetuses (Appendix 3-7). Similarly, the circumferential wall stress increased

significantly with increasing pressures, and was higher in the segments of femoral artery branch from UPE fetuses compared with controls (Figure 3-15B).

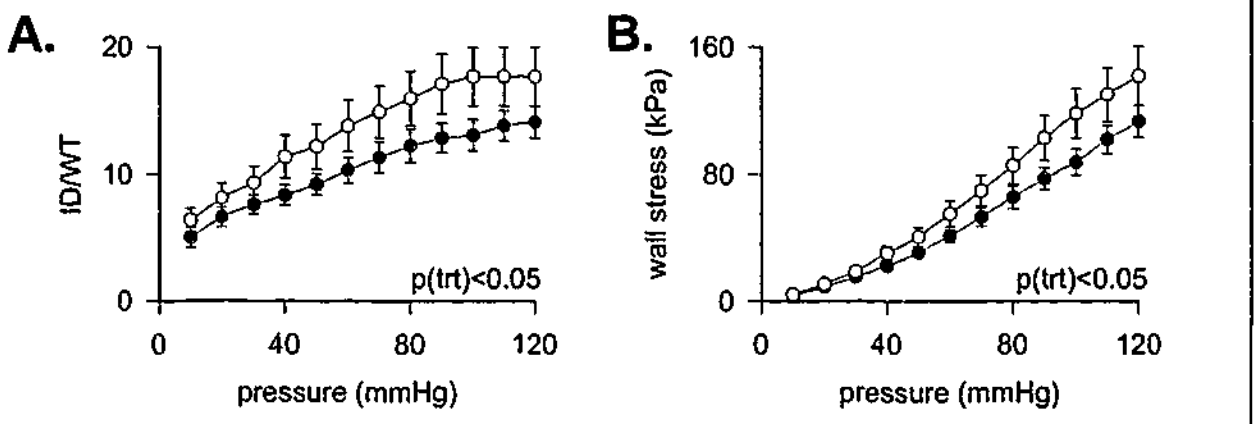


Figure 3-15. Arterial wall properties in the femoral artery branch during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of femoral artery branch from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

Compliance and distensibility The relative increase in luminal volume in segments of femoral artery branch from UPE fetuses was significantly less than that of arterial segments from controls (Figure 3-16A). Although there were no differences in the cross-sectional volume compliances of arteries from the two groups, the volume distensibility of the arterial segments from the UPE fetuses was lower than the distensibility of arteries from the controls (Figure 3-16C).

Stress-strain relationship Despite a leftward shift of the stress-strain curve for arteries from the UPE fetuses (Appendix 3-9), the overall rate constants for the stress-strain curves were not significantly different between the two groups (Table 3-8). There were also no differences in the slopes of the curves when low and high strain ranges were analysed separately.

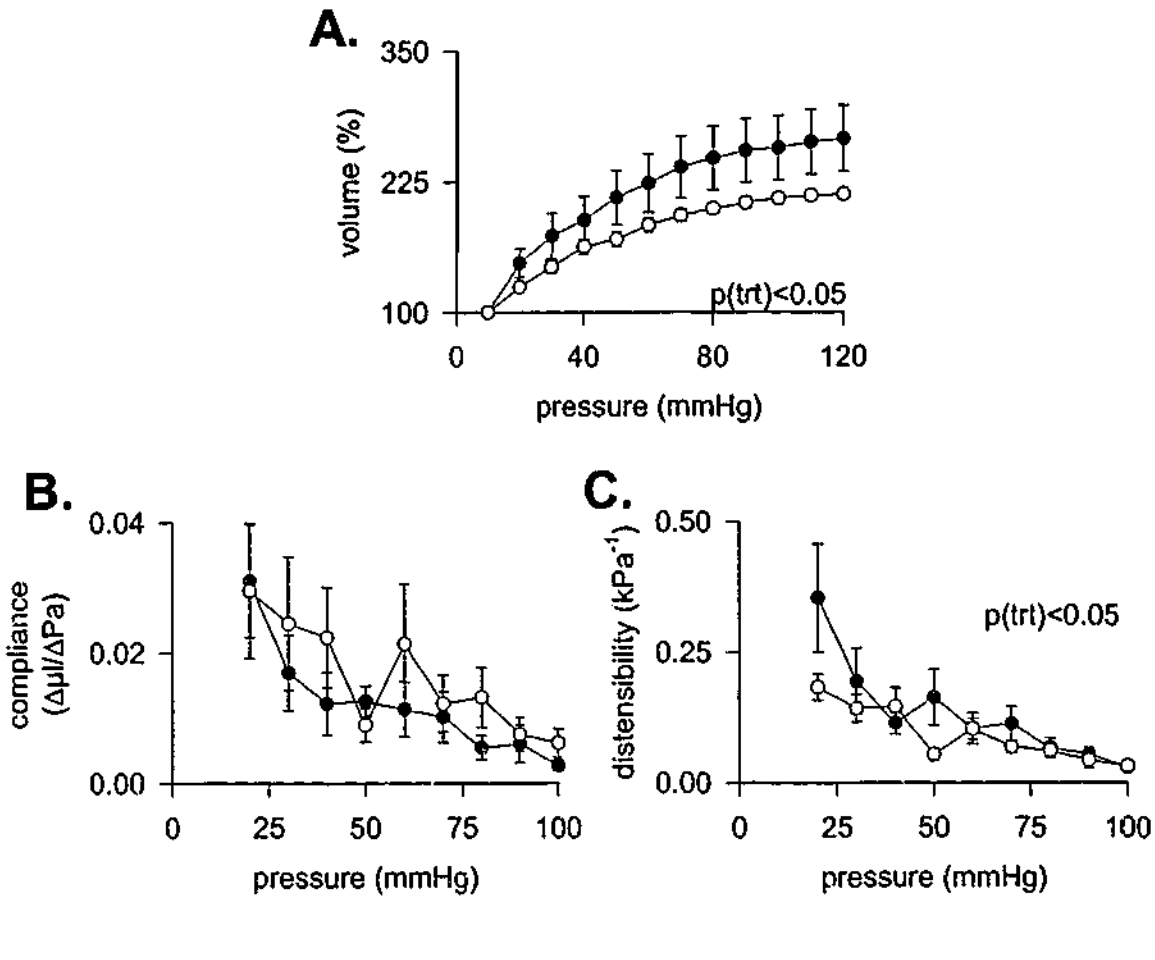


Figure 3-16. Compliance and distensibility of the femoral artery branch. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of femoral artery branch from control (•, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

Table 3-8. Incremental elastic modulus of the femoral artery branch. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries.

		Control (n=6)	UPE (n=6)
$y=ae^{kx}$	k	12.3±0.9	17.6±2.3
$y=ae^{k_1x}+be^{k_2x}$	k_1	8.9±0.8	9.6±2.3
	k_2	55.9±15.8	93.0±47.9

3.4.6d Branch of the mesenteric artery

Arterial dimensions (μm) during pressurisation There was a significant effect of treatment group on the diameters of the segments of mesenteric artery branch during pressurisation; arteries from UPE fetuses had smaller ID and OD compared with controls throughout pressurisation (Appendix 3-10) despite there being no significant difference in the initial OD (at 10mmHg intraluminal pressure, control: 367±58μm *vs* UPE: 341±19μm). Arterial wall thickness was not different between the two groups during pressurisation (Appendix 3-10C), nor were the cross-sectional areas of the lumen and arterial wall different between the control and UPE fetuses at 40mmHg (Table 3-9).

Table 3-9. Cross-sectional area (CSA) of the mesenteric artery branch at 40mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 40mmHg intraluminal pressure (~fetal MAP) for the two groups of fetuses.

	Control (n=6)	**UPE (n=6)**
Lumen CSA (mm^2)	0.114±0.038	0.086±0.014
Arterial wall CSA (mm^2)	0.0481±0.017	0.034±0.004

Relative changes in arterial dimensions during pressurisation Outer and inner diameter of the mesenteric artery branch from UPE fetuses showed smaller relative increases during pressurisation compared with the arterial segments from controls (Figure 3-17A, 3-17B). The relative decrease in wall thickness of arterial segments from the two groups were not significantly different (Figure 3-17C), nor was the relative lengthening of the arterial segments different in arteries from control and UPE fetuses (Figure 3-17D).

Arterial wall properties during pressurisation Both the ID/WT ratio and circumferential wall stress increased significantly during pressurisation; however, the ID/WT ratio and wall stress did not differ between the arteries from controls and UPE fetuses (Figure 3-18).

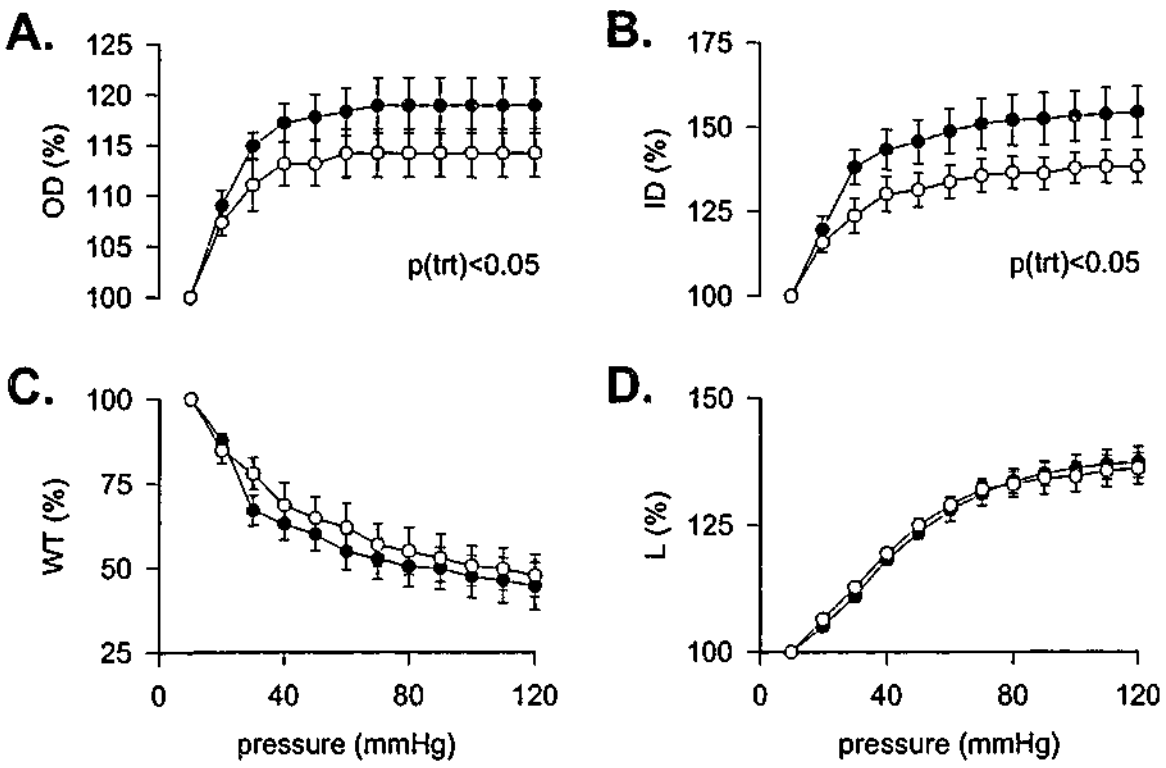


Figure 3-17. Relative dimension changes in the mesenteric artery branch during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of mesenteric artery branch from control (●, n=6) and UPE (○, n=6) fetuses. P(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

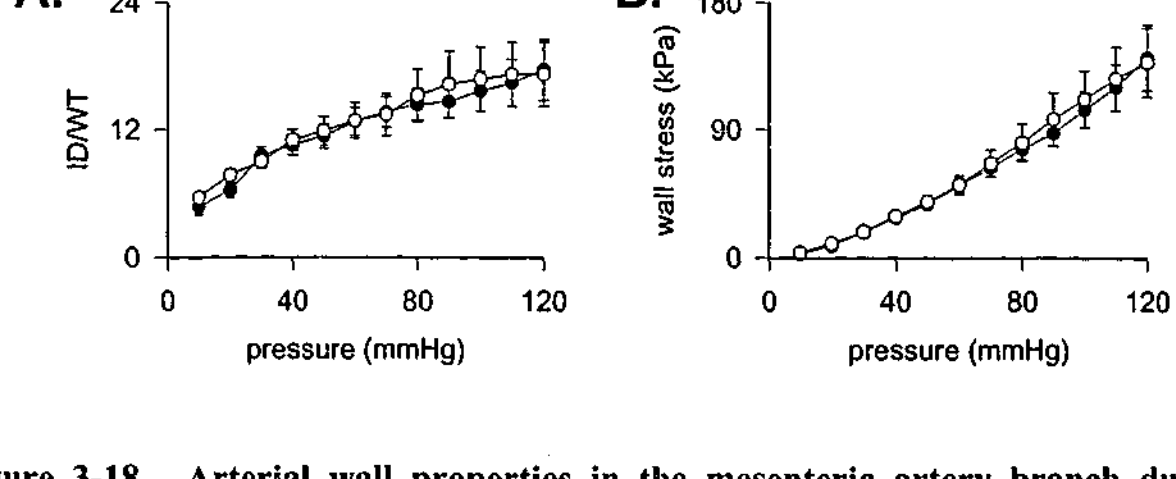


Figure 3-18. Arterial wall properties in the mesenteric artery branch during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of mesenteric artery branch from control (●, n=6) and UPE (○, n=6) fetuses.

Compliance and distensibility The segments of mesenteric artery branch from UPE fetuses had smaller relative increases in luminal volume during pressurisation compared with those from controls (Figure 3-19A). This reduced volume change is consistent with the UPE fetuses having less compliant arteries (Figure 3-19B); these arteries from UPE fetuses also had reduced volume distensibility compared with arteries from control fetuses (Figure 3-19C).

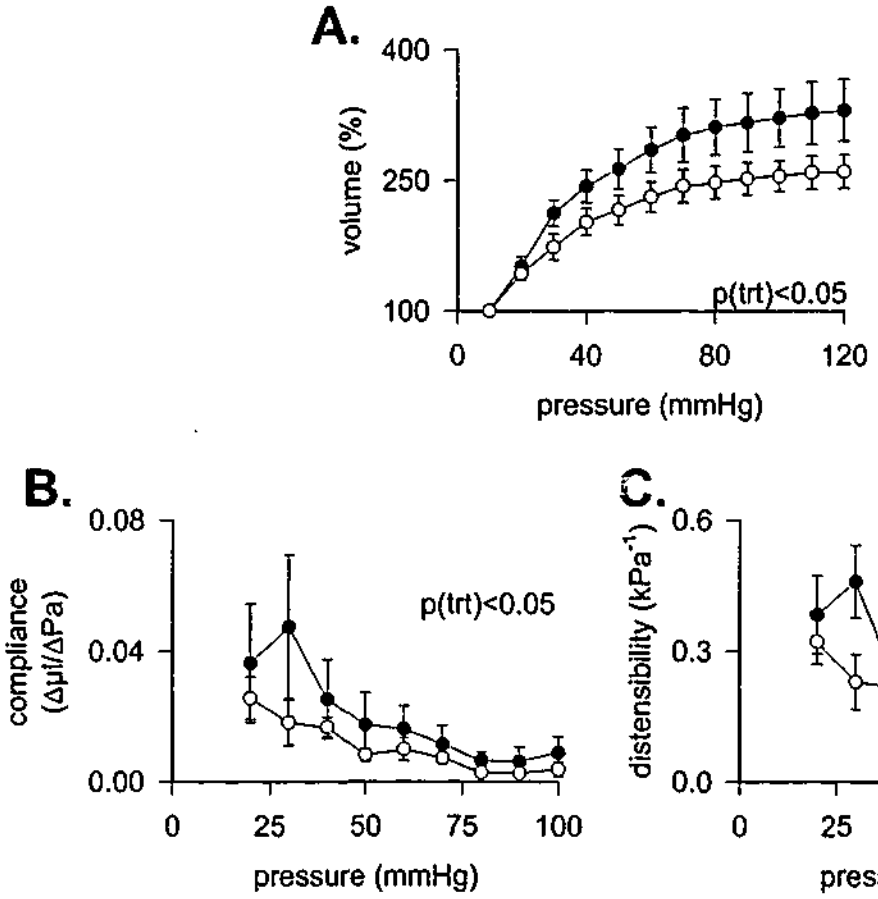


Figure 3-19. Compliance and distensibility of the mesenteric artery branch. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of mesenteric artery branch from control (•, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

Stress-strain relationship Despite a leftward shift of the stress-strain curve for mesenteric artery segments from UPE fetuses, suggesting increased arterial stiffness in these arteries (Appendix 3-12), the overall rate constants for the stress-strain curves of

the two groups were not significantly different (Table 3-10). The rate constants at the low and high strain ranges were also not different between the two groups (Table 3-10).

Table 3-10. Incremental elastic modulus of the mesenteric artery branch. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries.

		Control (n=6)	UPE (n=6)
$y=ae^{kx}$	k	17.4±1.8	20.7±2.1
$y=ae^{k_1x}+be^{k_2x}$	k_1	5.7±1.3	8.9±2.0
	k_2	41.1±5.7	61.3±14.6

3.4.6e Arcuate artery

Arterial dimensions (μm) during pressurisation The initial OD (pressurised at 10mmHg) of the segments of arcuate artery were not significantly different between the two groups (control: 394±32μm *vs* UPE: 337±37μm). However, there was a significant effect of treatment on both the OD and ID during pressurisation (Appendix 3-13); arcuate arteries from UPE fetuses had smaller diameters during pressurisation compared with controls. Arterial wall thickness was not different between the two groups of fetuses (Appendix 3-13). At 40mmHg, there was no significant difference between the two groups in the cross-sectional area of the lumen, nor was the cross-sectional area of the arterial wall different between the two groups (Table 3-11).

Relative changes in arterial dimensions during pressurisation Although the outer and lumen diameters of the arcuate arteries from UPE fetuses were smaller than those from controls during pressurisation (Appendix 3-13), the relative changes in OD and ID were significantly greater in arteries from UPE fetuses compared with controls (Figure 3-20A, 3-20B). The relative wall thickness was also greater in arteries from UPE fetuses (Figure 3-20C). The degree of vessel lengthening also differed between the two

groups; the segments of arcuate arteries from UPE fetuses lengthened less than those from control fetuses (Figure 3-20D).

Table 3-11. Cross-sectional area (CSA) of the arcuate artery at 40mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 40mmHg intraluminal pressure (~fetal MAP) for the two groups of fetuses.

	Control (n=6)	UPE (n=6)
Lumen CSA (mm^2)	0.109±0.020	0.082±0.009
Arterial wall CSA (mm^2)	0.042±0.007	0.034±0.005

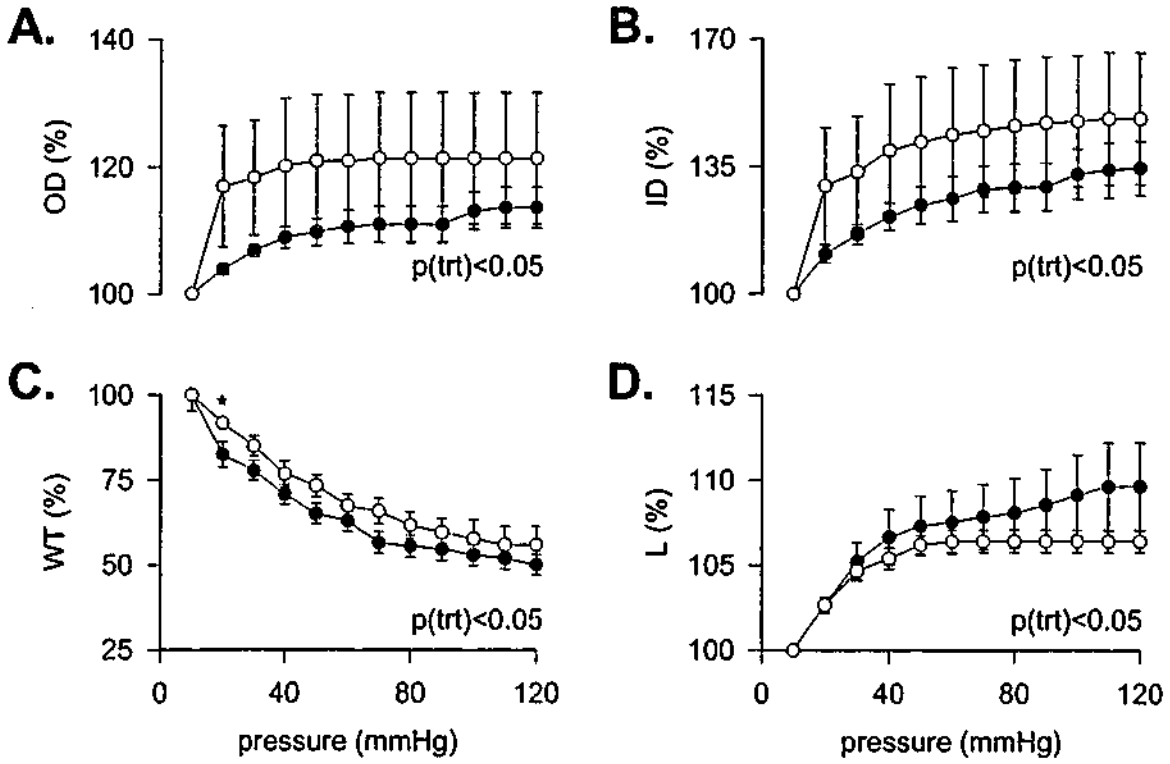


Figure 3-20. Relative dimension changes in the arcuate artery during pressurisation. The relative change (% of initial value at 10mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of arcuate artery from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Arterial wall properties during pressurisation ID/WT ratio was not different in the arcuate arteries of control and UPE fetuses (Figure 3-21A), nor did circumferential wall stress differ between the two groups during pressurisation (Figure 3-21B). Both ID/WT ratio and circumferential wall stress increased with increasing pressures in arteries from both groups of fetuses.

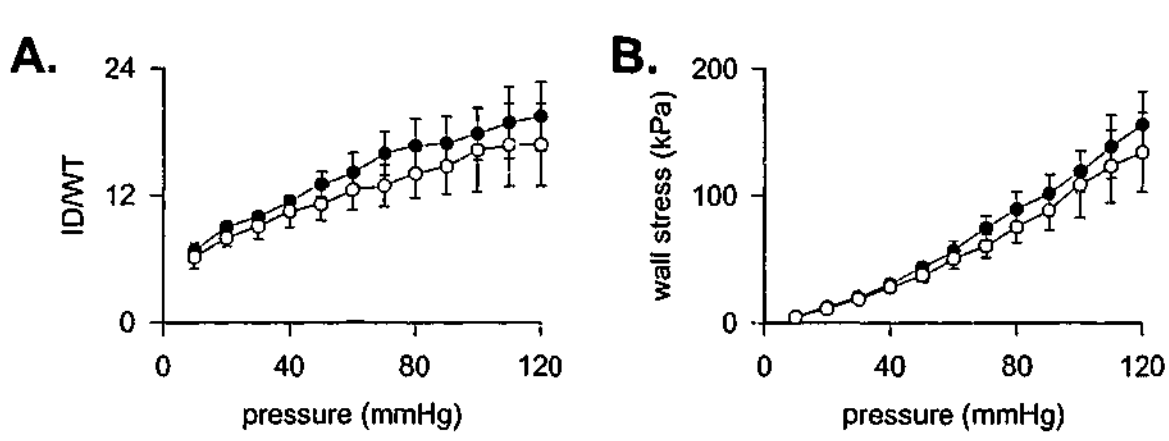


Figure 3-21. Arterial wall properties in the arcuate artery during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of arcuate artery from control (●, n=6) and UPE (○, n=6) fetuses.

Compliance and distensibility Although the relative change in luminal volume during pressurisation appeared to be greater in arcuate arteries from UPE fetuses than in those from controls, there was no significant difference in the relative volume change between the two groups (Figure 3-22A). There was also no significant difference in the volume compliance, or volume distensibility of the arcuate arteries from the two groups of fetuses (Figure 3-22B, 3-22C).

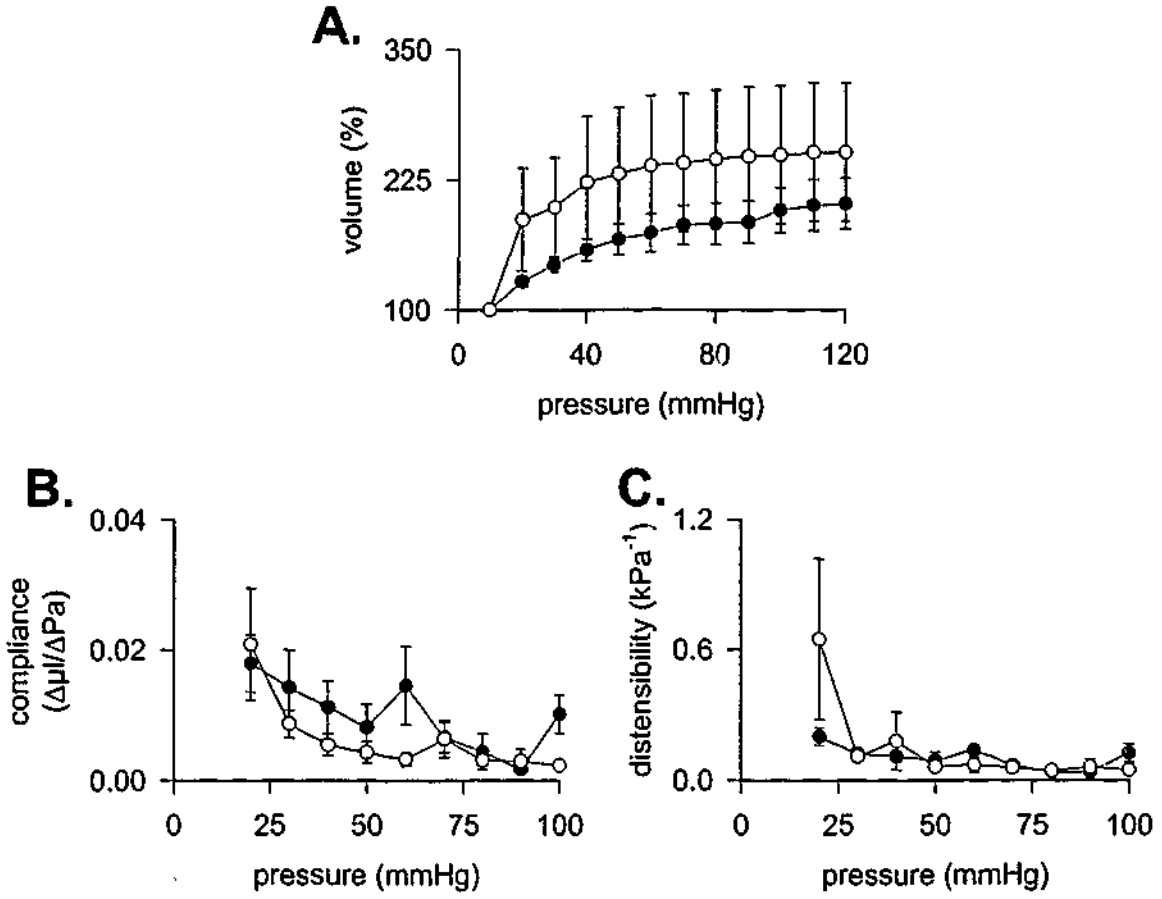


Figure 3-22. Compliance and distensibility of the arcuate artery. (A) Lumen volume (% initial volume at 10mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of arcuate artery from control (●, n=6) and UPE (○, n=6) fetuses.

Stress-strain relationship The stress-strain relationship for the arcuate arteries from control and UPE fetuses is shown in Appendix 3-22. The overall slope of the stress-strain curve was significantly greater for the arcuate arteries from UPE fetuses than those from controls (Table 3-12). In the low strain range, the rate constant for the arteries from UPE fetuses was significantly less than that of controls; this suggests that these arteries had greater elasticity in this range. Although not significantly different, the rate constant at the high strain range appeared to be higher for the arteries from UPE fetuses (57.0±17.6) than for controls (13.9±33.0) and appeared to have led to an increased overall rate constant for the stress-strain curve of arteries from UPE fetuses.

Table 3-12. Incremental elastic modulus of the arcuate artery. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 10mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. Asterisks (*) indicate a significant difference between the control and IUGR groups ($p<0.05$).

		Control (n=6)	UPE (n=6)
$y=ae^{kx}$	k	11.0±0.7	19.2±3.0*
$y=ae^{k_1x}+be^{k_2x}$	k_1	13.9±1.5	7.9±0.9*
	k_2	13.9±33.0	57.0±17.6

3.4.6f Summary

Relative changes in arterial segment dimensions between vascular beds For all arteries studied, OD, ID and volume, and L all significantly increased during pressurisation relative to initial dimensions at 10mmHg intraluminal pressure. The relative change in OD was similar in all four vascular beds, with OD increasing by 15–30% from the initial OD for the five arteries studied. Similarly, ID increased by 15–30% from the initial ID. However, this increase was greater for the cerebral surface arteries from control fetuses which was increased by 40% from initial ID during pressurisation. Arterial lengthening was similar in the segments of the basilar artery branch and arcuate artery, with segment length increasing by 5–10% of initial lengths; the cerebral surface, femoral and mesenteric segments showed greater lengthening, increasing from initial lengths at 10mmHg by 20–30%. Wall thickness decreased significantly relative to initial thickness during pressurisation in all arteries. The segments of basilar artery branch showed the greatest change in WT, decreasing to 30% of initial WT at 10mmHg; for the other arteries studied, the relative decrease in WT during pressurisation was 50%.

Effects of birth weight group on passive arterial wall mechanical properties Table 3-13 summarises the effects of birth weight group on arterial dimensions and wall mechanical properties. The segments of basilar artery from UPE fetuses had smaller relative increases in OD, ID and length, and relative WT was thicker than the relative

changes in basilar arteries from control fetuses. These changes are consistent with smaller changes in lumen volume in segments from UPE fetuses. However, there were no differences between the two groups in terms of volume compliance and distensibility. The slope of the stress-strain curve for the basilar arteries from UPE fetuses was greater than the control stress-strain curve suggesting increased arterial stiffness; the difference in the overall stress-strain relationship was most likely due to differences at the high strain range.

Like the basilar artery, the segments of cerebral surface artery from UPE fetuses had smaller relative increases in OD, ID and lumen volume compared with controls. The relative changes in WT and segment length were not different between groups. Arteries from UPE fetuses were less distensible than those from controls, and the overall slope of the stress-strain curve was increased in the arteries from UPE fetuses, suggesting increased arterial stiffness.

The relative increase in lumen volume was lower in the segments of femoral artery from UPE fetuses; this was the result of smaller relative increases in ID and segment length in these arteries compared with arteries from controls. There were no differences in compliance, distensibility or the stress-strain relationship between the arteries from the two groups of fetuses.

The segments of mesenteric artery from UPE fetuses showed lower relative increases in OD and ID during pressurisation; this led to a lower luminal volume change compared with controls. Volume compliance was also reduced in the arterial segments from UPE fetuses but distensibility and the stress-strain relationships did not differ between the two groups.

Relati··e increases in OD and ID were greater in the arcuate arteries from UPE fetuses; the arterial wall remained relatively thicker and segment lengthening was lower than in controls. This greater relative increase in lumen diameter, combined with reduced relative lengths of the arterial segments, resulted in no overall difference between the two groups in terms of lumen volume during pressurisation. Differences in the slopes of the stress-strain curves suggest increased arterial stiffness of arcuate arteries from UPE fetuses.

Table 3-13. Summary of effects of umbilico-placental embolisation on arterial dimensions and passive arterial wall mechanical properties in the five selected vessels. ↑ indicates an increase in the parameter in the UPE group compared with controls, ↓ indicates a decrease in the parameter in the UPE group compared with controls, and ↔ indicates no difference between control and UPE animals.

	Basilar	Cerebral surface	Femoral	Mesenteric	Arcuate
OD (μm)	↓	↔	↑	↓	↓
ID (μm)	↓	↔	↑	↓	↓
WT (μm)	↔	↓	↔	↔	↔
OD (%)	↓	↓	↔	↓	↑
ID (%)	↓	↓	↓	↓	↑
WT (%)	↓	↔	↔	↔	↓
L (%)	↓	↔	↓	↔	↓
ID/WT	↔	↑	↑	↔	↔
Wall stress	↔	↑	↑	↔	↔
Volume (%)	↓	↓	↓	↓	↔
Vol. Compliance	↔	↔	↔	↓	↔
Vol. Distensibility	↔	↓	↔	↔	↔
Stress-strain slope	↑	↑	↔	↔	↑
Stress-strain slope$_{low}$	↔	↔	↔	↔	↓
Stress-strain slope$_{high}$	↔	↔	↔	↔	↔

3.5 Discussion

The aims of this study were to investigate the effects of 20 days of late gestational placental insufficiency on fetal arterial pressure and passive arterial wall mechanics in the near-term fetus. Although mean arterial pressure was not significantly different between the control and UPE fetuses at 140d GA, alterations to the passive arterial mechanical properties indicate that the UPE fetuses had less compliant arteries.

3.5.1 Fetal blood parameters

In humans, fetal growth restriction has been associated with fetal hypoxemia, hypercapnia, acidemia, hypoglycemia, hyperlactemia (Nicolaides *et al.*, 1989; Economides *et al.*, 1991). Late gestational UPE in the sheep leads to changes in fetal blood gas status similar to those found in growth restricted human fetuses. The UPE fetuses described in this chapter were hypoxemic, hypercapnic, acidemic and hypoglycemic. Blood lactate concentrations, however, were not different to those in control fetuses. Similar changes to blood gas status have been reported during 20–26 days of UPE in sheep beginning at the same gestational age (Cock & Harding, 1997; Louey *et al.*, 2000).

Fetal growth restriction in humans has been associated with increased fetal cortisol concentrations (Economides *et al.*, 1991), and elevated cortisol concentrations have also been reported in fetal sheep following UPE. Cock *et al.* (2001b) reported significantly higher plasma cortisol concentrations in the UPE fetuses at 140d GA compared with controls. Cortisol concentrations might be expected to be increased in response to daily reductions in Sa_{O_2} by 50%. Interestingly, there was no difference between plasma cortisol concentrations measured prior to and two hours after daily UPE in the fetuses described in this chapter. While UPE was sufficiently severe to reduce fetal body weight by 37%, it did not significantly alter plasma cortisol concentrations. At 140d GA, mean cortisol concentrations in the UPE fetuses appeared to be higher than those of controls, but this difference was not statistically significant due to a high variability in the cortisol concentrations in the UPE fetuses. It is unclear whether this apparent increase in circulating cortisol in some UPE fetuses was the result of UPE, or altered timing of the pre-partum cortisol surge. The fetuses described in this chapter, and the fetuses described by Cock *et al.* (2001b) were studied up to 140d GA (~7 days before

term), at which time they were killed for tissue collection. The gestational age at which they would have been born is unknown. Given the absence of a difference between the daily pre- and post-UPE cortisol concentrations reported in this chapter, it is highly likely that the tendency for the UPE fetuses to have elevated cortisol at 140d GA was associated with an earlier pre-partum increase in cortisol levels. Plasma cortisol concentrations do not differ between term-born UPE fetuses and controls (Louey *et al.*, 2000), but elevated circulating cortisol levels at 140d GA compared with those in controls are associated with birth approximately one week preterm (Cock *et al.*, 2001a). Had the fetuses described in this chapter been allowed to be born, it is possible some may have been born preterm, soon after 140d GA.

3.5.2 Fetal growth

Body weights of the UPE fetuses were 37% lower than those of controls. Body length and ponderal index were also reduced in the UPE fetuses compared with controls. While 20d of UPE led to reductions in fetal body size, the majority of organ weights when adjusted for body weight were not different between the two groups of fetuses. The UPE fetuses showed evidence of asymmetrical growth restriction, having increased brain:liver weight ratio (i.e. greater growth of the brain, relative to "less vital" abdominal organs) and greater brain weights (g/kg body weight) compared with controls. The degree of growth restriction of UPE fetuses in this chapter was greater than previously reported following similar periods of UPE. Cock and Harding (1997) reported a 20% reduction in fetal body weight following 20 days of UPE and Murotsuki *et al.* (1997) reported a 28% reduction in body weight following 21 days of UPE. However, the degree of growth restriction was similar to that reported by Louey *et al.* (2000); in that study, 26 days of UPE led to a 33% reduction in birth weight.

3.5.3 Arterial pressure

During the period of placental embolisation, mean, systolic and diastolic pressures of UPE fetuses did not differ from those of controls. An increase in fetal arterial pressure might be expected to result from increased placental resistance associated with UPE and a possible increase in total peripheral vascular resistance (Trudinger *et al.*, 1987; Gagnon *et al.*, 1996). A significant increase in arterial pressure was not measured in the UPE fetuses described in this chapter; it is unclear why arterial pressure was not

elevated during this study when previous studies have reported either a transient, or persistent elevation in arterial pressure in response to UPE (Cock & Harding, 1997; Murotsuki *et al.*, 1997; Gagnon *et al.*, 2002). It is also not clear, given decreased arterial compliance in the fetuses described in this chapter, why UPE was not associated with fetal hypertension.

While MAP at 140d GA was not significantly different between UPE and control fetuses, these findings presented in this chapter are consistent with my previous study (Louey *et al.*, 2000) that reported MAP was not statistically different near term but tended to be lower in the UPE fetuses compared with controls; in this study, fetuses were allowed to be born and the tendency for relative hypotension persisted into the early postnatal period. Another study reported 20 days of UPE led to significantly lower MAP at 140d GA compared with controls (Cock & Harding, 1997). It is unclear why the MAP near term differed between these studies, given similar experimental protocols, the trends for lower arterial pressure in the UPE fetuses are present in all 3 studies.

In contrast to these studies, one study (Murotsuki *et al.*, 1997) found that 21d of UPE led to fetal hypertension within two days of the commencement of UPE. However, in that study, UPE was performed earlier in gestation, commencing at ~110d GA rather than 120d GA. In addition to fetal hypertension, the UPE fetuses showed evidence of left ventricular hypertrophy (Murotsuki *et al.*, 1997). The fetuses described in this chapter showed no evidence of left ventricular hypertrophy and left ventricular weight (control: 3.4±0.2g/kg body weight *vs* UPE: 3.4±0.12g/kg body weight) and left ventricular wall thickness (control: 7.8±0.5mm *vs* UPE: 7.3±0.3mm) were similar between control and UPE fetuses. This lack of left ventricular hypertrophy is consistent with the absence of a significant difference in the arterial pressure of control and UPE fetuses at 140d GA.

3.5.4 Passive arterial wall mechanics

Although mean arterial pressure was not significantly different between UPE and control fetuses at 140d GA, the postnatal arterial pressure of lambs following UPE had previously been reported to be lower than that of controls (Louey *et al.*, 2000).

Therefore, it might be expected that the arterial compliance in the near-term UPE fetuses is increased, leading to lower arterial pressures soon after birth. Conversely, vascular development, in particular the synthesis of elastin in the arterial wall, may be impaired by a sub-optimal intrauterine environment (Martyn & Greenwald, 1997), leading to decreased arterial compliance and potentially predisposing the fetus for later hypertension. In UPE fetuses, four of the five arteries studied had reduced relative luminal volume changes during pressurisation and three of the five arteries had higher rate constants for the stress-strain curves compared with controls. These differences suggest that the arteries from UPE fetuses had increased, rather than decreased, arterial stiffness. The mechanical properties (in particular the relative increases in OD, ID and lumen volume during pressurisation) of the arcuate artery from UPE fetuses were more variable than in control fetuses. It is possible that UPE had variable effects on vascular development in different fetuses with some fetuses more greatly affected by UPE than others; this variability may also partly explain different findings with respect to fetal arterial pressure between studies despite the use of the same experimental procedures (this chapter; Cock & Harding, 1997; Louey *et al.*, 2000).

Despite the absence of a difference in MAP pressure at 140d GA, and given increased arterial wall stiffness, it is possible the changes to vascular structure and mechanics may precede changes in arterial pressure. Van Gorp *et al.* (2000) found that alterations to aortic structure, compliance and distensibility precede the development of hypertension in spontaneously hypertensive rats (SHR). Inconsistencies between the arterial pressure and arterial mechanical properties investigated in the studies described in this chapter may be due to differences between the *in vitro* conditions in which the vascular mechanics were determined and the *in vivo* conditions in which arterial pressure was measured. Passive mechanical properties were determined in the arteries from UPE and control fetuses (i.e. in the absence of smooth muscle activity) whereas *in vivo*, vascular smooth muscle activity, may partially compensate for the observed alterations in arterial mechanical properties.

The fetal hypoxemia and hypoglycemia associated with UPE may have altered the synthesis and deposition of the structural proteins such as elastin and collagen, which are essential for the determination of vascular wall mechanical properties. Hypoxia has been shown to decrease both tropoelastin mRNA expression and protein synthesis

(Durmowicz *et al.*, 1991) and undernutrition has been shown to decrease collagen synthesis (Spanheimer *et al.*, 1991). It is not known how UPE affects the synthesis and deposition of elastin and collagen in blood vessels. However, differences in the mechanical properties of arteries from UPE fetuses compared with those from controls, strongly suggest UPE can affect collagen and elastin in the arterial wall. It is also not known whether elastin, collagen, or both are affected by UPE; even if only one of these proteins is altered by UPE, the collagen:elastin ratio could be altered, and this would be reflected in the altered arterial mechanics given that the mechanical properties are dependent on the combined properties of collagen and elastin in the arterial wall (Roach & Burton, 1957; Wolinsky & Glagov, 1963). Both the relative proportions and the arrangement of elastin and collagen in the vascular wall are important to arterial mechanical properties (Cox, 1978). In the absence of alterations to the amount of elastin and collagen synthesised *in utero*, it may be possible that UPE alters the cross-linkage of elastin and collagen which are also important for arterial wall mechanics (Wells *et al.*, 1999). Biochemical analyses will be required to determine if quantities of elastin and collagen (both absolute and relative proportions of each) differ in these resistance arteries to more fully understand the effect of late gestation placental insufficiency on arterial structure and mechanical properties. Histological studies are also required to determine if the arrangement of collagen and elastin fibres differ between control and UPE fetuses, even in the absence of differences in the relative amounts of elastin and collagen.

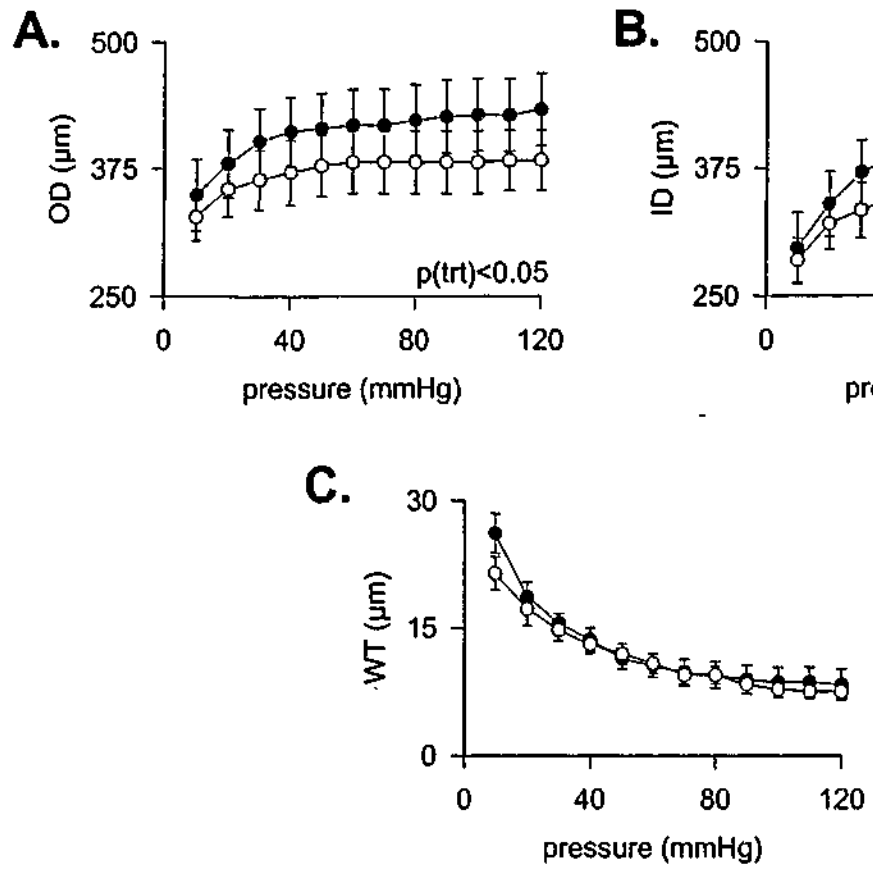
Increased stiffness of the arteries from UPE fetuses is not consistent with the lower postnatal arterial pressure previously reported in lambs born after 26d of UPE (Louey *et al.*, 2000). In sheep, it has been shown that the amount of elastin and collagen in arterial walls rapidly increases in the perinatal period (Bendeck & Langille, 1991) and is associated with hemodynamic changes between fetal and postnatal life (Bendeck *et al.*, 1994). Although in those studies (Bendeck & Langille, 1991; Bendeck *et al.*, 1994) there were regional differences in the timing of rapid elastin deposition, arterial elastin content was significantly greater at 21 days of postnatal age compared with 120d GA. In the abdominal aorta, the elastin deposition was greatest between 140d GA and 3 postnatal days whereas in the renal and carotid arteries, elastin deposition was greatest between 3 and 21 postnatal days (Bendeck & Langille, 1991; Bendeck *et al.*, 1994). While the hypoxemia associated with UPE may lead to decreased arterial elastin

synthesis, it is possible that this deficit may be reduced or eliminated by increased elastin deposition during the early postnatal period, after the cessation of UPE. In sheep, there is a significant increase in aortic elastin content between 119d GA and 21 postnatal days with no significant increase in aortic collagen in this same period (Wells *et al.*, 1999). If elastin synthesis was increased in the early postnatal period following UPE, arterial stiffness might be reduced, as there are no major changes in the amount of arterial collagen in this period (Wells *et al.*, 1999). Further studies investigating the amounts (and relative proportions) of arterial elastin and collagen in the late gestation fetus and early postnatal lamb following UPE would provide evidence for whether the increased arterial stiffness in UPE fetuses persists into the early postnatal period. If these changes in the mechanical properties of the resistance arteries do persist after birth, they may predispose the adult for later hypertension.

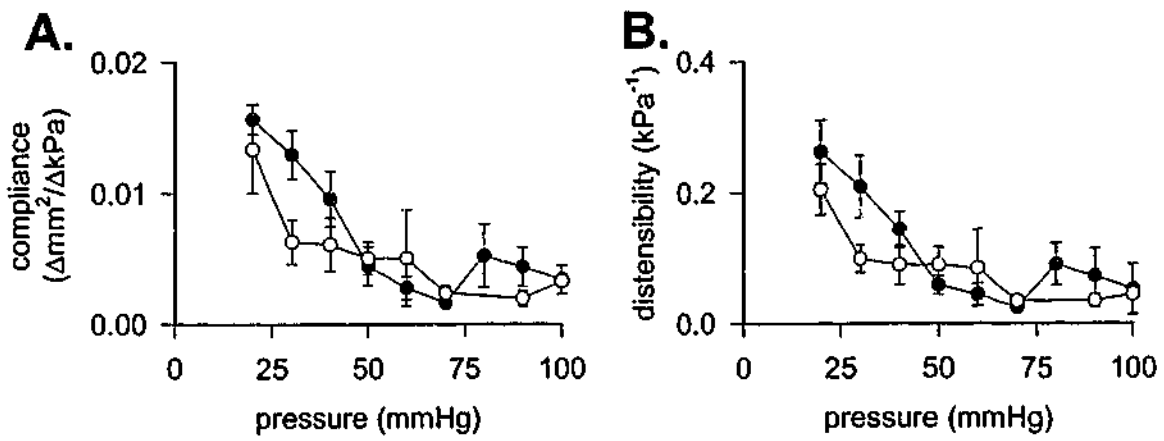
3.6 Conclusions

Late gestation UPE led to a 37% reduction in fetal birth weight. Although the growth restricted fetuses were hypoxemic, hypercapnic, acidemic and hypoglycemic, embolisation did not affect plasma cortisol concentrations. Despite there being no difference in arterial pressure (MAP, systolic and diastolic pressures) between control and growth restricted fetuses near term, the passive mechanical properties of resistance arteries suggest arteries from growth restricted fetuses are less compliant than those of controls. Although the *in vitro* arterial mechanical properties are not consistent with the measured arterial pressure, it is possible that increased arterial stiffness of resistance arteries from growth restricted fetuses may predispose these fetuses for later hypertension.

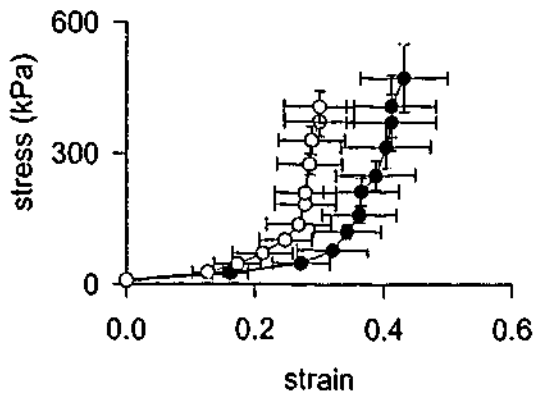
3.7 Appendices



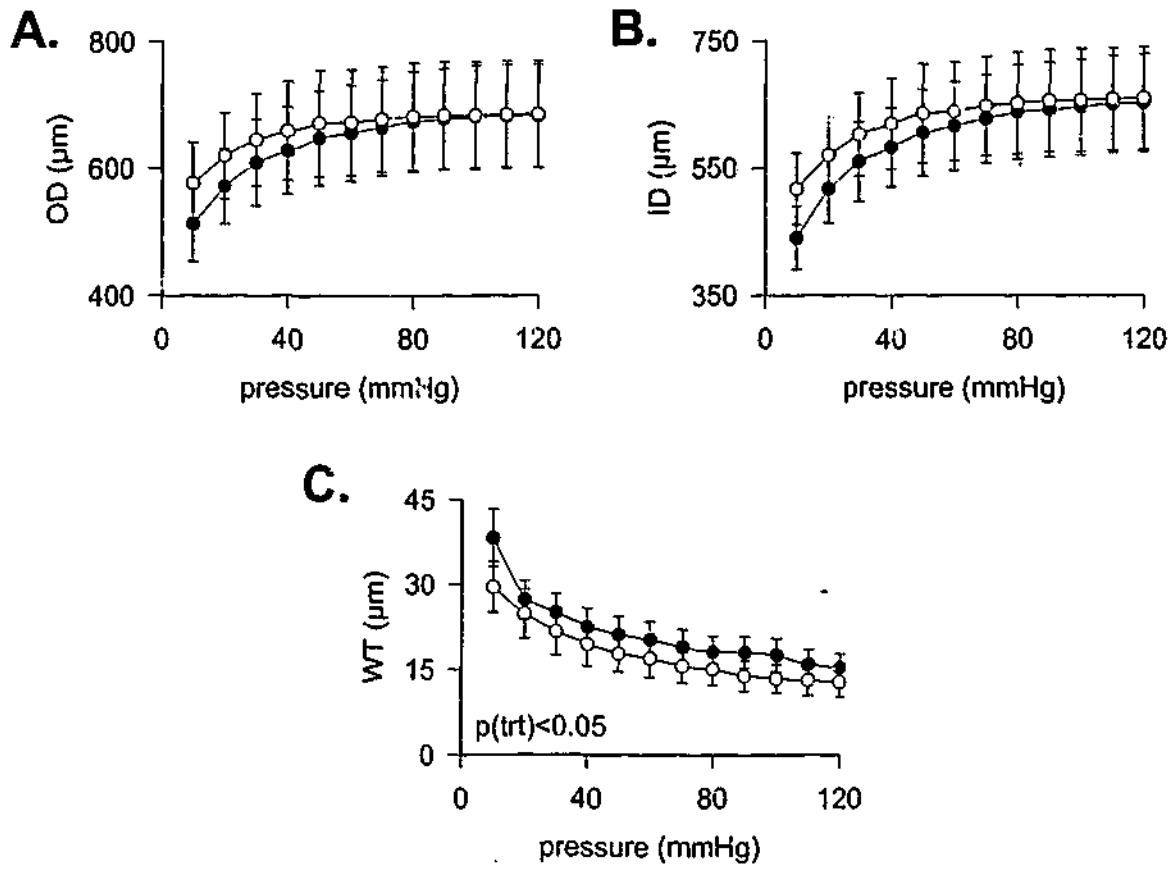
Appendix 3-1. Dimensions (µm) of the basilar artery branch during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of basilar artery branch from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.



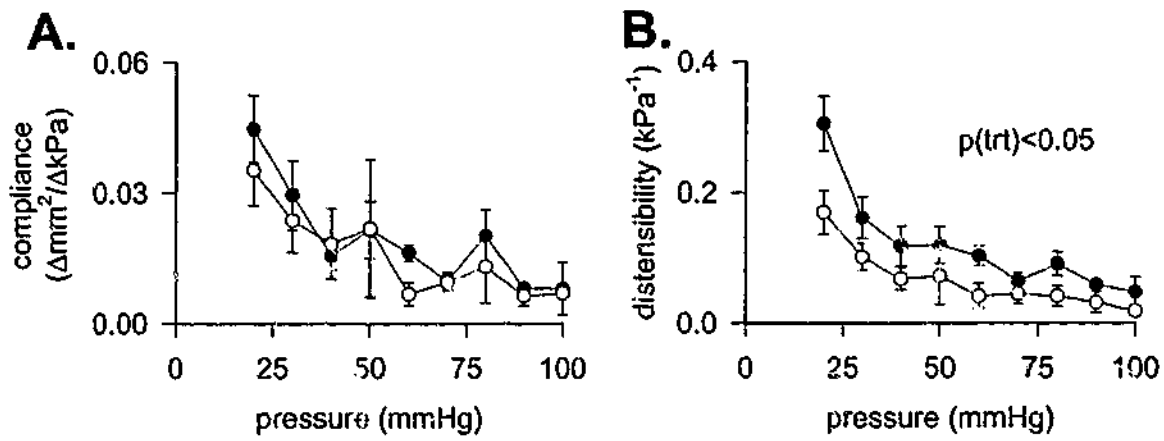
Appendix 3-2. Cross-sectional compliance and distensibility of the basilar artery branch. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of basilar artery branch from control (●, n=6) and UPE (○, n=6) fetuses.



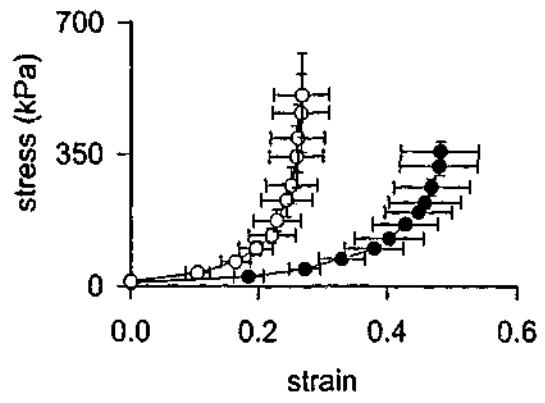
Appendix 3-3. Stress-strain relationships for the basilar artery. Stress-strain relationships for the segments of basilar artery branch from control (●, n=6) and UPE (○, n=6) fetuses.



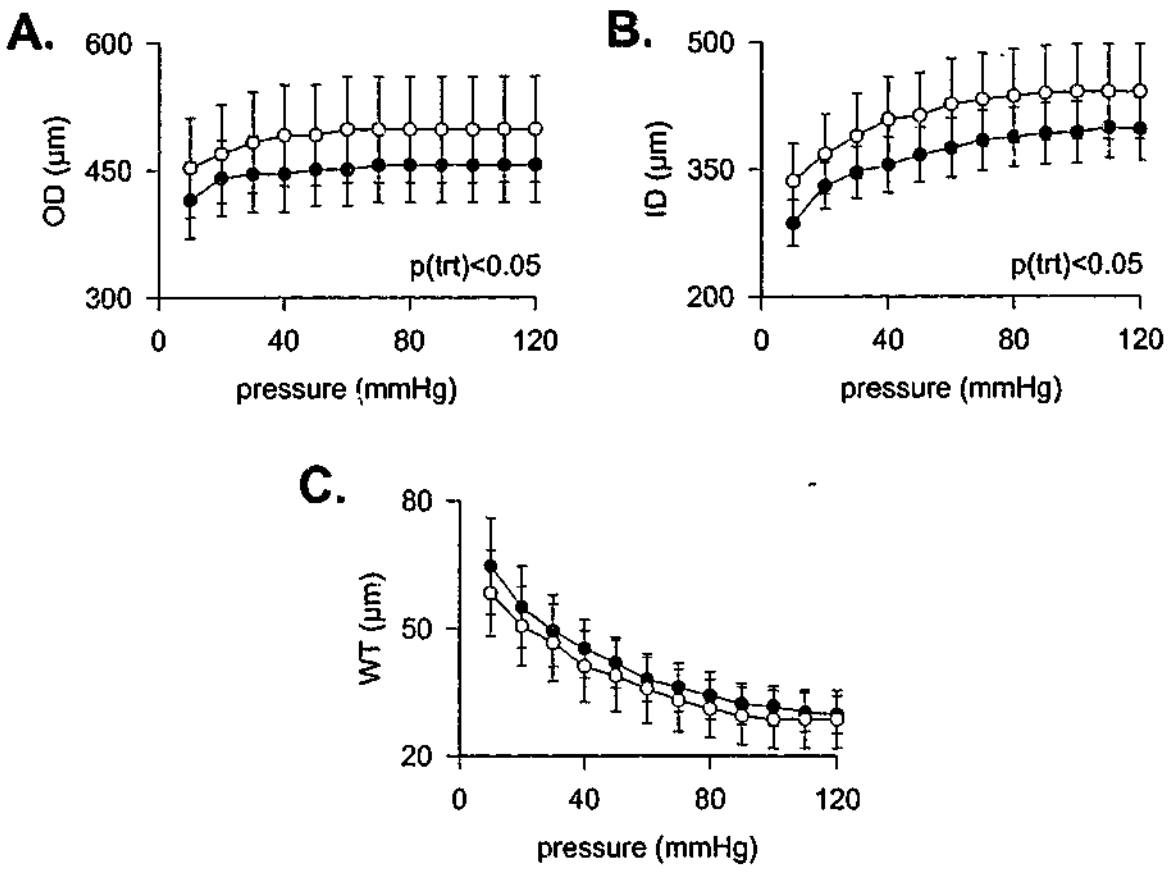
Appendix 3-4. Dimensions (µm) of the cerebral surface artery during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of cerebral surface artery from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.



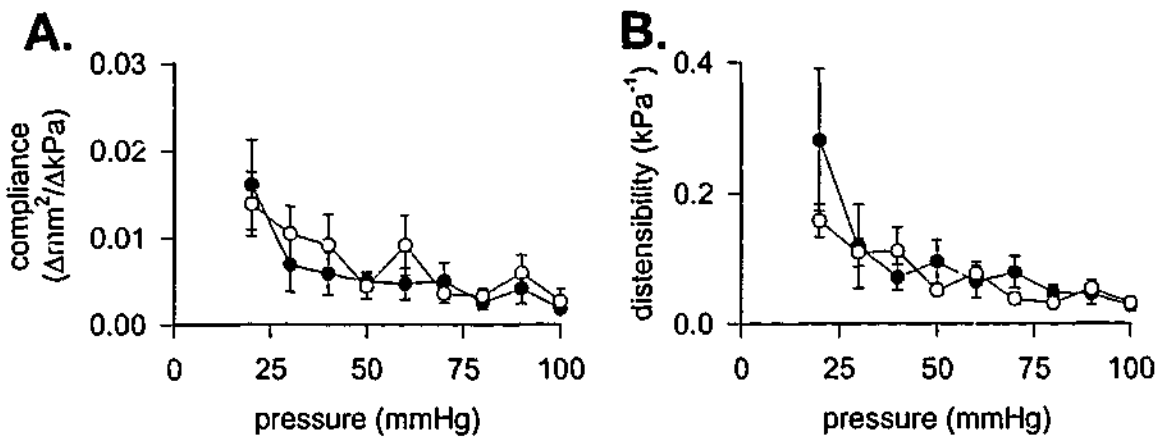
Appendix 3-5. Cross-sectional compliance and distensibility of the cerebral surface artery. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of cerebral surface artery from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.



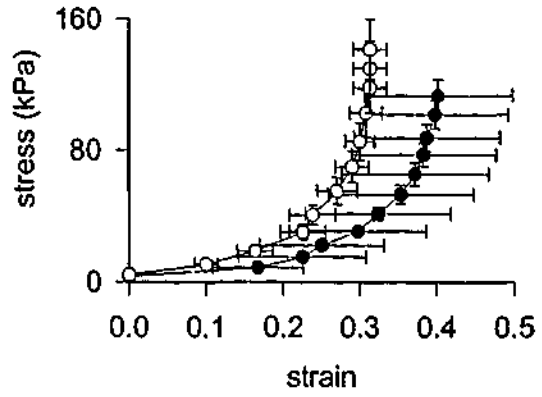
Appendix 3-6. Stress-strain relationships for the cerebral surface artery. Stress-strain relationships for the segments of cerebral surface artery from control (●, n=6) and UPE (○, n=6) fetuses.



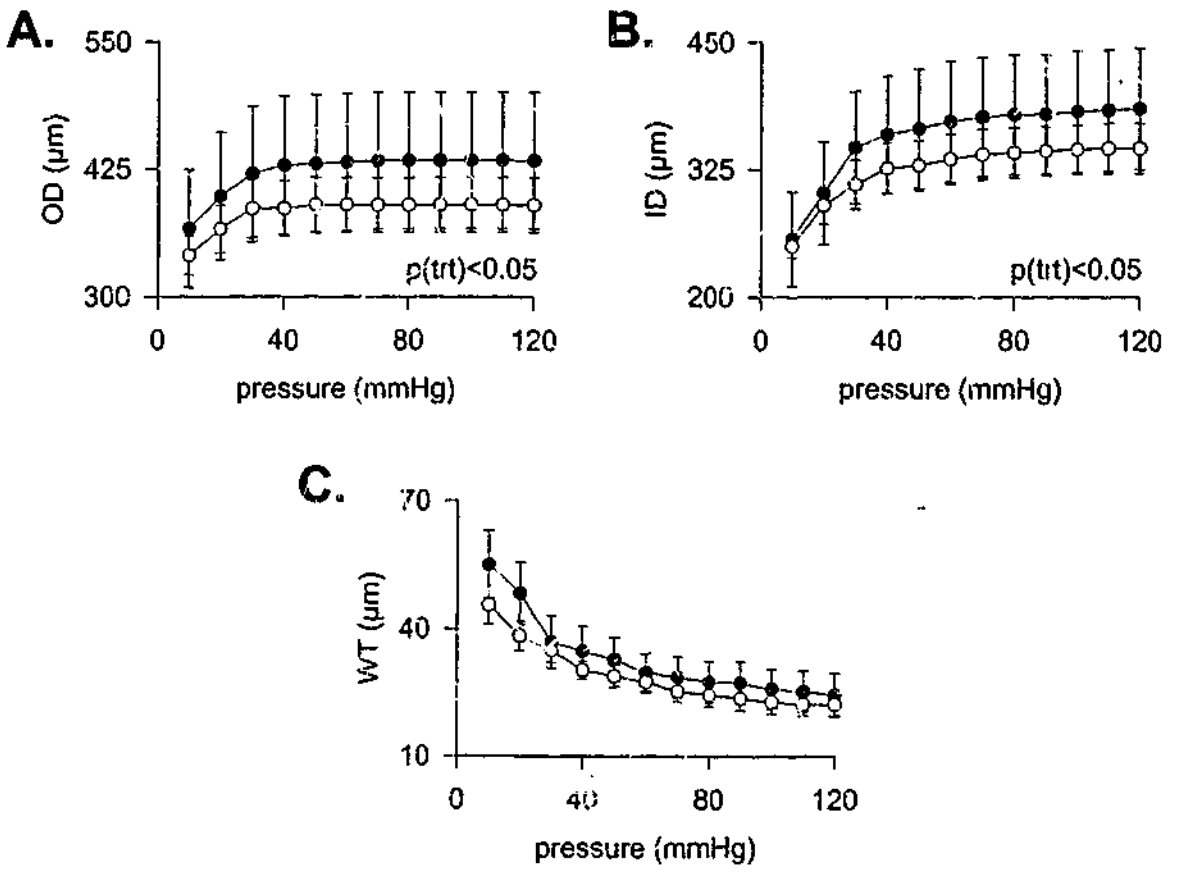
Appendix 3-7. Dimensions (µm) of the femoral artery branch during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of femoral artery branch from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.



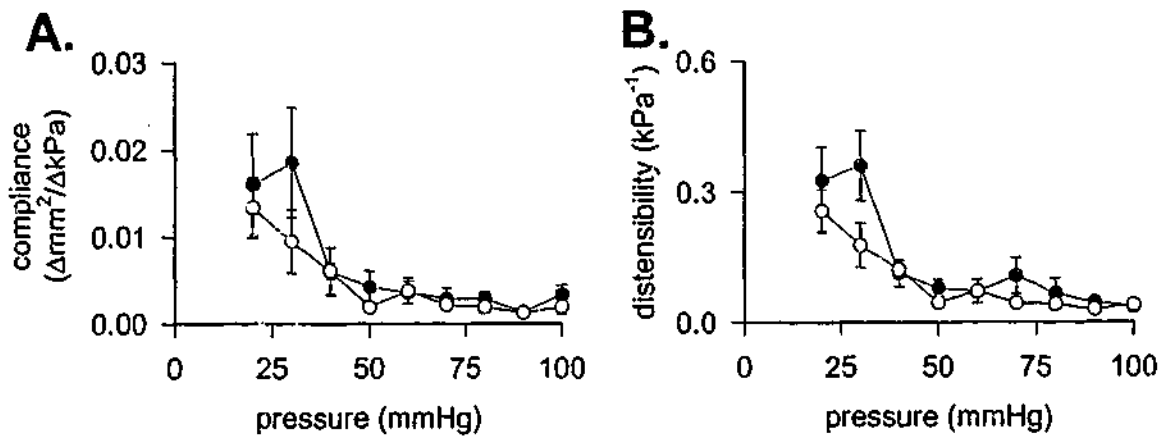
Appendix 3-8. Cross-sectional compliance and distensibility of the femoral artery branch. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of femoral artery branch from control (●, n=6) and UPE (○, n=6) fetuses.



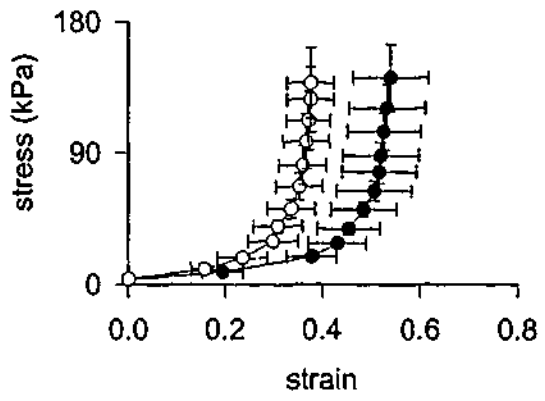
Appendix 3-9. Stress-strain relationships for the femoral artery. Stress-strain relationships for the segments of femoral artery branch from control (●, n=6) and UPE (○, n=6) fetuses.



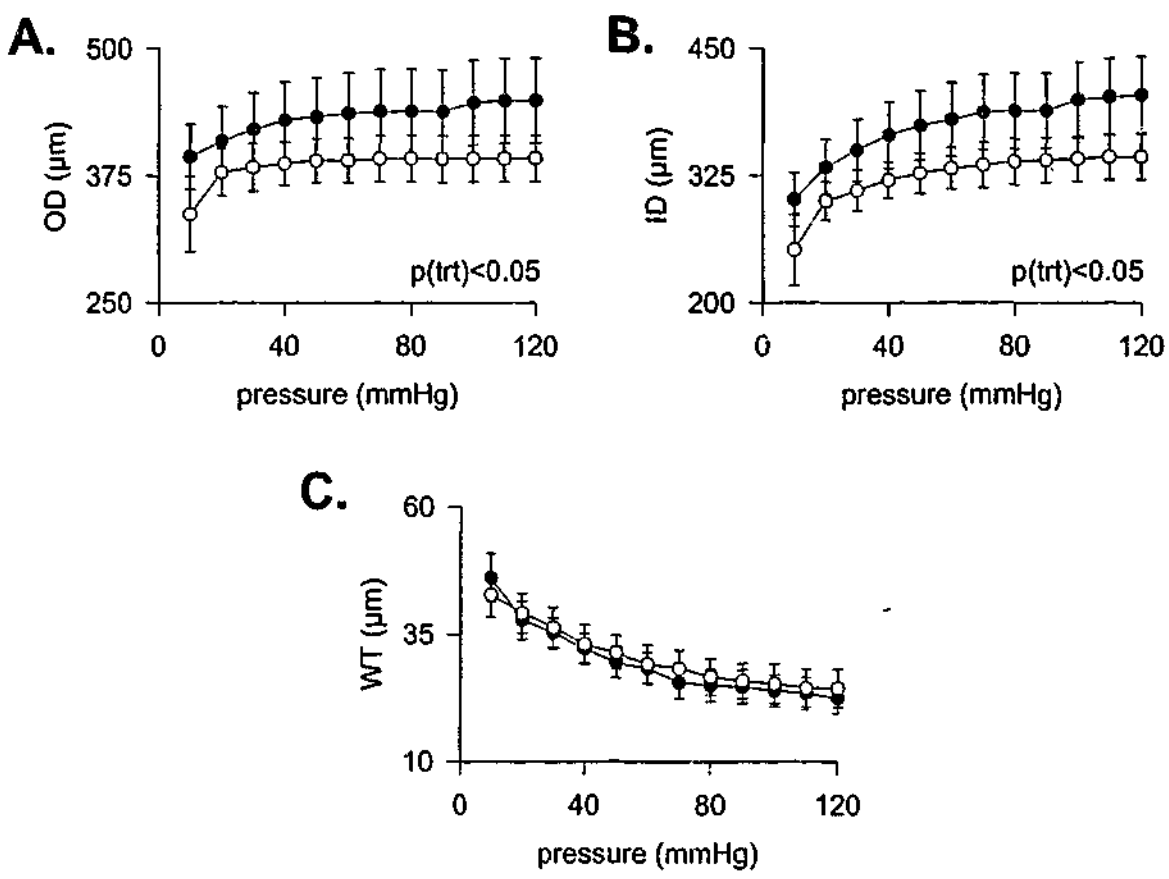
Appendix 3-10. Dimensions (µm) of the mesenteric artery branch during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of mesenteric artery branch from control (•, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.



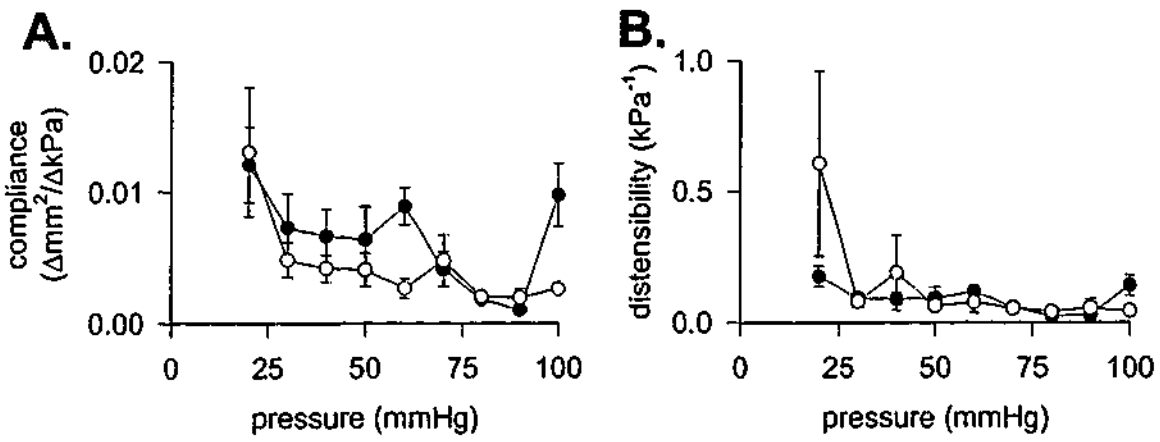
Appendix 3-11. Cross-sectional compliance and distensibility of the mesenteric artery branch. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of mesenteric artery branch from control (●, n=6) and UPE (○, n=6) fetuses.



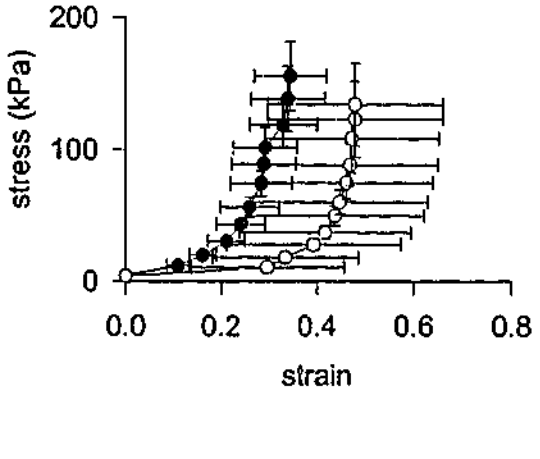
Appendix 3-12. Stress-strain relationships for the mesenteric artery. Stress-strain relationships for the segments of mesenteric artery branch from control (●, n=6) and UPE (○, n=6) fetuses.



Appendix 3-13. Dimensions (µm) of the arcuate artery during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of arcuate artery from control (●, n=6) and UPE (○, n=6) fetuses. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.



Appendix 3-14. Cross-sectional compliance and distensibility of the arcuate artery. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of arcuate artery from control (●, n=6) and UPE (○, n=6) fetuses.



Appendix 3-15. Stress-strain relationships for the arcuate artery. Stress-strain relationships for the segments of arcuate artery from control (●, n=6) and UPE (○, n=6) fetuses.

Chapter 4

The effects of intrauterine growth restriction on postnatal growth, arterial pressure and arterial wall mechanical properties in adult sheep

4.1 Introduction

This chapter describes a study of the long-term effects of intrauterine growth restriction (IUGR) in sheep on postnatal growth, arterial pressure and arterial wall mechanical properties. A number of sheep models (including glucocorticoid exposure, maternal undernutrition and altered placental size or function) have been used to investigate the postnatal effects of an altered *in utero* environment on arterial pressure. The perturbations to the fetal environment have differed between models and as a result, the effects in fetal growth and arterial pressure have differed between each model.

When the intrauterine environment is altered in the sheep during early gestation by mild maternal undernutrition or glucocorticoid exposure, fetal growth (and hence birth weight) are not altered, but lambs are hypertensive at 3–4 months postnatal age (Dodic *et al.*, 1998; Hawkins *et al.*, 2000a). These studies indicate that an altered *in utero* environment early in gestation can program sheep for postnatal hypertension without affecting size at birth. Alterations to the *in utero* environment that lead to growth restriction in lambs can lead to hypotension in the early postnatal period. For example, following pre-pregnancy caruncleclectomy, relative hypotension was evident in IUGR lambs in the early postnatal period but approximately two months after birth, the IUGR lambs became hypertensive relative to controls; this hypertension was maintained into adulthood (Robinson *et al.*, 1998). In another study, repeated exposure to glucocorticoids in mid-late gestation produced lambs that were hypotensive and lighter

than controls up to 3 months of age but these lambs became normotensive by 6 months of age (Moss *et al.*, 2001); it is unclear as to whether these lambs remain normotensive at ages greater than one year or will become hypertensive relative to controls. Late gestational placental insufficiency, induced by umbilico-placental embolisation (UPE), causes the growth restricted fetuses to have lower arterial pressure than controls in late gestation (Cock & Harding, 1997). The lower arterial pressures measured in the near-term IUGR fetus were maintained throughout the first 8 postnatal weeks; this relative hypotension was evident in IUGR lambs that were born at term, and approximately one week preterm (Louey *et al.*, 2000; Cock *et al.*, 2001a). These IUGR lambs also remained smaller than controls in this early postnatal period, and it is likely that the lower arterial pressure may be explained, in part, by their smaller size (Louey *et al.*, 2000). Since the lambs included in these studies were only followed up for the first 8 weeks after birth, their arterial pressure after this age is not known. The growth profiles of these IUGR lambs after 8 weeks of age is also unclear and it remained unknown as to whether these IUGR lambs would catch up in body size to controls. In humans, low birth weight has been associated with catch-up growth (Albertsson-Wikland *et al.*, 1993) and altered body proportions later in life, in particular increased abdominal adiposity (Law *et al.*, 1992). In turn, these altered body proportions have been associated with elevated arterial pressure (Daniels *et al.*, 1999). However, these associations have not been tested in a long-gestation animal model, in the absence of factors that can confound human follow-up studies.

The mechanisms for the persistent reductions in postnatal arterial pressure following late gestational UPE are unclear; the relative hypotension is apparently not due to altered circulating cortisol or plasma renin activity (Louey *et al.*, 2000). It is possible that arterial structure is altered by changes in the *in utero* environment which could have lasting effects on the mechanical properties of the arteries. The relative amounts of elastin and collagen, two major structural components of the arterial wall, are important in determining the mechanics of the arterial wall. Both of these proteins are synthesised *in utero*, with rapid rates of synthesis occurring in late gestation and the early neonatal period (Bendeck & Langille, 1991; Wells *et al.*, 1999). The synthesis and deposition of elastin and collagen can be altered by factors including undernutrition and hypoxia (Durmowicz *et al.*, 1991; Spanheimer *et al.*, 1991). Since umbilico-placental embolisation leads to fetal hypoxemia and hypoglycemia (Cock & Harding, 1997;

Louey *et al.*, 2000), it is possible that the development of elastin and collagen may be impaired in the arteries of the UPE fetuses which could lead to altered vascular mechanical properties. Changes to vascular structure maybe apparent in the absence of hypertension but can lead to the later development of elevated arterial pressure (Lee & Smeda, 1985; van Gorp *et al.*, 2000). Despite relative hypotension in the early postnatal period following umbilico-placental embolisation (Louey *et al.*, 2000; Cock *et al.*, 2001a), the potential for *in utero* alterations to the arterial structure and mechanics could predispose these animals for elevated arterial pressure in adulthood. Therefore, it was considered important to study the arterial pressures of IUGR animals into adult life.

4.2 Aims

1. To determine the postnatal growth profile of sheep following late gestational placental insufficiency.
2. To determine the effects of birth weight on postnatal arterial pressure in sheep during the first two postnatal years.
3. To determine the effects of birth weight on passive arterial wall mechanical properties in two year old sheep.

4.3 Methods

4.3.1 Overview of study

Data in the studies described in this chapter were obtained from animals that were studied both as fetuses (unless otherwise indicated) and postnatal lambs. To induce late gestational IUGR, UPE was performed from 120 days gestational age (GA) until birth. After birth, control and IUGR lambs were studied serially up to two years of age; at two years of age, sheep are past sexual maturity and can be classified as young adults, as they can live for up to 10–15 years. The arterial pressure of these lambs was measured via chronically implanted femoral artery catheters up to 8 weeks of age, and via temporary catheters inserted into an exteriorised carotid artery at 12 weeks, 6, 12 and 18 months after birth. At two years, arterial pressure was measured via chronically implanted carotid artery catheters and body composition was determined by dual emission x-ray absorptiometry. Following these *in vivo* measurements, sheep were killed and a selection of resistance arteries were collected for the *in vitro* determination

of arterial wall mechanical properties. All procedures involving animals were approved by the Monash University Animal Ethics Committee (Physiology).

4.3.2 Experimental animals

Studies were conducted on the fetuses of 22 Border Leicester × Merino ewes of known mating date. Prior to surgery, ewes were brought into the Department of Physiology (Monash University) animal house for acclimatisation. Before and after surgery, the ewes were housed in individual metabolic cages. They were exposed to 12 hour light and dark cycles and room temperatures of 18–20°C. They were provided daily with fresh lucerne-chaff and water.

Of the ewes that underwent surgery, 12 were carrying singletons and 10 were carrying twins. All twin fetuses were allocated to the IUGR group to maximise the degree of growth restriction. Twin animals were expected to be smaller than singletons at birth however, they were also were also subjected to late gestational umbilico-placental embolisation to further restrict their growth.

4.3.3 Fetal surgery

Fetuses underwent surgery for the chronic implantation of catheters at approximately 116d GA. The procedure was performed as previously described in Section 3.3.3. In brief, anaesthesia was induced in ewes with sodium pentothione and maintained with halothane (1.5–2%) in oxygen for the remainder of the surgery. Under aseptic conditions, the fetal hind quarters were exposed to allow the implantation of a femoral artery catheter; this catheter was inserted such that its tip was positioned in the abdominal aorta, 1–2cm below the level of the renal arteries. This catheter was used for blood sampling, measurement of arterial pressure, and also umbilico-placental embolisation. The catheter was tracked subcutaneously from the hind limb of the fetus to the flank, for exteriorsation through a small incision. Prior to returning the fetus to the uterus, a catheter was sewn to the fetal rump for the measurement of amniotic pressure. Electrodes were sewn to the uterine wall of the ewe to monitor labour via electromyography (EMG). The free ends of the catheters and electrodes were exteriorised through the flank of the ewe, sutured into position and fitted with metal

hubs and three-way taps. Following surgery, ewes and fetuses were allowed a 3–4 day recovery period before experimental procedures commenced.

4.3.4 Blood gas tensions, glucose and lactate concentrations

Catheters were flushed daily with sterile, heparinised saline (50IU/ml 0.9% sodium chloride) and an arterial blood sample (0.5ml) was collected to assess the general well-being of the fetuses. From these samples, arterial pH, blood gas tensions, glucose and lactate concentrations were measured using methods previously described in Section 3.3.3.

4.3.5 Umbilico-placental embolisation

The fetuses in the IUGR group underwent umbilico-placental embolisation (UPE) from 120d GA until birth, as described in Section 3.3.4. In brief, daily injections of microspheres (diameter 40–70μm) into the umbilico-placental circulation (via the fetal femoral artery catheter) reduced fetal arterial saturation to approximately 50% of control values. The control fetuses received saline injections only.

4.3.6 Fetal plasma cortisol concentrations

Arterial blood samples were taken weekly for the determination of fetal cortisol concentrations. A 2ml sample of whole blood (in a fluoride heparin tube) was spun in a centrifuge (Model J-6B, Beckman, USA) at 4°C for 15 minutes at 3000rpm. Plasma was stored at –20°C for later analysis by radioimmunoassay (performed by Mrs. Jan Loose). The assays were conducted using the method described in Bocking *et al.* (1986).

4.3.7 Delivery of lambs

At approximately 140d GA, uterine EMG traces were monitored for impending labour. When the EMG trace began to show the onset of labour, the free ends of the fetal catheters were cut and the lumen of each catheter was blocked with sterile pins. The ewe was then transferred to a large pen, with food and water available. We remained present from this stage onwards to assist in the delivery of the fetus.

Lambs were allowed to be born spontaneously, unless poor fetal health was observed from fetal arterial blood samples. In cases of poor fetal health and impending fetal death, preterm labour was induced by the administration of two doses (50mg each, 24h apart) of a 3β-dehydroxysteroid dehydrogenase inhibitor (Trilostane). This drug induces labour by the inhibition of the conversion of pregnenolone to progesterone. Trilostane was dissolved in 1ml of dimethyl sulphoxide, diluted with 4ml of heparinised saline and administered intravenously to the ewe. A 5mg intramuscular injection of betamethasone (Celestone Chronodose, Schering-Plough, Australia) was also administered at this time to stimulate fetal lung maturation and increase the chance of survival for the preterm lambs.

Once born, the lamb was weighed, then placed on a heated table and rectal temperature was measured. A three-way tap was fitted to the arterial catheter, and the catheter that was sutured to the fetal rump to measure amniotic sac pressure was removed. An arterial blood sample was taken for the measurement of pH, blood gases, glucose and lactate concentrations. The exteriorised catheter was stored in a plastic self-seal bag and secured beneath elasticised netting (Setonet, Seton Products Ltd, England) around the lamb's trunk. A protective jacket (Dialex, Australia) was placed around the lamb's trunk to further protect the catheter. The lamb was then returned to the ewe as quickly was possible to minimise the risk of rejection by the ewe. All lambs received colostrum either by feeding directly from the ewe or by bottle with expressed colostrum. Feeding habits, weight and rectal temperatures were monitored regularly for the first 24 hours after birth. If necessary, supplementary feeding by bottle of either expressed milk from the ewe or powdered milk (Veanavite Pty Ltd, Australia) was employed. Heat lamps were used where necessary to maintain the lamb's body temperature between 38.5–39.5°C.

Lambs were housed with their mothers in large pens in the Department of Physiology (Monash University) animal house until 8 weeks of age, when they were weaned. The lambs were housed with other lambs until 12 weeks of age, after which they were housed at the Prince Henry's Institute of Medical Research (PHIMR) large animal facility, Werribee, Victoria. There, the two genders were segregated into separate pastures. All catheters were removed from the lambs prior to housing at PHIMR. Lambs

remained at this facility until they were required for study at 6 monthly intervals. Prior to each study, the lambs were transported to the Department of Physiology (Monash University) for relevant studies before returning to PHIMR.

4.3.8 Catheterisation of lambs (<8 weeks of age)

Lambs underwent surgery for chronic implantation of catheters if they previously did not have any (unoperated controls) or for the replacement of existing catheters (fetal or postnatal lamb) that were no longer functional (i.e. unable to obtain a satisfactory arterial pressure recording). Implantation of a femoral arterial catheter was necessary for the recording of arterial pressure, and to allow blood sampling without causing discomfort to the animal.

4.3.8a Preparation of the lamb for surgery

Anaesthesia was induced in the lamb by halothane (3–4%) in a gas mixture of oxygen and nitrous oxide (O_2/N_2O, 50:50 v/v) administered via a face mask from an anaesthetic machine (Midget, CIG, Australia). After a satisfactory level of anaesthesia was achieved, the lamb was intubated with a cuffed endotracheal tube and connected to the anaesthetic machine. The halothane content of the inspired gas mixture was reduced to 2–2.5% for the remainder of the operation during which the lamb breathed spontaneously.

The left or the right side of the lamb's ventral abdomen, groin, upper inner aspect of the hind limb and flank were shorn and cleaned thoroughly as described in Section 3.3.2a. All drapes and surgical instruments were sterilised by autoclaving and aseptic conditions were maintained throughout the surgical operation, as previously described.

4.3.8b Surgical procedure

The femoral artery was exposed and a catheter (Dural Plastics, Australia, Cat. No. SV65, ID 0.85mm, OD 1.52mm, or Cat. No. SV116, ID 1.5mm, OD 2.7mm) was inserted 10–15cm into the vessel so that the tip of the catheter was located in the descending aorta. The catheter was then securely sutured to the vessel, tracked subcutaneously to the flank of the animal for exteriorisation through a small incision (1–2cm) and sutured securely to the skin. A loop of catheter was left subcutaneously to

allow for growth of the lamb. Incision sites were sprayed with antibiotic spray (Terramycin, Pfizer Agricare, Australia) before they were sutured with non-absorbable suture (Vetafil Bengen, 0.30mm, Clements Stansen Medical, Australia). The catheter was fitted with a three-way tap and kept inside a plastic self-seal bag secured beneath elasticised netting and a protective jacket.

4.3.8c Post operative care

After surgery and recovery from anaesthesia, lambs were returned to their mothers and given at least one day of recovery following surgery before studies were performed. At the completion of the surgery, a dose of antibiotics (Ilium Penstrep, procaine penicillin 250mg/ml, dihydrostreptomycin as sulphate 250mg/ml, procaine hydrochloride 20mg/ml, Troy Laboratories Pty Ltd, Australia) was administered intramuscularly, and a follow-up dose was also given on the day after surgery. Catheters were flushed with heparinised saline (100IU/ml 0.9% sodium chloride) every 2–3 days and the dead space of the catheter filled with heparin (Mulitiparin Heparin, 5000IU/ml, Fisons Pty Ltd, Australia) to maintain their patency.

4.3.9 Surgery to remove catheters and exteriorise a carotid artery

Prior to the lambs being transported to PHIMR for long term housing, all lambs underwent surgery for the removal of chronically implanted catheters and the relocation of the carotid artery into a subcutaneous skinfold. Subcutaneous relocation of the carotid artery was performed to facilitate future access to the artery to insert a temporary catheter for the measurement of arterial pressure at 6 month intervals. It also removed the need for the surgical insertion of catheters prior to each study, and the need to maintain the patency of chronic catheters between each study. This surgery was performed when the lambs were approximately 15 kg in body weight (usually between 8–12 weeks of age).

4.3.9a Preparation of the lambs for surgery

Lambs were allowed access to their lactating mothers, but both lamb and ewe were denied access to chaff for the 24 hours preceding the surgery. Water, however, was freely available. The anterior of the neck, the flank surrounding the exteriorisation site of existing catheters and the groin and upper inner aspect of the hind limb on the side of

the catheterised femoral artery were shorn. These sites were cleaned as previously described in Section 3.3.2a, and aseptic conditions were maintained throughout the surgical operation, as previously described. Lambs were anaesthetised and intubated as previously described in Section 4.3.8a.

4.3.9b Relocation of a carotid artery

A 10cm midline incision was made in the skin of the neck. Palpation of the carotid artery was made to determine its position before the surrounding connective tissue was carefully dissected from the artery. Special care was taken to avoid damage to the branches of the carotid artery, and to avoid the need to ligate these vessels. The artery was lifted into a position so that it lay between the muscle and the skin. A 5cm length of skin was then positioned such that it enveloped the artery. The two internal edges of the skin were brought together on either side of the artery and were sutured (Ethicon Chromic Gut, Surgical catgut suture 2/0, 3.5 metric) with a simple continuous stitch. The external skin incision was then sutured closed with non-absorbable suture. The external suture was removed 7–10 days after surgery, whereas the internal suture was absorbed over time so that the carotid artery was permanently held in its relocated subcutaneous position.

4.3.9c Removal of existing catheters

Following the completion of the carotid artery isolation surgery described above, all existing indwelling catheters were removed for two reasons: (1) they were no longer required since arterial pressure was to be measured via temporary catheters (2) indwelling catheters could lead to infections and other risks when these lambs were housed in a farm situation at PHIMR. Palpation of the catheterisation site in the upper inner aspect of the hind limb was made to determine the position of the existing femoral artery catheter. The femoral artery was carefully dissected, and a length of suture was placed around the artery. The catheter was removed and the suture was tightened around the artery. Incision sites were sutured closed with non-absorbable suture. At the conclusion of the surgery, the lambs were returned to an upright position in a sling and the endotracheal tube deflated. Once the lamb was able to breathe and swallow spontaneously, the endotracheal tube was deflated and removed.

4.3.9d Post operative care

After surgery and recovery from anaesthesia, lambs were returned to their pens. At the completion of the surgery, lambs were given an intramuscular dose of antibiotics (Ilium Penstrep), and received a follow-up dose the next day. All lambs remained in the Department of Physiology (Monash University) animal house for a minimum of one week following this surgery before transportation to PHIMR for long-term housing.

4.3.10 Catheterisation of two year old sheep

For the final measurement of arterial pressure at two years of age, indwelling catheters were surgically inserted into the carotid artery and jugular vein. Prior to this final surgery, sheep were brought into the Department of Physiology (Monash University) animal house from PHIMR for 3–4 days acclimatisation. The sheep were housed in individual metabolic cages in the company of other sheep, and were provided daily with fresh lucerne chaff and water.

4.3.10a Preparation of the sheep for surgery

Sheep were fasted for 12–24 hours prior to surgery, with only water available during this time. The animals were anaesthetised and intubated as described in Section 3.3.2. The anterior neck was shorn and cleaned, and strict aseptic conditions were maintained throughout the surgical operation, as previously described.

4.3.10b Surgical procedure

A 10cm incision was made in the skin overlying the site of the relocated carotid artery that had been isolated in the previous surgery performed between 8–12 weeks of age. Catheterisation of this site allowed the other carotid artery to remain untouched, thus allowing collection for later histological and biochemical analysis. Surrounding tissue was carefully dissected from the carotid artery before a catheter (Dural Plastics, Australia, Cat. No. SV116, Medical Grade, ID 1.5mm, OD 2.7mm, length 1.75m) was inserted. A catheter was also inserted into the jugular vein. Both catheters were secured to the neck before the two incision sites were sutured closed with non-absorbable suture. The exteriorised ends of the catheters were kept inside plastic self-seal bags and secured beneath elasticised netting around the sheep's neck. At the completion of the surgery, each sheep was given an intramuscular dose of antibiotics (Ilium Penstrep).

When the sheep was able to breathe and swallow spontaneously, the endotracheal tube was deflated and removed.

4.3.10c Post operative care

Following surgery, the sheep were returned to individual metabolic cages and 3–4 days were allowed for recovery before recording of arterial pressure. Both catheters were flushed every second day with sterile heparinised saline (50IU/ml 0.9% sodium chloride), to maintain their patency.

4.3.11 Postnatal arterial pressure recordings in lambs with catheters

The arterial pressure of lambs was measured via chronically implanted catheters at 2, 4 and 8 weeks of postnatal age. The arterial pressure of two lambs could be recorded simultaneously and each animal was placed prone in a sling for the duration of the recording (1–1½ hours). The animals were placed in a quiet room with low levels of light. The arterial catheter for the lamb was connected to a pressure transducer (TFN-R Disposable Transducers, Viggo-Spectramed, USA) positioned at a level approximating to the mid-heart region. The transducer was connected to a pressure amplifier to measure arterial pressure. Heart rate was derived from the arterial pressure signal via a ratemeter. Output signals from the pressure amplifier and ratemeter were logged by a digital data recording system (MacLab 8, ADInstruments Pty Ltd, Australia) connected to a computer using the program Chart for MacLab (Version 3.6, ADInstruments Pty Ltd, Australia). The data recording system was configured to record at a speed of 40 samples per sec up to a range of 10V; the time scale was set to 10 sec/division.

At the completion of the recording session, blood samples were taken for blood gas analysis (corrected for measured body temperature), blood lactate and glucose concentrations and plasma cortisol concentrations.

4.3.11a Analysis of arterial pressure recording

At the completion of the recording period, the data were digitally analysed in 1 minute blocks to obtain average diastolic pressure, average systolic pressure and average heart rate for each minute of recording. Data from periods when the lamb was bleating or moving excessively in the sling were excluded from the analysis; for the majority of the

recordings, all lambs remained still. Mean arterial pressure for each minute was calculated from the corresponding systolic and diastolic pressure readings using the formula:

$$MAP\ (mmHg) = diastolic + \tfrac{1}{3}\ (systolic - diastolic)$$

All valid data from the recording period were then averaged to obtain one value for each of diastolic, systolic and mean arterial pressure, and heart rate.

4.3.12 Arterial pressure recordings in sheep with a relocated carotid artery

Prior to each study at 6, 12 and 18 months of age, lambs were brought into the Department of Physiology (Monash University) animal house from PHIMR for 3–4 days acclimatisation. Before and during each study, the lambs were housed in individual metabolic cages, in the company of other sheep and had access to lucerne chaff and water.

On the study day, the area of the neck where the relocated carotid artery was isolated was shorn and palpation for a pulse was made to confirm the position of the artery. A subcutaneous injection of local anaesthetic (Lignocaine 20, lignocaine hydrochloride 20mg/ml, Troy Laboratories Pty Ltd, Australia) was given before a small incision 2–3mm was made in the skin. The underlying connected tissue was blunt dissected to expose the artery and a temporary catheter (Vialon Insyte IV catheter, 16G 1.77in. 1.7×45mm, Becton Dickinson Pty Ltd Australia) was inserted into the artery. An extension catheter (Dural Plastics, Australia, Cat. No. SV116, Medical Grade, ID 1.5mm, OD 2.7mm; length 1.75m) was connected to the end to the catheter and attached to a transducer for the measurement of arterial pressure and heart rate by the digital data logger, as previously described in Section 4.3.11. The transducer was positioned such that it was at the level of the heart (Figure 4-1). The catheter was held in place on the neck by Michel clips and elasticised netting was placed around the neck to further ensure security.

Arterial pressure was measured for 1–1½ hours, and at the conclusion of the recording period, blood samples were collected for the measurement of arterial blood gas tensions, blood glucose and lactate concentrations and plasma cortisol concentrations. Following blood collection, pressure was applied to the artery and the catheter removed. External

pressure continued to be applied until sufficient clotting had occurred so that bleeding from the carotid artery did not occur when the external pressure was removed. Each lamb was returned to PHIMR 2–3 days after each study. Data were analysed as previously described in Section 4.3.11a.

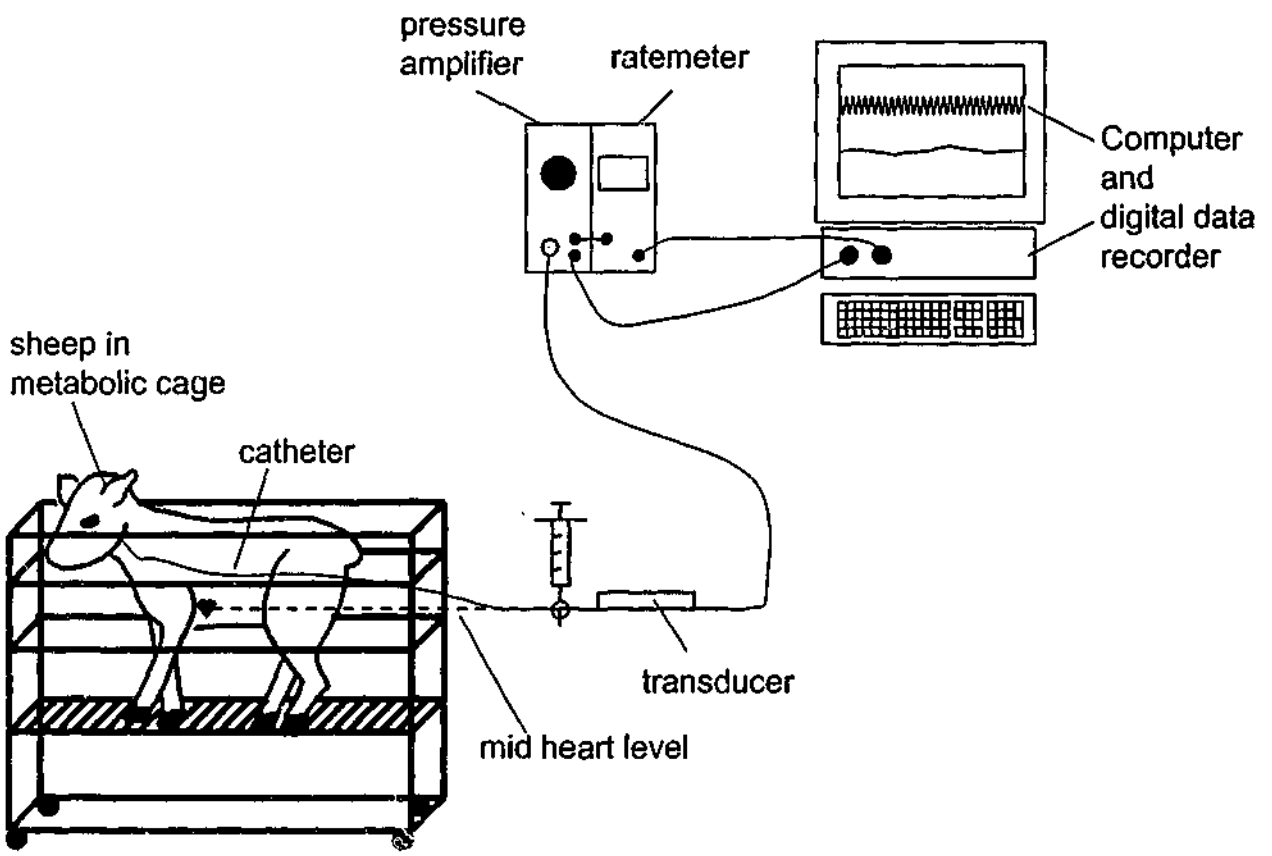


Figure 4-1. Measurement of arterial pressure in a sheep standing in a metabolic cage. A schematic diagram of the arrangement of recording equipment during arterial pressure measurements at 6, 12, 18 and 24 months of age. The pressure transducer was positioned at a level approximating heart. Data were logged using a digital data recorder (MacLab, ADInstruments, Australia).

4.3.13 Postnatal arterial pressure recordings in two year old sheep

Following 3–4 days of recovery after surgery for the implantation of catheters at two years of age, arterial pressure was recorded for 3 hours a day, over two consecutive days. Arterial pressure was recorded via the carotid artery catheter while sheep were standing in individual metabolic cages. The catheter was connected to a pressure transducer located at mid-heart level and recorded by a digital data acquisition system as previously described in Section 4.3.12 and shown in Figure 4-1.

At the conclusion of the recording period, blood samples were collected for the measurement of arterial blood gas tensions, blood glucose and lactate concentrations and plasma cortisol concentrations. Data were analysed as previously described in Section 4.3.11a.

4.3.14 Plasma cortisol concentration

A 2ml sample of whole blood was collected into a fluoride heparin tube at the conclusion of arterial pressure measurements. These samples were centrifuged, stored and analysed by radioimmunoassay as previously described in Section 4.3.6.

4.3.15 Body weight and postnatal growth rates

Lambs were weighed at birth, and also at intervals between birth and two years of age. Postnatal growth rates, with respect to body weight, were calculated as follows:

$$Weight\ increase/day = (x_2 - x_1) \div (age_1 - age_2)$$

$$Weight\ increase/day\ (\%) = (x_2 \div x_1) \div (age_1 - age_2) \times 100$$

where: x_1 = body weight at age_1

x_2 = body weight at age_2

age_1 = first age

age_2 = second age

e.g. to calculate the growth rate between 2 and 4 weeks of age, age_1 = 2 weeks, age_2 = 4 weeks.

4.3.16 Measurements of body dimensions

At two years of age, measurements of body dimensions were made to determine if there were differences in the body size of the control and IUGR sheep. Each measurement (in cm) was made in duplicate and included measures of body length, height, girths and bone lengths (Figure 4-2).

4.3.16a Body length and height

Body length was determined by measuring the crown-to-rump length (CRL) of the sheep. CRL was measured as the length along the vertebral column from the supraorbital margin to the coccyx (Figure 4-2, point A to point B); these bony

landmarks were identified by palpation and used as reference points for this measurement. The standing height of the sheep was determined by measuring the straight line distance between the vertebral margin of the scapula and the base of the hoof when the sheep was standing fully upright (Figure 4-2, point P to point Q).

4.3.16b Body girths

Thoracic girth, the circumference of the thorax, was measured at the level of the xiphoid process at the base of the sternum (Figure 4-2, C). Abdominal girth was measured as the circumference around the abdomen at the level of the navel (Figure 4-2, D), which approximated the point of the greatest abdominal circumference in each animal. The measured hip circumference was the circumference of the hip girdle; the iliac crest (Figure 4-2, E) was used as the reference point for the measurement of this girth.

4.3.16c Head size

All measurements of head size were made using calipers. The length of the head was determined as the straight line distance between the tip of the nose and the occipital protuberance at the rear of the head (Figure 4-2, point F to point G). The biparietal diameter was the straight line distance from the lateral margin of one eye (Figure 4-2, point H) to the lateral margin of the other eye.

4.3.16d Lengths of limb bones

The lengths of a number of bones in the front and hind limbs were measured; these measurements were made using calipers and palpation of the bones to determine the position of the bone ends to use as reference points (Figure 4-2). In the front limb, bone lengths for the humerus (point I to point J), ulna (point J to point K) and metacarpus (point K to point L) were measured. In the hind limb, the bone lengths for the tibia (point M to point N) and metatarsus (point N to point O) were measured.

4.3.16e Ponderal index

Ponderal index was calculated from the body weight and length measurements, using the formula:

$$ponderal\ index\ (kg/cm^3) = body\ weight \div CRL^3$$

A low ponderal index is indicative of an animal that is light for their body length, and is often used as a measure of thinness.

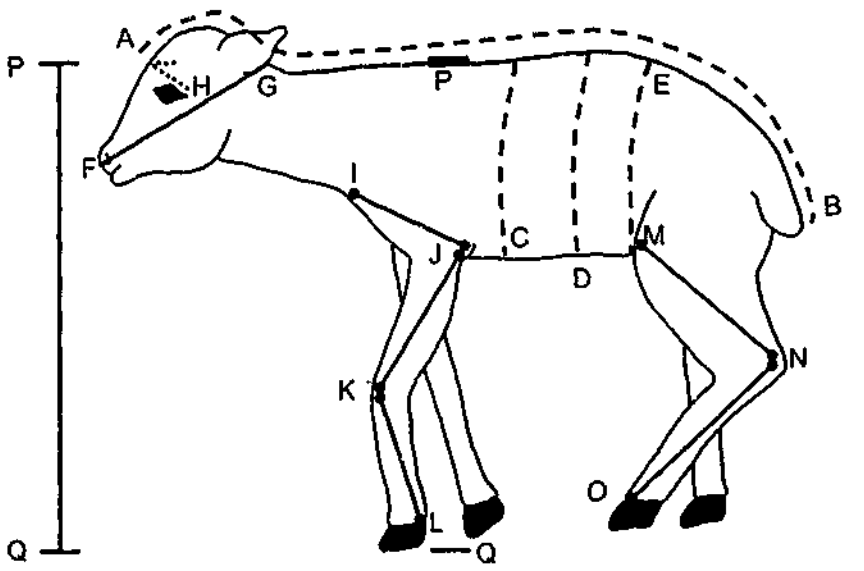


Figure 4-2. Measurement of body dimensions of the two year old sheep. A schematic diagram of the sites of measurement of body lengths and girths in the two year old sheep. Crown-to-rump length was measured as the length along the vertebral column from the supraorbital margin (A) to the coccyx (B). Thoracic girth, abdominal girth and hip circumference were measured as the circumference around the body at the level of the xiphoid process (C), navel (D) and iliac crest (E), respectively. Head length was measured from the tip of the nose (F) to the occiput (G); biparietal diameter was measured from the lateral margin of each eye (H). The lengths of a number of limb bones was also measured using the bone ends as reference points to determine the length of each specific bone (humerus: I–J, ulna: J–K, metacarpus: K–L, tibia: M–N, metatarsus: N–O). The standing height of the sheep was measured as the distance from the vertebral margin of the scapula (P) to the base of the hoof (Q). See text for more details of the reference points used for each measurement.

4.3.17 Dual emission x-ray absorptiometry

At two years of age, the *in vivo* body composition was determined by dual emission x-ray absorptiometry (DXA). This is a rapid, non-invasive method of measuring the relative amounts of lean tissue (mostly muscle), fat and bone mineral content in an intact animal. In addition, the DXA can also determine bone mineral density.

4.3.17a Principles of dual emission x-ray absorptiometry

The densitometer is able to determine the amounts of lean tissue, fat and bone in the body based on the principle that tissues of different densities absorb x-rays to differing degrees (Pietrobelli *et al.*, 1996; Fogelman & Blake, 2000). Given that bone is denser than muscle, x-rays are absorbed to a greater degree by bone than lean tissue. Thus, in an image from the densitometer, bone will appear a brighter white than muscle (Figure 4-3). Of bone, muscle (lean tissue) and fat, fat is the least dense and therefore x-rays will pass easily through this tissue (Figure 4-3). Both the amount of each tissue type (in grams) and also relative proportions of each tissue type can be determined by DXA. In addition to bone mineral content (BMC, in grams), bone mineral density (BMD, in g/cm^2) can also be calculated by dividing BMC by the surface area of the bones. Whole body composition may be measured by DXA, and grid lines may also used to digitally divide the body into specific regions for regional analysis of body composition.

An x-ray source located underneath the flatbed emits photons at two different energy levels and is received by an x-ray detector located in a movable arm above the flatbed. The subject is laid prone on the flatbed as the detector arm moves along the entire length of the subject a total of three times at angles of 45°, 90° and 135°. Each pass of the arm over the body creates a digital image of the subject, and the integration of the three images allows for the creation of a 3-dimensional image of the subject; this image is analysed for the amounts of bone mineral and soft tissue in the subject.

Measurements in sheep by the Hologic QDR 4500A densitometer are highly repeatable. The coefficients of variation for lean tissue, fat and bone mass have been shown to be 0.37%, 2.47% and 4.15% respectively (Hunter *et al.*, 2000). Chemically determined body composition has also been shown to be highly correlated with the body composition when determined by DXA for the measured tissues (lean muscle: r^2=0.98; fat: r^2=0.99; bone mineral content: r^2=0.97; Hunter *et al.*, 2000).

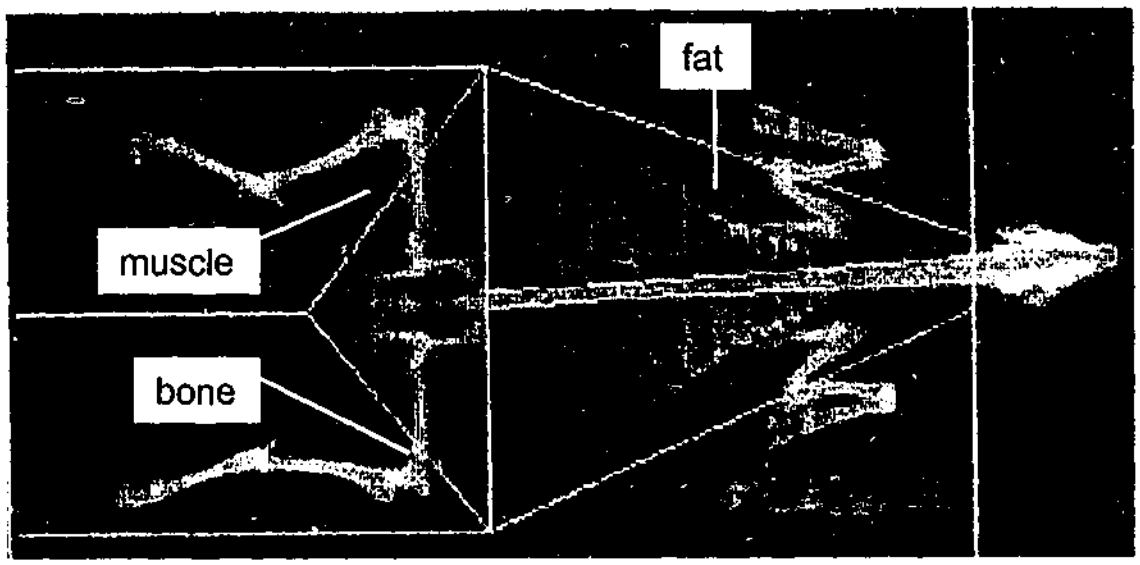


Figure 4-3. DXA scan image of two year old sheep. A live animal scan by the Hologic QDR 4500A, with grid lines set so as to determine regional body composition. The x-ray absorption by lean tissue (muscle), fat and bone differs due to their differing densities.

4.3.17b Determination of in vivo body composition

Sheep were shorn 2–3 days prior to scanning, and were fasted for 24 hours before scanning. A temporary venous catheter was inserted into the jugular vein of each sheep before sedation was induced with thiopentone sodium (Thiobarb, 1000mg/g thiopentone sodium, Jurox Pty Ltd, Australia); sedation for approximately 15–20 minutes was achieved using a dose of approximately 10–15mg/kg body weight. Once adequate sedation was achieved, the sheep was placed prone on the flat bed of the DXA scanner (Model QD 4500A, Hologic Inc., USA). The x-ray arm then made three passes over the length of the animal; data from these scans were relayed to the integrating computer to create a 3-dimensional image of the sheep. Data were integrated using the Hologic QDR operating system (V8.26a:3) and was set to determine the whole body composition, providing data of lean tissue, fat, BMC and BMD. At the completion of the three scans, the animal was returned to pasture to recover.

4.3.18 Post mortem

Following the completion of all *in vivo* studies, the two year old sheep were humanely killed and a selection of arteries collected for the *in vitro* determination of passive

arterial wall mechanical properties. A number of organs were also collected for various biochemical and histological analyses (not reported in this thesis).

Sheep were fasted for 24 hours prior to post mortem, with only water available during this period. Five minutes before the sheep were killed, they were given a 0.1ml/kg intravenous injection of vasodilator (papaverine hydrochloride, 120mg in 10ml, David Bull Laboratories, Australia) to maximally dilate blood vessels, followed by 5ml of heparin (Mulitiparin Heparin, 5000IU/ml, Fisons Pty Ltd, Australia) to minimise clotting in the blood vessels. The sheep were then killed with a 20ml intravenous injection of sodium pentobarbitone (Lethabarb, sodium pentobarbitone 325mg/ml, Virbac, Australia).

At post mortem, major organs were weighed and collected for later analysis (not presented in this thesis). In addition to the major organs, peri-renal and abdominal fat was collected and weighed. The left kidney, brain, a section of the ileum and a section of femoral muscle were collected and placed in oxygenated calcium-free physiological saline solution (PSS) until they could be dissected at the conclusion of the post mortem.

A number of conducting vessels were also collected for histological and biochemical analysis. Sections of thoracic aorta, inferior vena cava and carotid artery were removed and perfusion fixed at 80mmHg with 4% paraformaldehyde. Sections of aorta and carotid artery were also frozen in liquid nitrogen and stored at –70°C for future biochemical analysis of elastin and collagen content. These results are not reported in this thesis.

4.3.19 Measurement of passive arterial wall mechanical properties

4.3.19a Selection of arteries

Arteries were selected from four different vascular beds to investigate whether there were regional differences in the mechanical properties of arterial walls. Figure 4-4 illustrates the sites from which the segments of artery were collected. Arteries (~500μm) were taken from a similar area of each bed. While there was a degree of variation in the initial outer diameter (OD) of the vessels, there were no significant differences between the initial OD of each group for each vessel.

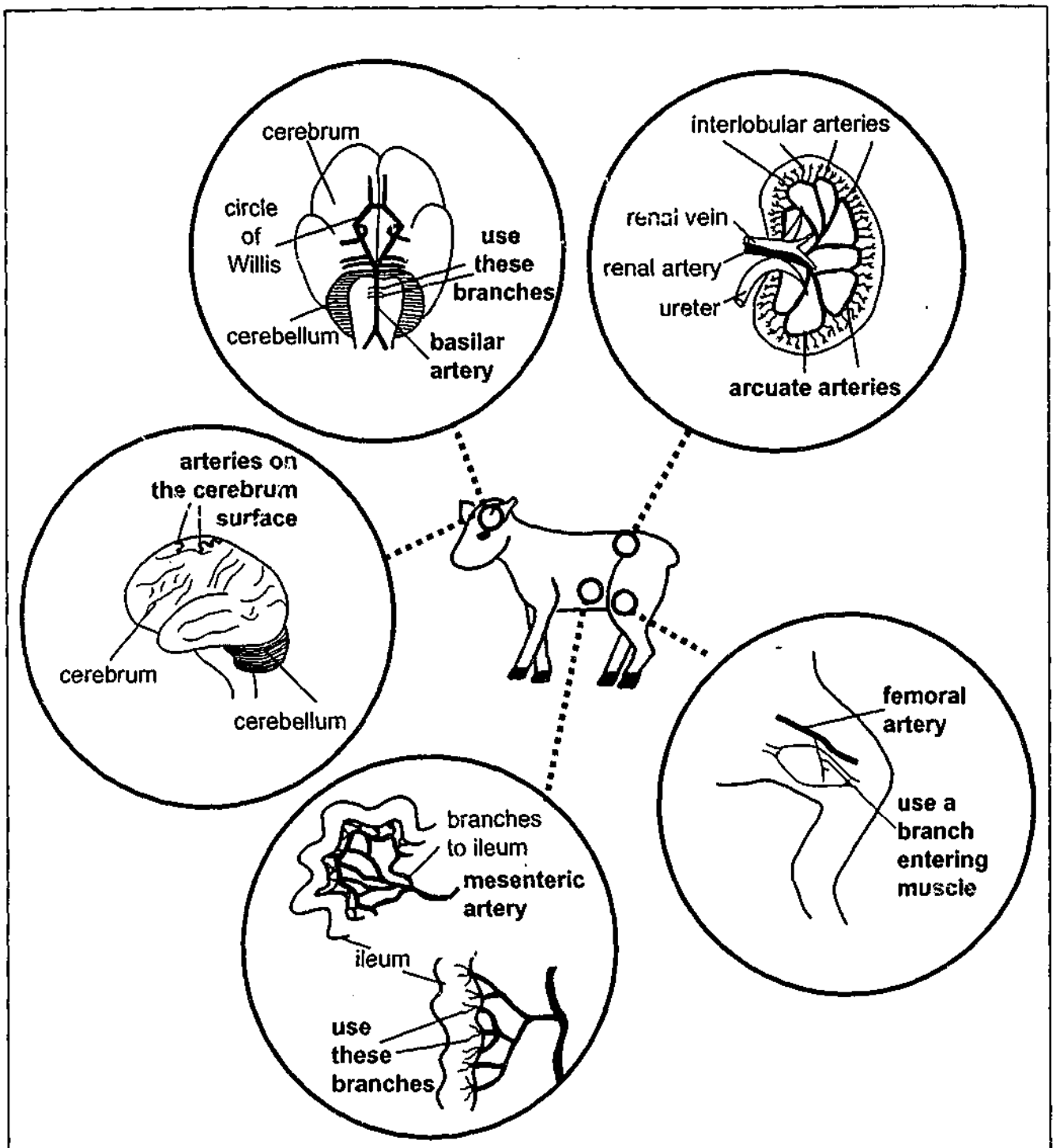


Figure 4-4. Arteries collected for the determination of passive arterial wall mechanics. Schematic diagrams showing the sites from which segments of artery were collected for the determination of passive arterial wall mechanics. Segments were taken from four different vascular beds: a cerebral surface artery and a branch of the basilar artery from the brain, the arcuate artery from the kidney, a branch of the femoral artery from the skeletal muscle, and a branch of the mesenteric artery.

Arteries from the brain A branch of the basilar artery (located at the base of the brain) and an artery from the surface of the cerebrum were collected to measure their dimensions during pressurisation and to determine passive mechanical properties of the arterial wall. One segment of the artery was dissected from each animal. The average

initial OD (pressurised at 5mmHg) of the basilar artery branch was 375±14μm; the average initial OD (pressurised at 5mmHg) of the cerebral surface artery was 518±11μm.

An artery from skeletal muscle A branch of the femoral artery was collected for the determination of the mechanical properties of its walls; this selected branch was a branch entering the muscle. One segment of the artery was dissected from each animal and was collected from the non-catheterised hind limb; the average initial OD (pressurised at 5mmHg) was 479±26μm.

An artery from the mesentery The mesenteric artery sends branches to the ileum, where they further divide before entering the gastrointestinal tract; the artery that was selected for study was the final branch before entry into the ileum. One segment of artery was collected from this region; the average initial OD (pressurised at 5mmHg) was 417±8μm.

An artery from the kidney The arcuate artery in the kidney was selected for study; these arteries are located at the inner border of the renal cortex, and run in a plane parallel to the cortical border, as opposed to perpendicular to the cortical border as do the other renal vessels. One segment of arcuate artery was collected; the average initial OD (pressurised at 5mmHg) was 456±15μm.

4.3.19b Dissection of arteries

Dissection of the arteries from the surrounding tissue has been previously described in Section 2.3.10b. In brief, organs were collected at post mortem and placed in oxygenated calcium-free PSS solution. Connective tissue was dissected from the target arteries before the artery was tied to the glass cannula of the pressure myograph. Blood contained in the arterial segment was gently flushed from the artery before the free end of the arterial segment was ligated. The segment was initially inflated to the maximum pressure (200mmHg) to ensure there were no major leaks in the artery before intraluminal pressure was returned to 5mmHg; the pressure-servo control system was able to compensate for minor leaks. A more detailed description of the pressure myograph and pressure-servo control circuit can be found in Section 2.3.10c. During

this time and during pressurisation, the artery was superfused with a warmed (35°C) and oxygenated solution of calcium-free EGTA-containing (1mM) PSS.

4.3.19c Measurements of arterial dimensions

Measurements (from a video monitor) of segment length (L) and outer diameter (OD) were made using a low magnification (×48), before wall thickness (WT) measurements were made at a higher magnification (×300); a more detailed description of these measurements can be found in Section 2.3.10d. Following the measurements at 5mmHg, the intraluminal pressure was increased to 10mmHg and measurements were repeated at each 10mmHg increment pressure up to 150mmHg.

4.3.19d Calculations

All measurements were converted from the values measured from the video monitor (in cm or mm) to the actual size (in μm). The calculations of arterial wall areas, wall stress and compliance (Hill & Ege, 1994; Pourageaud & De Mey, 1997; Crijns *et al.*, 1999) are described in more detail in Section 2.3.10e. In brief, the relative change from the initial measurement at 5mmHg intraluminal pressure has been calculated for each of:

outer diameter (OD)

inner diameter ($ID=OD-2WT$)

segment length (L)

wall thickness (WT)

and luminal volume ($V=\Pi(ID \div 2)^2 \times L$).

The following parameters were calculated from the measured OD, L and WT:

cross-sectional area of the lumen (μm^3) = $\Pi \times ID^2 \div 4$

cross-sectional area of the arterial wall (μm^3) = $\Pi(OD^2-ID^2) \div 4$

circumferential wall stress (kPa) = $(ID \div 2 \times P) \div (2 \times WT)$

cross-sectional compliance (mm^2/kPa) = $\Delta CSA/\Delta P$

cross-sectional distensibility (kPa^{-1}) = $\Delta CSA/CSA_i/\Delta P$

volume compliance (mm^3/kPa) = $\Delta V/\Delta P$

volume distensibility (kPa^{-1}) = $\Delta V/V_i/\Delta P$

wall strain = $(ID_x-ID_i) \div ID_i$.

The stress-strain relationship was analysed using Origin (Version 7.0, Microcal Software Inc., CA, USA) to calculate the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 5mmHg and x is strain at each pressure increment (Hill & Ege, 1994). This exponential function was used to describe the overall stress-strain relationship and the data fit this equation with a $r^2>0.94$. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries, and be^{k_2x} describes the high stress (collagenous) range of the arteries. The derived rate constants were compared using GraphPad Instat (Version 3.05, GraphPad Software Inc., CA, USA).

4.3.20 Statistical analysis

One-way analysis of variance (ANOVA) were used to analyse data when the IUGR animals were divided into those that were born at term, preterm or spontaneously IUGR; these were used compare controls with all three IUGR groups at birth (body weight, gestational age). T-tests for independent samples were performed to analyse data when all IUGR animals were combined as one group and compared with controls (birth data, body composition and body dimension at two years, post mortem data). Two-way repeated measures ANOVA were used to analyse body weight, postnatal growth rates, arterial pressure and heart rate measurements; factors were treatment and age. Two-way repeated measures ANOVA were also used to analyse the arterial wall mechanics data; factors were treatment and pressure. Pearsons correlation coefficients were calculated to correlate postnatal arterial pressure, body dimensions and body composition to fetal physiological measurements, measurement of size at birth and postnatal growth rates.

Data are presented as mean±SEM unless otherwise indicated. Statistical analyses were performed using SPSS for Windows (Version 10.0.5) with the level of significance taken at $p<0.05$, unless otherwise stated. All significant differences indicated by ANOVA were subjected to the Student-Newman-Keuls (SNK) post-hoc test to test for significant differences between individual means.

4.4 Results

4.4.1 Outcomes of umbilico-placental embolisation

Fetal surgery was performed on 22 pregnant ewes, bearing a total of 32 fetuses (12 singletons and 10 sets of twins). Of the singletons, 12 were assigned to the control group and the remaining 2 singleton fetuses underwent umbilico-placental embolisation. All 10 sets of twins underwent UPE; twins were expected to be smaller than singletons at birth and UPE restricted their growth further, thus maximising the difference in birth weight between controls and IUGR animals. Details of the animals used in the fetal component of this study are summarised in Table 4-1. Of the controls, all animals survived the fetal period to be delivered spontaneously and studied in the postnatal period. Of the fetuses that underwent UPE, two (both twins, in separate pregnancies) died *in utero*, with the cause of death most likely to have been from an accidental overdose of microspheres during UPE. Of the surviving twin of these pregnancies, both survived to be born (one delivered spontaneously, the other was induced). Two ewes were induced to deliver at 140d GA because their fetuses were at risk for fetal death; both of these ewes were carrying twins that had undergone UPE.

Of the animals that were born in 1998, some were included in studies investigating the effects of UPE on arterial pressure in the first 8 postnatal weeks (Louey *et al.*, 2000; Cock *et al.*, 2001a). Two of these animals were killed at 8 weeks age for the collection of tissues for other studies and their fetal and postnatal data have not been included in the results of this chapter. Similarly, animals that did not complete the majority of the two year study (due premature deaths) have not been included in the data analysis.

The majority of fetuses were born spontaneously and it became evident that some UPE fetuses would be born at term, while others would be born approximately one week preterm; UPE fetuses that delivered at term will be referred to as T-UPE fetuses and those that delivered preterm will be referred to as P-UPE fetuses. After birth, when it was confirmed by birth weight that these animals were growth restricted, they will be referred to as IUGR lambs.

Table 4-1. The histories of ewes and fetuses that underwent surgery at ~116d GA. This table summarises the identification numbers (ID) for the ewes and their fetuses, the date and GA at the time of surgery, the gender and the treatment group (Trt) of the fetus. "C" refers to control fetuses, and "UPE" refers to fetuses that underwent umbilico-placental embolisation. Twins are identified by [ewe ID].1 or [ewe ID].2 in their fetal ID. The cause of death in the fetuses that died *in utero* was most likely to be from an accidental overdose of microspheres from UPE.

Ewes were allowed to deliver spontaneously except when fetuses were in extremely poor health as indicated by fetal arterial blood samples; in this instance, labour was induced to avoid fetal loss (see Section 4.3.7 for details). Fetuses that delivered preterm (<142d GA), either spontaneously or induced, are indicated with an asterisk (*). The table also gives the date of birth (DOB) of the lamb and its postnatal identification number; fetal identification numbers were based on the identification number on the ear tag of the ewe, but after birth lambs were given their own numbered ear tag for identification.

The fetuses that have not been included in analyses are indicated with a †; these fetuses were excluded on the basis of death either *in utero* or within the first 9 postnatal months and therefore not completing at least 50% of the study period.

Ewe ID	Fetal ID	Sex	Trt	Surgery date	GA at surgery (d)	DOB	GA at birth (d)	Lamb ID	Comment
107	107.1	♂	UPE	06/07/98	116	30/07/98	140*	16	Induced
	107.2†	♀	UPE	06/07/98	116	30/07/98	140*	17	labour
108	108.1†	♂	UPE	07/07/98	117	139d GA died *in utero*			Induced
	108.2	♂	UPE	07/07/98	117	30/07/98	140*	18	labour
130	130	♀	C	27/07/98	116	30/07/98	140	13	
160	160.1†		UPE	24/08/98	116	122d GA died *in utero*			
	160.2	♀	UPE	24/08/98	116	14/09/98	137*	73	
214	214.1†	♀	UPE	12/10/98	116	10/11/98	145	–	8wk study
	214.2	♂	UPE	12/10/98	116	10/11/98	145	29	
215	215.1†	♂	UPE	13/10/98	117	13/11/98	148	–	8wk study
	215.2	♂	UPE	13/10/98	117	13/11/98	148	33	
224	224	♀	C	26/10/98	116	29/11/98	150	31	
233	233	♀	C	03/11/98	117	30/11/98	144	30	
243	243.1	♂	UPE	06/11/98	113	03/12/98	140*	35	
	243.2	♀	UPE	06/11/98	113	03/12/98	140*	34	
67	67	♂	C	18/05/99	117	19/06/99	149	52	
85	85.2	♂	UPE	31/05/99	115	29/06/99	144	53	
	85.1†	♀	UPE	31/05/99	115	29/06/99	144	54	
91	91	♀	C	07/06/99	117	08/07/99	148	55	
90	90	♀	C	08/06/99	118	10/07/99	150	56	
109	109.1	♀	UPE	21/06/99	116	18/07/99	143	57	
	109.2	♂	UPE	21/06/99	116	18/07/99	143	58	
129	129.2	♂	UPE	13/07/99	116	10/08/99	144	59	
	129.1	♂	UPE	13/07/99	116	10/08/99	144	60	
130	130.2	♂	UPE	14/07/99	117	09/08/99	143	61	
	130.1	♂	UPE	14/07/99	117	09/08/99	143	62	
146	146	♀	C	02/08/99	116	01/09/99	146	93	
147	147	♀	C	02/08/99	116	02/09/99	147	46	
209	209	♀	C	06/10/99	117	06/11/99	148	65	
218	218	♀	UPE	19/10/99	116	13/11/99	141*	66	
217	217	♀	UPE	19/10/99	116	16/11/99	144	67	
220	220†	♂	C	20/10/99	117	17/11/99	145	68	

4.4.2 Fetal arterial blood parameters

4.4.2a Arterial blood gas and pH status

Figure 4-5 shows the arterial blood gas and pH status in the period before the commencement of UPE, and during the period of UPE (120d GA until birth). For the UPE animals, the data from 120d GA until birth are the measurements taken at the completion of the daily embolisation.

Prior to UPE, there were no differences in the Sa_{O_2} and Pa_{O_2} of the animals. After the onset of UPE, the T-UPE fetuses were hypoxemic relative to controls and this hypoxemia was maintained by daily embolisation until birth (Figures 4-5A, 4-5B). Similarly, P-UPE fetuses were hypoxemic relative to controls during the period of UPE, but they were not different to controls at 140d GA, just prior to birth (Figures 4-5A, 4-5B). There were no differences in the degree of hypoxemia measured in T-UPE and P-UPE during the period of UPE.

T-UPE fetuses were hypercapnic compared with controls prior to the onset of UPE. This hypercapnia persisted for the remainder of the fetal study period until just prior to birth when there was no differences in the Pa_{CO_2} between the control and T-UPE fetuses (Figure 4-5C). There was no difference in the Pa_{CO_2} of control and P-UPE fetuses before the commencement of UPE, however, by 125d GA, P-UPE fetuses had higher Pa_{CO_2} than controls and remained hypercapnic until just prior to birth (Figure 4-5C). There was no difference in the Pa_{CO_2} of T-UPE and P-UPE fetuses between 125 and 135d GA.

Arterial pH of the P-UPE fetuses did not differ from that of the controls and the T-UPE fetuses during the period of embolisation (Figure 4-5D). The T-UPE fetuses were acidemic compared with controls at 130d GA, and again just prior to birth, but did not differ from controls at other times (Figure 4-5D).

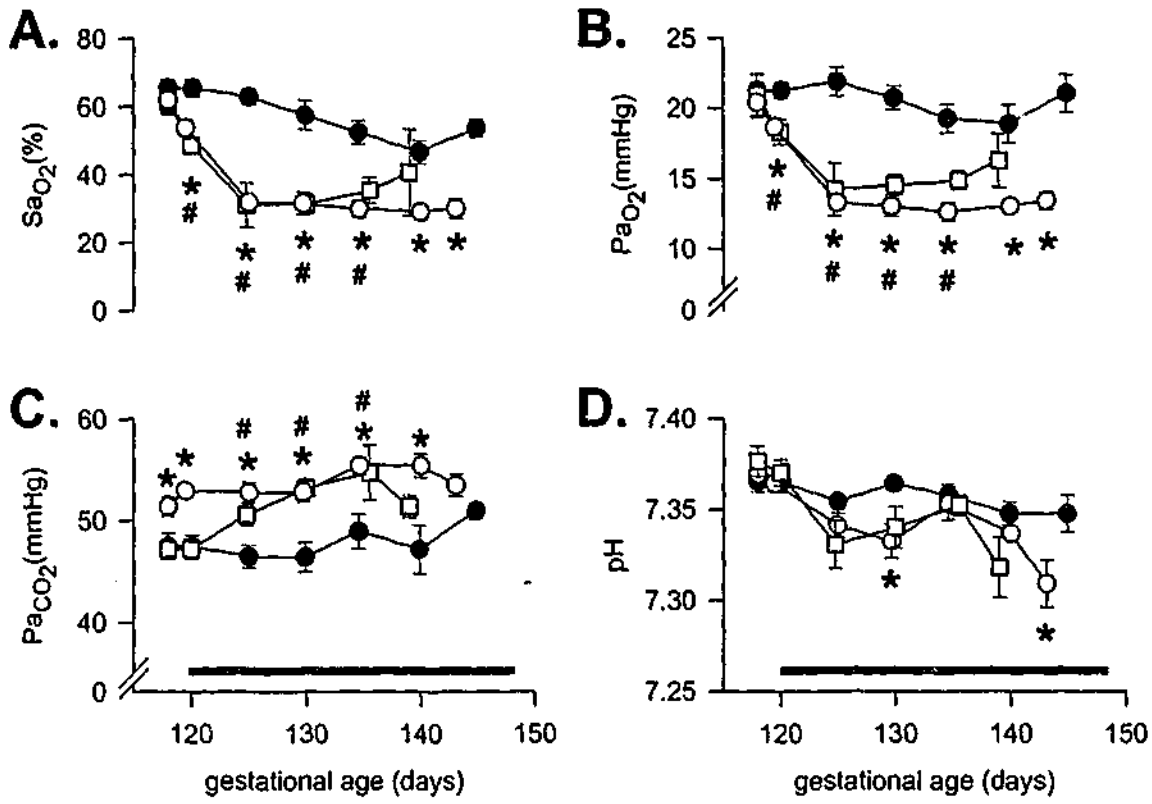


Figure 4-5. Fetal arterial blood gas and pH status from 118d until birth. (A) Arterial oxygen saturation (Sa_{O_2}), partial pressures of (B) oxygen (Pa_{O_2}) and (C) carbon dioxide (Pa_{O_2}) and (D) pH in control (●, n=9), T-UPE (○, n=10) and P-UPE (□, n=6) fetuses in the pre-embolisation (118–119d GA) and the umbilico-placental embolisation (120d GA until birth, indicated by the bar) periods. * indicates values that differ between control and T-UPE fetuses, # indicates values that differ between control and P-UPE fetuses ($p<0.05$).

4.4.2b Hemoglobin concentration and hematocrit

Fetal hemoglobin concentrations did not differ between the three groups of fetuses at the commencement of embolisation. However, the UPE fetuses had higher hemoglobin concentrations compared with controls at 125d GA and remained approximately 2g/dL higher than in controls until birth (Figure 4-6A).

Similarly, hematocrit did not differ between the three groups of fetuses prior to embolisation, but increased in the UPE fetuses soon after the onset of UPE (Figure 4-6B). The hematocrit of T-UPE fetuses was significantly higher than that of controls from 125d GA until birth, and the hematocrit of P-UPE fetuses was significantly higher than that of controls from 130d GA (Figure 4-6B). On average, the hematocrit of the

UPE fetuses was 6–7% higher than that of controls during this period, but did not differ between T-UPE and P-UPE fetuses.

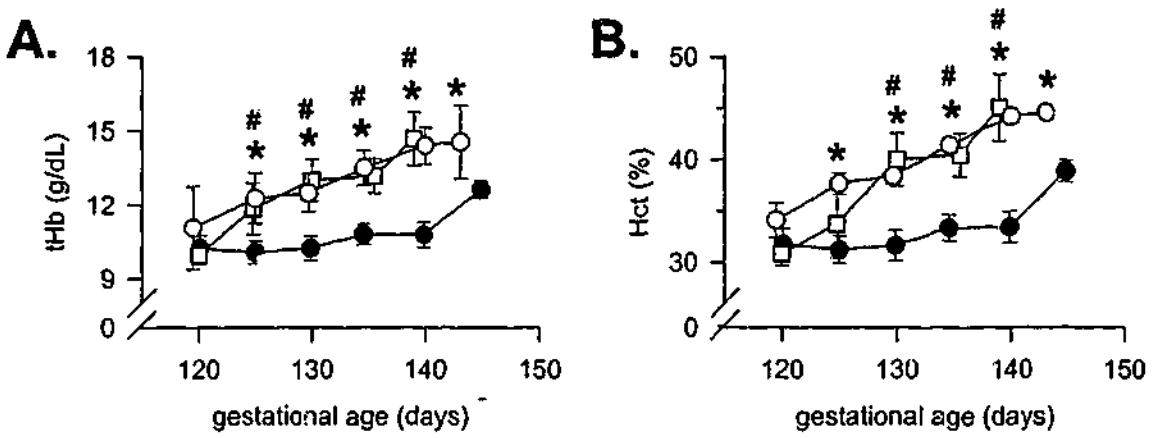


Figure 4-6. Fetal hemoglobin concentrations and hematocrit during umbilico-placental embolisation. (A) Hemoglobin concentration (tHb) and (B) hematocrit (Hct) in control (●, n=9), T-UPE (○, n=10) and P-UPE (□, n=6) fetuses during the period of umbilico-placental embolisation (120d GA until birth). * indicates values that differ between control and T-UPE fetuses, # indicates values that differ between control and P-UPE fetuses ($p<0.05$).

4.4.2c Blood glucose and lactate concentrations

There was a significant effect of treatment group on blood glucose concentrations during the period of embolisation. Throughout this period, T-UPE fetuses were hypoglycemic relative to controls (0.46±0.02mmol/l *vs* 0.68±0.03mmol/l, $p<0.05$; Figure 4-7A). In this same period, the blood glucose concentrations in P-UPE fetuses (0.64±0.06mmol/l) did not differ from that of control fetuses (Figure 4-7A). Blood glucose concentrations varied within the P-UPE group (n=6), with three of the fetuses being hypoglycemic relative to controls and the remaining three fetuses being normoglycemic. Blood lactate concentrations were not different in the three groups of fetuses during the period of umbilico-placental embolisation (Figure 4-7B).

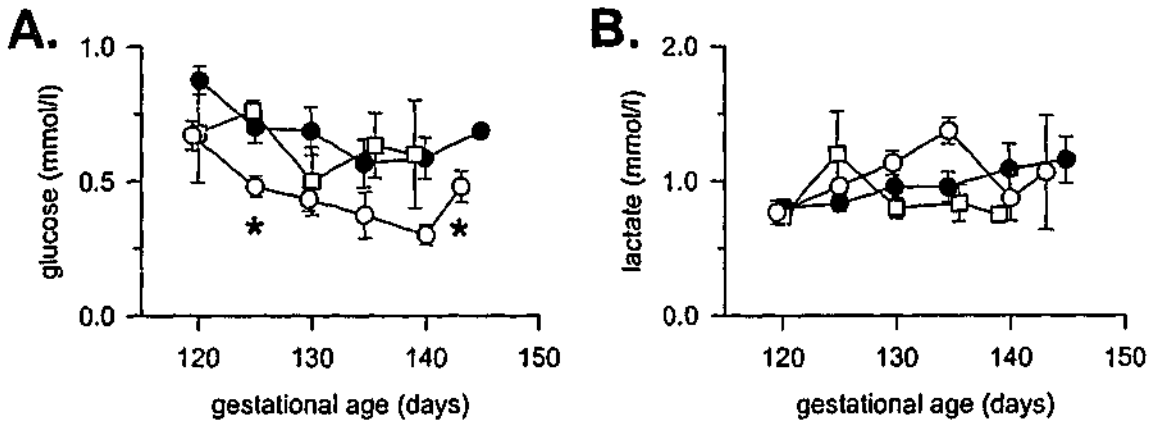


Figure 4-7. Fetal blood glucose and lactate concentrations during umbilico-placental embolisation. (A) Blood glucose and (B) blood lactate concentrations in control (●, n=9), T-UPE (○, n=10) and P-UPE (□, n=6) fetuses during the period of umbilico-placental embolisation (120d GA until birth). * indicates values that differ between control and T-UPE fetuses.

4.4.2d Plasma cortisol concentrations

Figure 4-8A shows the plasma cortisol concentrations from control, T-UPE and P-UPE fetuses during the period of UPE. While both the control and T-UPE fetuses showed an increase in their plasma cortisol concentrations with increasing gestation, there were no differences in the cortisol levels of the two groups at any of these ages. While the P-UPE fetuses, like the control and T-UPE fetuses, showed a pre-partum increase in cortisol concentration, this occurred approximately one week earlier than in the animals that delivered at term (Figure 4-8A). While the fetuses that delivered preterm had significantly higher circulating cortisol concentrations at 134d and 140d GA compared with the control and T-UPE groups, the maximal levels were similar between the three groups. When the cortisol concentration was plotted against age expressed as the number of days before birth rather than gestational age (Figure 4-8B), there were no differences in cortisol concentration in the three groups of animals.

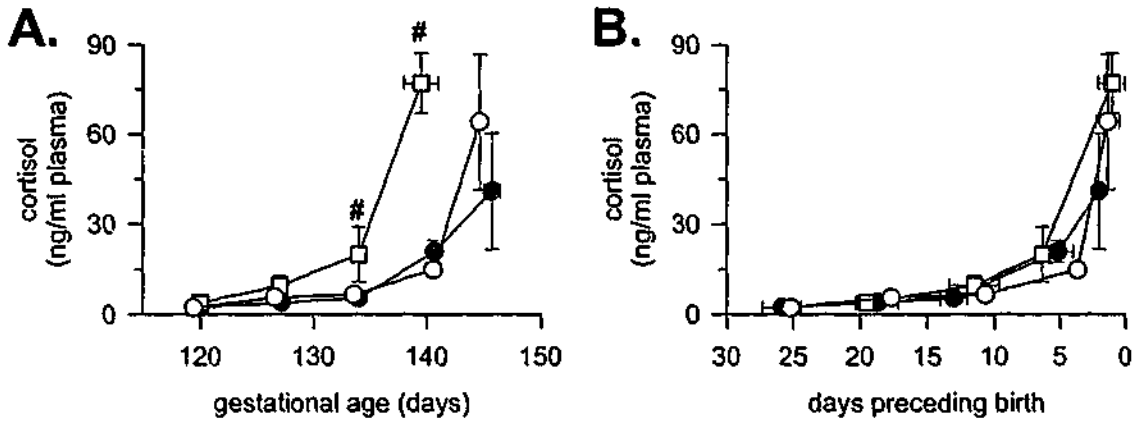


Figure 4-8. Fetal plasma cortisol concentrations from 120d GA until birth. (A) Fetal plasma cortisol concentrations in control (●, n=6), T-UPE (○, n=9) and P-UPE (□, n=4) during the period of umbilico-placental embolisation (120d GA until birth). (B) Fetal plasma cortisol concentrations in control, T-UPE and P-UPE fetuses plotted against age expressed as the number of days preceding birth. # indicates values that differ between control and P-UPE fetuses ($p<0.05$).

4.4.3 Lambs used for postnatal studies

Details of the lambs used in the postnatal component of this study are summarised in Table 4-2. In total, there were 37 lambs used in this study; 28 of these were studied throughout the two years, an additional 6 animals were studied at the two year age point only and the data from three animals were excluded from the studies (details below). Including the 6 sheep studied at two years only, there were 9 animals that were studied postnatally but were not studied *in utero*. One animal (#15), was a control animal that was studied from birth and throughout the two year period. Six control animals (#R1, #R2, #R3, #R4, #R5, #R6) were purchased from pasture at two years of age for the inclusion in the final studies (arterial pressure, body size, DXA, post mortem and arterial wall mechanical properties) to ensure gender equality between the control and IUGR groups. Two lambs included in the IUGR group had not undergone UPE but were naturally occurring low birth weight animals. These lambs were two in a set of triplets; one lamb was used by another researcher for another study and the remaining two lambs were included in this study.

Lambs that died or were killed before the completion of the two year study period are indicated in Table 4-2. Two lambs were killed prior to two years of age due to poor

health and have been excluded from the study results; animal #54 was killed at 91d due to a sudden onset condition that resulted in the lamb's loss of the ability to right itself and maintain its body temperature, and animal #68 was killed at 69d due to an untreatable leg infection. Animal #17 died at 7 months of age from a ruptured aortic aneurism; it was unclear when this aneurism developed and the data from this lamb has been excluded from the results of this study. Two sheep died just prior to the completion of the two year study. Lamb #33 died from exsanguination following the loss of a carotid artery catheter prior to the intended post mortem date; the *in vivo* studies involving this sheep were completed at the time of death and have been included in the results section. Lamb #31 died in pasture prior to its final arterial pressure measurement; all data from this animal (including DXA results) have been included in the results section.

Table 4-2. The histories of lambs used in the postnatal studies described in this chapter. This table summarises the identification numbers, gender, treatment (Trt) group, birth details and post mortem (PM) details for the lambs used in the postnatal studies. All lambs had undergone fetal surgery at ~116d GA unless indicated by an asterisk (*) – these animals were unoperated as fetuses and were studied postnatally only. Animals R1, R2, R3, R4, R5 and R6 were purchased from pasture at two years of age; their body weights and gestational age at birth are unknown and they were included in the final studies at two years of age only.

"C" refers to control lambs and "IUGR" refers to lambs that were growth restricted at birth, either as a result of umbilico-placental embolisation or spontaneous growth restriction. All lambs were born spontaneously except where indicated with a † – these lambs were induced to be born early due to poor fetal health.

Three of the lambs died spontaneously during the two year study period and two lambs were killed before the completion of the two years of study; refer to Section 4.4.3 for details of causes of death and reasons for premature post mortems.

Lamb ID	Sex	Singleton/ Twin	Trt	DOB	GA at birth (d)	Birth weight (kg)	PM date	Age at PM (d)
15	♀	Singleton	C*	17/07/98	148	6.15	09/03/01	966
16	♂	Twin	IUGR†	30/07/98†	140	2.20	10/04/01	985
17	♀	Twin	IUGR	30/07/98†	140	2.34	Died 23/02/99, 208d	
18	♂	Twin	IUGR	30/07/98†	140	2.10	10/04/01	985
13	♀	Singleton	C	20/08/98	140	3.05	09/03/01	932
25	♀	Triplet	IUGR*	19/10/98	144	2.75	08/03/01	871
24	♂	Triplet	IUGR*	19/10/98	144	2.85	12/04/01	906
73	♀	Twin	IUGR	14/09/98	137	2.29	08/03/01	906
29	♂	Twin	IUGR	10/11/98	145	3.60	04/04/01	876
33	♂	Twin	IUGR	13/11/98	148	3.74	Died 12/04/01, 881d	
31	♀	Singleton	C	29/11/98	150	5.02	Died 13/03/01, 835d	
30	♀	Singleton	C	30/11/98	144	3.48	19/04/01	871
35	♂	Twin	IUGR	03/12/98	140	2.25	19/04/01	858
34	♀	Twin	IUGR	03/12/98	140	2.88	19/04/01	858
52	♂	Singleton	C	19/06/99	149	4.85	23/07/01	765
53	♂	Twin	IUGR	29/06/99	144	2.09	23/07/01	755
54	♀	Twin	IUGR	29/06/99	144	2.52	Killed 28/09/98, 91d	
55	♀	Singleton	C	08/07/99	148	4.95	07/08/01	761
56	♀	Singleton	C	10/07/99	150	4.80	07/08/01	759
57	♀	Twin	IUGR	18/07/99	143	2.45	07/08/01	751
58	♂	Twin	IUGR	18/07/99	143	2.10	10/09/01	785
59	♂	Twin	IUGR	10/08/99	144	2.44	10/09/01	762
60	♂	Twin	IUGR	10/08/99	144	2.91	10/09/01	762
61	♂	Twin	IUGR	09/08/99	143	2.34	12/09/01	765
62	♂	Twin	IUGR	09/08/99	143	2.68	12/09/01	765
93	♀	Singleton	C	01/09/99	146	4.22	16/10/01	807
46	♀	Singleton	C	02/09/99	147	3.83	16/10/01	806
65	♀	Singleton	C	06/11/99	148	4.36	04/12/01	759
66	♀	Singleton	IUGR	13/11/99	'	2.74	04/12/01	752
67	♀	Singleton	IUGR	16/11/99	144	3.39	04/12/01	749
68	♂	Singleton	C	17/11/99	145	5.27	Killed 25/01/00, 69d	
R1	♂	Unknown	C*	Aug 1998	–	–	24/04/01	–
R2	♂	Unknown	C*	Aug 1998	–	–	24/04/01	–
R3	♂	Unknown	C*	Aug 1998	–	–	01/05/01	–
R4	♂	Unknown	C*	Aug 1998	–	–	01/05/01	–
R5	♂	Unknown	C*	Aug 1998	–	–	03/05/01	–
R6	♂	Unknown	C*	Aug 1998	–	–	03/05/01	–

4.4.4 Gestational age at birth

The distribution of gestational ages at birth of the control and IUGR animals is shown in Figure 4-9. All controls were born at term (147±1d GA), with the exception of one animal that was born spontaneously at 140d GA. Other than this animal being hypercapnic, acidemic and mildly hypoxemic for final 3–4 days *in utero*, prenatal and postnatal data from this animal does not differ from that of controls that delivered at term and has therefore been classed as a control animal.

The gestational ages at birth for the IUGR animals ranged from 137–148d GA. Of the IUGR cohort, 12 (including the 2 naturally occurring IUGR cases) were born at term (143–148d GA) and 6 animals were born preterm (137–141d GA). The distinction between term and preterm animals was made by calculating the gestational age that was 2 standard deviations below the mean GA at birth for control animals that have been studied in our laboratory – this was 142d GA and was based on data from 27 control animals. IUGR animals that were born at term (>142d GA) will be referred to as T-IUGR animals, those born preterm (<142d GA) will be referred to as P-IUGR animals and the naturally occurring IUGR animals will be referred to as U-IUGR animals. When these three groups of IUGR animals have been combined, they will be collectively referred to as IUGR animals.

The mean gestational age at birth for the total cohort of IUGR animals was less than that of controls (143±1d GA *vs* 147±1d GA). While the P-IUGR animals were born earlier (140±1d GA) than controls and the other IUGR lambs, the gestational age at birth of the T-IUGR (144±1d GA) and U-IUGR (144d GA) animals did not differ from that of controls.

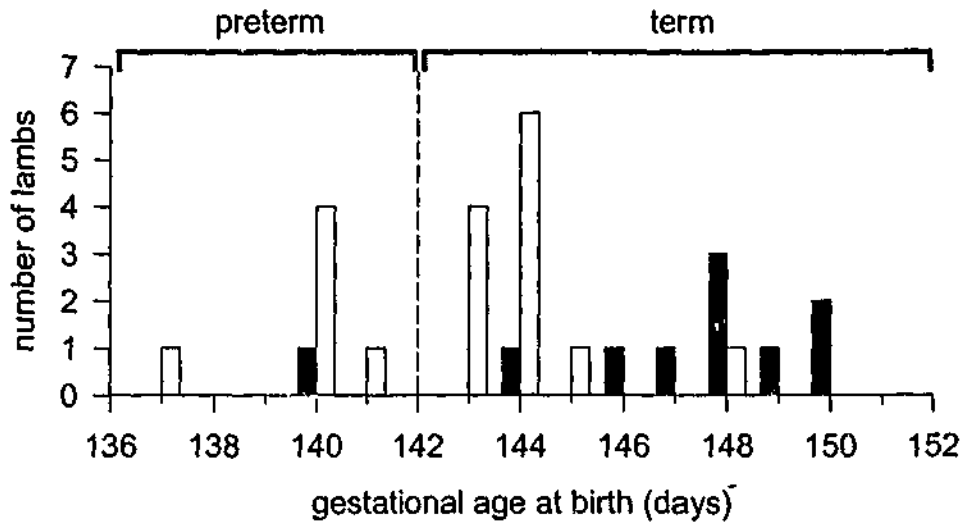


Figure 4-9. Distribution of gestational ages at birth. Gestational age at birth in the cohort of the control (■, n=10) and IUGR (□, n=18) lambs used in the postnatal studies. Lambs were classed as preterm if they were born before 142d GA; this age was determined by calculating the mean–2SD for control lambs born in our laboratory (n=27).

4.4.5 Body weight at birth

Figure 4-10 shows the distribution of birth weights in the control and IUGR lambs, which ranged from 2.09kg to 6.15kg. While there were not two distinct birth weight groups, all control animals had birth weights of at least 3kg and the majority (88%) of IUGR animals had birth weights less than 3kg. Lambs were classed as IUGR if they had undergone UPE in the fetal period (n=16) or were naturally of low birth weight, as in the case of the two U-IUGR animals. The mean (±SD) birth weight of a large series of control animals born in our laboratory was 4.5±0.8kg (n=27); the majority (n=14) of the IUGR animals in this cohort had birth weights less than 2SD below this mean birth weight and one lamb was marginally above this limit (2.91kg).

While it might be expected that the P-IUGR animals would have lower birth weights than those IUGR animals that were born at term, similar numbers of T-IUGR and P-IUGR lambs were classed in the birth weight groups <3.0kg; all P-IUGR and 75% of T-IUGR lambs had birth weight less than 3kg (Figure 4-10B).

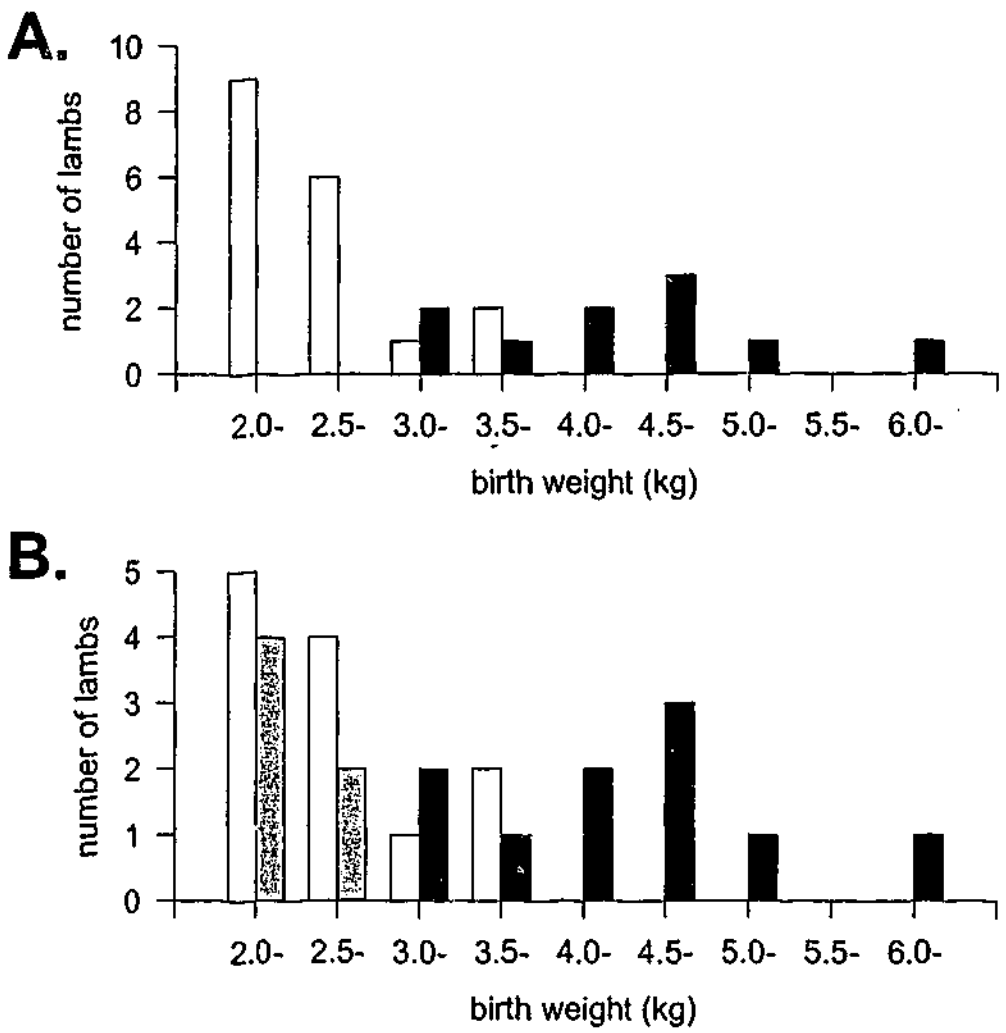


Figure 4-10. Distribution of body weights at birth. (A) Body weights at birth in the cohort of the control (■, n=10) and IUGR (□, n=18) lambs used in the postnatal studies. (B) Birth weights of the control (■) and IUGR lambs divided into those that were born at term (□, n=12) and preterm (▣,n=6).

Figure 4-11 shows the mean birth weights for the control, T-IUGR, P-IUGR and U-IUGR lambs. All three IUGR groups had significantly lower birth weights compared with controls (4.47±0.28kg); T-IUGR (2.77±0.19kg) and U-IUGR (2.80±0.05kg) lambs were 37–38% lighter than controls and P-IUGR lambs (2.41±0.13kg) were 46% lighter than controls at birth. The mean birth weights of the three groups of IUGR lambs were not significantly different to each other.

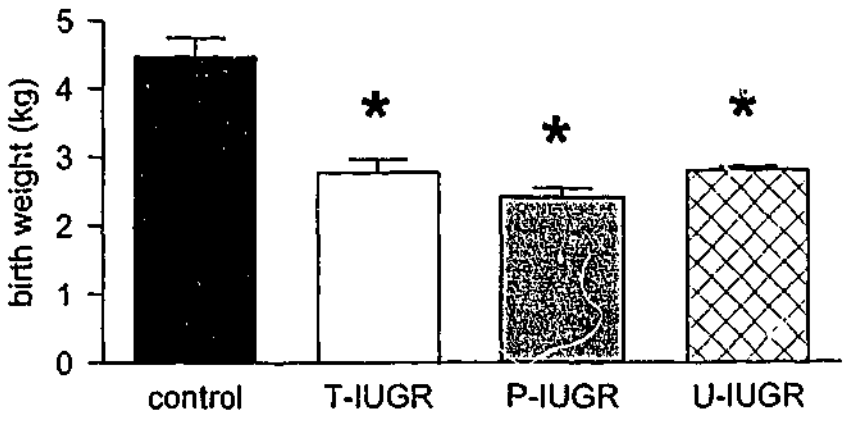


Figure 4-11. Body weight at birth of control and IUGR animals. Mean birth weights of control (■, n=10), T-IUGR (□, n=10), P-IUGR (▨, n=6) and U-IUGR (☒, n=2) lambs used in the postnatal studies. Asterisks (*) indicate significant differences when compared with controls ($p<0.05$).

4.4.6 Body size at birth

Within the complete IUGR cohort, there was a spectrum of birth weights ranging from 2.09kg to 3.74kg. Given that there was no statistical difference in the birth weight or gestational age at birth of the T-IUGR and U-IUGR lambs, these two groups were combined. While the mean gestational age at birth for the P-IUGR lambs was significantly less than that of the term lambs, their birth weights were not significantly different to the T-IUGR animals (Figure 4-11), nor did their fetal blood parameters differ greatly from those measured in the T-IUGR (Figures 4-5–4-8). Lambs were defined as preterm if their gestational age at birth was less than 142d GA, and this was an arbitrary limit. 80% of lambs classed as T-IUGR lambs were born within 2 days of this 142d limit, and 83% of P-IUGR lambs were born on, or after 140d. Given that of the total IUGR cohort (n=18), 15 of the lambs were born between 140–144d GA, it separation of lambs into preterm and term groups cannot be fully justified. For the remainder of this chapter, the results presented will be comparisons between the control (n=10) and combined IUGR (n=18) groups unless otherwise indicated.

Figure 4-12 shows the body weight, body length and ponderal index of control and IUGR lambs at birth. The mean birth weight of the total cohort of IUGR lambs (2.66±0.12kg) was 41% less than that of controls (Figure 4-12A). Body length, measured as crown-to-rump length (Figure 4-12B), was reduced by 9% in the IUGR

lambs at birth (51.5±1.3cm *vs* 46.7±1.2cm). IUGR lambs tended (p=0.1) to have lower ponderal indexes ($2.8e^{-5}$±$0.1e^{-6}$kg/cm^3) than control animals ($3.1e^{-5}$±$0.2e^{-6}$kg/cm^3) at birth, indicating that the IUGR lambs were light for their body length (i.e. thin compared with the controls; Figure 4-12C).

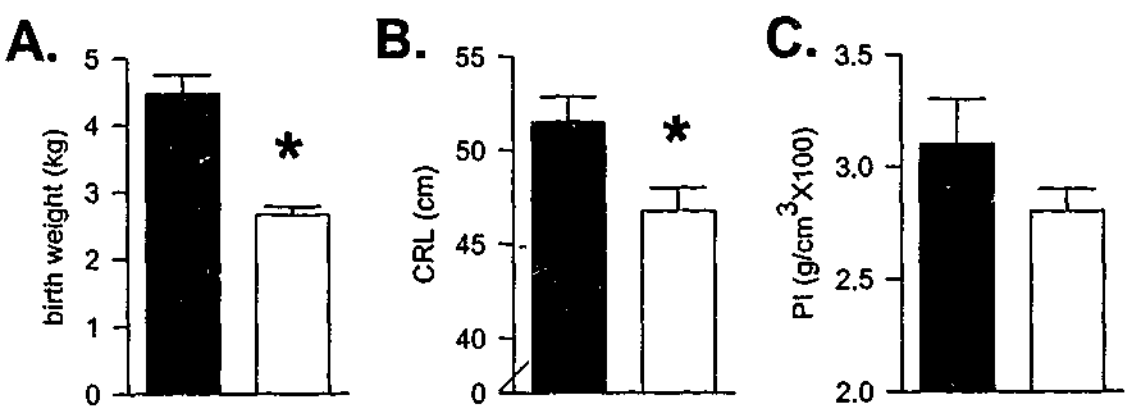


Figure 4-12. Body size at birth of control and IUGR animals. Mean (A) body weights, (B) crown-to-rump length (CRL) and (C) ponderal index (PI) of control (■, n=10) and IUGR (□, n=18) animals at birth. Asterisks (*) indicate significant differences when compared with controls (p<0.05).

4.4.7 Postnatal growth

4.4.7a Body weight

During the first 12 postnatal weeks, IUGR lambs had significantly lower body weights compared with controls. The IUGR lambs weighed less than controls at 12 weeks (3 months) of age (20.1±1.3kg *vs* 14.9±1.0kg; p<0.05), but by 6 months there was no difference between the body weights of the control (29.8±2.0kg) and IUGR lambs (26.1±1.5kg). After 6 months of age, the body weights of the IUGR lambs did not differ from those of control lambs (Figure 4-13).

Figure 4-14 shows the body weights of the two groups of sheep at two years of age. This figure includes the data from the 6 rams purchased from pasture. The mean body weight of the IUGR sheep (61.2±1.4kg) was not significantly different to that of the controls (66.6±3.4kg). When the data were analysed by gender, there was also no difference in the body weights of female controls (56.5±1.7kg) and female IUGR sheep

(58.7±1.2kg) but male IUGR sheep (62.4±1.9kg) remained significantly lighter than controls (78.2±3.3kg) at this age.

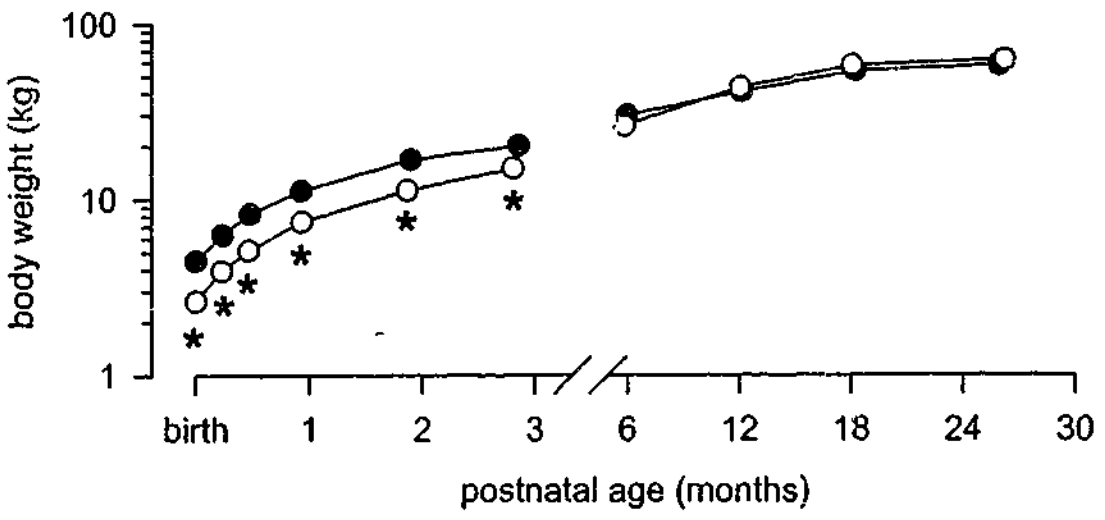


Figure 4-13. Body weights of lambs from birth to two years of age. Mean body weight in control (●, n=10) and IUGR (○, n=18) lambs from birth to two years of age. Asterisks (*) indicate values that differ between groups ($p<0.05$). N.B. The 6 rams purchased at two years of age have not been included.

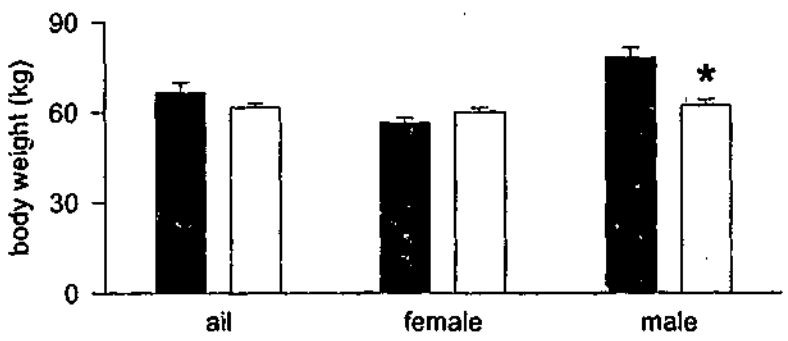


Figure 4-14. Body weight at two years of age. Mean body weight of control (■) and IUGR (□) sheep at two years of age for all animals (left; ■, n=15; □, n=18), females (centre; ■, n=8; □, n=6) and males (right; ■, n=7; □, n=12). Asterisk (*) indicates a significant difference between groups ($p<0.05$).

4.4.7b Growth rates

The absolute growth rates (g/day) for the control and IUGR lambs are shown in Figure 4-15A. The IUGR lambs gained less weight in the first 8 weeks compared with controls (Figure 4-15A), thus remained lighter than controls during this period (Figure 4-15A). With the exception of the period between 6 and 12 months during which IUGR lambs gained more weight/day compared with controls, there were no differences in the absolute growth rates of the two groups after 12 weeks of age.

Given that the IUGR lambs were smaller at birth, it might be expected that they gained less weight per day compared with controls, but if their smaller body size was accounted for, it might be expected that the weight gain would be similar between the two groups. Figure 4-15B shows the relative increase in body weight (i.e. weight increase expressed as a percentage of the preceding weight). There was no difference in the relative weight gain of the two groups of lambs during the first 8 postnatal weeks and therefore the IUGR lambs remained smaller than controls. For the IUGR lambs to "catch-up" in body weight to controls, they would have needed to exhibit greater growth rates than the controls. The IUGR lambs had greater relative increases in weight compared with controls for the period between 8 and 12 postnatal weeks (1.17±0.13%/day *vs* 0.68±0.07%/day, $p<0.05$). At 8 weeks, the IUGR lambs were 33% lighter than controls, and although they were still significantly lighter at 12 weeks, the body weights of the IUGR lambs were only 26% less than those of controls. By 6 months of age, IUGR lambs were only 12% lighter than controls. This catch-up in body weight was the result of a continued increased growth rate between 3 and 6 months of age in the IUGR lambs (0.83±0.08%/day *vs* 0.51±0.06%/day, $p<0.05$). The IUGR lambs continued to exhibit increased weight gain until 12 months of age, at which time they had fully caught up in body weight to the controls.

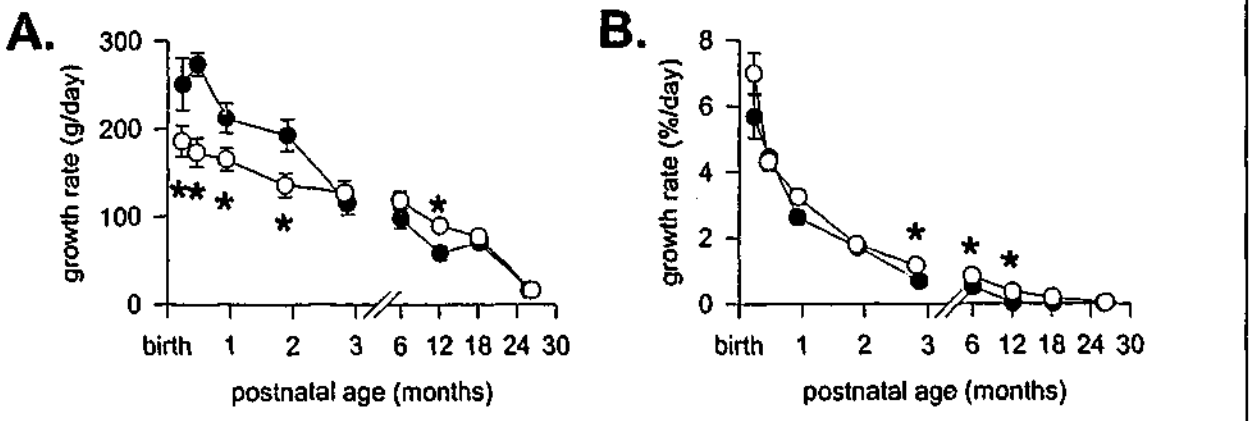


Figure 4-15. Postnatal growth rates from birth to two years of age. (A) Absolute growth rates (g/day) and (B) relative weight gain (%/day) in control (●, n=10) and IUGR (○, n=18) lambs between birth and two years of age. Asterisks (*) indicate values that differ between groups ($p<0.05$). N.B. The 6 rams purchased at two years of age have not been included.

4.4.7c Body dimensions at two years

Body length and height There was no difference in the crown-to-rump lengths of the control and IUGR sheep, however, analysis by gender showed that male IUGR sheep had shorter body lengths compared with the controls (142.1±1.5cm *vs* 134.0±3.0cm; Figure 4-16A). Similarly, while there were no overall differences in the heights of the two groups of sheep, the male IUGR sheep were shorter in stature compared with the male controls at two years, as indicated by a reduced shoulder height (77.4±1.2cm *vs* 82.4±1.4cm; Figure 4-16B).

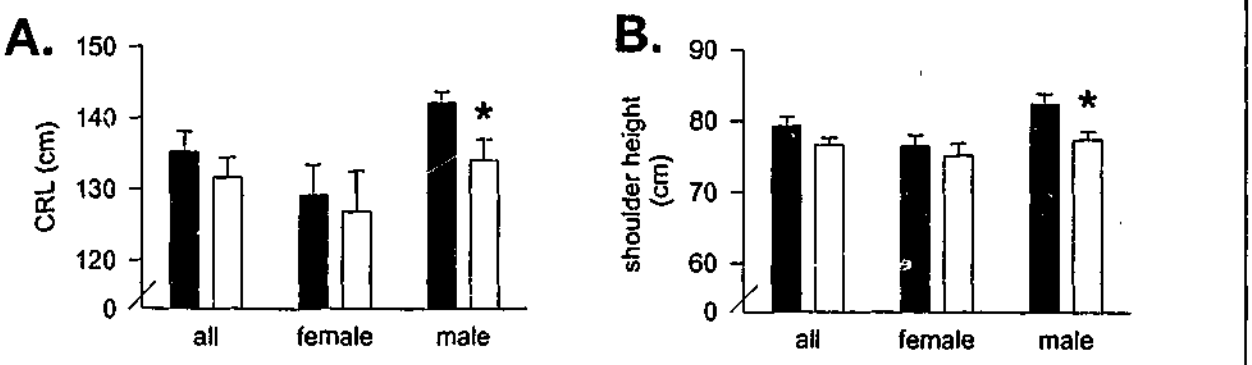


Figure 4-16. Body length and height of sheep at two years of age. (A) Crown-to-rump length (CRL) and (B) shoulder height in control (■) and IUGR (□) sheep at two years of age. Mean data for each parameter are shown for all animals (left; ■, n=15; □, n=17), females (centre; ■, n=8; □, n=6) and males (right; ■, n=7; □, n=11). Asterisks (*) indicate values that differ between groups ($p<0.05$).

Body girths There were no significant differences in the thoracic girth measurements between control (108.4±1.5cm) and IUGR (107.3±1.5cm) sheep at two years of age. Abdominal girth also did not differ between the control (123.5±1.4cm) and IUGR (121.5±1.5cm) sheep at this age, nor were the hip circumferences different between the two groups (control: 114.2±1.6cm, IUGR: 113.1±1.6cm).

Head size There were no significant differences in the measures of head size (length of the head, and biparietal diameter) between the control and IUGR sheep at two years (head length: control 28.5±0.5cm, IUGR 28.5±0.4cm; biparietal diameter: control 12.1±0.2cm, IUGR 12.0±0.2cm).

Lengths of limb bones There was no difference in the humerus length in the two groups of sheep (control: 20.6±0.5cm, IUGR: 19.9±0.4cm), however, there were some differences in the lengths of the other two forelimb bones, in particular in the males, consistent with the shorter shoulder heights of IUGR lambs. IUGR sheep tended ($p=0.07$) to have shorter ulnas than controls, and analysis by gender showed that while there was no difference between the female control and IUGR sheep (Figure 4-17A). Male IUGR sheep had significantly shorter ulna lengths compared with the male controls (Figure 4-17A). Similarly, there were no differences in the length of the metacarpal bones of female animals, but the metacarpus in male IUGR sheep was shorter than in male controls (Figure 4-17B). There were no differences between control and IUGR sheep in the lengths of the two hind limb bones that were measured (tibia: control 27.4±0.6cm, IUGR 26.7±0.9cm; metatarsus: control 19.5±0.5cm, IUGR 19.5±0.5cm).

Ponderal Index While IUGR sheep tended to be thinner at birth, as indicated by a reduced ponderal index, there was no difference in the mean ponderal index of the control ($2.7e^{-5}\pm0.1e^{-6}kg/cm^3$) and IUGR ($2.8e^{-5}\pm1.8e^{-6}kg/cm^3$) sheep at two years of age.

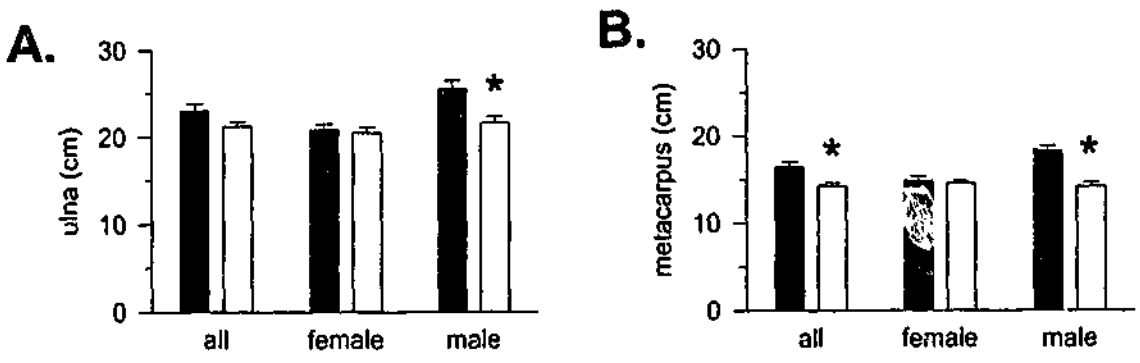


Figure 4-17. Limb bone lengths of sheep at two years of age. Length of the (A) ulna and (B) metacarpus in control (■) and IUGR (□) sheep at two years of age. Mean data for each parameter are shown for all animals (left; ■, n=15; □, n=17), females (centre; ■, n=8; □, n=6) and males (right; ■, n=7; □, n=11). Asterisks (*) indicate values that differ between groups ($p<0.05$).

4.4.7d Body composition at two years

Figure 4-18 shows the amounts (in kg) of lean tissue (muscle), fat and bone mineral in the two year old sheep, as determined by DXA. There was no difference in the amount of lean tissue in controls compared with IUGR sheep overall, however, analysis by gender showed that female IUGR sheep had significantly less lean muscle (38.3±1.1kg) compared with controls (35.3±0.5kg, $p<0.05$). Although IUGR animals in general tended to have less body fat compared with controls (9.0±0.8kg *vs* 11.1±1.0kg; $p=0.1$) there appeared to be gender-specific differences with respect to body fat (Figure 4-18B). While female IUGR sheep tended to have a greater fat mass compared with controls (12.0±1.0kg *vs* 9.6±0.9kg; $p=0.1$), male IUGR sheep had significantly less body fat than male controls (7.3±0.7kg *vs* 12.9±1.8kg, $p<0.05$). Male IUGR sheep also had lower bone mineral content compared with controls (13.7±0.1kg *vs* 18.2±0.1kg); this difference was not evident in females (Figure 4-18C).

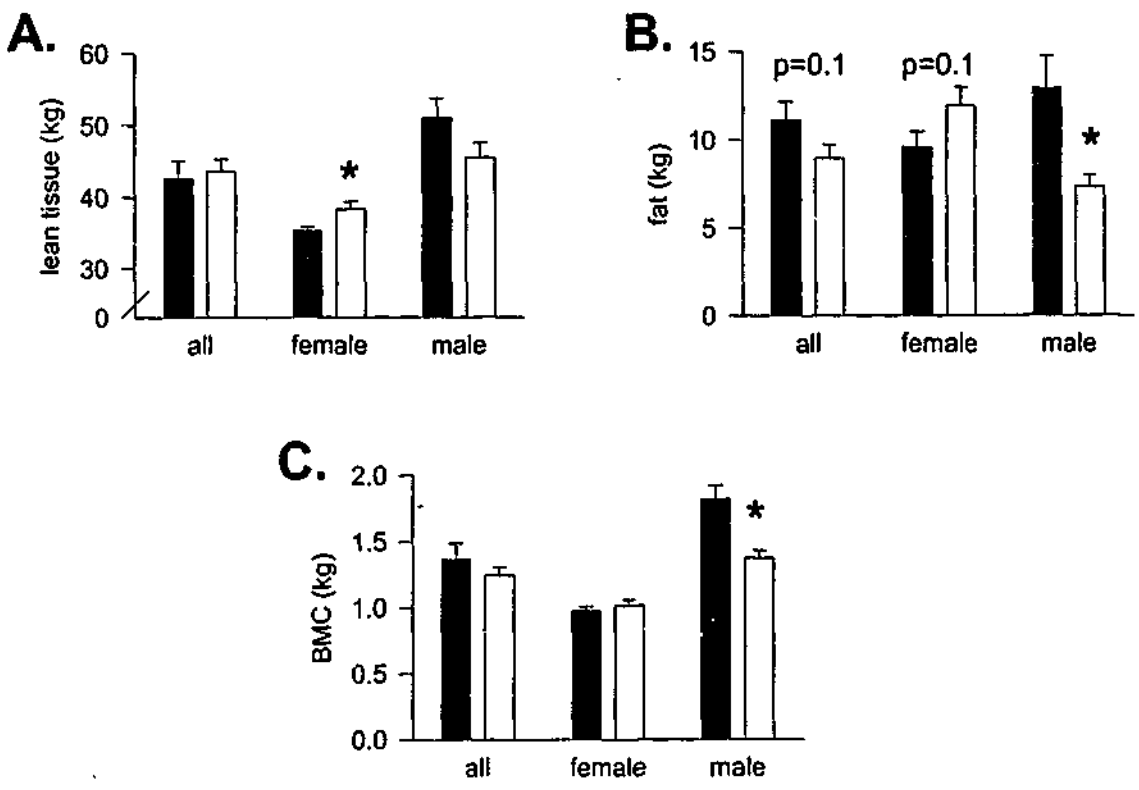


Figure 4-18. Amounts of lean tissue, fat and bone mineral in sheep at two years of age as determined by dual emission x-ray absorptiometry. (A) Lean tissue (muscle) mass, (B) fat mass and (C) bone mineral content of control (■) and IUGR (□) sheep at two years of age. Mean data for each parameter are shown for all animals (left; ■, n=15; □, n=17), females (centre; ■, n=8; □, n=6) and males (right; ■, n=7; □, n=11). Asterisks (*) indicate values that differ between groups ($p<0.05$).

Figure 4-19 shows the relative amounts of each tissue type, as measured by DXA. After adjustment for total body mass, IUGR tended to have a greater relative proportion of lean tissue compared with controls (81.0±1.3% *vs* 77.6±1.2%, p=0.1); this difference was significant in males (84.5±0.7% *vs* 78.1±2.0%, $p<0.05$; Figure 4-19A). Overall, IUGR sheep tended to have a smaller proportion of body fat than controls (16.7±1.4% *vs* 20.0±1.2%; p=0.08). Female IUGR sheep tended to have more body fat than controls (23.2±1.3% *vs* 20.7±1.5%; p=0.07), while male IUGR sheep had significantly lower proportions of body fat (13.1±0.7%) compared with controls (19.2±2.1%). There was no difference in the bone mineral content between controls and IUGR sheep overall, or in females (Figure 4-19C). However, male IUGR sheep had a reduced bone mineral content compared with controls (2.5±0.1% *vs* 2.8±0.1%).

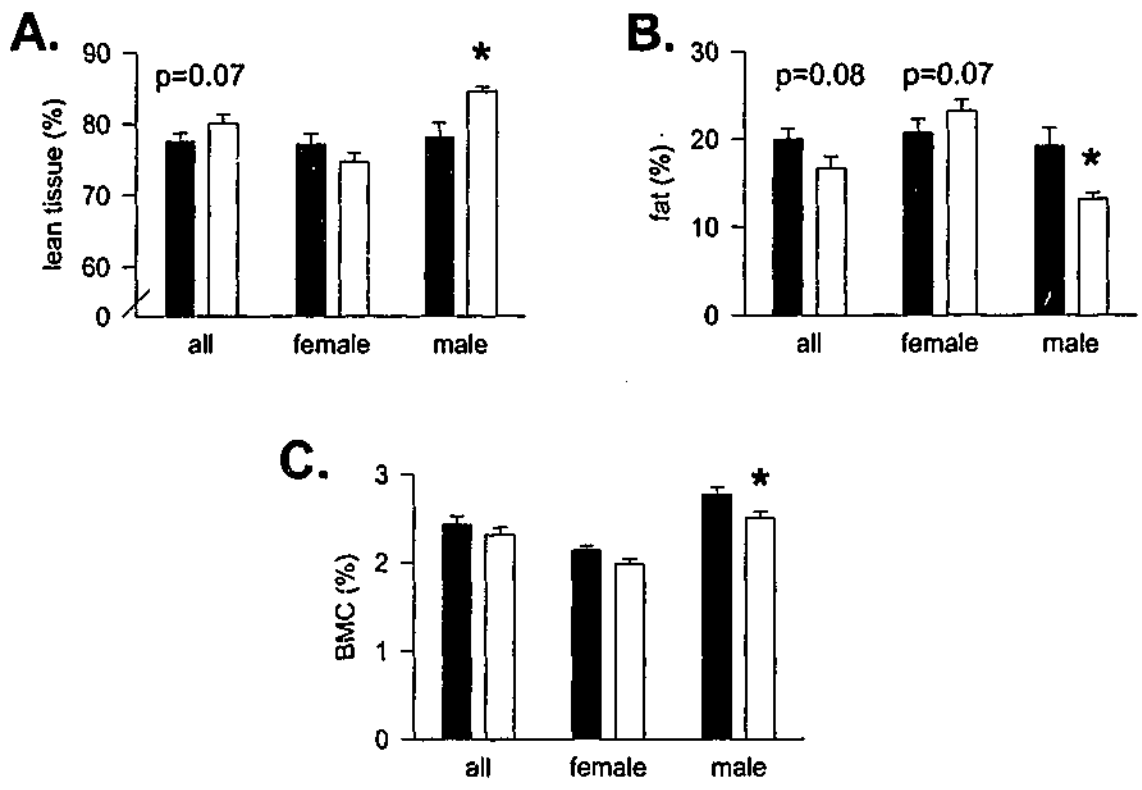


Figure 4-19. Relative amounts of lean tissue, fat and bone mineral in sheep at two years of age as determined by dual emission x-ray absorptiometry. Relative proportions of (A) lean tissue (muscle), (B) fat and (C) bone mineral in control (■) and IUGR (□) sheep at two years of age. Mean data for each parameter are shown for all animals (left; ■, n=15; □, n=17), females (centre; ■, n=8; □, n=6) and males (right; ■, n=7; □, n=11). Asterisks (*) indicate values that differ between groups ($p<0.05$).

Bone mineral density was determined by the DXA by dividing the bone mineral content by the surface area of the bones in the body. There was no overall difference in the bone densities of control and IUGR sheep (control: 0.99±0.04g/cm^2, IUGR: 0.99±0.02g/cm^2), or in the female sheep alone (control: 0.86±0.02g/cm^2, IUGR: 0.90±0.02g/cm^2). The male IUGR sheep had significantly lower bone mineral densities (1.03±0.02g/cm^2) compared with the male controls (1.1±0.03g/cm^2, $p<0.05$).

4.4.8 Postnatal arterial blood parameters

4.4.8a Arterial blood gases and pH

Figure 4-20 shows the arterial blood gases and pH for the control and IUGR lambs in the postnatal period between birth and two years of age. In the first postnatal week,

IUGR lambs had lower arterial P_{O_2} compared with controls (Figure 4-20B), but did not differ from controls after two weeks of age; there were no differences in the Sa_{O_2} between the two groups at any age (Figure 4-20A). The IUGR lambs were hypercapnic compared with controls at three days of age, but after one week, Pa_{CO_2} was not different between the two groups (Figure 4-20C). There was no difference in the arterial pH of the two groups of lambs, except at two weeks of age, when the IUGR lambs were acidemic relative to controls (Figure 4-20D).

4.4.8b Hemoglobin concentration and hematocrit

While the IUGR animals had chronically elevated hemoglobin concentrations and hematocrit in late gestation during UPE, there were no differences in the hemoglobin concentrations of the two groups of lambs except at 12 and 18 months of age (Figure 4-21A). There were no differences in the hematocrits between the two groups except at 12 months of age (Figure 4-21B).

4.4.8c Blood glucose and lactate concentrations

There was no difference in the blood glucose concentrations (non-fasted) in the two groups of lambs throughout the two year study period (control: 3.6±0.1mmol/l, IUGR: 3.4±0.1mmol/l). Similarly, there were no differences blood lactate concentrations of the two groups of lambs during this same period (control: 0.8±0.1mmol/l, IUGR: 0.8±0.1mmol/l).

4.4.8d Plasma cortisol concentrations

Throughout the postnatal study period, the plasma cortisol concentrations in IUGR lambs was lower than that in control lambs (11.5±0.9 *vs* 17.1±1.9ng/ml; Figure 4-22A) but there were no differences between the two groups at any age. There was also a significant effect of postnatal age on cortisol concentrations with the concentration decreasing in both groups with increasing age. There were no differences in the plasma cortisol concentrations in the female IUGR and control sheep, nor were there differences between two groups in males (Figure 4-22B).

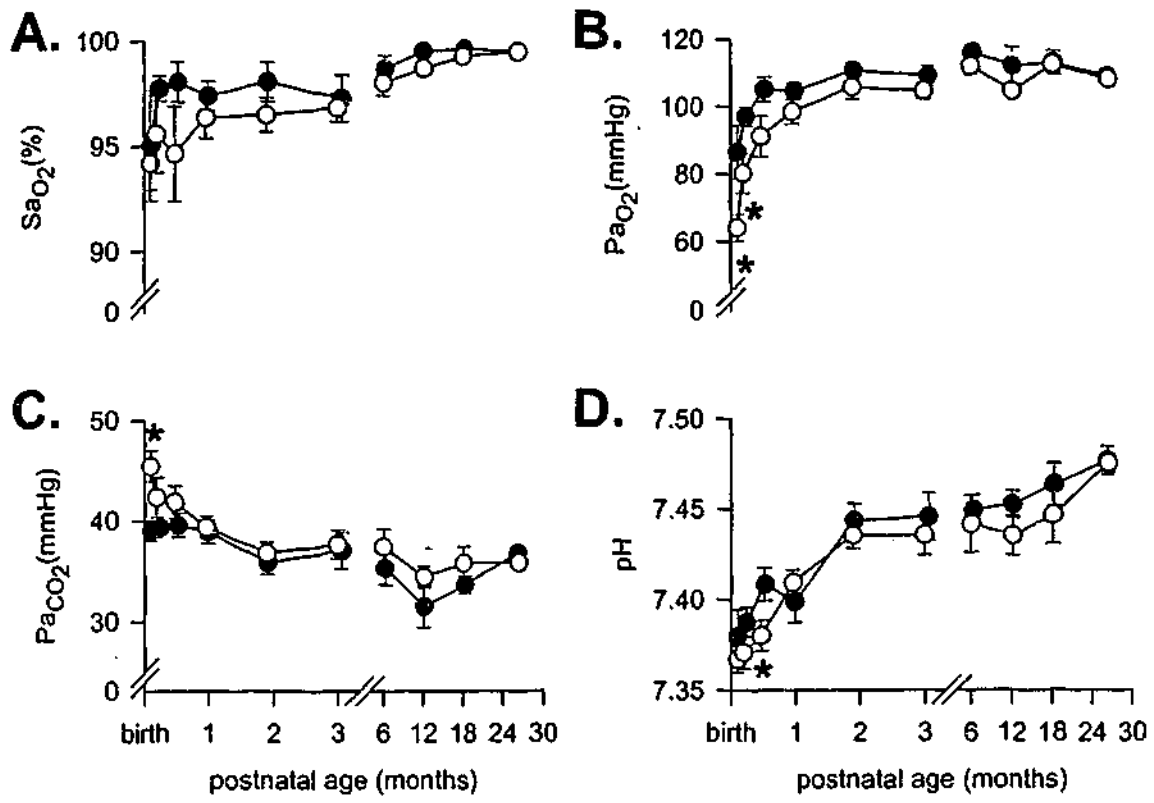


Figure 4-20. Postnatal arterial blood gas status. (A) Arterial oxygen saturation (Sa_{O_2}), partial pressures of (B) oxygen (Pa_{O_2}) and (C) carbon dioxide (Pa_{CO_2}) and (D) pH in control (●, n=10) and IUGR (○, n=18) lambs from birth to two years of age. Asterisks (*) indicate values that differ between groups ($p<0.05$).

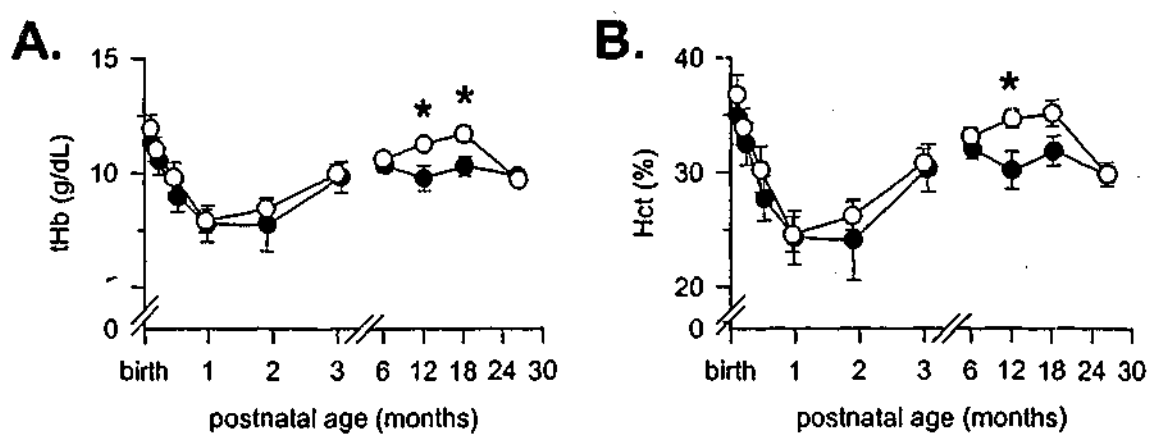


Figure 4-21. Postnatal hemoglobin concentration and hematocrit. (A) Hemoglobin concentration (tHb) and (B) hematocrit (Hct) in control (●, n=10) and IUGR (○, n=18) lambs from birth to two years of age. Asterisks (*) indicate values that differ between groups ($p<0.05$).

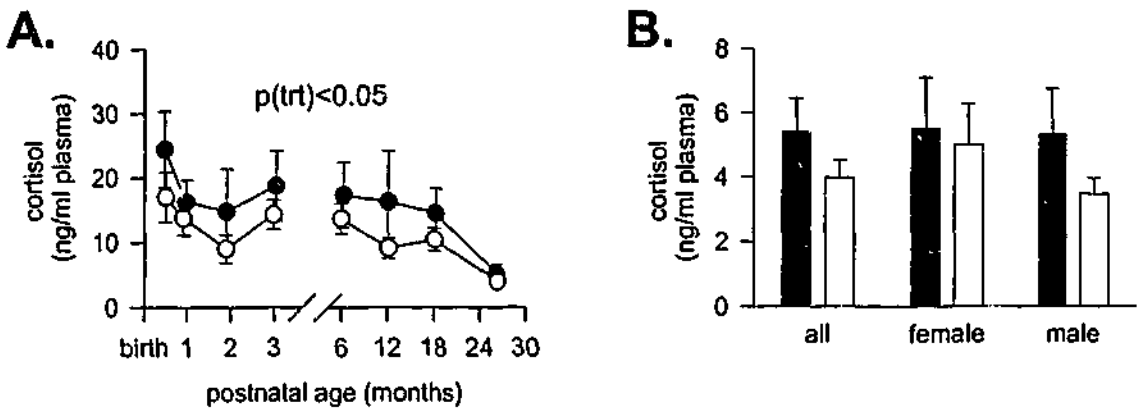


Figure 4-22. Plasma cortisol concentrations in lambs from birth to two years of age. (A) Plasma cortisol concentrations in control (●, n=10) and IUGR (○, n=18) lambs from birth to two years of age. p(trt)<0.05 indicates a significant effect of treatment as found by a two-way ANOVA. N.B. The 6 rams purchased at two years of age have not been included in the two year age point. (B) Plasma cortisol concentrations in control (■; includes 6 purchased rams) and IUGR (□) sheep at two years of age. Mean data is shown for all animals (left; ■, n=15; □, n=17), female (centre; ■, n=8; □, n=6) and male (right; ■, n=7; □, n=11).

4.4.9 Postnatal arterial pressure and heart rate

Arterial pressure and heart rate were recorded for 75.5±1.5 minutes at each study age up to 18 months. An average of 64.0±1.5 minutes (84.5±0.9%) of recording was included for analysis from each animal at these ages for analysis. At two years of age, arterial pressure and heart rate were recorded for 186.5±1.1 minutes per day, over two consecutive days. Thus, on average, 156.6±1.9 minutes (83.9±0.8 %) of each recording was included for analysis.

4.4.9a Arterial pressure

Figure 4-23 shows the arterial pressure and heart rate measurements in the control and complete IUGR cohort up to two years of age. There was a significant effect of treatment group (analysis by two-way ANOVA) on MAP, systolic and diastolic pressure throughout the two year study period (Figure 4-23). On average, MAP was 3.2±1.4mmHg lower in the IUGR animals throughout the two years compared with controls; MAP was significantly lower in the IUGR lambs compared with controls only at two months of age. Diastolic pressure was, on average, 2.3±1.4mmHg lower in the

IUGR sheep compared with controls throughout the two year period, with a significant difference between the two groups at two months of age. Systolic pressure was significantly lower in the IUGR lambs soon after birth compared with controls and was, on average, 6.4±1.9mmHg lower in the IUGR lambs throughout the two year period.

In Figure 4-24, the IUGR lambs have been divided into the T-IUGR, P-IUGR and U-IUGR groupings. Throughout the two year postnatal period, all lambs that had undergone UPE in late gestation had lower arterial pressure than controls. The mean difference in MAP, systolic and diastolic pressure between controls and these IUGR lambs (irrespective of whether they were born at term or preterm) were 4.0±1.4mmHg, 6.1±1.6mmHg and 3.0±1.4mmHg, respectively ($p<0.05$ for all). The arterial pressures of T-IUGR lambs did not differ from those of P-IUGR lambs during this period. The arterial pressures of the two U-IUGR lambs was not different to those of the controls during the two year study period. The average MAP for the two year period was 80.6±1.6mmHg in the U-IUGR group and 77.0±1.0mmHg for the controls.

4.4.9b Heart rate

Heart rate decreased with age in all animals, but was not different between the control and IUGR lambs during the two year study period (Figures 4-23D, 4-24D).

4.4.9c Arterial pressure at two years of age

Figure 4-25 shows the arterial pressure of the control and IUGR (separated groups) sheep at two years of age. There were no differences in MAP, systolic or diastolic pressures between the 4 groups at this age. While there was no difference in the MAP of the control and IUGR (complete cohort) sheep (control: 83.7±2.3mmHg, IUGR: 79.2±2.1mmHg) at two years of age (Figure 4-26), male IUGR sheep had significantly lower MAP than controls (78.7±2.8mmHg *vs* 89.4±3.0mmHg). Similarly, while there were no differences in the systolic and diastolic pressures of control and IUGR sheep at this age in females or in the total cohort, systolic and diastolic pressure were 12.3±5.1mmHg and 10.0±3.7mmHg lower ($p<0.05$) respectively in the male IUGR sheep compared with controls (Figure 4-26).

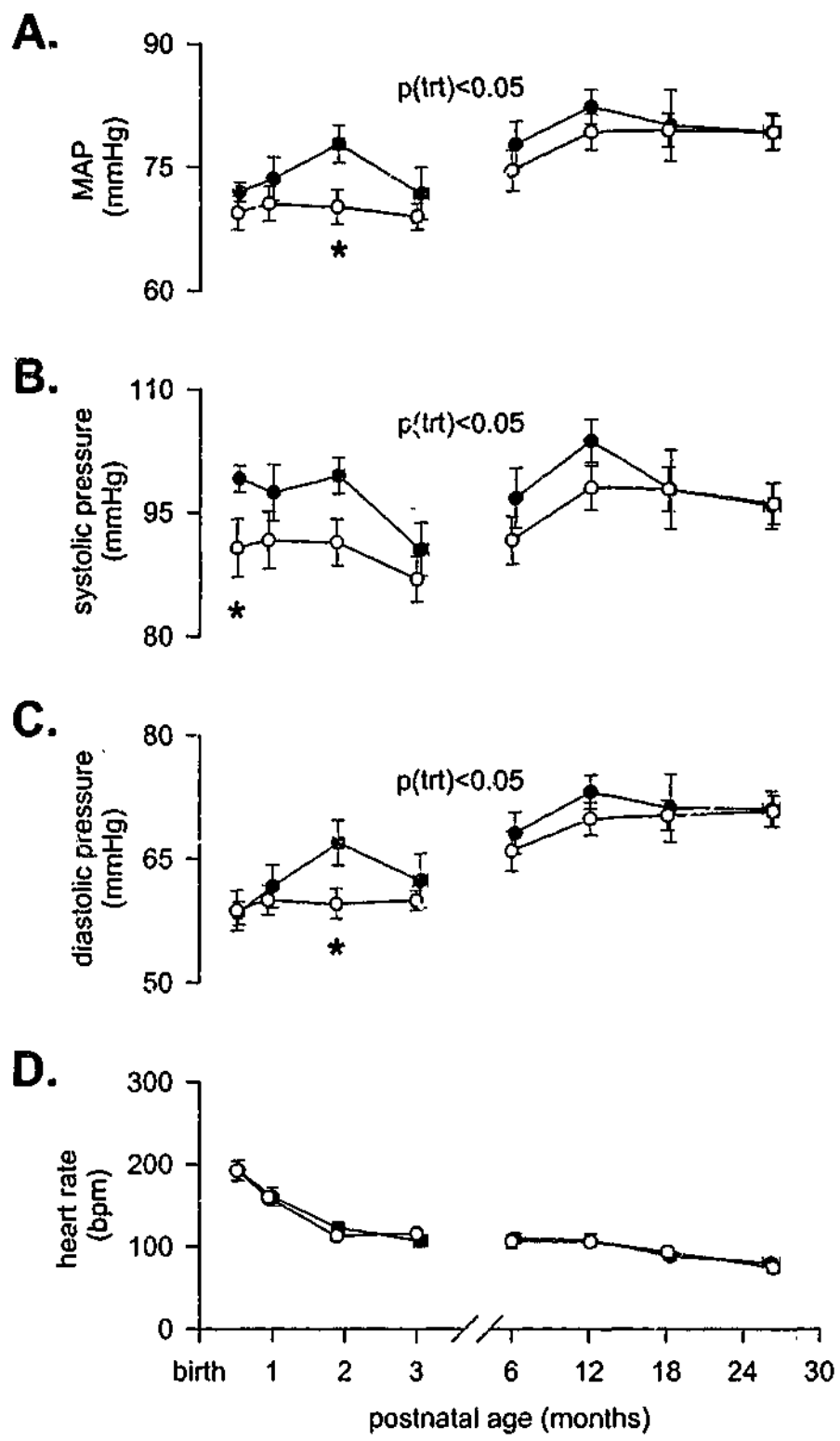


Figure 4-23. Arterial pressure and heart rate of lambs from birth to two years of age. (A) Mean arterial pressure (MAP), (B) systolic pressure, (C) diastolic pressure and (D) heart rate in control (●, n=10) and IUGR (○, n=18) lambs from birth to two years of age. p(trt)<0.05 indicates a significant effect of treatment as found by a two-way ANOVA; Asterisks (*) indicate values that differ between groups at individual ages ($p<0.05$). N.B. The 6 rams purchased at two years of age have not be included.

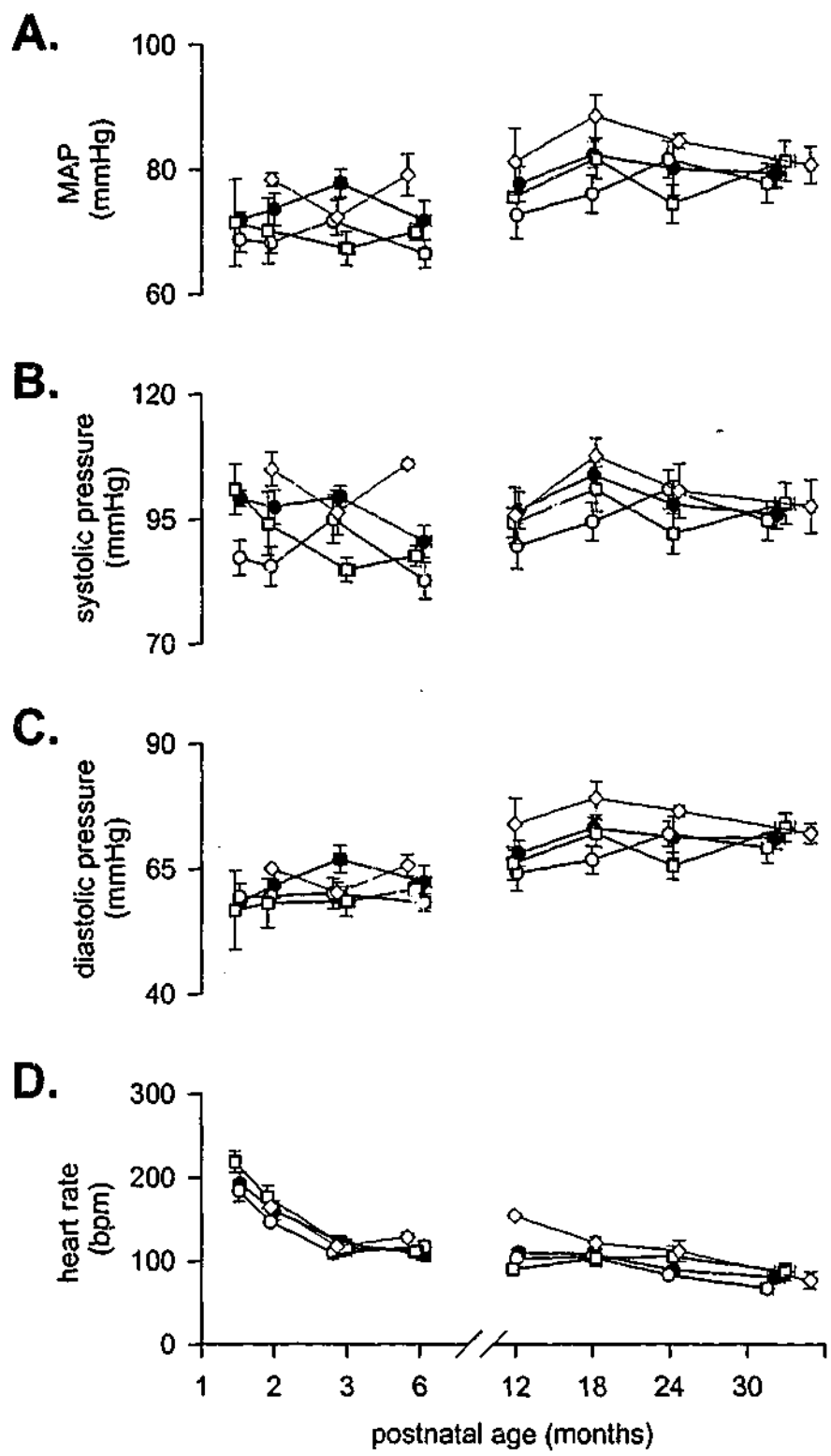


Figure 4-24. Arterial pressure and heart rate of IUGR lambs from birth to two years of age. (A) Mean arterial pressure (MAP), (B) systolic pressure, (C) diastolic pressure and (D) heart rate in control (●, n=10), T-IUGR (○, n=10), P-IUGR (□, n=6) and U-IUGR (◊, n=2) lambs from birth to two years of age. N.B. The 6 rams purchased at two years of age have not been included.

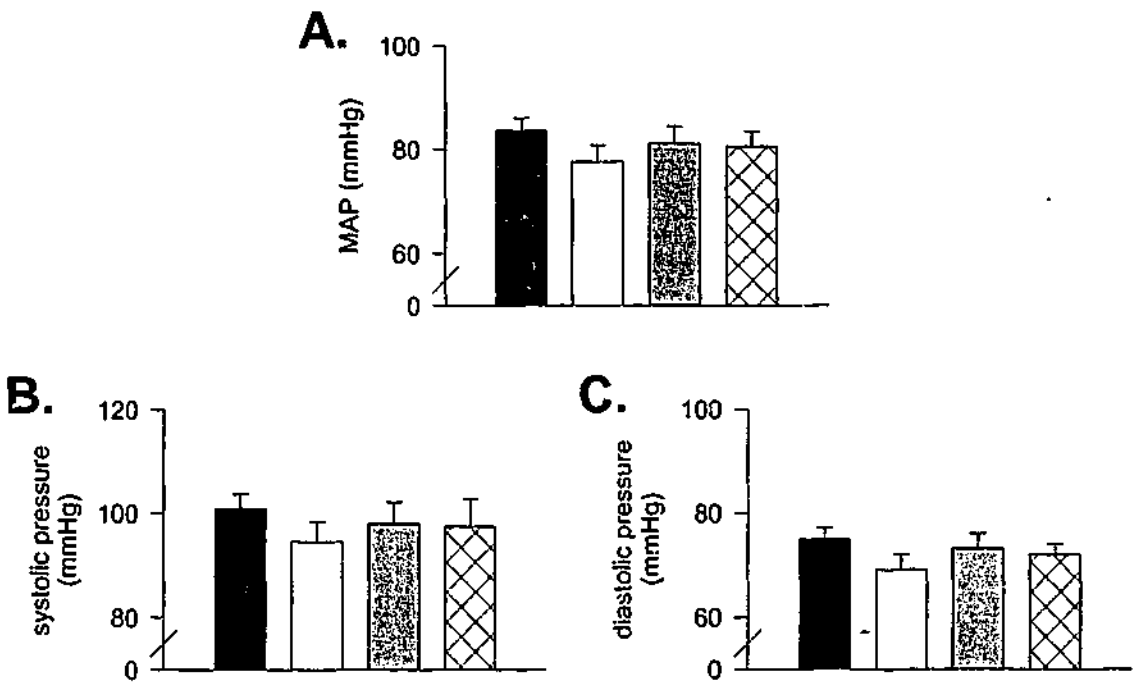


Figure 4-25. Arterial pressure of IUGR sheep at two years of age. (A) Mean arterial pressure (MAP), (B) systolic pressure and (C) diastolic pressure in control (■, n=15), T-IUGR (□, n=10), P-IUGR (▪, n=6) and U-IUGR (⊠, n=2) sheep at two years of age.

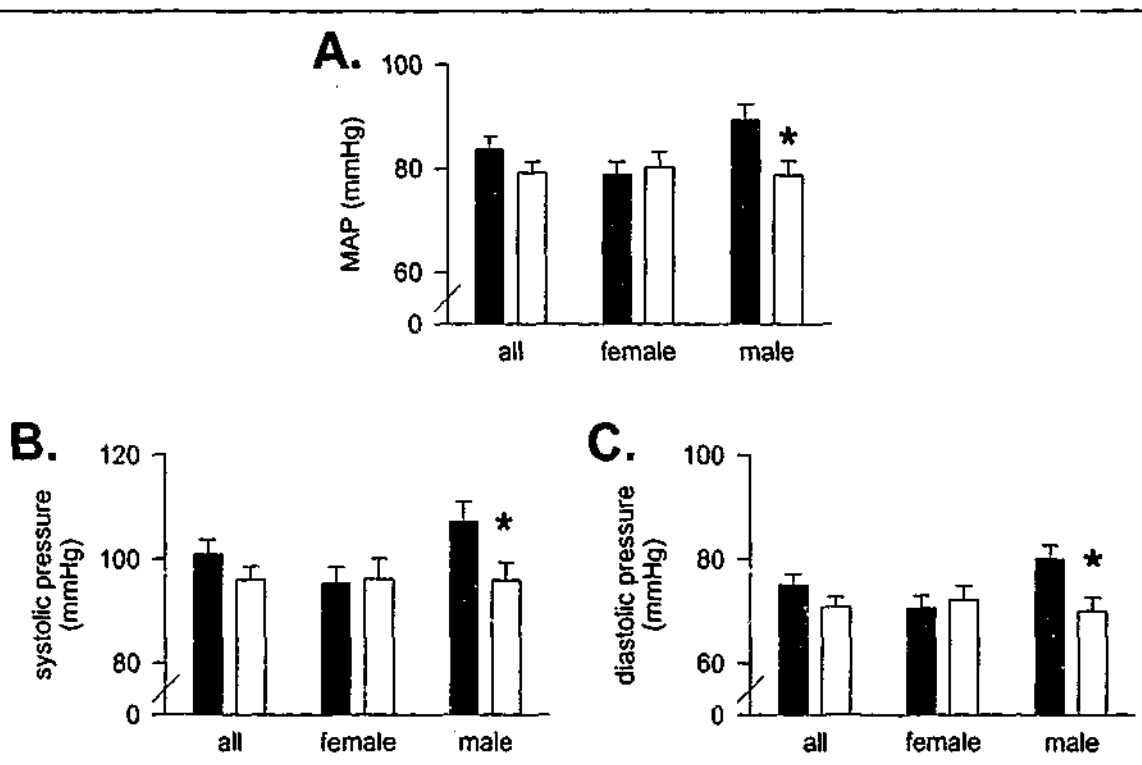


Figure 4-26. Effect of gender on arterial pressure of sheep at two years of age. (A) Mean arterial pressure (MAP), (B) systolic pressure and (C) diastolic pressure in control (■) and IUGR (□) sheep at two years of age. Mean data for each parameter are shown for all animals (left; ■, n=15; □, n=17), female (centre; ■, n=8; □, n=6) and male (right; ■, n=7; □, n=11). Asterisks (*) indicate values that differ between groups ($p<0.05$).

4.4.9d Correlations

While arterial pressure was lower in the IUGR animals throughout the two year period, mean arterial pressure at two years of age was not correlated with any of the fetal blood gas parameters, fetal glucose concentration or fetal cortisol concentration. Similarly, MAP was not correlated with birth weight (r=−0.16, p=0.3; Figure 4-27A). MAP at two years was also not correlated with postnatal growth rate (Figure 4-27B) or with body size and composition at two years of age. Cortisol concentrations at two years were not correlated to birth weight (r=0.07, p=0.7) or arterial pressure (correlation with MAP: r=0.07, p=0.7).

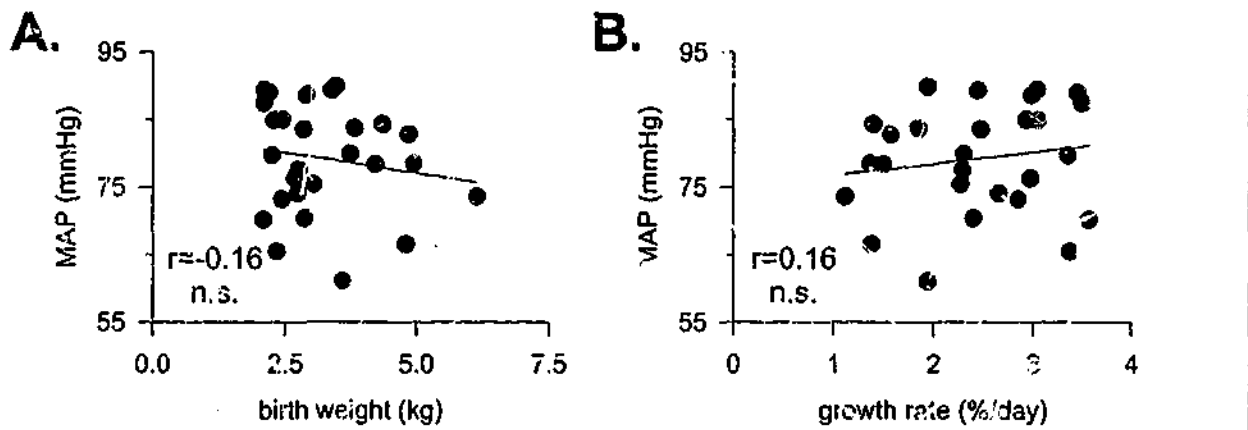


Figure 4-27. Mean arterial pressure at two years: Relationship to birth weight and postnatal growth rate. The relationship between MAP at two years of age and (A) birth weight and (B) overall growth rate throughout the two years (% weight increase/day). r value refers to Pearsons correlation coefficient for the data from the total cohort of animals; n.s. indicates correlation was not statistically significant.

Fetal Sa_{O_2} and birth weight were strongly correlated, indicating that the fetuses who had lower mean Sa_{O_2} had lower birth weights (r=0.79, p<0.01). A similar relationship was found with mean fetal Pa_{O_2} and birth weight (r=0.71, p<0.01). Postnatal growth rates were significantly correlated with fetal Sa_{O_2} and birth weight (Figure 4-28). The average arterial oxygen saturation between 120d GA and birth was calculated and was found to be strongly correlated (r=−0.93, p<0.01) with overall postnatal weight gain (Figure 4-28A). Thus, fetuses that had lower mean Sa_{O_2} (and also Pa_{O_2}) had higher postnatal growth rates than those fetuses with higher arterial oxygen. Similarly, birth weight was correlated (r=−0.84, p<0.01) with postnatal growth rate throughout the two years (Figure 4-28B), indicating that the lambs with lower birth weights exhibited

higher postnatal growth rates. These correlations are consistent with the finding that the IUGR lambs (that were hypoxemic fetuses and had lower birth weights) were able to catch up in body weight to control animals.

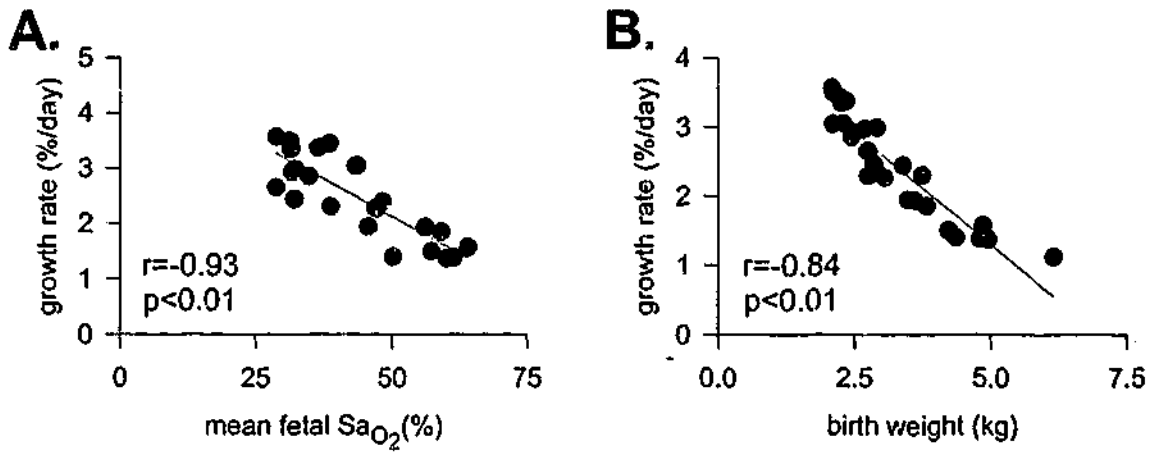


Figure 4-28. Postnatal growth rate: Relationship to fetal Sa_{O_2} and birth weight. The relationship between overall growth rate throughout the two years (% weight increase/day) and (A) mean fetal arterial saturation from 120d GA until birth and (B) birth weight. r value refers to Pearsons correlation coefficient for the data from the total cohort of animals; p values indicates the level of statistical significance.

4.4.10 Organ weights

The weights of the major organs weighed at post mortem are shown in Table 4-3. The combined (left and right) adrenal weight in the IUGR sheep was significantly greater than in controls; however, after adjustment for total body weight, the adrenal weights of the two groups were not statistically different. Lung weight (g/kg body weight) tended to be higher in the IUGR sheep compared with controls. There were no significant differences in the absolute, or adjusted weights of the other organs.

Table 4-3. Organ weights at two years of age from control and IUGR sheep. Data shown are absolute organ weights (g) and organ weights adjusted for body weight (g/kg BWt). Asterisks (*) indicate values that differ between groups ($p<0.05$). † includes rumen contents; # combined left and right organ weight.

		Control (n=15)	IUGR (n=17)
Heart	(g)	266.5±11.4	259.1±8.2
	(g/kg BWt)	4.06±0.15	4.22±0.11
Lung	(g)	643.2±44.7	664.2±38.0
	(g/kg BWt)	9.56±0.28	10.85±0.58
Liver	(g)	870.0±44.7	835.9±28.2
	(g/kg BWt)	13.18±0.48	13.61±0.35
Gut†	(kg)	11.7±0.4	11.1±0.4
	(g/kg BWt)	0.18±0.01	0.18±0.01
Kidneys#	(g)	182.6±8.0	181.0±5.6
	(g/kg BWt)	2.77±0.97	2.94±0.59
Adrenals#	(g)	3.9±0.3	3.1±0.1*
	(g/kg BWt)	0.06±0.003	0.05±0.003
Abdom. Fat	(g)	1956.7±210.9	1469.7±289.5
	(g/kg BWt)	30.42±3.61	24.15±4.75
Brain	(g)	103.2±2.3	99.0±1.4
	(g/kg BWt)	1.61±0.97	1.62±0.04

4.4.11 Passive mechanical properties of the arterial wall

4.4.11a Branch of the basilar artery

Arterial dimensions (μm) during pressurisation There was no significant difference in the mean starting OD of the basilar artery branches collected from the two groups of sheep (control: 371±15μm *vs* IUGR: 379±23μm; pressurised at 5mmHg). In both groups, vessel diameters increased, and wall thickness decreased with increasing pressures but the absolute size of the arteries with respect to OD, ID or WT did not differ between the groups at any pressure (Appendix 4-1). At 80mmHg, there were no significant differences in the cross-sectional areas of the lumen or arterial wall (Table 4-4).

Table 4-4. Cross-sectional area (CSA) of the basilar artery branch at 80mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 80mmHg intraluminal pressure (~MAP) for the two groups of sheep.

	Control (n=15)	IUGR (n=17)
Lumen CSA (mm^2)	0.128±0.010	0.146±0.025
Arterial wall CSA (mm^2)	0.028±0.002	0.031±0.004

Relative changes in arterial dimensions during pressurisation When expressed as a percentage of the initial dimensions at an intraluminal pressure of 5mmHg, the OD and ID of segments from the IUGR animals increased more than segments from controls (Figures 4-29A, 4-29B). The relative thickness of the arterial wall (Figure 4-29C) and the degree of lengthening of the arterial segments (Figure 4-29D) did not differ between the two groups.

Arterial wall properties during pressurisation In both groups, ID/WT ratio increased with increasing pressures, indicating an increase in the lumen of the vessels and a thinning of the arterial walls with increasing pressures. Given that there were no differences in the ID and WT between the two groups (Appendix 4-1), there was also no difference in the ID/WT ratio of the two groups during pressure (Figure 4-30A). There were no differences in the arterial wall tensions from the arterial segments from the two groups (Figure 4-30B).

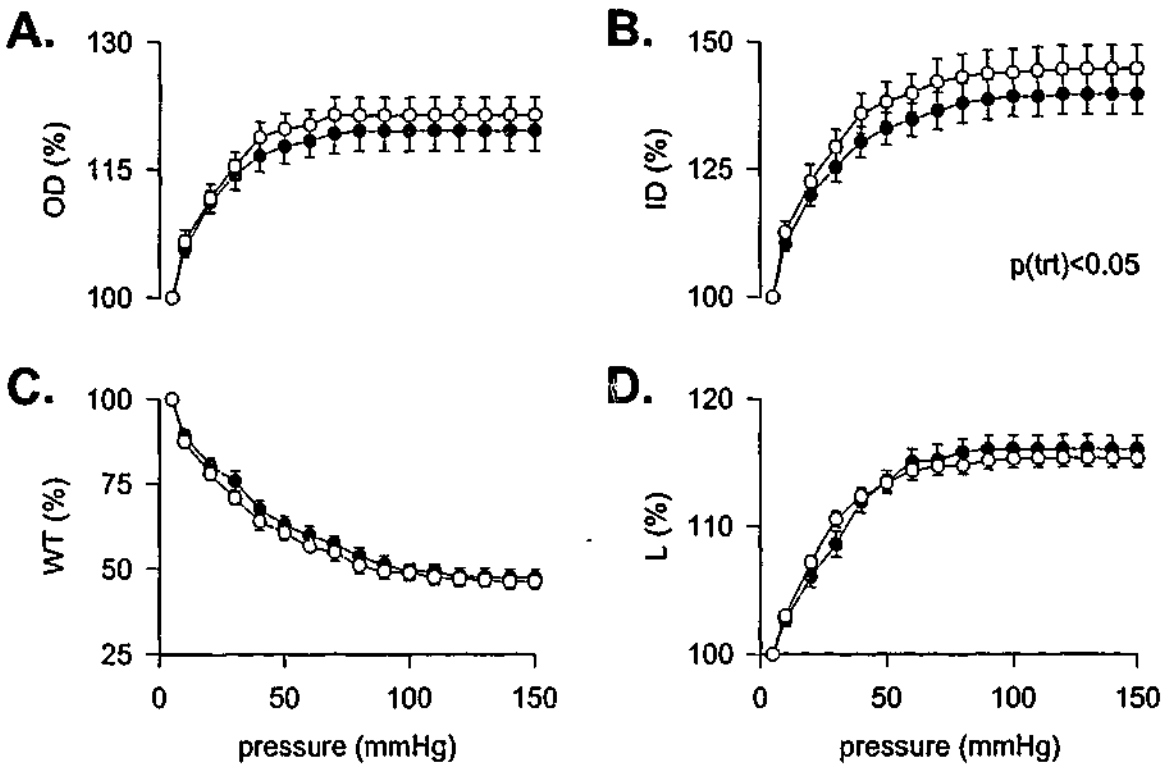


Figure 4-29. Relative dimension changes in the basilar artery branch during pressurisation. The relative change (% of initial value at 5mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of basilar artery branch from control (●, n=15) and IUGR (○, n=17) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

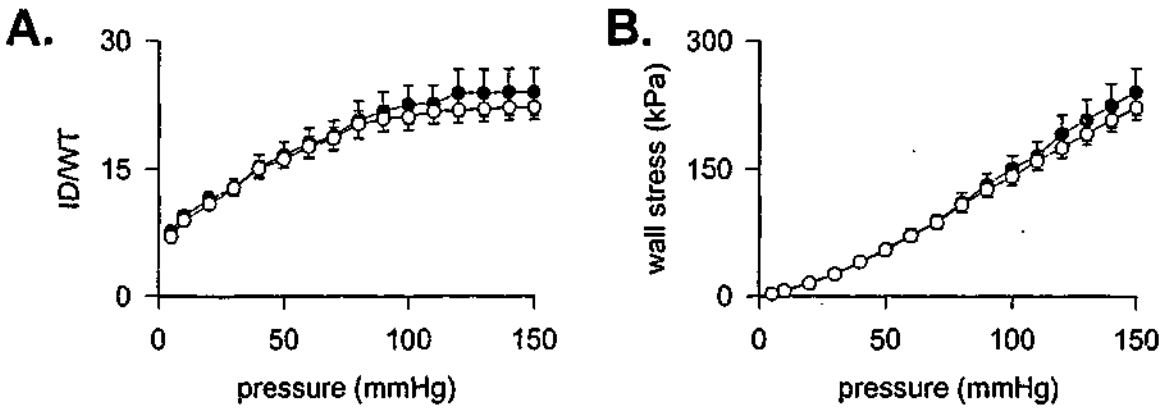


Figure 4-30. Arterial wall properties in the basilar artery branch during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of basilar artery branch from control (●, n=15) and IUGR (○, n=17) sheep.

Compliance and distensibility The relative increase in luminal volume was greater in the arterial segments from IUGR sheep than those from controls (Figure 4-31A). Cross-sectional compliance and distensibility (Appendix 4-2) and volume compliance and distensibility (Figure 4-31) decreased significantly with increasing intraluminal pressures but there were no differences between arteries from control and IUGR sheep with respect to these measures.

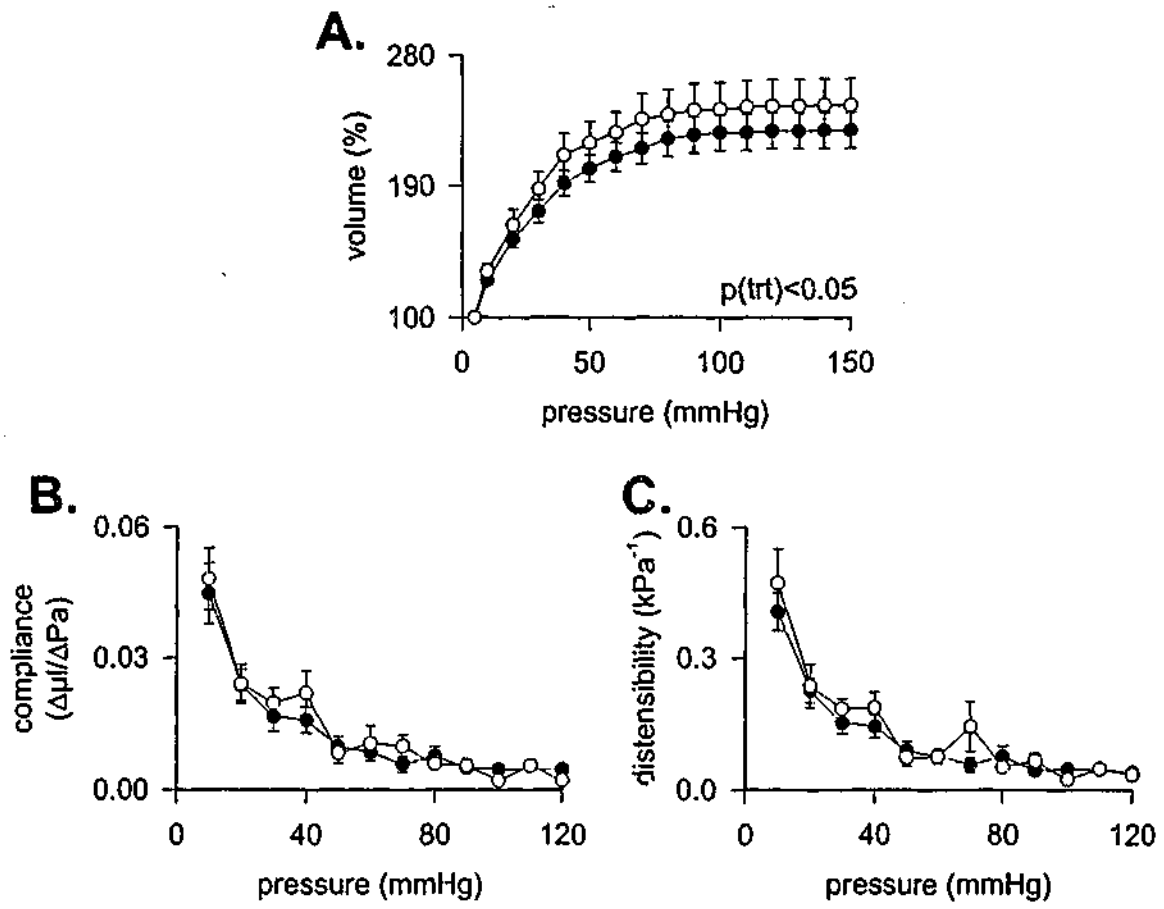


Figure 4-31. Compliance and distensibility of the basilar artery branch. (A) Lumen volume (% initial volume at 5mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of basilar artery branch from control (●, n=15) and IUGR (○, n=17) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

Stress-strain relationship The stress-strain relationships for the control and IUGR arteries are shown in Appendix 4-3. There was no difference in the overall rate constants for the two stress-strain curves, nor were there any differences in the rate

constants in the low strain, or high strain ranges, indicating that arterial stiffness was not different in basilar arteries from control and IUGR sheep (Table 4-5).

Table 4-5. Incremental elastic modulus of the basilar artery branch. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 5mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. Asterisks (*) indicate a significant difference between the control and IUGR groups ($p<0.05$).

		Control (n=15)	IUGR (n=17)
$y=ae^{kx}$	k	24.3±3.7	26.1±3.9
$y=ae^{k_1x}+be^{k_2x}$	k_1	12.0±1.5	10.1±1.4
	k_2	205±47	141±25

4.4.11b Cerebral surface artery

Arterial dimensions (μm) during pressurisation At 5mmHg intraluminal pressure, the initial OD of the segment of cerebral surface artery did not differ between the control (522±18μm) and IUGR sheep (516±15μm). In terms of the absolute dimensions of the segments cerebral surface artery, there were no differences in the OD, ID or WT of the segments of artery from the control or IUGR sheep (Appendix 4-4). There were also no differences in the lumen and arterial wall cross-sectional areas at 80mmHg between the two groups of sheep (Table 4-6).

Table 4-6. Cross-sectional area (CSA) of the cerebral surface artery at 80mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 80mmHg intraluminal pressure (~MAP) for the two groups of sheep.

	Control (n=15)	IUGR (n=17)
Lumen CSA (mm^2)	0.296±0.023	0.288±0.023
Arterial wall CSA (mm^2)	0.051±0.00-	0.049±0.003

Relative changes in arterial dimensions during pressurisation While there was no difference in the relative increase in OD between the control and IUGR cerebral arteries (Figure 4-32A), the ID increased less in the segments from the IUGR sheep compared with controls (Figure 4-32B). The thickness of the arterial wall also decreased to a lesser degree in the segments from the IUGR sheep (Figure 4-32C), indicating that relative to the WT at 5mmHg, the arterial walls of IUGR animals remained thicker than those from controls. The segments of artery from IUGR animals lengthened more than those from controls (Figure 4-32D).

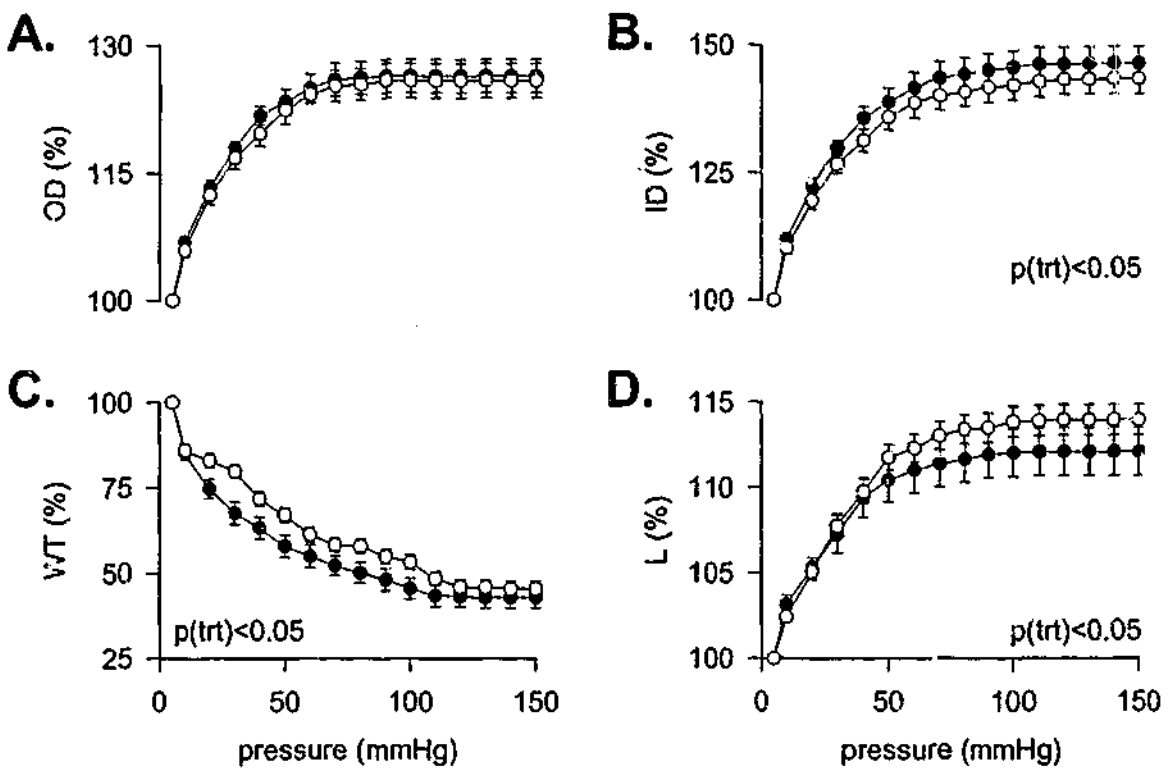


Figure 4-32. Relative dimension changes in the cerebral surface artery during pressurisation. The relative change (% of initial value at 5mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of cerebral surface artery from control (●, n=15) and IUGR (○, n=17) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

Arterial wall properties during pressurisation There was a significant effect of treatment group on the ID/WT ratio (Figure 4-33A) with the IUGR sheep having reduced lumen size relative to wall thickness compared with controls. There were no differences in the wall tensions of the two groups during pressurisation (Figure 4-33B).

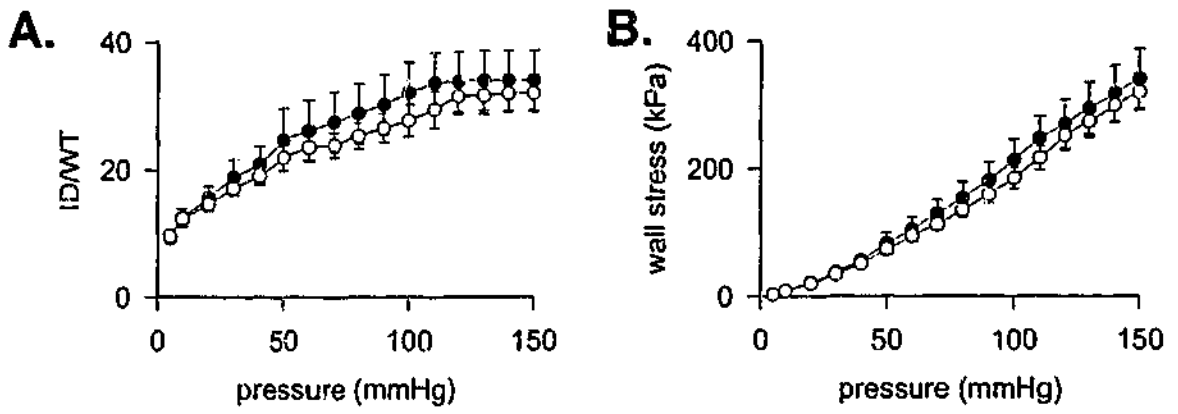


Figure 4-33. Arterial wall properties in the cerebral surface artery during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of cerebral surface artery from control (●, n=15) and IUGR (○, n=17) sheep.

Compliance and distensibility The pressure-volume relationship was not significantly different between controls and IUGR sheep (Figure 4-34A). Similarly, there were no significant differences between the cross-sectional or volume compliances and distensibility of cerebral surface arteries from control and IUGR sheep (Figure 4-34, Appendix 4-5).

Stress-strain relationship Consistent with no differences in compliance and distensibility of arteries from the two groups of sheep, the stress-strain relationships of cerebral surface arteries from control and IUGR sheep were not different (Appendix 4-6). There was also no difference in the overall slopes of the stress-strain curves of the two groups, nor were the slopes of the curves different between the two groups in the low, or high strain ranges (Table 4-7).

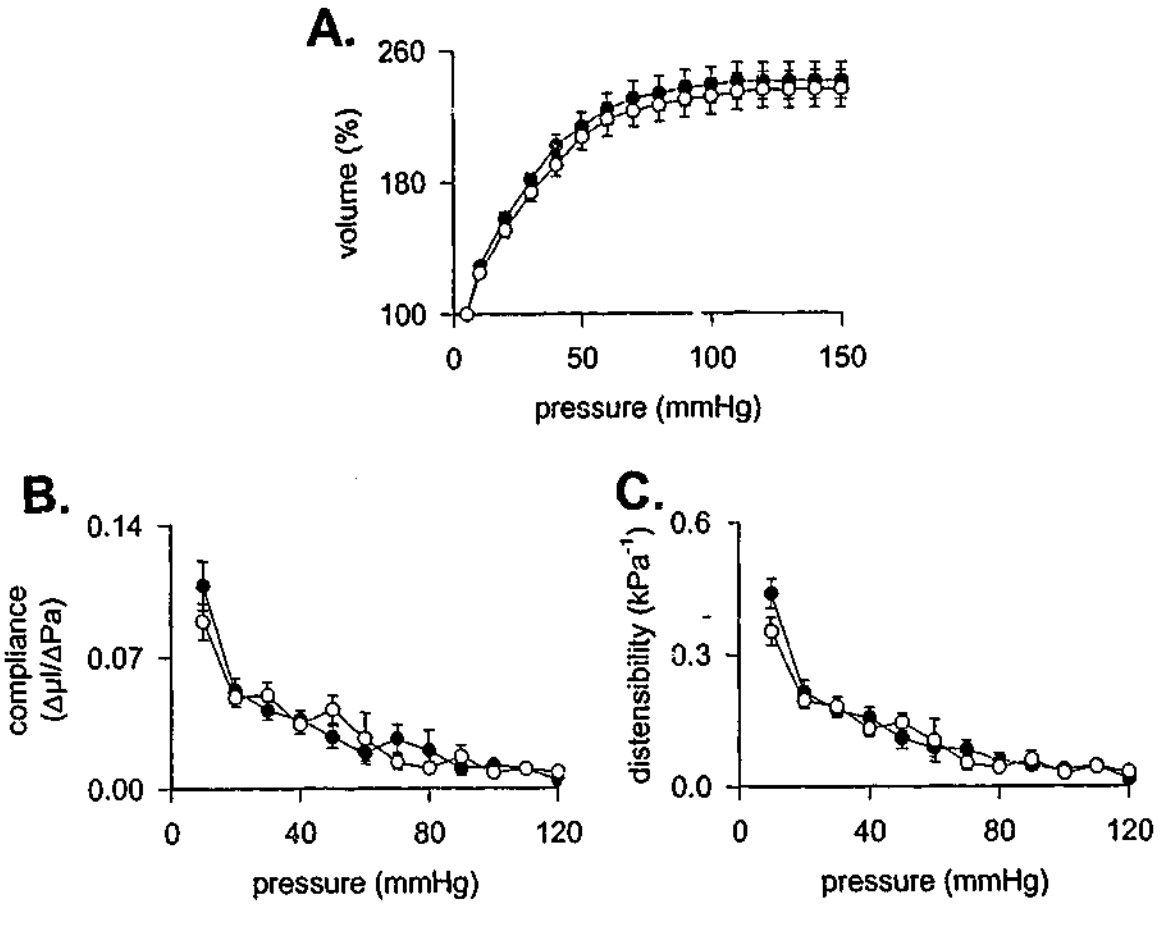


Figure 4-34. Compliance and distensibility of the cerebral surface artery. (A) Lumen volume (% initial volume at 5mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of cerebral surface artery from control (●, n=15) and IUGR (○, n=17) sheep.

Table 4-7. Incremental elastic modulus of the cerebral surface artery. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 5mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where $y=ae^{k_1x}$ describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. Asterisks (*) indicate a significant difference between the control and IUGR groups ($p<0.05$).

		Control (n=15)	IUGR (n=17)
$y=ae^{kx}$	k	24.3±3.7	24.8±3.1
$y=ae^{k_1x}+be^{k_2x}$	k_1	9.5±1.7	8.5±2.0
	k_2	146.1±42.3	84.5±23.2

4.4.11c Branch of the femoral artery

Segments of femoral artery were collected from a non-catheterised leg to eliminate any potential altered wall mechanics resulting from catheterisation of the main femoral artery. For animals that had had both femoral arteries catheterised during the study period due to the need to replace non-functional catheters for the measurement of arterial pressure, segments of femoral artery were not collected for study from these animals.

Arterial dimensions (μm) during pressurisation The OD of the femoral segments tended to be smaller in IUGR sheep at 5mmHg (429±23μm *vs* 518±40μm; p=0.09); at all pressures above 10mmHg, the OD of segments from IUGR sheep was significantly less than that of controls (Appendix 4-7). Similarly, while the lumen diameters were not different between the two groups initially, the ID of arterial segments from IUGR animals was significantly less than those of controls at pressures greater than 30mmHg. At 80mmHg, the cross-sectional area of the lumen tended (p=0.06) to be lower in the arterial segments from IUGR sheep (Table 4-8). The wall thickness of IUGR vessels was significantly less than those of controls at the lower pressures but was not significantly different to controls at pressures greater than 40mmHg (Appendix 4-7C). At ~MAP, the cross-sectional area of the arterial wall was not different between the two groups (Table 4-8).

Table 4-8. Cross-sectional area (CSA) of the femoral artery branch at 80mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 80mmHg intraluminal pressure (~MAP) for the two groups of sheep. # p=0.06

	Control (n=13)	**IUGR (n=10)**
Lumen CSA (mm^2)	0.286±0.045	0.173±0.028#
Arterial wall CSA (mm^2)	0.075±0.012	0.051±0.006

Relative changes in arterial dimensions during pressurisation In addition to the reduced OD and ID in absolute terms, the relative changes in both outer and inner diameters of femoral segments from IUGR animals were also less than the relative

changes arteries from control animals (Figures 4-35A, 4-35B). Similarly, the relative change in wall thickness was less in IUGR vessels compared with controls (Figure 4-35C) indicating that the arterial walls remained relatively thicker in the segments from IUGR animals compared with controls. The segments of femoral artery from IUGR sheep also showed reduced lengthening compared with controls (Figure 4-35D).

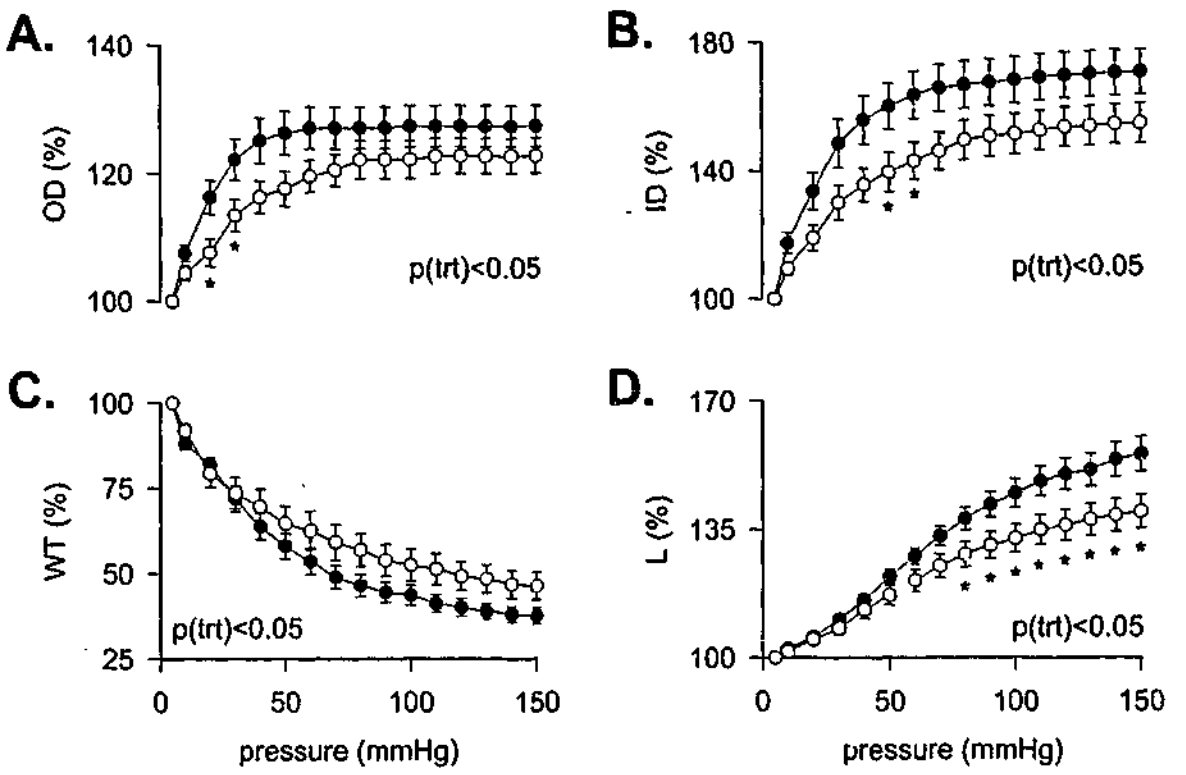


Figure 4-35. Relative dimension changes in the femoral artery branch during pressurisation. The relative change (% of initial value at 5mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of femoral artery branch from control (●, n=13) and IUGR (○, n=10) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Arterial wall properties during pressurisation Despite the arterial segments from IUGR sheep having smaller lumens and thinner walls (in absolute terms, Appendix 4-7), these two dimensions were reduced proportionately, as there were no differences in the ID/WT ratio of the two groups (Figure 4-36A). There were also no differences in the arterial wall tensions of the two groups during pressurisation (Figure 4-36B). Both of these parameters increased significantly during pressurisation.

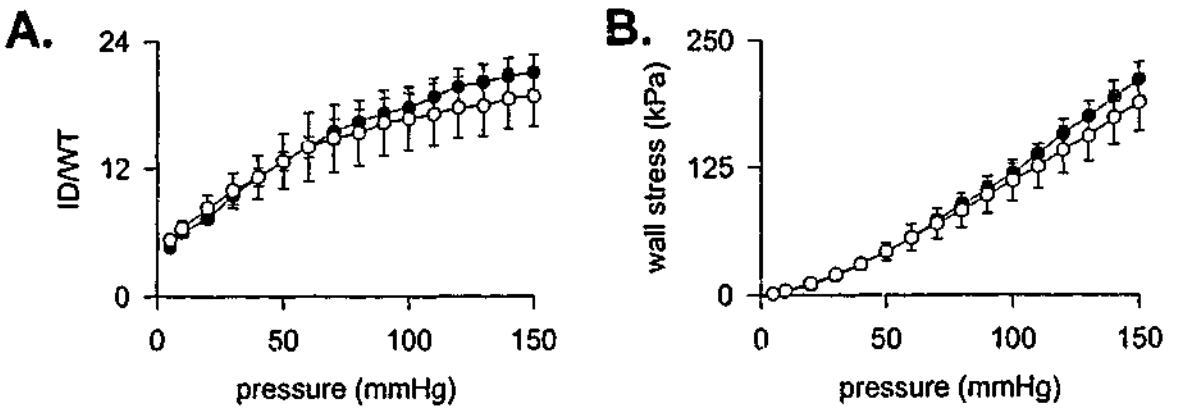


Figure 4-36. Arterial wall properties in the femoral artery branch during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of femoral artery branch from control (●, n=13) and IUGR (○, n=10) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Compliance and distensibility The relative increase in lumen volume with incremental pressure was significantly less in arteries from IUGR sheep, suggesting that femoral arteries from IUGR sheep were less compliant that those from controls (Figure 4-37A). Cross-sectional compliance (Appendix 4-8) and volume compliance (Figure 4-37B) were lower in arteries from IUGR sheep. Volume distensibility of IUGR arteries was also lower in the IUGR arteries compared with arteries from control sheep (Figure 4-37C).

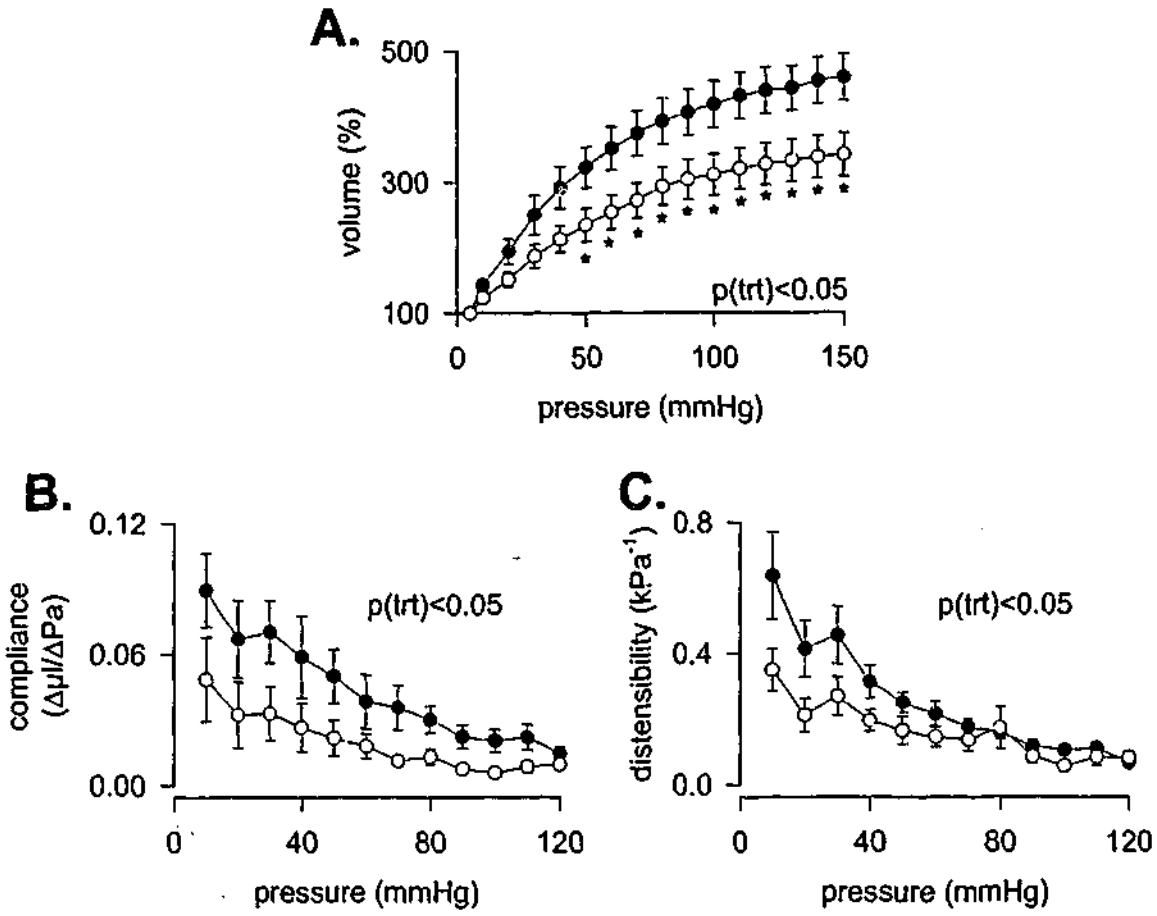


Figure 4-37. Compliance and distensibility of the femoral artery branch. (A) Lumen volume (% initial volume at 5mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of femoral artery branch from control (●, n=13) and IUGR (○, n=10) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

Stress-strain relationship Although the stress-strain curve (Appendix 4-9) for the femoral arteries from IUGR sheep was left-shifted, indicative of increased arterial stiffness (increased circumferential wall stress for a given strain, compared with controls), the overall rate constant for this curve was significantly less than the overall rate constant for the control stress-strain curve (Table 4-9). The stress-strain curves were then further analysed, and individual slopes for the low strain and high strain regions of the curves. It was found that at the low strain range, at which elastin is predominantly responsible for the mechanical properties, the arteries from IUGR sheep did not differ from those from controls. At the high strain range, at which collagen is the major determinant of arterial mechanics, the slope of the curve tended (p=0.06) to be

greater in the arteries from IUGR sheep (Table 4-9). This increased slope at the high strain range suggested increased amounts of collagen or collagen cross-linkage in arteries from IUGR sheep. This in turn led to increased arterial stiffness and decreased arterial compliance.

Table 4-9. Incremental elastic modulus of the femoral artery branch. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 5mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. Asterisks (*) indicate a significant difference between the control and IUGR groups ($p<0.05$).

		Control (n=15)	IUGR (n=17)
$y=ae^{kx}$	k	18.8±1.3	10.8±0.9*
$y=ae^{k_1x}+be^{k_2x}$	k_1	5.4±0.8	7.1±0.5
	k_2	35.7±3.0	55.6±11.0 (p=0.06)

4.4.11d Branch of the mesenteric artery

Arterial dimensions (μm) during pressurisation There was no significant difference in the starting OD (at 5mmHg intraluminal pressure) of the mesenteric artery branch collected from the two groups of sheep (control: 413±13μm *vs* IUGR: 420±11μm). Overall, throughout the pressurisation, there was a significant effect of treatment group on the OD and ID of the mesenteric artery segments, with the segments from IUGR sheep having larger diameters than those from control sheep (Appendix 4-10). At a pressure similar to MAP (~80mmHg), the cross-sectional area of the lumen was significantly greater in the IUGR arteries compared with those from control sheep (Table 4-10). While the diameters of the arterial segments differed between the two groups, there was no difference in the arterial wall thickness (Appendix 4-10), nor was there a difference in the cross-sectional area of the arterial wall of the two groups (Table 4-10).

Table 4-10. Cross-sectional area (CSA) of the mesenteric artery branch at 80mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 80mmHg intraluminal pressure (~MAP) for the two groups of sheep. Asterisk (*) indicates a significant difference between the control and IUGR groups ($p<0.05$).

	Control (n=15)	IUGR (n=17)
Lumen CSA (mm^2)	0.150±0.009	0.172±0.007*
Arterial wall CSA (mm^2)	0.035±0.002	0.037±0.003

Relative changes in arterial dimensions during pressurisation Compared with controls, the OD of the segments from IUGR animals increased to a greater extent relative to the initial OD measured at 5mmHg (Figure 4-38A). There was also an effect of treatment group on the change in ID during pressurisation, with the arterial segments from IUGR animals showing a greater relative increase in lumen size (Figure 4-38B). Despite increased relative diameters, the arterial walls of IUGR mesenteric arteries remained thicker (relative to initial thickness) than the arteries collected from the control animals (Figure 4-38C). There were no differences in the lengthening profiles of arterial segments from control and IUGR sheep (Figure 4-38D).

Arterial wall properties during pressurisation There was a significant effect of treatment group on the ID/WT ratio throughout pressurisation. The ID/WT ratio of mesenteric arteries from IUGR animals was greater than that of controls (Figure 4-9A), indicating these arteries from IUGR animals had larger lumens relative to the wall thickness. This finding is consistent with the IUGR artery segments having larger lumens than controls with no difference in the WT of the two groups (Appendix 4-10). Arterial wall tension (Figure 4-39B) was also greater in the segments from IUGR sheep, and is consistent with these segments having greater diameters compared with controls; La Place's law states that tension is the product of radius and pressure, so for a given pressure a vessel with a larger radius (or diameter) will have greater wall tensions.

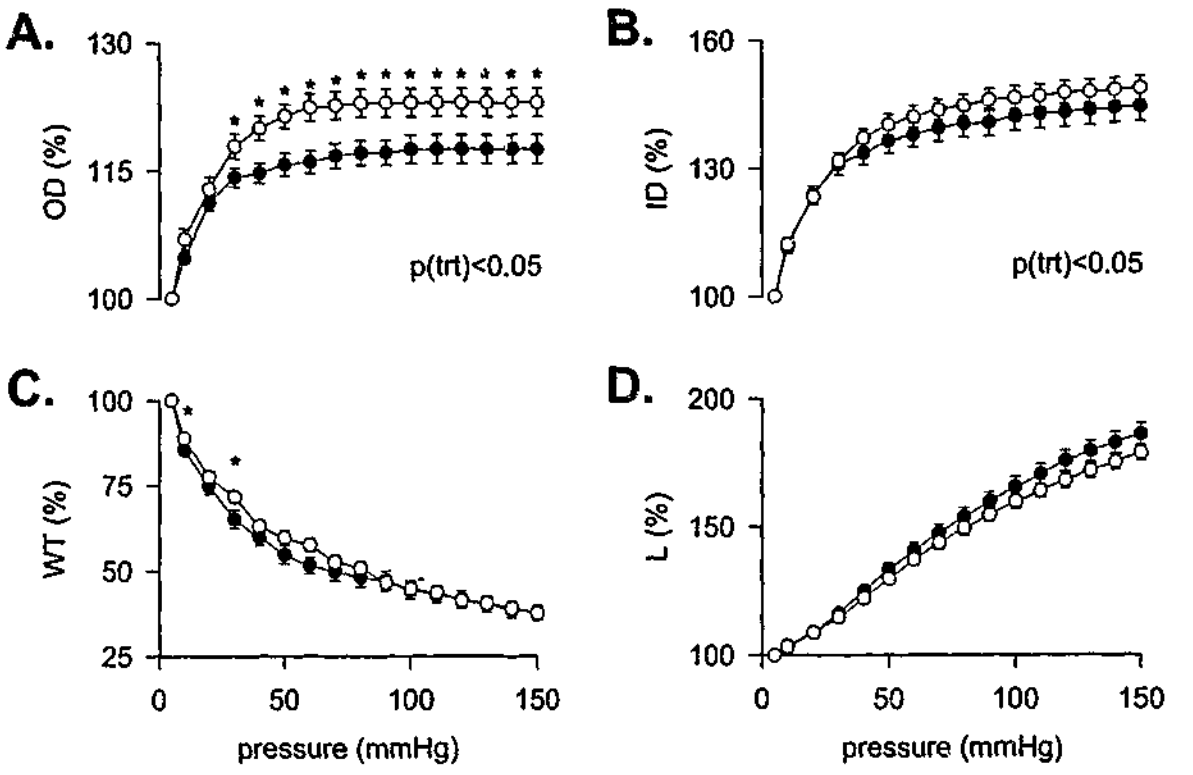


Figure 4-38. Relative dimension changes in the mesenteric artery branch during pressurisation. The relative change (% of initial value at 5mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of mesenteric artery branch from control (•, n=15) and IUGR (○, n=17) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).

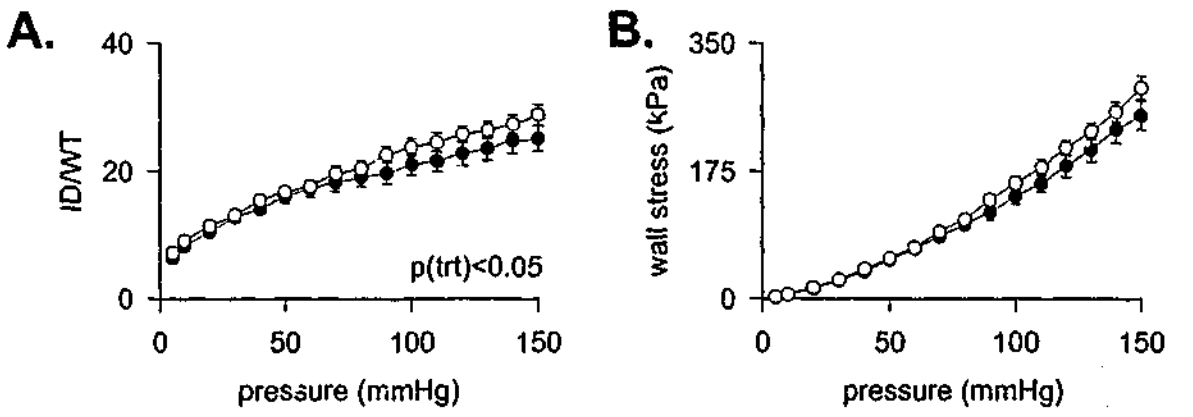


Figure 4-39. Arterial wall properties in the mesenteric artery branch during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of mesenteric artery branch from control (•, n=15) and IUGR (○, n=17) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

Compliance and distensibility The relative change in luminal volume was not different in the segments of mesenteric artery from IUGR and control sheep, nor was arterial compliance and distensibility different between the two groups of sheep (Figure 4-40).

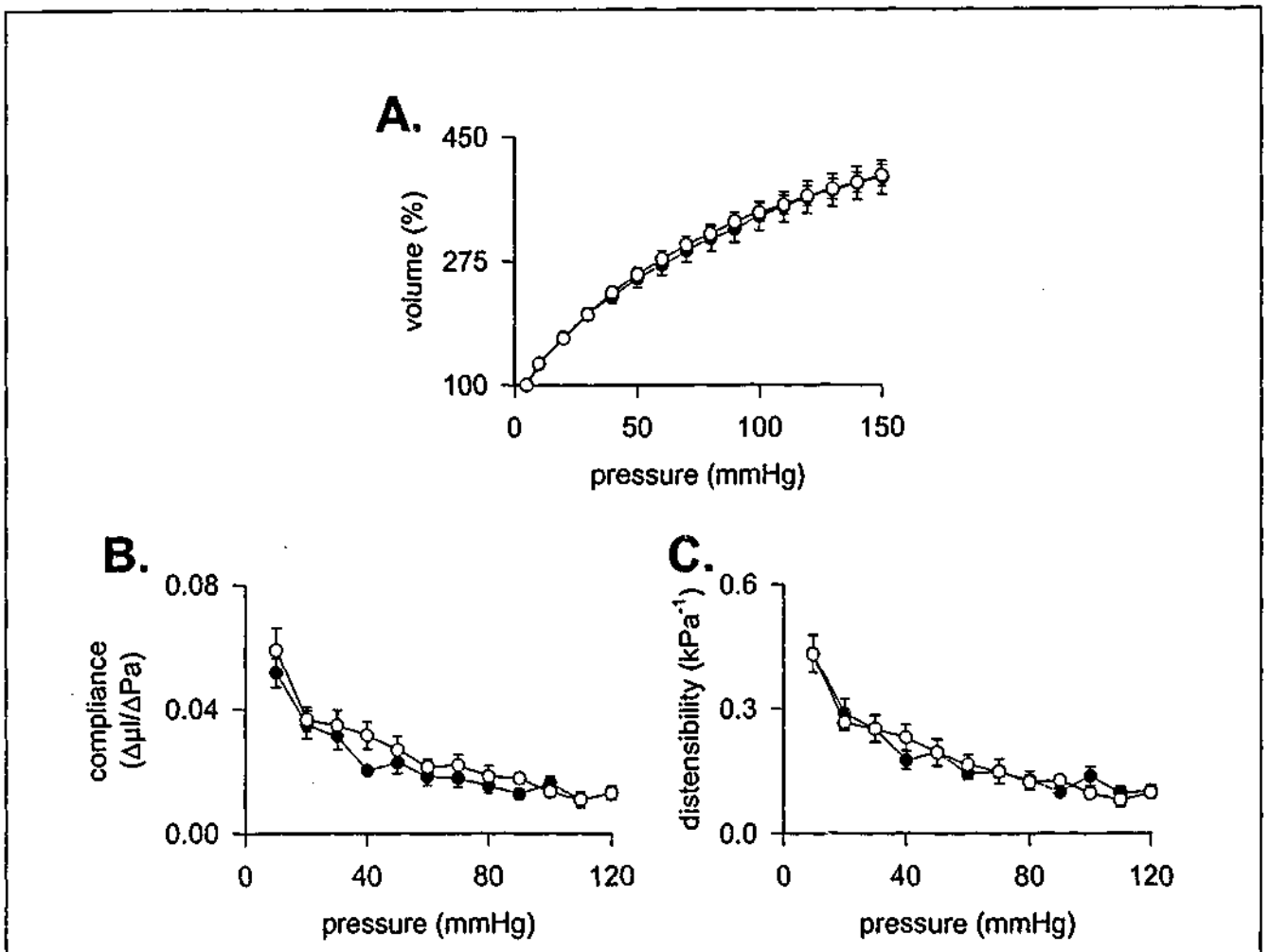


Figure 4-40. Compliance and distensibility of the mesenteric artery branch. (A) Lumen volume (% initial volume at 5mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of mesenteric artery branch from control (●, n=15) and IUGR (○, n=17) sheep. Asterisks (*) indicate values that differ between groups at individual pressures ($p<0.05$).

Stress-strain relationship As there were no differences between the IUGR and control sheep in terms of arterial compliance and distensibility, it is not surprising that the stress-strain relationships (Appendix 4-12) were not different between these two groups of sheep. There were also no differences between the two groups with respect to the overall slopes of the stress-strain curves, or in the slopes of the curves in the low and high strain ranges (Table 4-11).

Table 4-11. Incremental elastic modulus of the mesenteric artery branch. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 5mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. Asterisks (*) indicate a significant difference between the control and IUGR groups ($p<0.05$).

		Control (n=15)	IUGR (n=17)
$y=ae^{kx}$	k	20.6±0.9	22.4±1.3
$y=ae^{k_1x}+be^{k_2x}$	k_1	9.9±2.1	6.4±1.2
	k_2	36.8±5.5	36.3±2.8

4.4.11e Arcuate artery

Arterial dimensions (μm) during pressurisation The mean OD of the segment of arcuate artery from the IUGR sheep (425±20μm) was significantly less than the mean OD of segments collected from controls (492±20μm). The OD and ID of the segments of arcuate artery from IUGR animals were significantly smaller than the segments collected from control animals at all pressures between 5 and 150mmHg (Appendix 4-13). However, given the regular structure of the kidney, the consistency of the site of selection of the arcuate artery was able to be maintained. Therefore, the difference in the initial size of the segment is due to the IUGR animals having smaller arcuate arteries rather than the use of a different, smaller artery from the IUGR animals. Associated with smaller lumen diameters, the cross-sectional area of the lumen at 80mmHg was significantly less in the arcuate arteries from IUGR sheep than controls (Table 4-12). There was also a significant effect of treatment group on the wall thickness, with the arcuate arteries from IUGR animals having thinner walls throughout pressurisation (Appendix 4-13). Unlike arterial diameter, WT was not significantly different between the two groups at any given pressure increment. At a intraluminal pressure similar to MAP (80mmHg), the cross-sectional area of the arterial wall was not significantly different in the arcuate arteries between the two groups of sheep (Table 4-12).

Table 4-12. Cross-sectional area (CSA) of the arcuate artery at 80mmHg. Lumen cross-sectional area and cross-sectional area of the arterial wall at 80mmHg intraluminal pressure (~MAP) for the two groups of sheep. Asterisk (*) indicates a significant difference between the control and IUGR groups ($p<0.05$).

	Control (n=15)	IUGR (n=17)
Lumen CSA (mm^2)	0.210±0.018	0.157±0.016*
Arterial wall CSA (mm^2)	0.061±0.007	0.047±0.004

Relative changes in arterial dimensions during pressurisation Despite the outer and inner diameters of the arcuate arteries from IUGR animals being smaller than those from controls, when these dimensions were standardised for their size at 5mmHg, the relative increase in OD and ID during pressurisation was not different in the arcuate arteries collected from control and IUGR animals (Figures 4-41A, 4-41B). Similarly, the wall thickness of the arcuate arteries decreased to the same extent in control and IUGR animals (Figure 4-41C); however, the ability of the arteries from IUGR animals to lengthen was reduced (Figure 4-41D).

Arterial wall properties during pressurisation Despite smaller lumen diameters and thinner arterial walls in the IUGR arcuate arteries, the reduction in ID was greater than the reduction in WT. The ID/WT ratio was lower in IUGR arcuate arteries compared with controls, indicating the these arteries had a reduced lumen and a relatively thicker arterial wall compared with controls (Figure 4-42A). Wall tension was also lower in the IUGR arcuate arteries, consistent with the smaller arteries in the IUGR animals (Figure 4-42B).

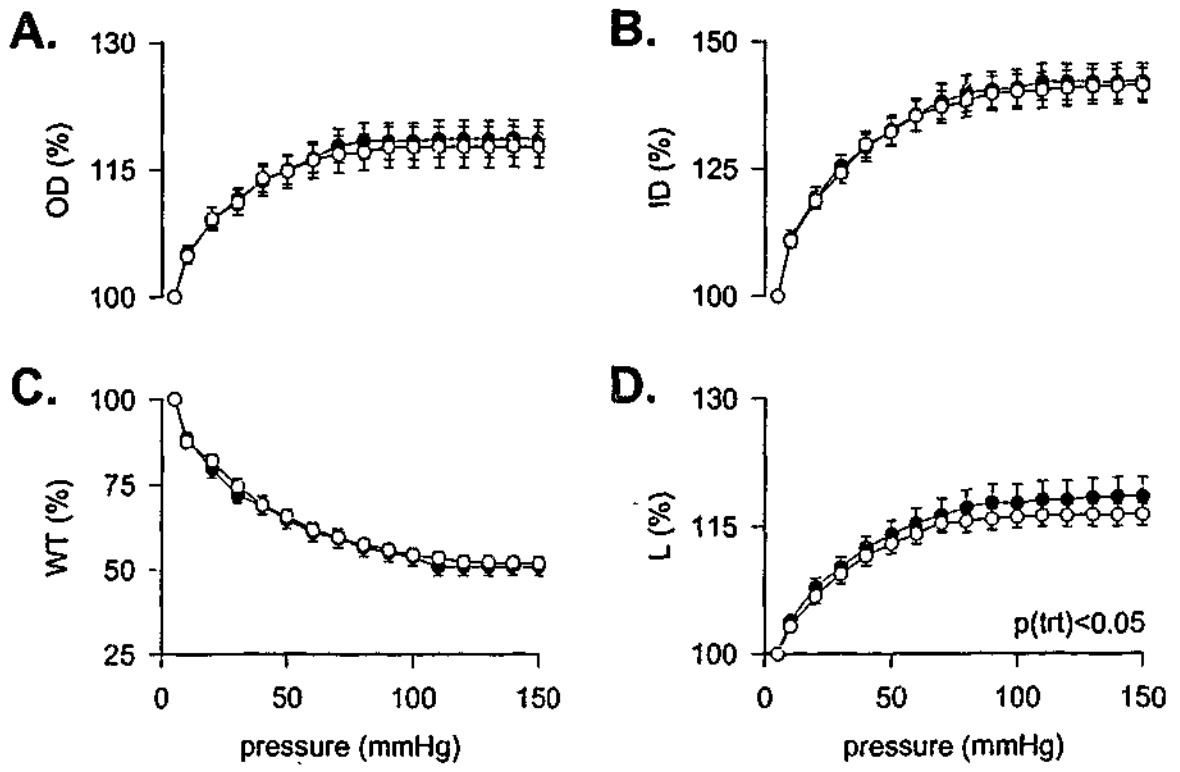


Figure 4-41. Relative dimension changes in the arcuate artery during pressurisation. The relative change (% of initial value at 5mmHg intraluminal pressure) in (A) outer diameter (OD), (B) inner diameter (ID), (C) wall thickness (WT) and (D) segment length (L) during pressurisation of the segments of arcuate artery from control (●, n=15) and IUGR (○, n=17) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

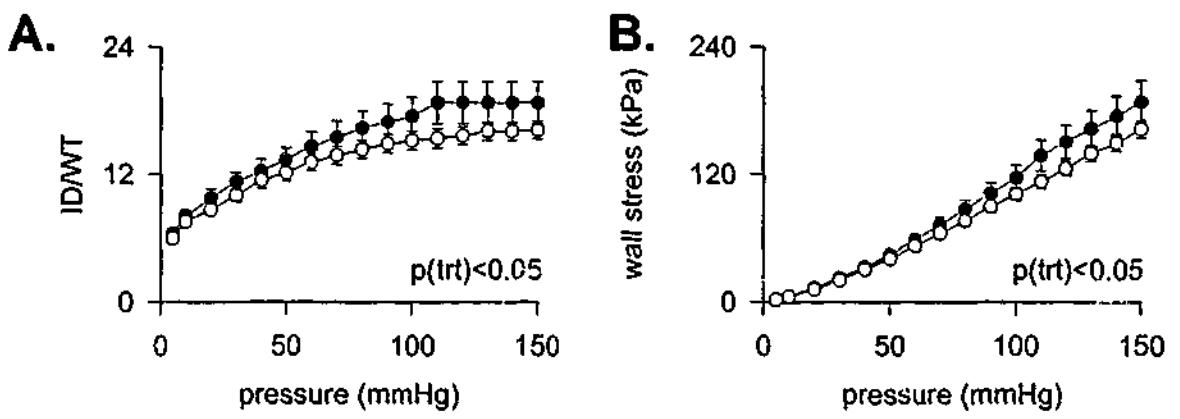


Figure 4-42. Arterial wall properties in the arcuate artery during pressurisation. (A) Lumen to wall thickness ratio (ID/WT) and (B) wall stress during pressurisation of the segments of arcuate artery from control (●, n=15) and IUGR (○, n=17) sheep. P(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.

Compliance and distensibility There were no differences in the pressure-volume relationship of the arcuate arteries collected from controls and IUGR sheep (Figure 4-43), suggesting the arterial compliance was similar between the groups. Arterial compliance and distensibility were not different in the segments of arcuate artery from control and IUGR sheep (Figure 4-43).

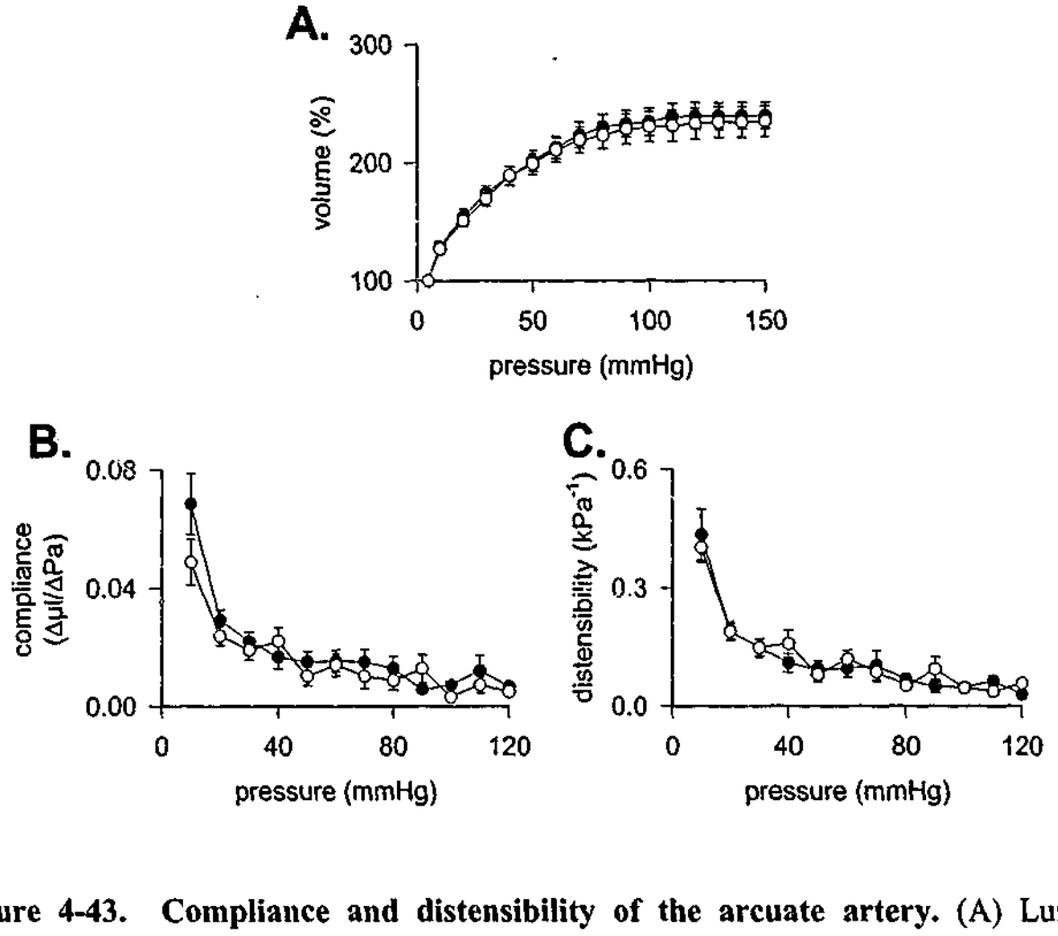


Figure 4-43. Compliance and distensibility of the arcuate artery. (A) Lumen volume (% initial volume at 5mmHg intraluminal pressure), (B) volume compliance and (C) volume distensibility during pressurisation of the segments of arcuate artery from control (●, n=15) and IUGR (○, n=17) sheep.

Stress-strain relationship The stress-strain curves for the arcuate arteries from IUGR sheep and control sheep did not appear to be significantly different from each other (Appendix 4-15) and the overall slopes of these curves were not different (Table 4-13). However, at the high strain range of the curve, the arcuate arteries from IUGR animals tended ($p=0.08$) to have higher slopes, suggesting an increase in collagen in these arteries from IUGR sheep compared with arteries from controls (Table 4-13).

Table 4-13. Incremental elastic modulus of the arcuate artery. The stress-strain relationship for the two groups were linearised by calculating the rate constant (k) in the equation $y=ae^{kx}$, where y is stress, a is the initial strain at 5mmHg and x is strain at each pressure increment. Data were further analysed by fitting the stress-strain curves to the equation $y=ae^{k_1x}+be^{k_2x}$, where ae^{k_1x} describes the low stress (elastic) range of the arteries and be^{k_2x} describes the high stress (collagenous) range of the arteries. Asterisks (*) indicate a significant difference between the control and IUGR groups ($p<0.05$).

		Control (n=15)	IUGR (n=17)
$y=ae^{kx}$	k	17.1±2.4	18.6±2.1
$y=ae^{k_1x}+be^{k_2x}$	k_1	7.4±3.6	9.4±0.7
	k_2	52.3±30.1	109.2±14.9 (p=0.08)

4.4.11f Summary

Relative changes in arterial segment dimensions between vascular beds Relative increases in arterial diameter were similar between the five arteries studied. The OD increased by 15–25% from initial OD at 5mmHg intraluminal pressure, and ID increased by 30–40%. In all five arteries, WT decreased to approximately 50% of initial WT. The relative increase in arterial segment length varied between vascular beds. The arterial segments from the brain and kidney increased in length by 10–20%, femoral segments increased by approximately 30–50% but the mesenteric segments showed the greatest lengthening, increasing by approximately 80% from the initial length (at 5mmHg intraluminal pressure). Similarly, the relative changes in luminal volume differed between vascular beds, and may have been related to different lengthening between the five arteries studied. While the luminal volume doubled during pressurisation of arterial segments from the brain and kidney, the volume of arterial segments from the femoral muscle and mesentery increased to 300% and 400% of their initial volume when pressurised to 5mmHg.

Effects of birth weight group on passive arterial wall mechanical properties Table 4-14 summarises the effects of IUGR on arterial dimensions and wall properties. The mechanical properties of the basilar artery branch segments were similar between the control and IUGR groups. During pressurisation, the relative increase in lumen diameter in these segments from IUGR sheep was greater than those from control sheep; this led

to a greater relative increase in luminal volume in basilar arteries from IUGR sheep. There were no differences between the two groups in the other calculated parameters.

The relative increase in ID was less in cerebral surface artery segments from IUGR sheep, and the relative thickness of the arterial wall was greater in IUGR sheep compared with controls. However, the segments of cerebral surface artery from IUGR sheep showed greater lengthening than arterial segments from controls. There were no differences between the two groups in the pressure-volume relationship, compliance, distensibility or stress-strain relationship.

The dimensions (OD, ID, WT, in μm) of the segments of femoral artery branch from IUGR sheep were smaller than the arterial segments from control sheep. The relative change in these dimensions, and also segment length, were also lower in IUGR sheep compared with controls. Overall, the segments of femoral artery branch from the IUGR sheep had lower relative lumen volume changes, were less compliant and distensible, and had a higher rate constant for the stress-strain relationship (i.e. the IUGR sheep had stiffer femoral arteries).

The outer and lumen diameters of the mesenteric artery branches from IUGR sheep were larger than the mesenteric arteries from control sheep, and the relative increase in these diameters during pressurisation was also greater in these animals. The mesenteric arteries of the two groups did not differ in their pressure-volume relationships, arterial compliance and distensibility or in their stress-strain relationships.

Although the arcuate arteries of IUGR sheep were smaller than those from controls in terms of OD, ID and WT, the relative changes in these parameters during pressurisation did not differ between the two groups. The arcuate arteries from IUGR sheep lengthened more than those from controls but the relative volume increase during pressurisation were not different between the two groups, nor was the arterial compliance, distensibility or stress-strain relationship.

Table 4-14. Summary of effects of IUGR on arterial dimensions and passive arterial wall mechanical properties in the five selected vessels. ↑ indicates an increase in the parameter in the IUGR group compared with controls, ↓ indicates a decrease in the parameter in the IUGR group compared with controls, and ↔ indicates no difference between control and IUGR animals.

	Basilar	Cerebral surface	Femoral	Mesenteric	Arcuate
OD (μm)	↔	↔	↓	↑	↓
ID (μm)	↔	↔	↓	↑	↓
WT (μm)	↔	↔	↓	↔	↓
OD (%)	↔	↔	↓	↑	↔
ID (%)	↑	↓	↓	↑	↔
WT (%)	↔	↓	↓	↔	↔
L (%)	↔	↑	↓	↔	↓
ID/WT	↔	↓	↔	↑	↓
Wall stress	↔	↔	↔	↑	↓
Volume (%)	↑	↔	↓	↔	↔
Vol. Compliance	↔	↔	↓	↔	↔
Vol. Distensibility	↔	↔	↓	↔	↔
Stress-strain slope	↔	↔	↓	↔	↔
Stress-strain slope$_{low}$	↔	↔	↔	↔	↔
Stress-strain slope$_{high}$	↔	↔	↔	↔	↔

4.5 Discussion

The aims of this study were to determine the effects of late gestational placental insufficiency on the postnatal growth, arterial pressure and arterial wall mechanics in young adult sheep. Late gestational placental insufficiency which led to fetal hypoxemia and hypoglycemia, produced IUGR in lambs that were capable of postnatal catch-up growth. However, this catch-up was gender-specific. Body composition in the young adult sheep also differed between control and IUGR sheep, and between males and females. IUGR lambs were hypotensive compared with controls soon after birth. Arterial pressure of male IUGR sheep remained lower than that of male controls at two years, but at this age the arterial pressure of females IUGR and controls were not different. At two years, the femoral arteries from IUGR sheep were less compliant than those from controls but there were no major changes to the arterial mechanical properties of resistance arteries from the brain, mesentery and kidney.

4.5.1 Fetal blood parameters

Placental insufficiency was induced in fetal sheep for 24±1 days, from 120d GA until birth at, or near term. Fetal Sa_{O_2} was reduced to levels that were similar to those reported in previous studies of UPE in sheep (Cock & Harding, 1997; Murotsuki *et al.*, 1997; Louey *et al.*, 2000; Cock *et al.*, 2001a); alterations to other parameters including pH and lactate concentration were also similar to those previously reported. Umbilical cord blood samples from human IUGR fetuses have found them to be hypoxemic, hypercapnic, acidemic and hypoglycemic (Nicolaides *et al.*, 1989; Economides *et al.*, 1991), and the UPE fetuses had similar alterations to arterial blood gases and pH. In contrast to human IUGR in which fetuses can be hyperlactemic (Nicolaides *et al.*, 1989; Economides *et al.*, 1991), the blood lactate concentrations in UPE fetuses were not different to those of controls.

The fetal arterial blood parameters for the term-born IUGR animals described in this chapter are consistent with those measured in the term-born IUGR lambs used in the study by Louey *et al.* (2000). Of the animals included in this chapter, there were two control animals (lambs #30 and #31) and two IUGR animals (lambs #29 and #33) that were common to the study by Louey *et al.* (2000).

The arterial blood gases and pH status of the UPE fetuses that were born preterm were not different from those measured in those UPE fetuses that were born at term; these findings differ from those described by an earlier study (Cock *et al.*, 2001a). Of the six animals classed as P-IUGR animals in this chapter, four were common to this earlier study (Cock *et al.*, 2001a) which reported no differences in the Pa_{CO_2} and blood glucose concentrations of term-born and preterm-born IUGR fetuses, but the preterm IUGR fetuses were more acidemic, hypoxemic and had higher hemoglobin concentrations than term-born fetuses. Of the P-IUGR animals described in this chapter, there were no differences in any parameter when compared with T-IUGR animals except fetal blood glucose concentration. While the T-IUGR fetuses were hypoglycemic (0.46±0.02mmol/l) compared with controls (0.68±0.03mmol/l), there was no difference in the blood glucose concentrations between the P-IUGR fetuses (0.64±0.06mmol/l) and controls. However, blood glucose concentrations varied within the P-IUGR group; three fetuses were hypoglycemic relative to controls but the remaining three did not undergo UPE to the desired level of hypoxia as the other three P-IUGR fetuses, and as a result, were not hypoglycemic. One P-IUGR fetus (fetus #243.1/lamb#35) had high lactate concentrations throughout the fetal period (1.2±0.1mmol/l, max. concentration 1.8mmol/l), and to reduce the risk of fetal death, Sa_{O_2} was reduced to levels between 30–40% rather than 25–35%; i.e. this fetus was less hypoxemic than the other IUGR fetuses. The arterial catheter of its twin (fetus #243.2/lamb #34) became blocked during the UPE period and was only occasionally functional, so was unable to be used daily for the injection of microspheres or blood sampling. The twin of fetus #160.2 (lamb#73) died at 122d GA and to reduce the risk of death of the other fetus, Sa_{O_2} was reduced to levels between 30–40% during UPE. While their blood glucose concentrations were not different to controls, these fetuses were hypoxemic (fetus #243.2 was mildly hypoxemic) compared with controls and were growth restricted at birth; the birth weights for the lambs #35, #34 and #73 were 2.25kg, 2.88kg and 2.29kg, respectively.

It is not clear why the arterial blood measurements of the P-UPE fetuses reported by Cock *et al.* (2001a) differ from those in this chapter, or why the P-IUGR lambs described in this chapter were born earlier than the T-IUGR lambs. The P-IUGR lambs in the study by Cock *et al.* (2001a) were born 7 days earlier than the T-IUGR lambs (139±1d *vs* 146±1d GA) while the P-IUGR lambs described in this chapter were born

only 4 days earlier than the T-IUGR lambs (140±1d *vs* 144±1d GA). Given that T-IUGR lambs described in this chapter were born earlier than the previously reported T-IUGR lambs (Louey *et al.*, 2000; Cock *et al.*, 2001a), it is possible that rather than the P-IUGR and T-IUGR lambs being two separate groups, these lambs (based on their gestational age at birth) are part of the same population. This may explain, in part, why the fetal blood parameters did not differ between the T-UPE and P-UPE fetuses.

In sheep, *in utero* exposure to exogenous glucocorticoids can increase arterial pressure in the fetus (Tangalakis *et al.*, 1992) and postnatal lamb (Dodic *et al.*, 1998). However, for the animals described in this chapter, there were no differences in blood cortisol concentrations in control and IUGR fetuses. Therefore, it is unlikely that any differences in the postnatal arterial pressure of the control and IUGR lambs was the result of elevated cortisol concentrations during the fetal period. The mean cortisol concentration in the days leading up to birth was variable in control and IUGR fetuses, depending on the number of days between plasma collection and birth. However, there was no difference between control and IUGR fetuses when the concentrations were expressed as the number of days preceding birth. Elevated cortisol concentrations have been measured in human IUGR fetuses (Economides *et al.*, 1991); in contrast, cortisol concentrations did not differ between controls and IUGR fetuses when standardised for gestational age at birth. Given cortisol concentrations were not different before or two hours after UPE (refer to previous chapter). Thus the findings in this chapter are consistent with UPE not having a significant effect on circulating cortisol concentrations.

In humans, SGA babies can be born at term or preterm (O'Callaghan *et al.*, 1997; Bukowski *et al.*, 2001). Although UPE does not alter fetal plasma cortisol concentrations, this gestational insult can affect the timing of the pre-partum cortisol surge and as a result, some IUGR lambs are born at term and others are born approximately one week before term. Two of the P-IUGR fetuses included in this chapter were induced to deliver preterm due to indications of poor fetal health and the belief that the lambs would not survive to term. Early labour was induced by the injection of a 3β-dehydroxysteroid dehydrogenase inhibitor to the ewes #107 and #108 at 139d GA, together with an injection of betamethasone (to the ewe) to mature the fetal

lung and increase the chance of survival of the newborn preterm lamb. While previous studies have shown that synthetic glucocorticoids during gestation can programme the postnatal arterial pressure of lambs, there appears to be a critical time during early prenatal development when this programming occurs (Dodic *et al.*, 1998), and exposure to exogenous glucocorticoids later in gestation not appear to programme postnatal hypertension (Moss *et al.*, 2001). Therefore, it is unlikely that the administration of betamethasone to these ewes on the day prior to delivery would have programmed the postnatal arterial pressure of their lambs.

4.5.2 Postnatal blood parameters

Although fetuses were acidemic, hypercapnic and hypoxemic during UPE, after birth, the arterial pH and blood gas status of the control and IUGR lambs were similar. IUGR lambs had lower Pa_{O_2} and higher Pa_{CO_2} than controls in the early postnatal period. After the first postnatal week, there were no significant differences in the arterial blood gases of the two groups. Similarly, the elevated hemoglobin concentrations and hematocrit measured in the hypoxemic fetuses did not persist into the postnatal period; after birth, there were no major differences in these measures between IUGR and control lambs. Altered postnatal respiratory function has been shown in UPE fetuses in the early postnatal period (Joyce *et al.*, 2001). A lower diffusing capacity of the lung (measured by a carbon monoxide rebreathing method) in IUGR lambs in the first postnatal month (Joyce *et al.*, 2001) may have accounted for the mild hypercapnia and hypoxemia soon after birth in the IUGR lambs described in this chapter. Therefore, late gestational UPE did have effects on the postnatal body composition and arterial pressure in the IUGR animals, these postnatal effects were not the result of altered postnatal blood gas.

4.5.3 Size at birth and postnatal growth

The majority of the IUGR lambs were twins, and while multiple gestations alone can lead to reduced fetal weight compared with singletons in late gestation (Min *et al.*, 2000), all twins included in this study (with the exception of the two naturally occurring IUGR lambs) also underwent UPE in late gestation. UPE in twins was performed to maximise the difference in birth weight between lambs classed as IUGR and those classed as controls. The mean birth weight of the UPE twins described in this chapter was 2.64±0.13kg (n=16), and was lower than that of twins from our laboratory that have

not undergone UPE (3.73±0.18kg at 148±1d GA, n=4; $p<0.01$). Therefore, the twins in this study that underwent UPE were small, even in comparison to twins that had not undergone UPE. The use of twins in studies of the fetal origins of adult disease hypothesis in humans remains controversial. Dwyer *et al.* (1999) has shown in children that birth weight is inversely related to arterial pressure when twins and singletons are included in the analysis together, or the analysis contains twins alone. Christensen *et al.* (2001) has also shown birth weight in adolescent twins to be inversely related to arterial pressure; Loos *et al.* (2001b) found this inverse relationship in female twins, but did not find a relationship between birth weight and arterial pressure in male twins aged between 18 and 34 years. Another study in adults reported no relationship between birth weight and arterial pressure (Baird *et al.*, 2001); in contrast, children who were low birth weight due to twinning had lower arterial pressure than singleton children (Williams & Poulton, 1999). Barker (1995a) suggested that fetuses that were subjected to disproportionate fetal growth (during mid-late gestation) were at an increased risk for later adult disease. Even though twins are expected to have lower birth weights than singletons, the twins included in this chapter also underwent UPE; therefore, the studies described in this chapter investigated the effects of late gestational placental insufficiency rather than the effects of twinning on postnatal arterial pressure.

In the human, IUGR can be classified as symmetrical or asymmetrical; this difference is thought to be due to the timing of the insult that leads to reduced fetal size. Growth restriction early in gestation leads to reductions in fetal body length and weight such the fetus is proportionately small (Villar & Belizan, 1982). In contrast, fetal growth restriction in late gestation affects body weight greater than body length, leading to a fetus that has a appropriate length but is thin, often characterised by a reduced ponderal index (Villar & Belizan, 1982). While the body composition of lambs in my study was not measured at birth, the findings they were lighter, shorter in length and thinner are consistent with a study in which body composition was determined in SGA human infants at birth. Lapillonne *et al.* (1997) found that not only were SGA infants lighter and shorter at birth (for all gestational ages from 36–41 weeks), lean tissue, fat and bone mineral weights were also reduced in these infants. A reduction in the lean tissue and fat mass at birth would be consistent with the observation that the IUGR lambs had a wasted appearance at birth. The shorter body lengths and lower ponderal index in the IUGR lambs at birth are also consistent with the asymmetrical growth restriction

exhibited by human infants that are at an increased risk for later hypertension (Barker *et al.*, 1992).

Despite the IUGR lambs being 41% lighter than controls at birth, by 6 months of age, there was no difference in the body weights of the control and IUGR lambs. The catch-up in postnatal body weight was the result of increased growth rates (% increase/day) in the IUGR lambs between 3 and 12 months. This period corresponded to the period at which the differences in body weight between control and IUGR lambs were decreasing. Following late gestational UPE, it has been shown that IUGR lambs remain lighter than controls in the early postnatal period (Louey *et al.*, 2000; Cock *et al.*, 2001a), but long-term studies have shown that these lambs are able to catch up to controls in terms of body weight. The period of catch up is also consistent with the catch up observed in IUGR lambs following maternal betamethasone exposure in mid-late gestation (Moss *et al.*, 2001). In the human, the majority of SGA infants catch up in weight and height during the postnatal period. Height catch-up occurs in 76% of SGA infants by 2 postnatal months, and 92% have caught up in height by 18 years of age (Karlberg *et al.*, 1997). Similarly, catch-up in body weight has been shown to occur in SGA babies; at 7 postnatal weeks, 75% of SGA children remain lighter than controls but by 18 years of age, 95% of SGA individuals are within the normal weight range (Karlberg *et al.*, 1997). The catch-up growth seen in the sheep described in this chapter, however, was apparently gender-specific. While the female IUGR sheep had fully caught up in body size to controls by two years, male IUGR sheep had significantly lower weights and were shorter in terms of height and body length compared with controls. These findings in the male sheep are in contrast with the evidence of catch-up growth in male human SGA infants; Albertsson-Wikland *et al.* (1993) reported differences in the timing of catch-up growth in males and female SGA infants, with only 5% and 11% of male SGA infants remaining more than 2SD below mean weight and height, respectively, at 4 years of age. The difference in body weight between male controls and male IUGR sheep at two years may have been due to greater body weights in the 6 rams that were purchased for inclusion in the study at two years. Although they were of the same breed as our operated sheep, these rams were heavier (range: 73.0–87.5kg) than the single male control studied from gestation through to two postnatal years (62.5kg at 2 years); the mean weight of the IUGR rams at two years was 62.4±1.9kg.

Epidemiological studies have shown associations between low birth weight and increased abdominal adiposity later in life (Law *et al.*, 1992; Eriksson *et al.*, 2001). Gale *et al.* (2001) has also found that after adjustment for age, sex, height and weight, total body fat in human adults (determined by DXA) was negatively correlated with birth weight; that is, low birth weight individuals had significantly more body fat at 70–75 years of age. In contrast, human fetuses that were exposed to undernutrition during the Dutch famine either during the third trimester of pregnancy or early postnatal life had reduced birth weights and a lower incidence of obesity at 19 years of age compared with non-famine exposed fetuses (Ravelli *et al.*, 1976). Female IUGR sheep described in this chapter tended to have more (both absolute and relative amounts) body fat (as determined by DXA). This trend was also found at post mortem when female IUGR sheep tended to have larger fat deposits in the abdominal cavity (49.01±2.82g/kg body weight *vs* 38.74±4.00g/kg body weight, p=0.058). In contrast, male IUGR sheep had significantly less (both absolute and relative amounts) body fat than controls (as determined by DXA) and like the females, these reduced amounts of fat were reflected in the amounts of fat collected at post mortem (IUGR: 10.59±1.24g/kg body weight *vs* control: 20.92±3.97g/kg body weight, $p<0.05$). It has been proposed that a fetus can adapt to reductions in fetal nutrition, but modifications to its physiology that ensure fetal survival are not beneficial in the postnatal period when nutrition is not compromised (Hales & Barker, 1992). It is possible, therefore, that metabolism is slowed during fetal undernutrition and this alteration in metabolism continues into postnatal life, predisposing the individual to later obesity, which in turn may increase the risk for cardiovascular disease (Daniels *et al.*, 1999; Tanaka *et al.*, 2002). While this may account for the trend in female IUGR sheep to have more body fat, it does not appear to hold true for the male IUGR sheep.

Adults of low birth weight have been shown to have less lean tissue mass than normal birth weight adults; this has been shown in 18–34 year old males (Loos *et al.*, 2001a) and in 70–75 year old men and women (Gale *et al.*, 2001). In my study, there was no difference in the amount of lean tissue (muscle) mass (in g) in the control and IUGR sheep. Further analysis revealed that, while there was a trend for the male IUGR sheep to have less lean tissue than male controls, female IUGR sheep had a greater muscle mass than female controls. When the muscle mass was adjusted for body weight, there

was an overall trend (p=0.07) for IUGR sheep to have a greater relative lean tissue mass compared with controls; this difference was significant in the male sheep.

In humans, SGA infants have been shown to have lower bone mineral content (both total body and standardised for body weight) at birth compared with appropriately grown infants (Petersen *et al.*, 1989; Pohlandt & Mathers, 1989; Lapillonne *et al.*, 1997). Bone mineral content has been shown to be lower in 70–75 year old low birth weight men and women (Gale *et al.*, 2001), and it has been suggested that this reduction in bone mass can predispose the low birth weight individual to bone fractures and osteoporosis later in life. Gale *et al.* (2001) found reduced bone mineral content (whole body) at 70–75 years of age in both males and females that were born low birth weight; however, bone mineral density was correlated with birth weight in females only. There was evidence of altered bone development in the IUGR sheep used for studies described in this chapter. In addition to lower body weights in the IUGR rams at two years, these animals were also shorter in terms of shoulder height and body length compared with control rams at this age. The reduction in shoulder height may have been due to 2 of the 3 front limb bones (ulna and metacarpus) in male IUGR sheep being significantly shorter than in male controls, and these reduced bone lengths have been related to altered deposition of bone in these animals. Although there were no differences in the bone mineral content or density between female control and IUGR sheep, the male IUGR sheep had both reduced bone mineral content and density compared with male controls, also suggesting that the deposition of calcium and other bone minerals may have been impaired as a result of UPE in these animals. Uterine artery ligation in the rat has been shown to reduce placental calcium transport of IUGR rats. Total fetal calcium content was reduced in the IUGR rats compared with controls. However, when total calcium was standardised to body weight, there were no differences between the IUGR and control groups (Mughal *et al.*, 1989). It is possible that during UPE, in addition to reducing oxygen and glucose supply to the fetus, delivery of other substrates, such as calcium, may have also been compromised.

4.5.4 Postnatal arterial pressure

It has previously been shown that late gestational placental insufficiency leads to lower arterial pressure in sheep in the early postnatal period (Louey *et al.*, 2000; Cock *et al.*,

2001a), and the long term studies described in this chapter show that this relative hypotension persists for the first postnatal year. At two years of age, there were no overall differences in the arterial pressure of controls and IUGR sheep. These findings differ from those of Robinson *et al.* (1998) who also found relative hypotension in the early postnatal period in IUGR lambs born to placentally restricted ewes but after 60 days, the lambs became hypertensive relative to controls. Moss *et al.* (2001) also found that lambs that were growth restricted from repeated glucocorticoid exposure in mid-late gestation had lower arterial pressure than controls at 3 months of age but this hypotension was not found to persist to 6 months of age. Therefore, while the majority of epidemiological studies, and experimental studies in rats have found low birth weight to be associated with postnatal hypertension, in the sheep, IUGR induced during late gestation appears to be associated with postnatal hypotension that can either persist, become normal (Moss *et al.*, 2001) or progress to hypertension (Robinson *et al.*, 1998).

Two other ovine models have shown that an altered *in utero* environment leads to hypertension in the postnatal sheep, but neither of these show low birth weight to be associated with hypertension. Both early glucocorticoid exposure (Dodic *et al.*, 1998) and mild maternal undernutrition during early gestation (Hawkins *et al.*, 2000a) lead to hypertension in offspring at 3–4 months of age, but neither of these insults reduced birth weight. While the arterial pressure of the lambs from nutrient restricted ewes have not been reported after 3 months of age, it has been shown that the hypertension in lambs from ewes exposed to dexamethasone persists from 4 months up to 80 months of age (Dodic *et al.*, 2001). Therefore, using five different methods to compromise the *in utero* environment of the ovine fetus such that fetal growth was impaired, only one (caruncleotomy; Robinson *et al.*, 1998) has found IUGR lambs to develop postnatal hypertension which, interestingly, was preceded by a period of postnatal hypotension. The relative hypotension measured in the early postnatal IUGR lamb (Robinson *et al.*, 1998; Louey *et al.*, 2000; Cock *et al.*, 2001a; Moss *et al.*, 2001) is consistent with hypotension measured in healthy low birth weight infants in the first week after birth (Contis & Lind, 1963; Lee *et al.*, 1976; O'Sullivan *et al.*, 1996). In my previous study (Louey *et al.*, 2000), it was unclear whether the relative hypotension in the IUGR lambs would persist or would develop into hypertension. These lambs were studied for the first 8 postnatal weeks, and the period of cross-over from relative hypotension to relative hypertension in the placentally restricted lambs (Robinson *et al.*, 1998)

occurred at approximately 2 postnatal months. It is now known that following UPE, IUGR lambs do not become hypertensive after 2 postnatal months; however, it remains unclear whether these sheep will become hypertensive after two years. It is possible that the normotension observed at two years is an intermediate stage between the hypotension prior to 18 months and later hypertension. This could be resolved by studying the sheep until older ages, but this was not possible in my study due to time constraints.

In humans, arterial pressure has been related to current body height and weight (Voors *et al.*, 1977; Law & Shiell, 1996), and it has been argued that the lower arterial pressure in the IUGR (induced by UPE) lambs was due to their smaller size in the early postnatal period (Louey *et al.*, 2000). The lower arterial pressure of IUGR lambs (from repeated glucocorticoid exposure) was also measured at an age at which the IUGR lambs were lighter than controls and in the subsequent measurement of arterial pressure, neither body size nor arterial pressure were different between IUGR and control lambs (Moss *et al.*, 2001). These results suggest there may be a positive relationship between arterial pressure and current body weight. For the lambs described in this chapter, MAP was correlated to current body weight at all ages, with lighter animals having lower arterial pressure ($r=0.526$, $p<0.01$ for all lambs; Figure 4-44). This relationship between MAP and current body size was significant in the controls alone ($r=0.542$, $p<0.01$) and also the IUGR lambs alone ($r=0.502$, $p<0.01$). However, the regression line is shifted in the IUGR lambs such that they have lower arterial pressure than controls for a given weight (Figure 4-44). If the lower arterial pressure in the IUGR lambs is due to smaller current body size, the regression lines of the two groups should be superimposed on each other but they are not, suggesting that another factor may responsible for the lower arterial pressure in IUGR lambs.

The IUGR lambs used in my earlier study had lower mean arterial pressure in the first 8 postnatal weeks, and this relative hypotension may have been due to reduced body weights in the IUGR lambs (Louey *et al.*, 2000). Analysis of MAP data in Chapter 4 by two-way ANOVA revealed a significant effect of treatment group, with the IUGR lambs having lower arterial pressure than controls but analysis by t-test at each study age did not reveal significant differences between the two groups at any age except at 8 weeks. It is possible that arterial pressure in these lambs is related to current body size

in the early postnatal period; however, the trend for lower arterial pressure was apparent until 12 months, despite body weights of the IUGR lambs (male and female data combined) not being different after 6 months.

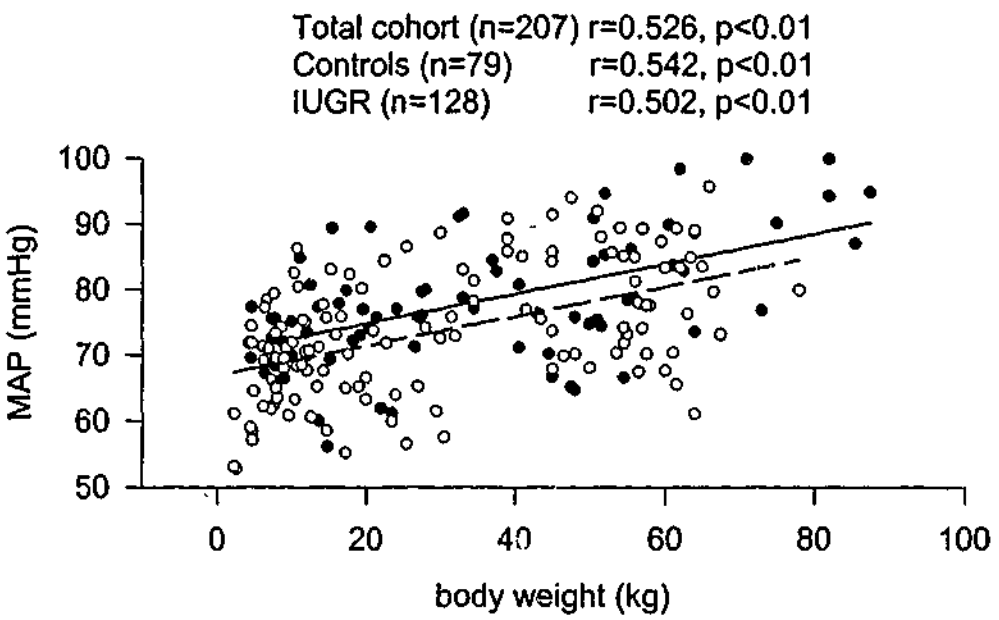


Figure 4-44. Relationship between mean arterial pressure (MAP) and current body weight. The relationship between MAP and body weight at the time of MAP measurement in control (●) and IUGR (○) lambs. Regression lines for the control (—) and IUGR (– –) lambs are also shown. Data are from all MAP measurements from two weeks to two years postnatal age. r value refers to Pearsons correlation coefficient for all of the data, data from control lambs alone and data from IUGR lambs alone; p values indicate significant correlations ($p<0.01$)

Differences in the postnatal circulating cortisol concentrations did not account for the differences in arterial pressure between IUGR and controls. Although there was a significant effect of treatment group on postnatal cortisol concentration, there were no significant differences between the two groups at any individual age, nor was cortisol concentrations correlated with MAP during the postnatal study period ($r=-0.032$, $p=0.65$). While there were no significant differences in the cortisol concentrations between the two groups, it is possible that the response to a stressor may have elicited a different cortisol response in the IUGR sheep compared with the controls. In another study. basal cortisol concentrations did not differ between control lambs and lambs from ewes that were undernourished for the first 70 days of ovine gestation; however, the cortisol response to a challenge that activated the hypothalamo-pituitary-adrenal

(HPA) axis was greater in the lambs from undernourished ewes (Hawkins *et al.*, 2000a). This indicates that basal measurements may not be altered between controls and treated animals but responses to stressors may be altered as a consequence of an compromised *in utero* environment. Future studies of the effects of late gestational UPE on postnatal physiological function could investigate the responses of the IUGR lambs to challenges to different systems including the HPA axis.

Epidemiological studies have shown associations between birth weight and postnatal arterial pressure in both males (Nilsson *et al.*, 1997) and females (Curhan *et al.*, 1996). Both male and female sheep were included in the studies described in this chapter, with a mixture of each gender in each treatment group. Due to the presence of more females than males in the control group, 6 rams of the same breed of sheep were purchased from pasture for inclusion in the final studies at two years of age. For the studies at this age, there were 7 male and 8–9 female controls and 11–12 male and 6 female IUGR sheep. While arterial pressures were not significantly different between controls and IUGR sheep at two years, analysis by gender found that IUGR males had lower arterial pressure than male controls, while female IUGR and control sheep had similar arterial pressures. The difference in arterial pressure in the male sheep may, in part be due to the male IUGR sheep being smaller than male controls (in terms of body weight, length and height). Increased postnatal growth rates that lead to catch-up growth have been associated with an increased risk of later hypertension in humans (Huxley *et al.*, 2000). As the male IUGR sheep were smaller than male controls at 2 years, elevated arterial pressure may not be expected to be increased in these sheep. It appears that IUGR has different long-term effects in males than females. While the female IUGR sheep were able to catch up in body size and did not have altered arterial pressure compared with female controls, the male IUGR sheep showed persistent effects of IUGR on arterial pressure and postnatal body size and composition. However, the possibility that the 6 purchased rams that were included in the studies at two years may have confounded the analysis of data (both arterial pressure and body weight data) from male sheep cannot be excluded.

4.5.5 Passive arterial wall mechanics

If the relative hypotension observed in the IUGR lambs during the first 8 postnatal weeks (Louey *et al.*, 2000) had persisted to two years of age, the arterial wall mechanical properties might be expected to reflect more compliant arteries. Given that there was no significant difference between the arterial pressures of control and IUGR sheep at two years, arterial mechanics between the two groups were not expected to be different.

It is possible that changes to arterial structure and mechanics preceded the increase in arterial pressure (van Gorp *et al.*, 2000). Late gestational UPE has the potential to affect arterial wall mechanics as both elastin and collagen synthesis and deposition are affected by alterations to oxygen and nutrient supply (Durmowicz *et al.*, 1991; Spanheimer *et al.*, 1991). In the previous chapter, increased arterial stiffness was measured in the arteries from IUGR fetuses but this increased stiffness was not translated into hypertension in the postnatal period. The alterations to the passive mechanical properties were also not consistent with the early postnatal hypotension, nor did the majority of alterations to arterial mechanical properties evident in the near-term fetus persist to adulthood. Increased arterial stiffness in the near-term ovine fetus, in the absence of elevated arterial pressure suggests that alterations in arterial structure and mechanical properties might predispose the individual to later hypertension, as shown in the spontaneously hypertensive rat (van Gorp *et al.*, 2000). The alterations to arterial mechanical properties also lend support to the hypothesis that hypertension is initiated *in utero* and is amplified with age (Law *et al.*, 1993). Arterial compliance has been shown to decrease with age (de Simone *et al.*, 1997), which could lead to increasing arterial pressure with age. If arterial compliance was reduced in the late gestation UPE fetus, it might be expected that compliance would continue to decrease with age, resulting in elevated arterial pressure that is amplified with age. This did not occur, as the arterial pressure of the early postnatal IUGR lamb was lower than that of controls (Louey *et al.*, 2000).

It is possible that the IUGR lambs had greater rates of elastin synthesis in the early postnatal period to compensate for decreased *in utero* deposition that may have led to the increased arterial stiffness in the near-term IUGR fetuses. In the sheep, elastin

synthesis and deposition continue into the early postnatal period (Bendeck & Langille, 1991; Wells *et al.*, 1999), and increased synthesis rates after birth could lead to increased arterial compliance. Quantification of elastin and collagen in arteries from IUGR lambs between 140d GA and 3–4 postnatal weeks would indicate whether (a) the amounts of arterial elastin and collagen differ between control and IUGR lambs during a period at which the IUGR lambs are hypotensive, (b) the timing of deposition of elastin and collagen differ between control and IUGR lambs, and (c) if elastin and/or collagen synthesis is reduced during late gestational placental insufficiency or merely delayed until a time when substrate availability is not compromised (i.e. the early postnatal period). Wells *et al.* (1999) have shown that aortic elastin and collagen contents are not significantly different between 21 day old lambs and adult sheep, so there may be a critical window for the accumulation of elastin and collagen, after which the arterial content of these proteins does not further increase.

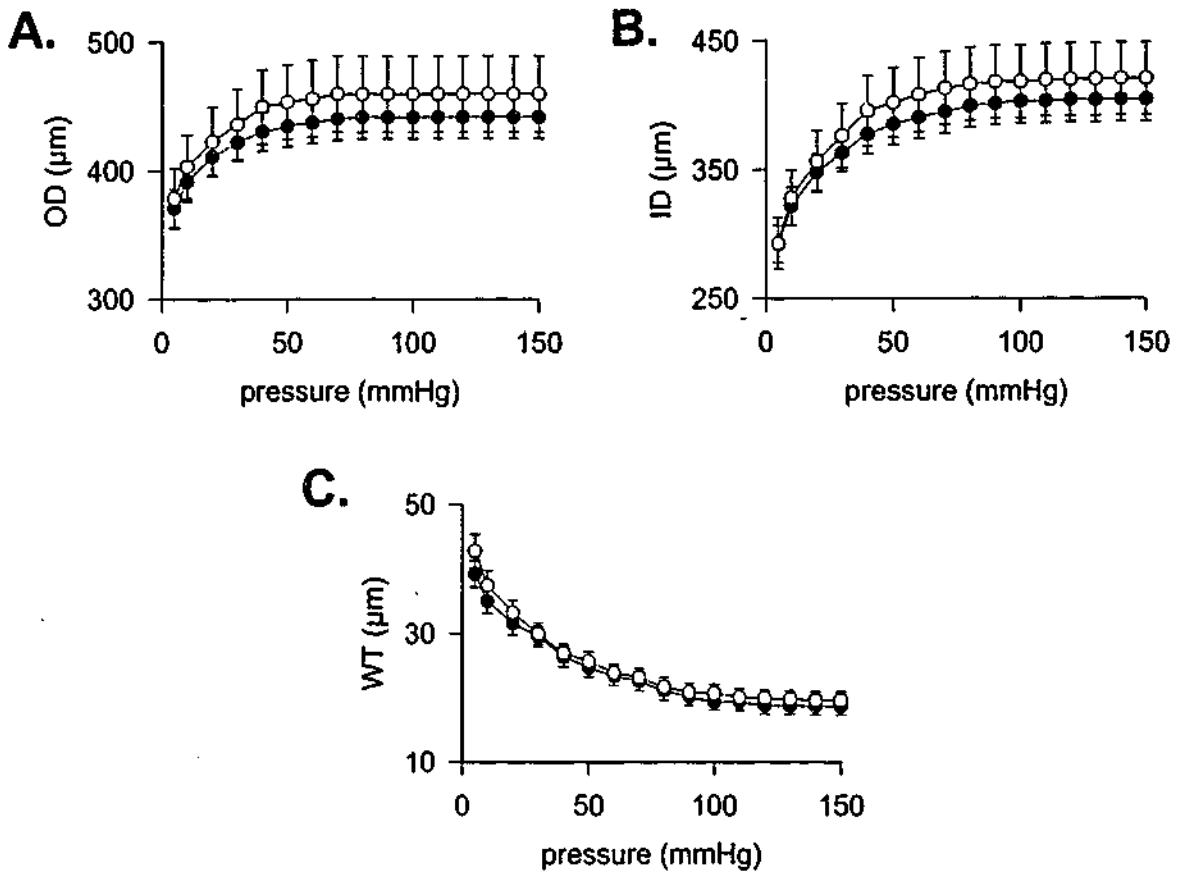
The branch of the femoral artery from adult IUGR sheep was less compliant than those from control sheep. There were no major effects of IUGR on the compliance of the other arteries studied. Relative changes in arterial dimension differed between control and IUGR arteries from the brain, kidney and mesentery but overall compliance, distensibility and the stress-strain relationship of these arteries did not differ between the IUGR and control groups. Since arterial pressures of adult control and IUGR sheep were not different when the arteries were collected, it is not unexpected that there were no major differences in the arterial mechanical properties of the two groups of sheep. The femoral arteries studied from the UPE fetuses had smaller relative increases in lumen diameter, lumen volume and segment length and these alterations persisted to two postnatal years. At this age, arterial compliance and distensibility were also found to be reduced in the femoral artery segments from IUGR sheep. Interestingly the increased stiffness of the basilar, cerebral surface and mesenteric arteries of the 140d GA UPE fetuses did not persist to two postnatal years. It is unclear why the altered mechanics measured in the near-term fetus did not persist, or become less compliant with age. An ontogenetic study of elastin and collagen synthesis and deposition from the late gestation fetus to early postnatal life (as described earlier) may provide evidence of altered synthesis and deposition of the extracellular matrix proteins in IUGR lambs.

In my study, arterial mechanical properties were determined in the absence of smooth muscle activity, and therefore may differ from the *in vivo* mechanical properties. However, as there were minimal differences in the passive arterial mechanical properties of the two groups, and there were no differences in arterial pressure at this age, it is unlikely that *in vivo* arterial function would significantly differ from the passive mechanical properties studied.

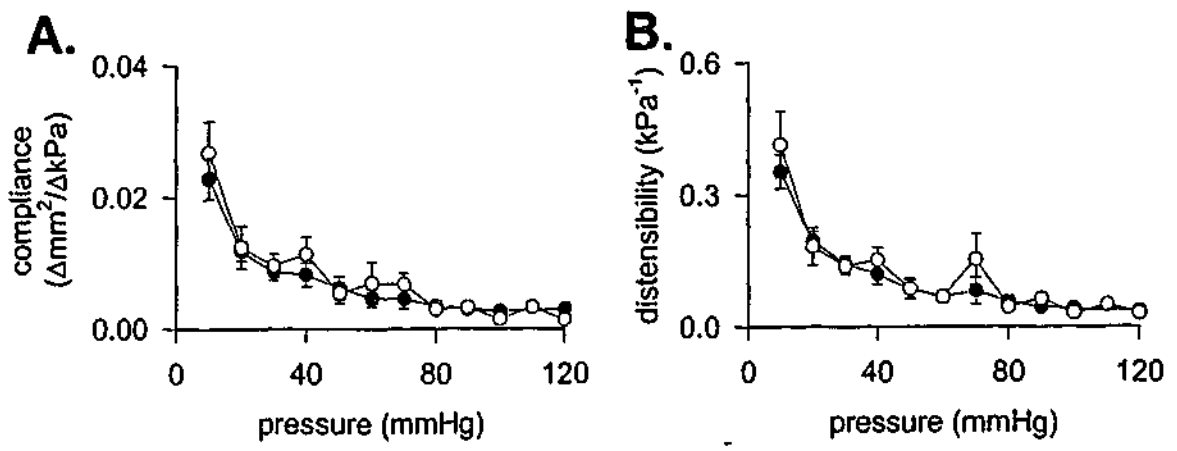
4.6 Conclusions

Following late gestational placental insufficiency, IUGR lambs are able to catch up in terms of body weight within the first 6 months of age. This catch-up in body weight appears to be gender-specific; there were also gender-specific differences in body composition and body size at two years of age in these sheep. Late gestational placental insufficiency leads to persistent alterations to arterial pressure; however, using the placental embolisation technique to induce IUGR does not lead to hypertension in the postnatal sheep. Arterial compliance was reduced in the femoral arteries from IUGR sheep at two years of age. However, IUGR had no major effects on the passive mechanical properties of resistance arteries from the brain, kidney or mesentery. These arterial mechanical properties are consistent with similar arterial pressures of controls and IUGR sheep at this age.

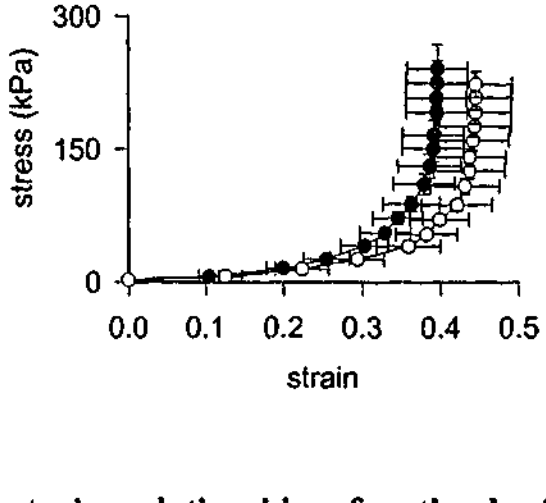
4.7 Appendices



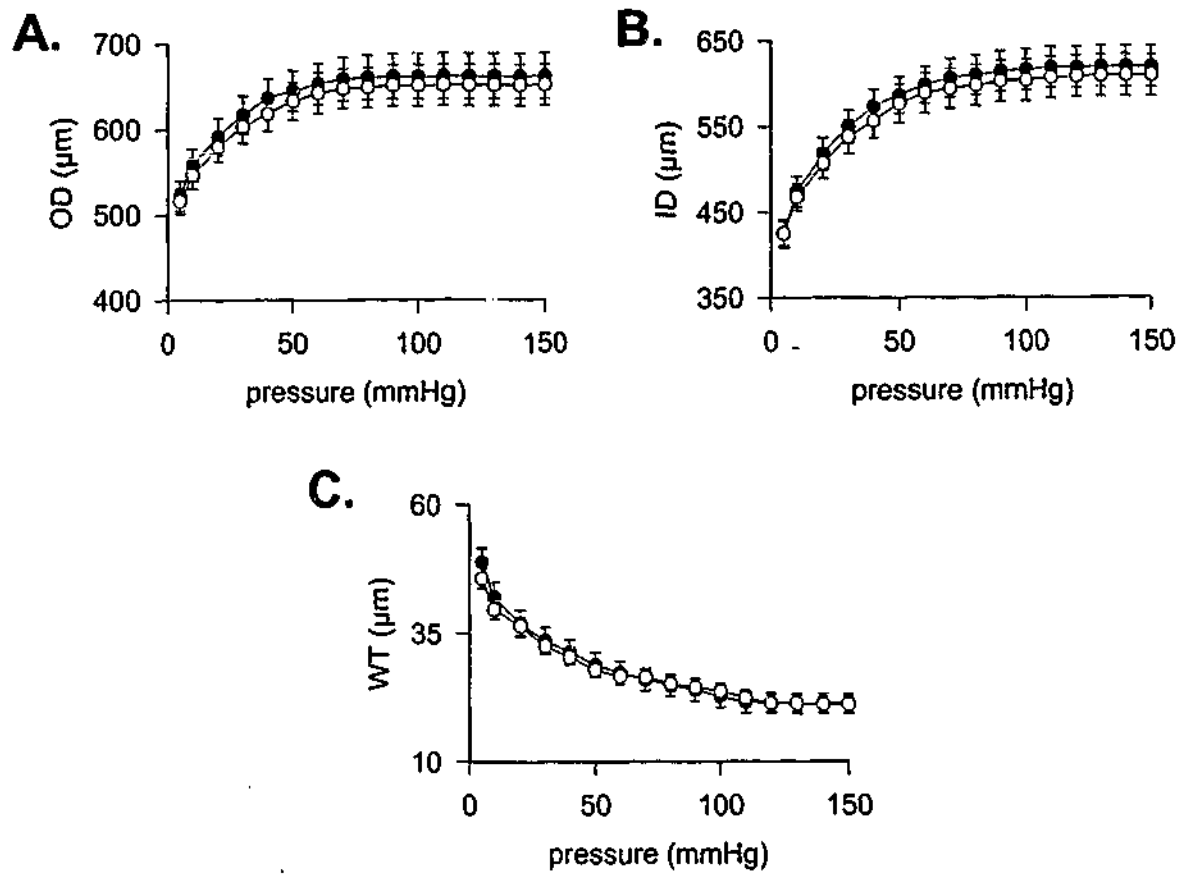
Appendix 4-1. Dimensions (µm) of the basilar artery branch during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of basilar artery branch from control (●, n=15) and IUGR (○, n=17) sheep.



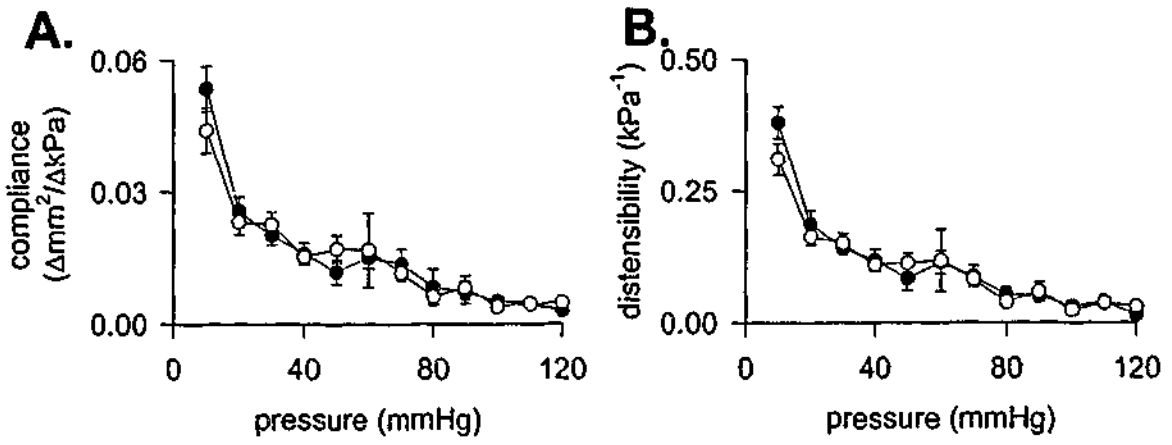
Appendix 4-2. Cross-sectional compliance and distensibility of the basilar artery branch. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of basilar artery branch from control (●, n=15) and IUGR (○, n=17) sheep.



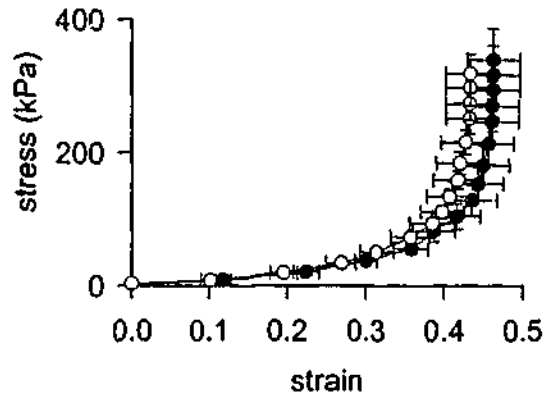
Appendix 4-3. Stress-strain relationships for the basilar artery. Stress-strain relationships for the segments of basilar artery branch from control (●, n=15) and IUGR (○, n=17) sheep.



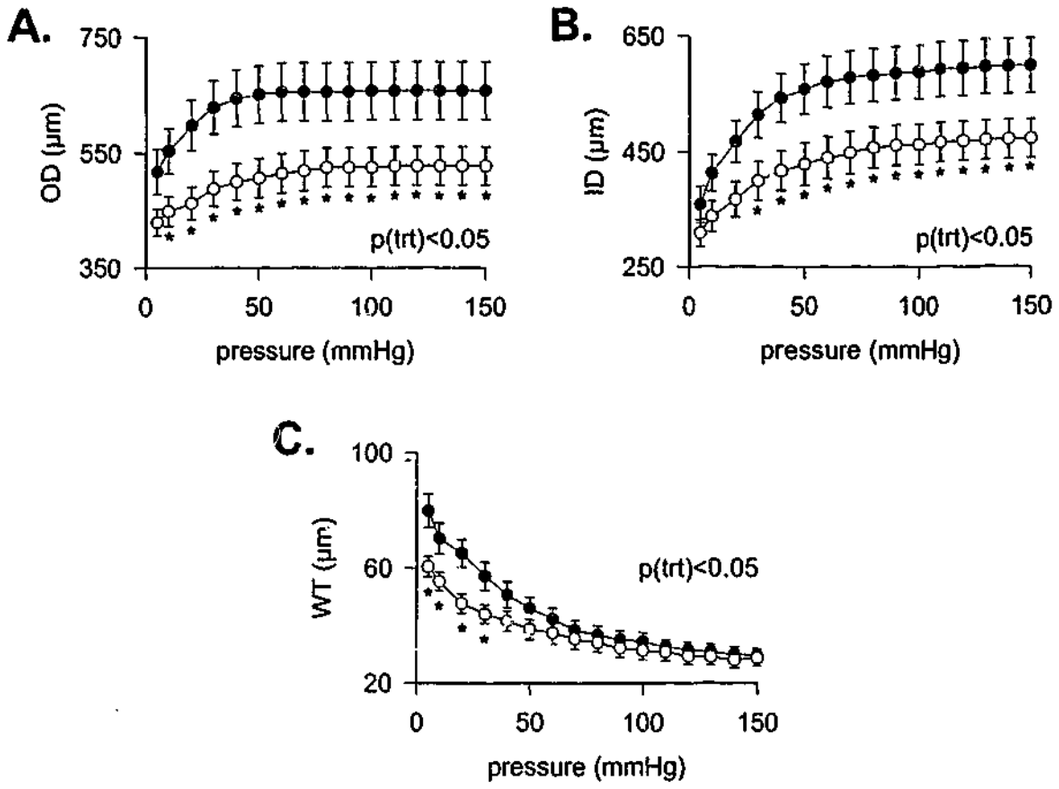
Appendix 4-4. Dimensions (µm) of the cerebral surface artery during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of cerebral surface artery from control (●, n=15) and IUGR (○, n=17) sheep.



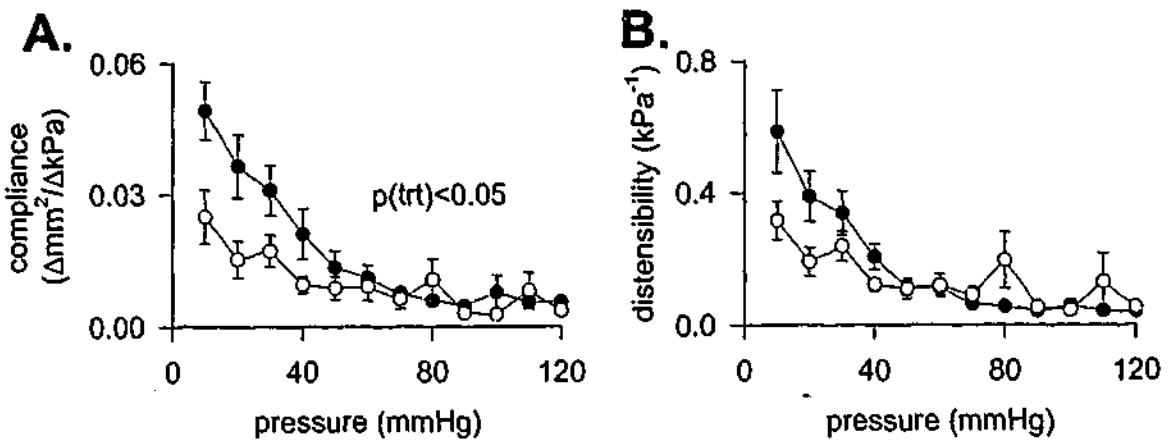
Appendix 4-5. Cross-sectional compliance and distensibility of the cerebral surface artery. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of cerebral surface artery from control (●, n=15) and IUGR (○, n=17) sheep.



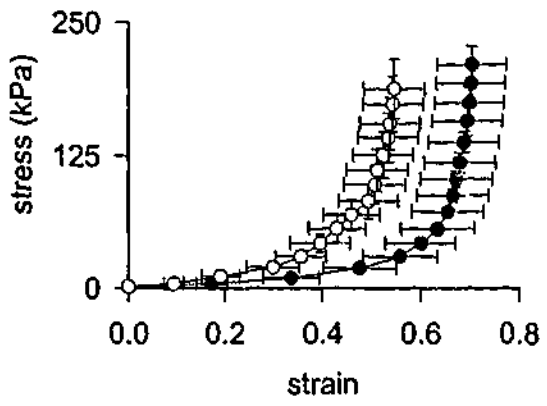
Appendix 4-6. Stress-strain relationships for the cerebral surface artery. Stress-strain relationships for the segments of cerebral surface artery from control (●, n=15) and IUGR (○, n=17) sheep.



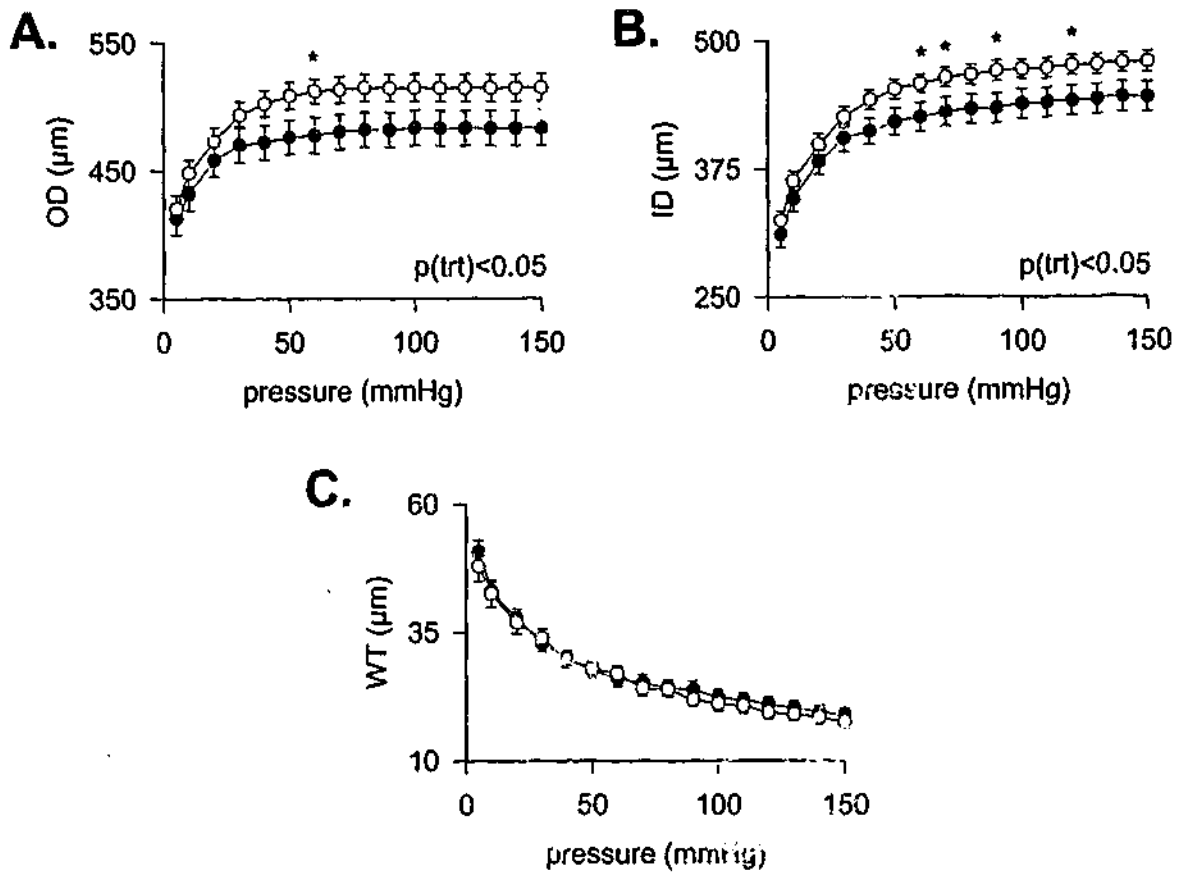
Appendix 4-7. Dimensions (µm) of the femoral artery branch during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of femoral artery branch from control (●, n=13) and IUGR (○, n=10) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).



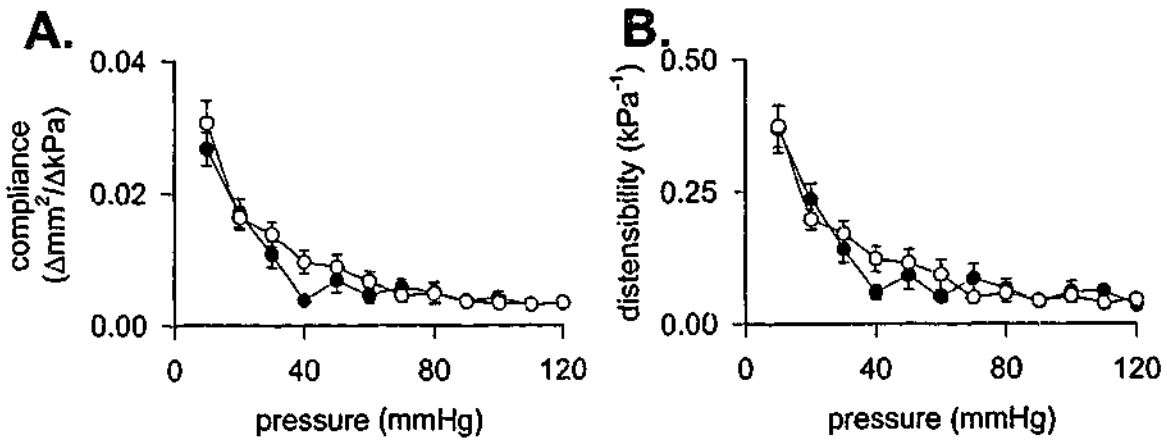
Appendix 4-8. Cross-sectional compliance and distensibility of the femoral artery branch. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of femoral artery branch from control (●, n=13) and IUGR (○, n=10) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA.



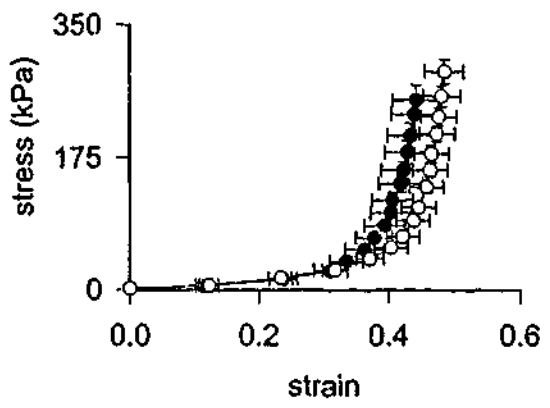
Appendix 4-9. Stress-strain relationships for the femoral artery. Stress-strain relationships for the segments of femoral artery branch from control (●, n=13) and IUGR (○, n=10) sheep.



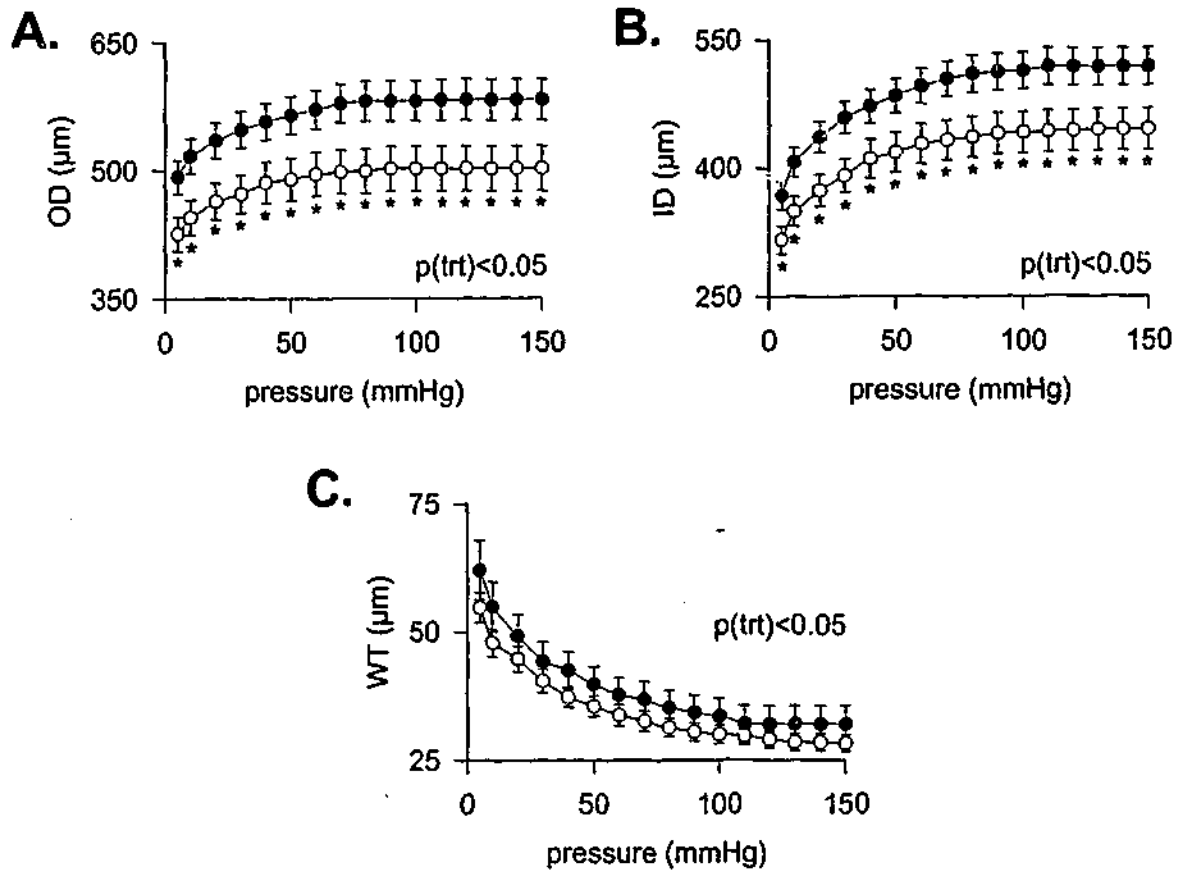
Appendix 4-10. Dimensions (µm) of the mesenteric artery branch during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of mesenteric artery branch from control (●, n=15) and IUGR (○, n=17) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures ($p<0.05$).



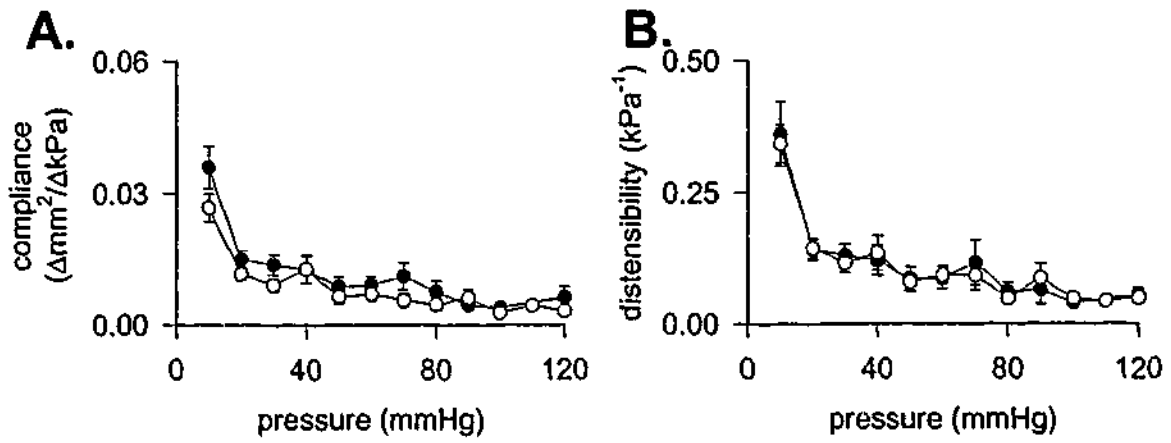
Appendix 4-11. Cross-sectional compliance and distensibility of the mesenteric artery branch. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of mesenteric artery branch from control (●, n=15) and IUGR (○, n=17) sheep.



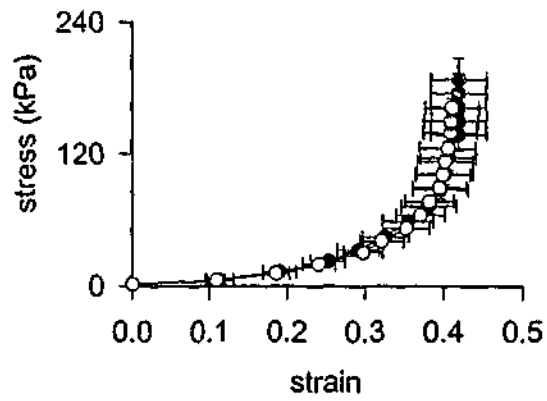
Appendix 4-12. Stress-strain relationships for the mesenteric artery. Stress-strain relationships for the segments of mesenteric artery branch from control (●, n=15) and IUGR (○, n=17) sheep.



Appendix 4-13. Dimensions (µm) of the arcuate artery during pressurisation. (A) Outer diameter (OD), (B) inner diameter (ID) and (C) wall thickness (WT) during pressurisation of the segments of arcuate artery from control (●, n=15) and IUGR (○, n=17) sheep. p(trt)<0.05 indicates a significant effect of treatment group as found by two-way ANOVA; asterisks (*) indicate values that differ between groups at individual pressures (p<0.05).



Appendix 4-14. Cross-sectional compliance and distensibility of the arcuate artery. (A) Cross-sectional compliance and (B) cross-sectional distensibility during pressurisation of the segments of arcuate artery from control (●, n=15) and IUGR (○, n=17) sheep.



Appendix 4-15. Stress-strain relationships for the arcuate artery. Stress-strain relationships for the segments of arcuate artery from control (●, n=15) and IUGR (○, n=17) sheep.

Chapter 5

General Discussion

The overall aim of this thesis was to determine the effects of fetal growth restriction on postnatal growth, arterial pressure, and the passive mechanical properties of resistance arteries. These aims were investigated in long-term follow-up studies using two animal models of late gestational intrauterine growth restriction (IUGR): placental restriction in guinea pigs and umbilico-placental embolisation (UPE) in sheep.

5.1 Birth weight is not related to adult arterial pressure in guinea pigs

At the commencement of the studies described in this thesis (March 1999), there was little known of the long-term effects of a sub-optimal fetal environment on somatic growth and arterial pressure in guinea pigs. Persson and Jansson (1992) showed that unilateral uterine artery ligation led to hypertension in IUGR offspring compared with their appropriately grown littermates. The authors speculated that the relationship between birth weight and arterial pressure would be amplified with age but to date, the effect of unilateral artery ligation on arterial pressure beyond 3–4 months has not been reported. More recently, Kind *et al.* (2002) reported that mild maternal feed restriction leads to hypertension in young adult male IUGR offspring; this prenatal insult did not lead to IUGR or postnatal hypertension in female offspring. Studies in this thesis included the investigation of the relationship between birth weight and arterial pressure at one year of age in guinea pigs. My study differed from previous studies in guinea pigs (Persson & Jansson, 1992; Kind *et al.*, 2002) not only in the age at which arterial pressure was measured, but also in that low birth weight offspring were not hypertensive as adults.

In my study, fetal mortality following the placental restriction procedure was high, and it was possible that birth weights differed due to naturally occurring variations in birth weight (i.e. from differences in genetic factors or placentation between individual offspring), rather than the result of any experimental intervention. Despite this possibility, a relationship between birth size and later arterial pressure might still be expected to exist. In humans, an inverse relationship between birth weight and arterial pressure has been demonstrated within the normal birth weight range (Barker *et al.*, 1992; Law *et al.*, 2002). Similarly, systolic pressure of offspring from *ad libitum* fed guinea pigs is inversely related to birth weight (Kind *et al.*, 2002). This inverse association between birth weight and adult arterial pressure was not found in the guinea pig offspring described in Chapter 2 of this thesis.

Although the guinea pig is a relevant species in which to investigate the mechanisms involved in the fetal programming of arterial pressure, with similarities to humans in terms of placentation (Enders, 1965) and prenatal renal development (Merlet-Benichou *et al.*, 1981; Hinchliffe *et al.*, 1991), there are few reports of this species being used in follow-up studies. Both uterine artery ligation and maternal feed restriction have been shown to elevate arterial pressure in guinea pig offspring (Persson & Jansson, 1992; Kind *et al.*, 2002) but neither study has investigated the underlying mechanisms. In Chapter 2, no relationship between birth weight and arterial pressure was found, but the modifications to postnatal arterial pressure may have been subtle and unable to be detected by the methods used. Given the low fetal survival rate, the low incidence of IUGR and a lack of any effect of size at birth on later arterial pressure, the ablation of utero-placental arteries in guinea pigs does not appear to be a favourable model to use to investigate the mechanistic basis underlying fetal programming. These mechanisms are more likely to be revealed in a model in which postnatal cardiovascular function is modified by a gestational insult such as maternal undernutrition and unilateral uterine artery ligation. However, a relationship between birth weight and arterial pressure is not always obvious in studies using guinea pigs; maternal undernutrition leads to hypertension only in male offspring (Kind *et al.*, 2002) and IUGR induced by unilateral uterine artery ligation is associated with hypertension only after transformation of the data (Persson & Jansson, 1992). Further studies in guinea pigs could examine the effects of the gestational insult on the structural development of specific tissues (e.g. arteries, kidney, heart) that may be evident prior to the onset hypertension which

appears to be established by 3–4 postnatal months (Persson & Jansson, 1992; Kind *et al.*, 2002) and that may predispose the individual to later cardiovascular dysfunction. In addition, longer follow-up studies could investigate whether the hypertension at 3–4 months is amplified with age, as suggested by Persson and Jansson (1992), or whether female offspring from feed-restricted mothers develop hypertension at ages older than 3–4 months (Kind *et al.*, 2002).

5.2 Long term consequences of IUGR in sheep

The studies in sheep presented in this thesis contribute to the growing evidence that has accumulated over the past 5–6 years indicating that perturbations to the ovine intrauterine environment can alter postnatal physiological function, without necessarily having effects on size at birth or leading to postnatal hypertension. Follow-up studies in sheep presented in this thesis show that late gestational placental insufficiency (during which the fetus is hypoxemic and hypoglycemic) reduces fetal growth, but does not lead to postnatal hypertension, as in the guinea pig.

5.2.1 Effects on postnatal somatic growth

Epidemiological studies indicate that postnatal catch-up growth can increase the risk for development of cardiovascular disease, with the greatest risk in individuals who were of low birth weight and who had increased postnatal growth rates (Huxley *et al.*, 2000). In the present studies, umbilico-placental embolisation during the final 20–28d of gestation led to a 41% reduction in birth weight; catch-up in body weight occurred in the IUGR sheep within the first 6 postnatal months. This catch-up was gender-specific, as male IUGR lambs were 20% lighter than male controls at two years; however, this may have been an effect of the rams purchased for inclusion in studies at this age (refer to Section 5.2.3c). Although the female IUGR sheep had caught up in body weight, they were not hypertensive compared with female controls at two years, indicating that in this cohort of females, small size at birth and postnatal catch-up growth was not associated with postnatal hypertension at maturity.

Unlike associations in humans (Law *et al.*, 1992), sub-optimal intrauterine conditions in the sheep did not lead to increased abdominal obesity. As with body weight, there were gender-specific differences in body composition at two years. In contrast with human

data, IUGR rams had less body fat (total body fat assessed by DXA and abdominal fat measured at post mortem) than control rams. In terms of postnatal growth between birth and two years, only body weight was recorded, which is a crude measure providing no indication of whether catch-up growth was due to proportionate increases in body fat, muscle and bone mass, or greater relative increases in one of these tissue types (e.g. fat). Determination of body composition between birth and two years may have provided insight as to whether age-related changes in body composition differed between controls and IUGR sheep. It is possible, in the female sheep at least, that IUGR was associated with reduced fat mass at birth and the catch-up in body weight was due to increased deposition of fat, such that, at two years, there was no significant difference in the total body fat mass in control and IUGR sheep. At two years, the female IUGR sheep tended ($p=0.07$) to have a greater relative fat mass compared with female controls and this may be a consequence of increased fat accumulation between birth and this age. However, with no measure of body composition at birth or before two years, the existence of relative changes in body composition in IUGR and control sheep in the early postnatal period are not known.

5.2.2 Effects on arterial pressure

The findings of the studies in this thesis do not support the hypothesis that a compromised intrauterine environment causing restriction of fetal growth during late gestation programmes postnatal hypertension. Several ovine models indicate that perturbations to the intrauterine environment can result in postnatal hypertension that is preceded by a period of relative hypotension (Robinson *et al.*, 1998; Hawkins *et al.*, 1999; Hawkins *et al.*, 2000c). Repeated prenatal glucocorticoid exposure also results in a transient postnatal hypotension, but does not lead to hypertension; IUGR lambs were normotensive at 6 months (Moss *et al.*, 2001). It is possible that the ages at which Moss *et al.* (2001) and I completed our studies (one and two years respectively), arterial pressure was in a period of cross-over from hypotension to hypertension. It is currently not known if, or when these young adult IUGR sheep will become hypertensive at an older age.

There may be a "critical window" during fetal life during which postnatal hypertension may be programmed. It is possible that this period occurs early in gestation, or at least

earlier in gestation than the age at which IUGR was induced in guinea pigs and sheep for studies in this thesis. In rats, brief maternal undernutrition during embryonic development (Kwong *et al.*, 2000) or throughout gestation (and therefore including the early gestational period; Woodall *et al.*, 1996; Woods *et al.*, 2001), can programme persistent hypertension in offspring, but maternal undernutrition only in the second half of gestation does not alter the arterial pressure of the offspring (Holemans *et al.*, 1999). Similarly, in sheep, maternal undernutrition (Hawkins *et al.*, 2000a) or exogenous glucocorticoid exposure (Dodic *et al.*, 1998) early in gestation can result in persistent postnatal hypertension in offspring, whereas perturbations including UPE (Chapter 4) and exogenous glucocorticoid exposure (Moss *et al.*, 2001) later in gestation are not associated with postnatal hypertension. There may also be a critical period during which UPE can induce persistent hypertension in offspring. Several studies have investigated the effects of UPE on fetal arterial pressure, but only one (Murotsuki *et al.*, 1997) has found UPE to be associated with persistent fetal hypertension. Interestingly, UPE in that study (Murotsuki *et al.*, 1997) was commenced earlier in gestation than other similar studies (108d GA *vs* 120d or 125d GA) that found no persistence of fetal hypertension (Gagnon *et al.*, 1994; Cock & Harding, 1997; Louey *et al.*, 2000; Chapters 3 and 4, this thesis). While UPE fetuses in the study by Murotsuki *et al.* (1997) were not studied in the postnatal period, it is possible that the fetal hypertension associated with UPE at this earlier gestational age would have persisted into the postnatal period.

It is possible that, rather than causing alterations to postnatal resting arterial pressure and hormonal levels, a sub-optimal *in utero* environment may alter responses to physiological challenges. In rats, manipulations of the maternal diet can lead to exaggerated stress responses in offspring (Tonkiss *et al.*, 1♀ ♂) and postnatal hypercaloric feeding amplifies the hypertension associated with IUGR (Vickers *et al.*, 2000). In sheep, maternal undernutrition does not alter resting cortisol concentrations in the late gestation fetus or early postnatal lamb; however, these offspring showed altered cortisol responses to HPA axis challenges (Hawkins *et al.*, 2000a). In all studies described in this thesis, physiological measurements were made only in resting conditions. It is possible that while the resting arterial pressure in the adult guinea pigs and sheep was not altered; exaggerated endocrine and/or pressor responses to physiological challenges may have resulted from the sub-optimal prenatal conditions.

5.2.3 Confounding factors of FOAD studies

5.2.3a Twins and multiple gestation

In humans, the evidence for FOAD in twins remains controversial (refer to Section 1.2.2c). For the long-term study in sheep (Chapter 4), the majority of animals in the IUGR group were twins and control sheep were singletons. While the use of twins in the IUGR group may have had effects on postnatal growth and arterial pressure, the study investigated the effects of an imposed late gestational insult rather than the effects of IUGR due to twinning on postnatal growth and arterial pressure. Interestingly, the two lambs that were naturally growth restricted (due to triplet gestation) were not hypotensive in the early postnatal period whereas the IUGR lambs that had been subjected to UPE were hypotensive relative to controls (Figure 4-24). At present, the effects of a multiple gestation in sheep, in the absence of an additional late gestational insult, on postnatal physiological function remains unknown.

The use of polytocous species such as rats, guinea pigs and pigs has advantages for studies investigating fetal programming as both control and IUGR offspring may be present within a litter, thus allowing for within-litter comparisons. Similar studies may be performed in sheep, with one fetus subjected to UPE, while its twin acts as an appropriately grown control. However, even within-twin pair studies do not account for genetic differences between offspring, or differences in placentation (leading to discordant fetal growth) that can occur within monozygotic twin pairs (refer to Section 1.2.2c).

In Chapter 3, early postnatal nutrition in the twin IUGR offspring may have differed from singleton controls, and therefore, may have had effects on postnatal somatic growth. All lambs were housed with their mothers prior to weaning, and each IUGR lamb may have had to compete with its twin for access to the lactating ewe, while singleton controls had free access to feed from the ewe. Competition between twin offspring may have imposed a postnatal nutritional limitation, and may explain the reduced postnatal growth rates (g/day) in the IUGR lambs during the first two postnatal months, and why postnatal catch-up was not exhibited until after weaning.

5.2.3b Gestation length

In humans, estimations of gestation length can be inaccurate. Furthermore, the effects of reduced birth weight due to preterm birth on postnatal arterial pressure have not been fully investigated (refer to Section 1.2.2b). In animal studies, gestation length is known in offspring from date-mated pregnancies, thus the effects of preterm birth may be accounted for. The majority of the guinea pig offspring used for the studies in this thesis were born at term; only one of these offspring was born prior to term and inclusion of this animal did not affect analyses. In the long-term sheep study, lambs were born at a range of gestational ages. Data from the preterm animals (<142d GA at birth; mean GA at birth–2SD for total cohort) could have been analysed separately to data from IUGR lambs born at term. At birth, there was a range of body weights and gestation lengths in both the control and IUGR group. In the IUGR group, gestational age at birth ranged from 137d GA to 148d GA (mean 143±1d GA), thus it did not appear logical to class an animal born at 141d GA as preterm, while another born at 143d GA was classified as term-born. For this reason, data from all IUGR animals were combined and analysed as one group. In the preterm IUGR lambs, birth weight was presumably not reduced due to preterm birth, but rather reduced weight as a result of UPE. Birth weights of the preterm IUGR and term-born IUGR lambs were not different from each other. The arterial pressures of lambs born an average of one week prior to term were not different to those of IUGR lambs born at term (Figure 4-24).

5.2.3c Gender, current body size and composition

In humans, an inverse relationship between body weight at birth and later arterial pressure has been shown in males and females. Similarly, *in utero* perturbations, including maternal protein restriction in rats (Langley-Evans *et al.*, 1994) and early prenatal glucocorticoid exposure in sheep (Dodic *et al.*, 2002c), can lead to hypertension in both male and female offspring. In contrast to these studies, UPE has gender-specific effects on arterial pressure, body weight, body dimensions and body composition in the young adult sheep (Chapter 4). At two years of age, the female IUGR sheep were not different from female controls, with no significant differences in terms of body size, body composition or arterial pressure. This is consistent with findings of Kind *et al.* (2002), who reported that maternal undernutrition in guinea pigs had no effect on birth weight or arterial pressure in female offspring, whereas male

offspring were growth restricted at birth and hypertensive at 100 postnatal days. For male sheep described in Chapter 4, IUGR led to persistent changes in somatic growth and arterial pressure. These alterations, however, were not consistent with the hypothesis that IUGR would lead to postnatal catch-up growth, increased body fat and elevated arterial pressure. The opposite effect was found, and IUGR in male sheep was associated with reduced body weight, length and height, reduced body fat and lower arterial pressure at maturity. While it is not clear why IUGR only had persistent effects in male offspring, or why these alterations were not in the direction that was initially expected, it may be related to the 6 rams purchased for the two year studies. These purchased rams were heavier than the one control ram studied serially from birth to two years, and this may have affected results regarding body dimensions and composition. Also, given the possibility that arterial pressure may be related to current body size, this may also be a contributing factor to explain the significantly lower arterial pressure in the IUGR rams.

5.3 IUGR and the arterial wall: Function and structure

A variety of animal models have been used to investigate the relationship between the prenatal environment and postnatal health. It is apparent the gestational timing, duration, and severity of the insult can have different effects on pre- and postnatal somatic growth and physiological function (refer to Table 1-1–Table 1-4). Studies to determine the mechanistic basis for programmed changes are in their infancy and potential mechanisms for the fetal programming of hypertension that have been studied include impaired renal development and alterations to the HPA axis.

In 1997, Martyn and Greenwald proposed that alterations to arterial wall structure (in particular, impaired elastin synthesis) resulting from a sub-optimal intrauterine environment, may predispose an individual for later hypertension. These impairments to arterial wall structure, and the subsequent alterations to arterial mechanical properties could be a key mechanism underlying the inverse relationship between size at birth and arterial pressure (Martyn & Greenwald, 1997). Despite the plausibility of this hypothesis, neither the immediate or long-term effects of sub-optimal intrauterine environments on arterial wall structure or mechanics have been investigated in animal models. One of the aims of this thesis was to determine the effects of reduced fetal

growth on arterial mechanical properties; short-term effects on arterial mechanics were investigated in the near-term sheep fetus, and long-term effects were studied in adult sheep and guinea pigs.

5.3.1 Passive mechanical properties of resistance arteries

In guinea pigs (Chapter 2), a sub-optimal intrauterine environment in which fetuses were potentially hypoxemic and hypoglycemic had no major effects on adult arterial pressure or passive arterial mechanical properties. In contrast, resistance arteries from the brain, femoral muscle and mesentery were less compliant in near-term IUGR sheep fetuses than controls (Chapter 3). This finding appears to support the hypothesis that reduced arterial compliance *in utero* initiates hypertension in IUGR individuals; however, this model of IUGR did not apparently result in persistent hypertension (at least not to 2 years of age) nor did the majority of alterations to fetal arterial mechanical properties persist to adulthood. Arterial mechanical properties were investigated under passive conditions, in the absence of smooth muscle effects. This provided the advantage of assessing the mechanical capabilities of arterial wall structural components, but may not have provided an accurate representation of the *in vivo* arterial mechanics. *In vivo*, smooth muscle makes active contributions to vascular mechanics (Folkow & Karlstrom, 1984) and vascular responses to pharmacological agents can be modified in offspring by maternal feed restriction (Ozaki *et al.*, 2000; Ozaki *et al.*, 2001; Franco *et al.*, 2002), even in the absence of alterations to arterial pressure (Holemans *et al.*, 1999). The effects of other models of prenatal compromise, including UPE, on vascular responsiveness to pharmacological agents are not known. Future studies may include investigation of the effects of IUGR on resistance artery responsiveness, and also the determination of regional peripheral resistances *in vivo*, which may also be altered as a consequence of compromised prenatal environmental conditions (Hawkins *et al.*, 2000a).

Although UPE led to less compliant resistance arteries in the near-term fetal sheep, these alterations did not persist to maturity and it is possible that compensatory vascular development may have occurred after birth when the external environment was no longer sub-optimal. Determination of arterial mechanical properties (also arterial structure and composition, refer to next section) in young lambs will indicate whether

the decreased arterial compliance evident in the near-term IUGR fetus is present in early postnatal life, and whether compensatory arterial development occurs soon after birth to "correct" any deficits in arterial wall structural protein content. Arterial pressure was not significantly different between IUGR lambs and controls at 140d GA or two postnatal years when the mechanical properties of the arteries were determined. The optimal time in the early postnatal period to investigate the short term effects of UPE on postnatal arterial mechanical properties may be at 4 weeks when the relative hypotension in IUGR lambs was at its greatest.

5.3.2 Structural alterations to the arterial wall

Late gestational IUGR does not appear to have adverse long-term effects on resting arterial pressure or passive arterial mechanical properties. At present, the effects of late gestational IUGR on arterial wall structure is not known. Morphological and immunohistochemical studies of lung (Maritz *et al.*, 2002) and retinas (Loeliger *et al.*, in press) from the two year old sheep described in this thesis indicate that this prenatal insult can induce persistent structural alterations in tissue structure. Biochemical and histological studies on conduit and resistance arteries collected from animals used for studies in this thesis may provide direct evidence for a possible effect of UPE on the development of structural proteins in the arterial wall. These analyses may reveal that UPE induces structural alterations to the arterial wall with no immediate functional consequences. Alterations to arterial structure and mechanical properties are evident in spontaneously hypertensive rats prior to the onset of hypertension (van Gorp *et al.*, 2000), and reduced glomerular number is evident in offspring from feed-restricted rat dams prior to the development of postnatal hypertension (Vehaskari *et al.*, 2001). It is therefore possible that alterations to arterial structure may exist in the IUGR sheep described in this thesis, even in the absence of increased arterial pressure; such changes may be a predisposing factor for later development of hypertension.

In sheep, significant increases in aortic elastin content occur between late gestation and the early postnatal period, with no further increase evident in the adult (Wells *et al.*, 1999). The developmental increase in the elastic modulus in the low tensile (elastic) stress range parallels the increases in elastin content (Wells *et al.*, 1999). Given the absence of any change in aortic elastin content between 3 postnatal weeks and

adulthood (Wells *et al.*, 1999), it may be sufficient to measure the arterial elastin content in the 140d GA fetuses and two year old sheep described in this thesis to determine the immediate and long-term effect of IUGR on elastin content. However, if IUGR does lead to lower arterial elastin in the late gestation fetus, delayed or compensatory elastin synthesis may have occurred after birth in the absence of a sub-optimal environment. Such delayed or compensatory elastin synthesis may then lead to increased or not different arterial elastin content in the two year IUGR sheep. This could explain why the reduced arterial compliance evident in the late gestation IUGR fetus did not persist to two years.

In contrast to elastin, developmental increases in the elastic modulus of the aorta in the high tensile (collagenous) stress range reflect increases in aortic collagen cross-linkage rather than increases in collagen content; these changes occur between the early postnatal period and adulthood (Wells *et al.*, 1999). The postnatal, rather than the perinatal period appears to be the critical period for the development of functional arterial collagen. Therefore, measurement of arterial collagen in an early postnatal group of lambs may be important to understand the effects of UPE on the development of this structural protein. As the mechanical properties of arteries at high tensions appear to be more dependent on cross-linkages in collagen rather than the amount of collagen present (Wells *et al.*, 1999), it would also be important to determine whether UPE has adverse effects on collagen cross-linkage. Given the importance of relative amounts of collagen to elastin in arterial mechanical properties (Roach & Burton, 1957; Wolinsky & Glagov, 1963), calculation of the relative proportions of arterial collagen and elastin may provide a valuable insight as to how the ratio of these structural proteins might be altered by IUGR.

5.4 Concluding remarks

IUGR was induced using clinically relevant models of late gestational placental insufficiency in both guinea pigs and sheep. As with humans, the majority of the IUGR offspring described in this thesis exhibited postnatal catch-up in body weight and length, with the timing of the catch-up growth being gender-specific. Catch-up in body weight occurred earlier in male IUGR guinea pigs than females. In contrast, female

IUGR sheep exhibited catch-up growth by early adulthood, but male IUGR sheep remained lighter and shorter, and had less body fat than male controls.

The studies presented in this thesis do not support the hypothesis that a sub-optimal intrauterine environment, specifically late gestational placental insufficiency, programmes postnatal hypertension. In the guinea pig, arterial pressure in the adult was not related to birth weight, nor were there significant associations between birth weight and the passive mechanical properties of resistance arteries. In the sheep, late gestational placental insufficiency was associated with reduced arterial compliance in several resistance arteries in the fetus, potentially predisposing offspring to hypertension. The reduced arterial compliance did not persist to adulthood, nor did hypertension develop in the IUGR sheep. The reasons why the reduced arterial compliance in the IUGR fetal sheep did not persist to adulthood are not clear, but it is possible that delayed or compensatory arterial development of extracellular matrix proteins may have occurred in the early postnatal period. It is presently unclear why, despite reduced arterial compliance in the near-term sheep fetus, these alterations were not associated with prenatal or early postnatal hypertension. Further in-depth studies of the immediate and long-term consequences of a late gestational placental insufficiency on arterial structure and *in vivo* function are required to more thoroughly understand how postnatal physiological function and health may be modified by the fetal environment.

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