THE UNIVERSITY OF AUCKLAND

The Long Term Consequences of Preterm Birth and Neonatal Growth Rates in Lambs

Mary Judith Berry

A thesis submitted in partial fulfilment of the requirements

for the degree of Doctor of Philosophy in Biomedical Science.

The University of Auckland, 2012

Abstract
Preterm birth is associated with adverse health outcomes. However, it is not known whether preterm birth itself causes these outcomes, or whether they are due to other factors that frequently co-exist including antenatal corticosteroid exposure, nutritional supplementation and altered postnatal growth rates. The experiments described in this thesis investigated the effects of gestation length, antenatal corticosteroid exposure, nutritional supplementation and postnatal growth rates on later cardiovascular and metabolic outcomes in sheep. Ewes with singleton lambs (n=208) were randomised to corticosteroid-induced preterm birth (preterm, n=60), spontaneous term birth (term-spont, n=73), corticosteroid-induced term birth (term-dex, n=52), or non-corticosteroid induced term birth (term-Alizin, n=16). Term-spont, term-dex and preterm lambs were also randomised to receive nutrient or water supplements from birth to 2 weeks. Pilot studies of *in utero* growth restriction were also undertaken to investigate the effects of poor fetal growth.

Preterm birth in male sheep and reducing gestation length in female term-spont sheep led to increased cardiac sympathetic and reduced parasympathetic activity in adulthood; changes associated with increased cardiovascular risk in humans. Antenatal corticosteroid exposure had no effect on cardiovascular or metabolic outcomes. Nutritional supplementation had different sex-specific effects following preterm and term birth. In term-spont males, supplementation decreased weight gain and weight-for-length between 2 weeks of age and weaning and reduced adult fat mass by ≈ 40% (not statistically significant). Conversely, in preterm males supplementation increased weight-for-length between 2 weeks and weaning without affecting adult fat mass. In term-spont females, supplementation reduced dextrose- and arginine-stimulated insulin secretion by 50-60% at 18 weeks, whereas in preterm females, supplementation increased arginine-stimulated but not dextrose stimulated insulin secretion by ≈ 50%. These effects of preterm birth and nutritional supplementation were not mediated by variations in postnatal growth rate.

These findings demonstrate that in sheep, reduced gestation length alters cardiac autonomic function and that a brief nutritional intervention has consequences for growth and pancreatic function into adulthood. Research in humans is needed to investigate whether the cardiovascular risk following preterm birth is mediated by altered autonomic function, and to assess the long-term metabolic consequences of nutritional strategies aimed at increasing neonatal growth rates.

Acknowledgements

My supervisors Associate Professor Frank Bloomfield, Professor Jane Harding and Doctor Anne Jaquiery have been, and continue to be, a tremendous source of knowledge, skill and expertise, and without their continued guidance and encouragement none of this would have been possible. I am also grateful to the HRC for the scholarship that has enabled me to undertake this research.

During my time at Ngapouri Research Station, Doctor Mark Oliver's assistance was invaluable in helping me to learn all the necessary aspects of sheep husbandry, surgical and experimental manipulation, for which I am very grateful. Also at Ngapouri, Diane Morse worked tirelessly to help me with all aspects of animal work; her humour and spirit kept us going through many long cold winter days on the farm. Bridget Clarke, Serina Digby, Anna Harding, Maggie Honeyfield-Ross, Basil Koberstein and Keith McCallum have all provided help and technical assistance throughout the project, as have our summer students Daphne Meijler, Ruchira Seneviratne, Ravi Ram and Mustafa Saffi.

Eric Thorstensen and his team in the analytical laboratory at the Liggins institute, and Michael Tavendale at Palmerston North (D_2O assays) have worked like Trojans to deal with the vast numbers of samples generated by the project with unflagging cheerfulness and efficiency.

To Professor Ivor Mason, I owe a vast amount, and am grateful. So too for the unstinting support of the Zeds, the Mothers of the Tyburn Monastery and our many friends that continue to rally around us, whichever way our lives take us. I especially want to acknowledge the unwavering confidence and pride that Ruggero had in my work, and the great sadness we all have that he isn't with us now to see my 'book'.

And finally my special thanks to Colin, Sophie and James. I couldn't wish for a more supportive or tolerant family; between us, all things are possible.

Table of Contents

List of Figures

List of Tables

Abbreviations

°C	degrees centigrade
11βHSD1	11 beta hydroxysteroid dehydrogenase 1
AA	arachadonic acid
ACC	acetyl-coenzyme A carboxylase
ACTH	adrenocorticotrophic hormone
AG	abdominal girth
AGA	appropriate for gestational age
AgRP	agouti related peptide
AIR	acute insulin response
Akt	serine/threonine protein kinase
ANOVA	analysis of variance
ANS	autonomic nervous system
ART	assisted reproduction technology
AUC	area under the curve
BIA	bioelectrical impedance analysis
BMC	bone mineral content
BMF	breast milk fortifier
BMI	body mass index
BP	blood pressure
BWT	birth weight
CA	corrected postnatal age
CBS	carbonate buffered saline
CG	chest girth
CGA	corrected gestational age
CIDR	controlled internal drug release
cm	centimetre
CNS	central nervous system
CO_2	carbon dioxide
CRH	corticotrophin-releasing hormone
CRL	crown rump length
cv	co-efficient of variation
d	gestational day
D_2O	deuterium labelled water
DBP	diastolic blood pressure
DHA	docosahexaenoic acid

DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
DI	glucose disposition index
DNA	deoxyribonucleic acid
DOHaD	Developmental Origins of Health and Disease
DRWG	daily relative weight gain
DXA	dual-energy X-ray absorptiometry
EBM	expressed breast milk
ECG	electrocardiogram
EM	ewe milk
EMS	ewe milk supplement
EPA	eicosapentaenoic acid
ETT	endotracheal tube
FAS	fatty acid synthase
FAUC	fatty acid area under the curve
FFA	free fatty acids
FMD	flow mediated vasodilatation
FMI	fat mass index
g	gram
G/I	fasting glucose to insulin ratio
G6Pase	glucose 6 phosphatase
GAUC	glucose area under the curve
GH	growth hormone
GLUT	glucose transporter proteins
GTT	glucose tolerance test
GV	growth velocity
GV_{0-2}	GV between birth and 2 weeks
GV_{0-TEA}	GV between birth and term equivalent age
GV_{2-W}	GV between 2 weeks and weaning
GV_{TEA-W}	GV between term equivalent age and weaning
GV_{W-1Yr}	GV between weaning and 1 year
HDL	high density lipoprotein
HEC	hyperinsulinaemic euglycaemic clamp
HF	high frequency component of HRV frequency domain analysis (0.15-0.4 Hz)
HGC	hyperglycaemic clamp
HMF	human milk fortifier

HOMA-IR	homeostatic model assessment for insulin resistance
HPAA	hypothalamic pituitary adrenal axis
HR	heart rate
HRV	heart rate variability
HSD	hydroxysteroid dehydrogenase
HTL	hock toe length
Hz	hertz
ID	internal diameter
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IL6	interleukin 6
im	intramuscular
IMT	intima-medial thickness
IQR	interquartile range
IR	insulin receptor
IRS	insulin receptor substrate
IUGR	intrauterine growth restriction
iv	intravenous
JNK	c-Jun amino-terminal kinases
kcal	kilocalorie
kg	kilogram
LBW	low birth weight
LCPUFA	long chain polyunsaturated fatty acids
LDL	low density lipoprotein
LF	low frequency component of HRV frequency domain analysis (0.04-0.15 Hz)
LGA	large for gestational age
LIRKO	liver-specific insulin receptor knockout
LL	leg length
LMP	last menstrual period
ln	log transformation
MDT	minimum detection threshold
mg	milligram
ml	millilitre
mmol	millimole
mRNA	messenger ribonucleic acid
mTOR	mammalian target of rapamycin
n	number

NN50%	percent of difference between adjacent R-R intervals that are greater than 50 ms
NO	nitric oxide
NPY	neuro-peptide Y
NZ	New Zealand
OD	outer diameter
PAR	predictive adaptive response
PDX-1	pancreatic duodenal homeobox 1
PEPCK	phosphoenolpyruvate carboxykinase
PFA	paraformaldehyde
PGE2	prostaglandin E2
$PGF2_{\alpha}$	prostaglandin F2α
PGHS-II	prostaglandin H synthase type 2
PI	ponderal index
PKCζ	protein kinase C zeta
PM	post mortem
POMC	pro-opiomelanocortin
PPARGC1A	peroxisome proliferator-activated receptor gamma co-activator 1-alpha
PR	progesterone receptor
PSNS	parasympathetic nervous system
PTL	preterm labour
PWV	pulse wave velocity
RAS	renin angiotensin system
RDS	respiratory distress syndrome
RIA	radioimmunoassay
RM ANOVA	repeated measures analysis of variance
RMSSD	square root of the mean of the sum of the squares of differences between adjacent R-R intervals
R-R interval	interval between successive normal 'R' waves on an ECG
S	supplemented
SAN	sinoatrial node
SBP	systolic blood pressure
sc	subcutaneous
SD	standard deviation
SDΔNN	standard deviation of differences between adjacent R-R intervals (ms)
SDNN	standard deviation of all R-R intervals (ms)
SEM	standard error of the mean
SGA	small for gestational age

S_i	insulin sensitivity
SNS	sympathetic nervous system
SVS	Southern Veterinarian Supplies
TEA	term equivalent age
TNFα	tumour necrotic factor alpha
U	units
UAC	uterine artery catheter
UCP	uncoupling protein
UK	United Kingdom
ULF	ultra low frequency component of HRV frequency domain analysis (<0.003 Hz)
UN	un-supplemented
USA	United States of America
USS	ultrasound scan
VLDL	very low density lipoprotein
VLF	very low frequency component of HRV frequency domain analysis (0.003 - 0.04 Hz)
W/L	weight for length
WHO	World Health Organisation
WPI	whey protein isolate
Wt	weight
α	alpha
β	beta
Δ PI	change in PI between birth and adult challenges
ζ	zeta

Chapter 1. Introduction

1.1. The Developmental Origins of Health and Disease

1.1.1. David Barker and the evolution of the 'Developmental Origins' hypothesis:

In the late 1980s Barker and his colleagues revolutionised the established sequence of disease causality with their 'fetal origins of adult disease' hypothesis. In their landmark papers they demonstrated a significant link between small size at birth and later cardiovascular morbidity and mortality (Barker, Osmond, Golding, Kuh, & Wadsworth, 1989; Barker, Winter, Osmond, Margetts, & Simmonds, 1989). These findings created much interest from epidemiologists, clinicians and scientists around the world, and formed the foundations of the 'Barker' or 'fetal origins' hypothesis.

Prior to Barker's publications, medical dogma held that adult disease was a reflection of genetic propensity, augmented by lifestyle choices made in adulthood. For instance, cigarette smoking, dietary overindulgence and physical inactivity were held to be the prime mediators in the pathogenesis of atherosclerotic cardiovascular disease. Despite this, it had been noted in a large study of British civil servants that while these aetiological risk factors undoubtedly played a part in disease progression, it was possible that they were neither the sole, nor possibly even dominant, influence on disease expression. It was proposed that other as yet unidentified risk factors were responsible for differences in rates of coronary heart disease between men of differing social class (Rose & Marmot, 1981).

In addition to the disparities in social class that existed in England in the last century, it had been recognised that there was a marked regional variation in death due to ischaemic heart disease. When county mortality records were examined, it became apparent that the regional variation in adult cardiac mortality during 1968-78 mirrored the regional variation in rates of infant mortality in the early part of the twentieth century, such that areas reporting high infant mortality also had high rates of death due to ischaemic heart disease in the adults of that generational cohort (Barker & Osmond, 1986). Explanations for this phenomenon that fitted conventional medical dogma were sought; proxies for poor socio-economic status with their implications for poor adult cardiovascular health were explored, but failed to correlate with infant mortality rates (Barker & Osmond, 1986). Mortality records showed that most early

infant deaths occurred within the first days of life, and were often associated with low birth weight (Woolf, 1996). Given the limitations in perinatal care available at that time, it is impossible to determine whether death and low birth weight were due to prematurity, fetal growth restriction or other causes. It was inferred that in this birth cohort, an adverse *in utero* environment, manifest as smallness at birth, rather than an adverse *ex utero* environment, was key to the observed early infant mortality rate, and hence there was a link between *in utero* events and adult outcomes (Barker & Osmond, 1986; Barker, Osmond, & Law, 1989).

The argument then followed that infants born after a similarly challenging *in utero* environment, but who survived, would potentially form an adult cohort at increased risk of cardiovascular disease. In Hereford, England, midwives attended all births from 1911 onward and recorded birth weight. Health visitors monitored infant health and recorded infant weight at one year of age. Through analysis of these records it was found that small size at birth and at one year correlated with an increased risk in adulthood of coronary heart disease (Barker, Winter, et al., 1989) and type 2 diabetes (Hales et al., 1991). In an attempt to find the link between birth size and cardiovascular morbidity and mortality, a further group of younger individuals were studied. An association between birth weight and blood pressure was already present by age 10, becoming more pronounced with age, whereby small size at birth was correlated with increased blood pressure, independent of current weight and other potentially confounding factors (Barker, Osmond, Golding, et al., 1989). This was interpreted as evidence that intrauterine life exerted effects on blood pressure in later life, which might in turn explain the geographical variation in cardiovascular death rates in England.

The relationship between small size at birth, impaired glucose tolerance, raised blood pressure and increased cardiovascular risk has since been replicated in both sexes and from a range of different populations and ethnic groups (Eriksson, Forsen, Tuomilehto, Osmond, & Barker, 2001; Leon et al., 1998; Lithell et al., 1996; Rich-Edwards et al., 1999; Rich-Edwards et al., 1997; Stein et al., 1996).

Barker's group though, was not the first to comment on the association between early life events and disease in adult life. Forsdahl had published a paper in 1977 in which he drew attention to the unexpected correlation between Norway's regional infant mortality rates and rates of adult death from atherosclerotic heart disease. He had concluded that poverty in childhood and adolescence, if followed by an increase in prosperity, predisposed to atherosclerotic heart disease. He postulated that nutritional deficits in early life may in some

way cause permanent biological damage, and that this might be the mechanism underlying the association between early life conditions and adult pathology (Forsdahl, 1977). Other investigators too had observed an association between conditions experienced *in utero* and adult sequelae. For instance, body mass index (BMI) of adults *in utero* during the 1944-5 Dutch Winter Famine was shown to vary according to the stage of gestation when maternal famine was experienced (higher BMI in those exposed to famine early in gestation, lower BMI for those exposed late in gestation compared to a reference group who had not experienced famine *in utero*) (Ravelli, Stein, & Susser, 1976).

While epidemiological studies provide an invaluable framework highlighting associations between events and outcomes, they do not provide robust evidence of causality, or offer potential biological mechanisms. In addition, human epidemiological cohorts have to contend with the presence of many confounding factors, loss of original cohort members to follow up and a time lag of many decades between perinatal events and their possible consequences. Experimental studies in animals can control for many of these variables, and may also allow assessment of long-term outcomes within a reasonable time-frame.

1.1.2. Mechanistic constructs for the developmental origins phenomenon

Although size at birth is a useful measure it neither establishes causality for later outcomes, nor explains the pathophysiological mechanisms underlying the final manifestation of disease or disease risk. Many conceptual frameworks have therefore been developed to try and explain this link between size at birth and later outcomes.

1.1.2.1. The thrifty fetus phenotype

In this construct an adverse uterine environment leads to fetal growth restriction and small size at birth. Physiological adaptation in the fetus favours survival in an environment with limited food or energy resources (Hales & Barker, 1992); however, during postnatal life if there is a mismatch with fetal nutritional environment, previously adaptive responses such as increased insulin sensitivity may favour increased adiposity. With increased fat mass comes an increased propensity to insulin resistance and Type 2 Diabetes (Leunissen et al., 2008). The thrifty fetus phenotype, however, only refers to the metabolic sequelae of *in utero* growth restriction, and therefore may be too constrained to encompass the broad range of adaptive responses encompassing what are now widely regarded as the developmental origins of adult disease (McMillen & Robinson, 2005).

1.1.2.2. Predictive adaptive response model

Another perspective is proposed by the 'predictive adaptive response (PAR)' model. Rather than assuming a match between the postnatal environment and the prenatal, this model draws on fetal adaptive responses to an unknown, but anticipated, postnatal environment (Gluckman & Hanson, 2004), the effects of which may span several generations.

1.1.2.3. 'Programming'

Both the thrifty fetus phenotype and the PAR model exemplify the concept of 'programming' to explain the late effects of physiological choices made in fetal life. Lucas used the term 'programming' to describe the way in which a stimulus, provided at a critical window of postnatal life can permanently alter physiological function throughout the life course of the individual (Lucas, Fewtrell, & Cole, 1999). For instance, female rat pups given a single dose of androgen during the first five days of postnatal life develop male patterns of gonadotropin secretion after puberty and fail to develop normal patterns of female sexual behaviour; if exogenous androgen exposure is initiated at day ten of life, no such late effects are seen (Barraclough, 1961). Numerous other examples of this phenomenon have been described in a range of species (McMillen & Robinson, 2005).

Similar critical periods exist in fetal ontogeny such that an adverse *in utero* environment leads to changes in physiological homeostatic mechanisms. This may be manifest externally by gross markers such as size at birth, but may also cause far reaching metabolic perturbations only manifest in adult life, without a major influence on birth size. It is likely that fetal nutrition (as distinct from maternal nutrition) is a major determinant of fetal physiological perturbation and adaptation (Harding, 2001).

Fundamentally, much of this 'programming' must be due to alterations in the way that genes are expressed. Epigenetic regulation is the process through which DNA and histone interactions are manipulated by nutritional or other factors so that gene expression is permanently changed, without altering DNA sequence (Godfrey, Lillycrop, Burdge, Gluckman, & Hanson, 2007; Waterland & Jirtle, 2004).

In humans, the epigenetic methylation status of offspring from women acutely exposed to famine has been shown to depend on their sex and on the period of peri-conception or gestation during which their mothers were starved (Tobi et al., 2009). Similarly, adults born small for their gestational age (SGA) at term have altered methylation of the promoter region

of the peroxisome proliferator-activated receptor gamma coactivator 1-alpha gene (PPARGC1A, an important regulator of glucose metabolism and energy and temperature homeostasis) (Puigserver & Spiegelman, 2003), compared to normal birth weight, gestation length, age and sex matched controls (Brons et al., 2010). It has therefore been suggested that epigenetic modification represents a mechanism through which *in utero* events may influence metabolic function decades later.

1.1.3. Summary

Although the claims of the original 'Barker', or fetal origins hypothesis initially met with some criticism (Tu, West, Ellison, & Gilthorpe, 2005), the complex interplay between fetal and early life events and later physiological function and health outcomes is now widely accepted, even by those initially sceptical (Gillman & Rich-Edwards, 2000; Joseph & Kramer, 1996). Current terminology, however, refers to the 'Developmental origins of health and disease' (DOHaD) to reflect the importance of events in both fetal and early postnatal life on later outcomes.

1.2. Measures of metabolic and cardiovascular health

1.2.1. Overview

Events in perinatal health have been linked to components of the Metabolic Syndrome which comprises a constellation of impaired glucose tolerance or insulin resistance, obesity, dyslipidaemia and hypertension (Grundy et al., 2005), although precise diagnostic criteria vary (National Institutes for Health, 2002; World Health Organisation, 1999). The fundamental point, however, is that it refers to a *syndrome*, not a discrete clinical entity, and that individuals with metabolic syndrome are at increased risk of cardiovascular morbidity and mortality, and of death from any cause (Hu et al., 2004; Malik et al., 2004; Ninomiya et al., 2004). No unifying pathophysiological process has been defined, and it is possible that one may not exist.

Ethnicity, genetic and environmental factors also modify the expression of different components of Metabolic Syndrome. For instance, individuals who have a first degree relative with type 2 diabetes are themselves at increased risk of becoming insulin resistant (Danadian et al., 1999; Einhorn et al., 2003) and children whose parents have Metabolic Syndrome have increased rates of obesity and insulin resistance compared to children without

a parental history of Metabolic Syndrome (Pankow, Jacobs, Steinberger, Moran, & Sinaiko, 2004). Compared to Caucasians, increments in abdominal girth have a more deleterious effect on insulin sensitivity and the risk of Metabolic Syndrome in those of Asian descent (Shah, Jonnalagadda, Kicklighter, Diwan, & Hopkins, 2005; WHO Expert Consultation, 2004). In young people, individuals of Hispanic or Afro-Caribbean descent have less visceral adiposity for a given BMI (Bacha, Saad, Gungor, Janosky, & Arslanian, 2003) and a greater insulin response during glucose tolerance tests and hyperglycaemic clamps (Arslanian & Suprasongsin, 1996; Arslanian, Suprasongsin, & Janosky, 1997; Svec et al., 1992) than those of Caucasian descent.

Obesity and associated co-morbidity are increasingly prevalent in children. Attempts to define paediatric Metabolic Syndrome (de Ferranti et al., 2004; Huang, Nansel, Belsheim, & Morrison, 2008; Weiss et al., 2004) are hampered by the variable patterns of growth and physiological maturation during childhood and adolescence (Steinberger et al., 2009). Preliminary findings however are salutary; in contemporary North American paediatric cohorts over 10% of all children (Lambert et al., 2004) and one third of overweight/obese children had features of Metabolic Syndrome (de Ferranti, et al., 2004).

Longitudinal studies have shown that diet, physical activity and lifestyle patterns adopted during childhood persist into adulthood (Mikkila et al., 2007; Mikkila, Rasanen, Raitakari, Pietinen, & Viikari, 2005). Although the long term implications for children with paediatric Metabolic Syndrome is unknown, these data suggest that individuals may be at risk of adverse cardiovascular or metabolic health from a young age.

Adult survivors of preterm birth or fetal growth restriction may be at greater risk of developing components of the metabolic syndrome than their non growth-restricted or term-born peers, and this risk may be further modified by postnatal diet and growth rate.

1.2.2. Glucose – Insulin axis function

1.2.2.1. Introduction

Under normal conditions a glucose load stimulates insulin secretion from pancreatic beta (β) cells. Insulin has classically been regarded as the key mediator of glucose disposal, playing a pivotal role in promoting glucose uptake by fat and skeletal muscle, and modulating hepatic gluconeogenesis. It is now recognised that these actions represent only a fraction of the vast

repertoire of insulin mediated activity. Other more recently described actions of insulin include signal transduction within the central nervous system (Porte, Baskin, & Schwartz, 2005), cardiovascular function (Muniyappa, Montagnani, Koh, & Quon, 2007), gene transcription, and cell growth and differentiation (Accili, 2004).

1.2.2.2. Insulin synthesis, secretion and action

Insulin is a peptide hormone, synthesised and secreted by pancreatic β cells, that is critical to the regulation of energy homeostatic mechanisms. Insulin is synthesized as preproinsulin, cleaved to form proinsulin, transported to the Golgi apparatus and packaged into secretory vesicles close to the cell surface. Proinsulin is cleaved to yield equimolar amounts of C-peptide and insulin (Steiner et al., 1969). Insulin secretion requires fusion of these secretory vesicles to the cell membrane and exocytosis of insulin, C-peptide and a small amount of biologically inactive proinsulin. Insulin circulates unbound, and has a half-life of about 5 minutes.

Glucose is the principal regulator of insulin secretion, both through a direct action on pancreatic β cells and indirectly via effects on other insulin secretogogues. When high concentrations of glucose are present in the extracellular fluid, glucose is transported into β cells via the glucose transporters GLUT1 and GLUT2 (Unger, 1991). Islet-specific glucokinase then phosphorylates glucose to form glucose-6-phosphate and starts a cascade of intracellular events culminating in an increase of cellular ATP, closure of ATP dependent membrane potassium channels, membrane depolarisation and influx of calcium (Berggren & Larsson, 1994). This in turn promotes margination of secretory vesicles and exocytosis of insulin. Glucokinase is therefore an important regulator of insulin secretion (Grupe et al., 1995), and alteration in enzyme function is responsible for some forms of diabetes (Froguel et al., 1993). Amino acids, fatty acids, ketone bodies, gut hormones and the autonomic nervous system all play a role in modulating insulin secretion (Ahren, 2000; Deeney, Prentki, & Corkey, 2000; Newsholme, Bender, Kiely, & Brennan, 2007; Nolan & Prentki, 2008).

Sudden increases in plasma glucose concentration elicit a biphasic insulin response. First-phase response describes a burst of insulin secretion that peaks within 3 – 5 minutes and probably reflects the release of stored insulin from secretory granules immediately adjacent to the β cell membrane (Henquin, Ishiyama, Nenquin, Ravier, & Jonas, 2002). A second-phase sustained release of both stored and newly synthesized insulin then develops if glucose concentrations remain high (Ferrannini & Pilo, 1979; Wang & Thurmond, 2009). Abnormal

first phase responses have been proposed as the first pre-diabetic abnormality in glucose-insulin axis homeostasis (Gerich, 2002).

The insulin receptor is a membrane glycoprotein comprising two extracellular α-subunits and two transmembrane β-subunits. The conformational change induced by insulin binding to the α-subunits leads to autophosphorylation of the β-subunits at their tyrosine sites (Kasuga, Karlsson, & Kahn, 1982) and activation of the Insulin Receptor Substrate (IRS); insulin signal transduction is primarily initiated via IRS1 and IRS2 (Sun et al., 1992; Sun et al., 1995).

Beta cell mass continues to increase in postnatal life (Ackermann & Gannon, 2007). The majority of this increase may be accounted for by proliferation of differentiated β cells (Dor, Brown, Martinez, & Melton, 2004) as the pancreas has little capacity for β cell neogenesis (Butler et al., 2003). Thus it appears that the final complement of β cells at birth may play an important part in determining the later ability of the endocrine pancreas to increase its β cell complement; fewer β cells at the end of pancreatic organogenesis, such as may occur after preterm birth or fetal growth restriction, may limit the extent to which postnatal pancreatic β cell replication can occur.

1.2.2.3. Glucose homeostasis

Glucose is principally derived from enteral feeding, glycogenolysis (breakdown of glycogen, the storage form of glucose) and gluconeogenesis (synthesis of glucose from substrates derived from carbohydrate, fat and protein metabolism). While both liver and kidney possess glucose-6-phosphatase (G6Pase, the terminal enzyme in both gluconeogenesis and glycogenolysis) (Burchell, Allan, & Hume, 1994) and are therefore capable of glycogenolysis and gluconeogenesis, the vast majority of *de novo* glucose production is in the liver (Stumvoll, Meyer, Mitrakou, Nadkarni, & Gerich, 1997). Blood glucose concentrations must be tightly controlled to ensure that sufficient glucose is available for cellular metabolism, whereas excess blood glucose concentrations result in toxicity and damage to many tissues and organ systems. Post prandial increases in blood glucose concentrations lead to increased circulating insulin concentrations.

Glucose disposal is principally mediated through cellular uptake which is facilitated by a large family of glucose transporter (GLUT) proteins. Of the 13 isoforms currently described, GLUT1-4 are perhaps the best characterised (Wood & Trayhurn, 2003).

Facilitative glucose transporters 1-3 (GLUT1-3) promote insulin-independent passage of glucose into cells. During fetal life the high affinity GLUT1 is the predominant fetal isoform, is ubiquitously expressed (Olson & Pessin, 1996), and appears to be critical for embryonic development (Wang et al., 2006).

Following birth there is a more tissue-specific expression of GLUT isoforms (Olson & Pessin, 1996; Postic et al., 1994). GLUT1 is still ubiquitously expressed, but is most abundant in the blood-brain-barrier (Leen et al., 2010) and in erythrocytes (Montel-Hagen et al., 2008). GLUT3 is a high affinity transporter localised mainly in the neuronal tissue of the brain. GLUT1 therefore promotes glucose transport across the blood brain barrier and into to the interstitial spaces of the brain, whereas GLUT3 facilitates the transport of glucose across neuronal membranes thus ensuring that the high metabolic needs of the CNS are met. GLUT1 also plays a part in insulin-independent whole body glucose homeostasis and glucose disposal. Transgenic mice in which GLUT1 is over expressed in skeletal muscle have reduced plasma glucose concentrations in both the fed and fasted states, and enhanced clearance of a glucose load compared to control mice (Marshall et al., 1993).

GLUT2 appears to have a unique role in the pancreatic beta cell where it acts as part of the glucose sensing insulin secretion pathway (Unger, 1991). Experimental models have shown that GLUT2 null mice develop hyperglycaemia and relative hypoinsulinaemia and, unless treated with exogenous insulin, die within the first weeks of life (Guillam et al., 1997). *In vitro* studies of the pancreata from these animals demonstrate loss of the first phase insulin response to glucose (Guillam, et al., 1997). In addition to, and independent from, its function as a glucose transporter, it has recently been shown that GLUT2 signalling in the brain may be an important mediator in the central regulation of both food intake (Stolarczyk et al., 2010) and thermoregulation (Mounien et al., 2010).

GLUT4 is primarily expressed in heart, skeletal muscle and adipose tissue, and is the only insulin-dependent glucose transporter (Huang & Czech, 2007). Skeletal muscle is able to store glucose as glycogen and, as glucose uptake is the rate limiting step in glucose metabolism, skeletal muscle is key to glucose disposal (Ren et al., 1993), accounting for approximately 75% of glucose disposal after an intravenous glucose load (DeFronzo et al., 1981). Defects in glucose uptake by skeletal muscle are one mechanism underlying peripheral insulin resistance (Cline et al., 1999; DeFronzo, Gunnarsson, Bjorkman, Olsson, & Wahren, 1985).

Activation of the insulin receptor on the skeletal muscle cell stimulates a complex cascade in which GLUT4 translocates from intracellular vesicles to the cell membrane where it acts as a glucose transporter (Thong, Dugani, & Klip, 2005). In the absence of insulin-induced signalling, GLUT4 is endocytosed and recycled, so that in the basal un-stimulated state only a small percent of total cellular GLUT4 is located in the cell membrane (Rea & James, 1997). This insulin-induced GLUT4 translocation is significantly reduced in the skeletal muscle of individuals with type 2 diabetes (Ryder et al., 2000). Insulin resistance in humans has also been associated with a reduction of GLUT4 expression in adipocytes while normal expression is maintained in muscle (Shepherd & Kahn, 1999). Transgenic mice in which GLUT4 is selectively reduced in adipocytes also become glucose intolerant and insulin resistant (Abel et al., 2001).

In the liver the insulin signalling cascade leads to a reduction in the expression of phosphoenolpyruvate carboxykinase (PEPCK) and G6Pase, both of which are necessary for hepatic gluconeogenesis (Matsumoto, Han, Kitamura, & Accili, 2006), but promotes expression of genes such as fatty acid synthase (FAS), and acetyl-coenzyme A carboxylase (ACC), required for fatty acid and triglyceride synthesis (Horton, Goldstein, & Brown, 2002).

In adipose tissue, insulin promotes the flux of glucose into adipocytes; glucose is then converted to glycerol, which combines with fatty acids to form triglyceride, the storage form of fat. Additionally, insulin inhibits the actions of hormone-sensitive lipase, thus preventing the hydrolysis of stored triglycerides and preventing fatty acid release from adipocytes (Large, Peroni, Letexier, Ray, & Beylot, 2004).

Insulin receptors have also been found in the arcuate nucleus of the hypothalamus. Through inhibition of neuropeptide Y (NPY)/agouti related peptide (AgRP) neurons (neurons involved in stimulating food intake and reducing energy expenditure) and stimulation of proopiomelanocortin (POMC) neurons (anorexic effects) insulin mediated signalling co-ordinates a reduction in both food intake and hepatic glucose production when insulin concentrations are raised (Porte, et al., 2005; Rother, Konner, & Bruning, 2008; Schwartz, Woods, Porte, Seeley, & Baskin, 2000).

1.2.2.4. Insulin resistance and glucose intolerance

Insulin resistance refers to reduced responsiveness of target organs to the metabolic effects of insulin (Muniyappa, Lee, Chen, & Quon, 2008) and is believed to be a central component of

the metabolic syndrome (Reaven, 1993). In both adults (Haffner, Stern, Mitchell, Hazuda, & Patterson, 1990) and children (Weiss et al., 2003), the progression from insulin resistance to impaired glucose tolerance and frank type 2 diabetes has been well documented.

With increased β cell metabolic requirements, such as in obesity and insulin resistance an initial compensatory hyperinsulinaemia, either through increased secretion or reduced clearance of insulin, or both (Mittelman et al., 2000), develops in order to maintain euglycaemia. Increased secretion may be mediated by an increase in overall β cell mass (Butler, et al., 2003; Pick et al., 1998; Unger & Zhou, 2001). In some individuals compensatory hyperinsulinaemia is not sustainable, and the balance of β cell proliferation, neogenesis (differentiation from precursor cells), hypertrophy and apoptosis becomes significantly altered with a relative increase in the rates of β cell apoptosis (Rhodes, 2005). There are many possible routes through which β cell apoptosis may be triggered, although ultimately many pathways appear to converge on IRS2; increased IRS2 activity has been shown to promote β cell survival (Hennige et al., 2003), whereas loss of IRS2 mediated signalling is associated with an increase in β cell apoptosis (Lingohr et al., 2003). Chronic hyperglycaemia (Briaud et al., 2005; Werner, Lee, Hansen, Yuan, & Shoelson, 2004), chronic hyperlipidaemia (Unger & Orci, 2001; Wrede, Dickson, Lingohr, Briaud, & Rhodes, 2002), and pro-inflammatory cytokines (Maedler et al., 2002) all promote β cell apoptosis through increased serine phosphorylation and inactivation of the IRS2 complex.

Once β cell capacity is exceeded, hyperglycaemia and type 2 diabetes develop (Sinha et al., 2002). Individuals at risk of a smaller β cell complement at birth, such as those born either SGA (Van Assche, De Prins, Aerts, & Verjans, 1977) or born during a period of pancreatic organogenesis (Green, Rozance, & Limesand, 2010) (such as individuals born preterm) might therefore be at increased risk of developing diabetes. Impaired insulin signalling and altered glucose transport and metabolism are also thought to play a role in the evolution of type 2 diabetes, but despite many possible candidates, the mechanisms responsible remain unclear (Coletta et al., 2008).

While obesity and type 2 diabetes frequently co-exist, the relationship is neither invariable nor permanent. Weight loss has been shown to reverse the process so that some type 2 diabetics can revert to the less pathological state of insulin resistance (Wing et al., 1991).

Many animal models of insulin resistance exist, and the very fact that so many have been developed is in itself an indication of the complexity and uncertainty regarding the

underlying pathophysiology of insulin resistance (Leroith & Accili, 2008). For example, in the ob/ob mouse (a leptin deficient model of insulin-resistant type 2 diabetes), hyperphagia leads to hyperinsulinaemia and, although plasma insulin concentrations are high, transcription of PEPCK and G6Pase persists with consequent continuation of hepatic gluconeogenesis (Shimomura et al., 2000). Others have tried to isolate the contribution of different tissues to the development of insulin resistance, and therefore their relative contribution to hyperglycaemia and hypertriglyceridaemia. In the liver-specific insulin receptor knockout mouse (LIRKO), Biddinger and colleagues have shown that hepatic insulin resistance is sufficient to produce hyperglycaemia, hyperinsulinaemia, and atheromatous vascular lesions. Altered lipid profile was also observed, although triglyceride concentrations were not different from those seen in control animals (Biddinger et al., 2008). It has therefore been speculated that there is a key, as yet undetermined, regulatory step in which the insulin regulated hepatic gluconeogenic and lipogenic pathways diverge (Brown & Goldstein, 2008).

Hyperinsulinaemia is also independently associated with adverse cardiovascular outcomes (Despres et al., 1996). Possible mechanisms include insulin-mediated increases in sympathetic autonomic nervous system activity (Lembo et al., 1992; Rowe et al., 1981), or insulin-stimulated secretion of endothelin-1 (ET-1) (Potenza et al., 2005) and mitogen-activated protein kinase (Begum, Ragolia, Rienzie, McCarthy, & Duddy, 1998) from vascular endothelium, with consequent increase in vascular smooth muscle proliferation (Nagai et al., 2003), vascular tone and blood pressure (Stout, 1992).

1.2.3. Obesity and dyslipidaemia

Overweight and obese are defined by the National Institute for Health on the basis of BMI (calculated as body weight in kilograms divided by height in metres squared) (National Institutes of Health, 1998). Obesity is increasing in both the paediatric and adult population. Ten percent of infants and twenty percent of children and adolescents in the USA are overweight or obese (Ogden et al., 2006), and it has been confirmed that obesity tracks from childhood through into adult life (Steinberger, Moran, Hong, Jacobs, & Sinaiko, 2001). Obesity-related death in the UK is estimated at 30,000 per annum and in the USA is set to overtake smoking as the leading preventable cause of death (Haslam & James, 2005). Obesity and related co-morbidities are projected to lead to the first decline in life expectancy in first world nations in many decades (Olshansky et al., 2005).

The pattern of fat distribution is an important factor in the association between obesity and poor cardiovascular and metabolic outcomes; an 'android' pattern of obesity (upper body or abdominal obesity) confers a higher risk than 'gynoid' obesity (gluteal or lower body obesity) (Yusuf et al., 2004). Abdominal fat comprises two metabolically distinct compartments; the visceral fat mass, which is associated with metabolic and cardiovascular risk (Fox et al., 2007) and the subcutaneous fat mass. Visceral fat is thought to promote metabolic risk through a number of different pathways. Adipocytokines, vasoactive substances and free fatty acids (FFA) secreted by visceral fat drain through the portal circulation directly to the liver (Bjorntorp, 1990; Jensen, Haymond, Rizza, Cryer, & Miles, 1989; Wajchenberg, 2000) and promote aberrant lipid deposition in liver and muscle, inhibit insulin-stimulated glucose metabolism in skeletal muscle and suppress hepatic glycogenolysis, all of which promote the development of insulin resistance (Boden & Shulman, 2002; Petersen & Shulman, 2002; Yu et al., 2002).

In experimentally manipulated rats it has been shown that transplantation of donor subcutaneous fat to the intra-abdominal cavity led to a significant reduction in body weight, attributable to reduced total fat mass, increased insulin sensitivity and reduced hepatic gluconeogenesis (Tran, Yamamoto, Gesta, & Kahn, 2008). Donor subcutaneous fat transplanted to a subcutaneous site has similar, but less pronounced consequences whereas visceral fat transplanted to subcutaneous sites does not lead to altered growth or insulin-glucose axis function. These data suggest that there are depot-specific characteristics of adipocytes that are reliant on proximity to other tissues, such as the liver, to exert their metabolic effects.

The distinction between 'healthy' and 'unhealthy' obesity has been proposed (Bluher, 2010) to acknowledge those obese individuals who do not have diabetes, hypertension or dyslipidaemia (Sims, 2001). It is suggested that increased visceral fat mass independent of BMI or total fat mass is the main predictor of metabolic dysfunction, and that in 'healthy' obese individuals the ability to expand the subcutaneous fat depot prevents ectopic visceral fat deposition and therefore maintains a more favourable metabolic phenotype (Bluher, 2010).

Obesity is recognised as an inflammatory state in which adipocytes themselves secrete an array of pro-inflammatory cytokines (e.g. tumour necrosis factor a (TNFα), interleukin 6 (IL-6)) and adipocytokines including adiponectin and leptin (You, Yang, Lyles, Gong, & Nicklas,

2005). It has been shown that there are higher levels of IL-6 secretion from visceral than subcutaneous fat stores (Fried, Bunkin, & Greenberg, 1998), and circulating IL-6 concentrations reflect overall adiposity in adults (Belza, Toubro, Stender, & Astrup, 2009) and obese children (Roth, Kratz, Ralston, & Reinehr, 2011). Plasma concentrations of TNFα are also elevated in obese adults (Dandona et al., 1998).

IL-6 and TNFα interact directly with the insulin receptor to reduce insulin signal transduction, and therefore enhance insulin resistance (Klover, Zimmers, Koniaris, & Mooney, 2003; Marette, 2002). In addition they indirectly enhance lipolysis and thus promote hepatic synthesis of fatty acids (Khovidhunkit, Memon, Feingold, & Grunfeld, 2000). Experimental blockade of TNFα improves the metabolic profile of humans with insulin resistance and chronic inflammatory disease (Huvers, Popa, Netea, van den Hoogen, & Tack, 2007). Other anti-inflammatory agents, such as salsalate (an inhibitor of NFκB, a proinflammatory cytokine also implicated in insulin resistance (Cai et al., 2005)) have also been shown to improve glycaemic profile in type 2 diabetes (Goldfine et al., 2010).

1.2.4. Hypothalamic pituitary adrenal axis activity

Individuals with Cushing Syndrome, whether as a result of excessive endogenous or exogenous corticosteroid exposure, are often hypertensive (Magiakou, Smyrnaki, & Chrousos, 2006), hyperglycaemic (Friedman et al., 1996) with inappropriately increased hepatic gluconeogenesis (Reinartz, Angermaier, Buchfelder, Fahlbusch, & Georgieff, 1995), and dyslipidaemic (Colao et al., 1999) with increased truncal fat deposition (Rebuffe-Scrive, Krotkiewski, Elfverson, & Bjorntorp, 1988). It has therefore been suggested that a relative increase in cortisol or hypothalamic pituitary adrenal axis (HPAA) activity might be the principal pathophysiological basis for the co-existence of components of the Metabolic Syndrome (Anagnostis, Athyros, Tziomalos, Karagiannis, & Mikhailidis, 2009).

Increased prevalence of subclinical hypercortisolism has been demonstrated in Type 2 diabetics and, in a large cohort of young men, a high cortisol:dehydroepiandrosterone (DHEAS) ratio was positively associated with the presence of metabolic syndrome. Increased morning plasma cortisol concentrations have also been reported in children with the metabolic syndrome, even after correction for body fat and degree of insulin resistance. Observational studies such as these are, however, unable to determine whether increased HPAA activity in metabolic syndrome is a primary or secondary phenomenon (Anagnostis, et al., 2009; Walker, 2006).

Experimental evidence has, however, suggested a causal role for increased HPAA activity in the Metabolic Syndrome. Eleven beta hydroxysteroid dehydrogenase type 1 (11βHSD1) catalyses a bidirectional conversion between the biologically inactive cortisone and the biologically active cortisol, but as it has a higher affinity for cortisone the usual direction is from inactive to active forms of corticosteroid (Stewart, Murry, & Mason, 1994). 11βHSD1 is widely expressed in metabolically active tissue including liver, muscle and fat (Ricketts et al., 1998), and is therefore thought to regulate local exposure to corticosteroids and hence have a role in regulating tissue responses to glucocorticoids. Rodents lacking 11βHSD1 have reduced hepatic gluconeogenesis and do not become hyperglycaemic when obese or stressed (Kotelevtsev et al., 1997), whereas transgenic mice over-expressing 11βHSD1 in adipose tissue develop many of the features of metabolic syndrome (Masuzaki et al., 2001). In humans, hepatic expression of 11βHSD1 is significantly greater in obese individuals with features of Metabolic Syndrome than in those without features of Metabolic Syndrome (Torrecilla et al., 2011) .

Pharmacological blockade of 11βHSD1 in clinical trials of Type 2 diabetics leads to improved lipid and glycaemic profile, and a reduction in body weight (Rosenstock et al., 2010). Pharmacological inhibition of enzymes in the cortisol biosynthetic pathway reduces systemic corticosteroid concentrations and leads to improvement in the metabolic profile of patients with Cushing Syndrome (Liu, Fleseriu, Delashaw, Ciric, & Couldwell, 2007) and Metabolic Syndrome (Arakaki & Welles, 2010).

1.2.5. Cardiovascular disease

Insulin resistance, diabetes and even elevated fasting plasma glucose concentrations in non-diabetics have been associated with hypertension (Ferrannini et al., 1987) and poor cardiovascular outcomes (Sarwar et al., 2010).

Endothelial dysfunction, in which altered insulin signalling may play a part, is an early event in the formation of atherosclerosis (Ross, 1993), which itself is integral to the development of cerebrovascular and cardiovascular disease. Post-mortem examination of children and young adults enrolled in the Bogalusa Heart Survey, who had died principally as a result of either accident or homicide has demonstrated the presence of atheromatous lesions at all ages. The severity increased with age across the cohort, but was also positively related to antemortem measures of adiposity, blood pressure, serum LDL and triglycerides, and cigarette smoking (Berenson et al., 1998). Sudden cardiac death accounts for 300,000 - 400,000 deaths annually

in the USA alone, is often the first clinical sign of cardiac disease, and may account for up to 50% of the overall cardiovascular mortality (Zipes & Wellens, 1998). These data highlight the presence of 'silent' cardiovascular pathology in paediatric as well as adult populations. One of the challenges in public health is the need to identify both at-risk populations, and robust pre-symptomatic markers for cardiovascular disease so that appropriate interventions can be initiated prior to overt disease expression.

1.2.5.1. Markers of cardiac autonomic nervous system activity

The integrity of the autonomic nervous system (ANS) is a major contributor to cardiovascular health, acting as a key mediator in the transition between physiological adaptation and pathological maladaptation. The ANS comprises two parts: the parasympathetic nervous system (PSNS) acting via the vagal nerve to reduce heart rate, and the sympathetic nervous system (SNS) which exerts its effects via pre- and post-ganglionic neurons of the sympathetic chain and acts to increase heart rate (Bailey, Fitzgerald, & Applegate, 1996). These different limbs of the ANS have mutually antagonistic effects that regulate key homeostatic functions, including heart rate and blood pressure.

Scrutinising autonomic function directly is complex. A range of biological 'markers' or proxies for ANS function have therefore been developed to try and elucidate the relative contribution of, and relationships between, the different elements involved. Heart rate (HR), and heart rate variability (HRV) are perhaps the most clinically useful and accessible of these measures. HR reflects the overall balance of sympathovagal tone, HRV gives an indication of the beat by beat modulation of heart rate by shifts in relative contribution between SNS and PSNS innervation of the sino-atroal node (Lahiri, Kannankeril, & Goldberger, 2008).

Heart Rate analysis

Heart rate is principally determined by both the intrinsic rate of spontaneous depolarisation of the sinoatrial node (SAN), and extrinsic influences of the SNS and PSNS. The intrinsic rate is further subject to modification by factors such as temperature and oxygenation. A denervated heart has an intrinsic rate of about 100 bpm and a healthy adult has a resting HR of 60-70 bpm, although this varies to reflect age, gender and exercise conditioning. The cumulative influence of autonomic innervation is to reduce the resting intrinsic heart rate. In steady state, the effects of SNS and PSNS are in equilibrium, but the balance of that set point shifts to reflect physiological perturbations. As HR lies on a continuum, the point at which a

given rate indicates pathology is difficult to determine in isolation, although increasingly the prognostic value of resting heart rate is being explored (K. Fox et al., 2007). For instance, even after correction for possible confounding factors such as BMI, tobacco use, diabetes and other traditional cardiac risk factors, asymptomatic individuals with a resting heart rate above 75 beats per minute have a 3.5 times higher relative risk of sudden cardiac death than those with a resting heart rate of less than 60 beats per minute (Jouven et al., 2005). Although women have a higher resting heart rate than men, the relationship between resting heart rate and cardiovascular morbidity and mortality is present in both sexes (Palatini et al., 2002), although less strong in women than men (Tverdal, Hjellvik, & Selmer, 2008).

Causality between HR increase and adverse outcome has not been fully identified, although evidence suggests that increased HR is an integral part in the evolution of pathological change. Artificially increasing heart rate in rats results in a progressive reduction in arterial wall compliance and distensibility (Mangoni, Mircoli, Giannattasio, Ferrari, & Mancia, 1996), possibly mediated through an increase in fluid shear stress on the vascular endothelium, which in turn promotes a pro-inflammatory response (Traub & Berk, 1998). In cynomolgus monkeys, increased heart rate is proportional to the degree of coronary artery atherosclerosis (Kaplan, Manuck, & Clarkson, 1987). In these monkeys, if heart rate is reduced through ablation of the SAN, the distribution and severity of atheroma is reduced (Beere, Glagov, & Zarins, 1992). More recently the progression of atheromatous lesions in genetically susceptible mice has been significantly reduced through selective SAN ion channel blockade-mediated reduction in heart rate (Custodis, Baumhakel, Schlimmer, & al, 2008).

Others have proposed that increased heart rate reflects unbalanced autonomic activity, with over-activity of the sympathetic and decreased activity of the parasympathetic nervous systems as the primary pathology. It has been suggested that re-addressing this balance through increased vagal activity reduces myocardial oxygen demand and work by reducing both heart rate and contractility (Lewis et al., 2001). Additionally, increased vagal activity confers protection from fatal cardiac arrhythmias in animal models of myocardial ischaemia (De Ferrari et al., 1993; Vanoli et al., 1991). Noradrenaline, a mediator of sympathetic nerve activity, is cardiotoxic, and high levels result in cardiac myocyte hypertrophy (Mann, Kent, Parsons, & Cooper, 1992) and apoptosis (Communal, Singh, Sawyer, & Colucci, 1999).

Heart rate variability

HRV is a measure of the variation seen between sequential R waves on ECG, and is an estimation of the effects of sympathetic versus vagal activity on a beat-to-beat basis. In health, HRV is high, inferring an intact autonomic system that is able to alter cardiovascular function to match minute physiological changes. A blunted or diminished HRV implies impaired responsivity of the autonomic nervous system, with increased risk of adverse health outcomes (Dekker et al., 2000). There are many different techniques available to measure HRV. The two main techniques used are time and frequency domain analysis.

Time domain analysis is based on statistical manipulation of R-R intervals, either individually or with reference to the adjacent R-R interval contained in an ECG, and therefore does not convey any information about the underlying periodicity or structure of the ECG. The conventional nomenclature is to describe R-R intervals as normal-to-normal (NN) intervals. Commonly used measures include SDNN [(ms) standard deviation of all R-R intervals]; SDΔNN [(ms) standard deviation of differences between adjacent R-R intervals]; RMSSD [(ms) square root of the mean of the sum of the squares of differences between adjacent R-R intervals] and NN50 [(%) percent of difference between adjacent R-R intervals that are greater than 50 ms]. Measures calculated in this way principally reflect parasympathetic effects (Malliani, 2005; Pumprla, Howorka, Groves, Chester, & Nolan, 2002; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Frequency domain analysis describes the periodic oscillation of heart rate signal at different frequencies and amplitudes, and therefore gives information on the structure and sequence of the underlying ECG. The usual approach involves using non-parametic transformation of the raw ECG using the fast Fourier transformation technique. This transforms the individual R-R intervals into bands of different spectral frequencies, which are then converted into Hertz by dividing by the mean R-R interval. Spectral components are therefore evaluated in terms of their frequency (Hz) and also their amplitude, which is expressed as the area (or spectral density) for each component. Thus, absolute values are referred to in ms^2. The total power of R-R interval variability refers to the total variance and corresponds to the sum of the spectral bands which are usually divided into high frequency (HF 0.15-0.4 Hz), low frequency (LF; 0.04-0.15 Hz), and very low frequency (VLF 0.003-0.04 Hz). Ultra low frequency spectra [<0.0003 Hz] are only calculated from data collected over at least 24 hours. Expression of LF and HF as their contribution to the total power allows better comparison between them.

When the data are normalised in this way (normalised units; nu), the VLF contribution is conventionally subtracted from the total power as follows: LFnu = LF(ms^2) * 100/(total power(ms^2) – VLF (ms^2)). LF reflects primarily sympathetic activity, and HF parasympathetic activity (Malliani, 2005; Pumprla, et al., 2002; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Real time analysis of HRV has been validated as a marker of fetal wellbeing (Lange et al., 2009) as reduced HRV is associated with fetal morbidity and mortality (Hon & Lee, 1963). In adults, reduced HRV has been associated with cardiovascular and all-cause mortality (Dekker, et al., 2000; Tsuji et al., 1996; Wolf, Varigos, Hunt, & Sloman, 1978).

Cardiac autonomic imbalance, manifested as increased heart rate, increased sympathetic activity and reduced parasympathetic activity, has also been associated with an unfavourable cardiovascular risk profile (Dekker, et al., 2000; Seccareccia et al., 2001; Tsuji, et al., 1996), with a diagnosis of Metabolic Syndrome (Licht et al., 2010) and with the dynamic of weight gain. Rapid or recent increases in adiposity are associated with increased sympathetic and reduced parasympathetic activity (Rabbia et al., 2003) whereas once fat mass is established and maintained, sympathetic activity declines while parasympathetic activity remains depressed (Verwaerde et al., 1999). Increased sympathetic activity has been shown to predict increased risk of insulin resistance (Masuo, Kawaguchi, Mikami, Ogihara, & Tuck, 2003), weight gain, reduced vascular endothelial function (Kaufman, Kaiser, Steinberger, & Dengel, 2007) and elevated blood pressure (Flaa, Aksnes, Kjeldsen, Eide, & Rostrup, 2008). Conversely, increasing parasympathetic activity has been shown to reduce markers of sympathetic activation and systemic inflammation, to improve HRV parameters in an animal model of heart failure (Zhang et al., 2009), and to reduce weight in obese subjects (Pardo et al., 2007; Val-Laillet, Biraben, Randuineau, & Malbert, 2010). Whether excess sympathetic activation, parasympathetic withdrawal or the overall balance of cardiac ANS function underlies these findings is unclear.

Hypertension is a also major public health problem (Pagani & Lucini, 2001) and is associated with increased risk of myocardial infarction, stroke and sudden death (The Joint National Committee on prevention detection evaluation and treatment of high blood pressure, 1997). On a population basis it is recognised that even small changes in blood pressure may be of significant public health importance (Erlinger, Vollmer, Svetkey, & Appel, 2003). The

autonomic nervous system has been implicated in the evolution of hypertensive change (Greenwood, Stoker, & Mary, 1999), as both reduced HRV and increased sympathovagal tone have been found to precede overt hypertension (Lucini, Mela, Malliani, & Pagani, 2002; Singh et al., 1998). For instance, normotensive individuals with a family history of hypertension had increased sympathetic activation and slower vagal re-activation during dynamic tests of cardiac autonomic function than normotensive individuals without a family history of hypertension (Davrath & Goren, 2003). Altered autonomic function in parallel with increases in blood pressure has also been described such that, even within the normal range, an increase in blood pressure is associated with an increase in LFnu (sympathetic) component and reduction in HFnu (parasympathetic) component of the total spectral power (Lucini, et al., 2002). In those with clinical hypertension, the changes in LFnu and HFnu were even more pronounced (Lucini, et al., 2002).

By comparison, the 4 year follow-up of the Framington Heart Study group showed that amongst normotensive men, a reduced LF component of spectral HRV was a stronger predictor of the development of hypertension than body mass index, and that a 1SD decrement in LF was associated with an increase in blood pressure of 1.95 mmHg (Singh, et al., 1998). Additionally they reported that in those known to be hypertensive at baseline, all markers of HRV were lower than those of normotensive individuals. Importantly, the LF and HF spectral components were not 'normalised' relative to the total power of the spectrum, which may account for the apparent discrepancy between studies.

1.3. Patterns and Regulation of Fetal Growth and Development

The fetus lies at the end of a long and often precarious supply line and is dependent on the maternal hormonal and metabolic milieu, uterine blood supply and adequate placental function for nutrient provision and growth. Factors influencing the fetus, mother or the placenta are capable of causing major disruption to fetal wellbeing, growth and long-term health.

1.3.1. Species dependent patterns of growth and development

Experimental manipulation of the maternal and fetal environment has been performed in, amongst others, rodents, sheep and non-human primates in an attempt to establish a causal pathway from *in utero* insult to postnatal phenotype. An important consideration of the choice of species is both the rate at which organ maturation occurs and its timing in prenatal

and postnatal life. In humans and sheep a comparatively long gestation results in a much higher degree of organ development and functional maturity at birth than is found in rodents. For example, although development of the endocrine pancreas follows a similar sequence in all mammalian species, the precise timing of maturational events differs between species (Sarkar et al., 2008). In atricial species such as the rat and mouse, pancreatic development is characterised by discrete, temporally defined clusters of differentiation and maturation: differentiation of cells destined to form part of the pancreatic cell lineage does not start until mid-gestation, with the first appearance of fully differentiated endocrine cells and islet formation in late gestation (Slack, 1995). In the rat, remodelling of the pancreas to replace fetal-type β cells (which respond well to amino acids, but poorly to glucose) with adult-type β cells (highly glucose responsive) happens in the second to third postnatal week, at the time of weaning (Scaglia, Cahill, Finegood, & Bonner-Weir, 1997). By contrast, in both sheep and human, β cells and islet formation are demonstrable in early gestation (Green, et al., 2010), and considerable similarities exist with respect to the tempo and regulation of cellular differentiation and maturation (Cole, Anderson, Antin, & Limesand, 2009). Unlike the rodent, apoptosis and re-modelling occurs in late fetal life so that pancreatic organogenesis is largely complete by term gestation (Fowden & Hill, 2001; Rahier, Wallon, & Henquin, 1981), although much lower rates of apoptosis and β cell remodelling do continue during postnatal life. Similar species-specific patterns are evident in other organ systems: in humans (Koutcherov, Mai, & Paxinos, 2003), non-human primates (Grayson et al., 2006) and sheep (Muhlhausler et al., 2005), hypothalamic neural networks involved in appetite regulation are established by mid-to-late gestation, whereas in rodents development of these neuronal networks is a postnatal event, in which postnatal nutrition and leptin play an important role (Bouret, Draper, & Simerly, 2004; Grove & Smith, 2003; Vickers et al., 2005). Similarly, nephrogenesis in humans, non-human primates and sheep is complete by the end of gestation (Abbott, Barnett, Colman, Yamamoto, & Schultz-Darken, 2003; Moritz & Wintour, 1999) whereas in rats it is not complete until the second postnatal week (Larsson & Maunsbach, 1980).

The validity of some rodent paradigms of perinatal environment has, however, been challenged by the observation that ‘control’ laboratory animals, raised over successive generations in standardized but possibly sub-optimal conditions may actually have significantly altered metabolic, neuronal and behavioural profiles secondary to limited

environmental stimulation and exercise, and current *ad libitum* feeding standards (Martin, Ji, Maudsley, & Mattson, 2010).

1.3.2. Role of the placenta during pregnancy

The placenta represents the interface between the maternal and fetal circulations, and plays a crucial part in maintaining fetal growth and wellbeing. It co-ordinates metabolic and endocrine signalling between mother and fetus, and is the site of fetal nutrient acquisition and waste removal. It is, therefore, not merely an inert bystander providing an open channel between the two; rather it has been described as a physiological sensor, detecting and responding to, changes in the maternal nutritional endocrine and hormonal milieu and shifts in fetal requirements (Jansson & Powell, 2006).

Placental function is reliant on uterine and umbilical blood flow, placental growth, the concentration gradient of nutrients between maternal and fetal circulations and the expression and activity of transporters on both maternal and fetal sides of the placenta. Passive diffusion of oxygen, carbon dioxide (CO_2) and urea across the placenta are constrained by both placental villous surface area and placental blood flow. Facilitated diffusion of glucose is reliant on a concentration gradient across the placenta (Bozzetti et al., 1988); thus diabetic mothers with excessively low or high blood glucose concentrations during pregnancy have an increased incidence of IUGR and fetal macrosomia respectively (Leguizamon & von Stecher, 2003). Active transport of other nutrients such as amino and fatty acids requires energy consumption and contributes to the high oxygen and glucose utilisation rate of the placenta itself (Bauer et al., 1998; Bell, Kennaugh, Battaglia, Makowski, & Meschia, 1986).

1.3.2.1. Nutrient transfer

Glucose

During pregnancy, placental growth hormone (GH) is released at high enough concentrations to obliterate the usual pulsatile release of GH from the maternal pituitary. Growth hormone is a powerful anabolic hormone and acts to promote lipolysis and protein synthesis, and oppose the actions of insulin. This induces a state of relative maternal insulin resistance, which in turn increases the availability of glucose and other nutrients to the fetus (Lacroix, Guibourdenche, Frendo, Muller, & Evain-Brion, 2002).

Although most gluconeogenic and glycolytic enzymes are detectable in fetal life, fetal glucose production is negligible in normal conditions (Girard, 1986; Kalhan, D'Angelo, Savin, & Adam, 1979). In fetal sheep, gluconeogenesis can be provoked experimentally by inducing maternal and fetal hypoglycaemia either by prolonged maternal fasting (Hay, Sparks, Wilkening, Battaglia, & Meschia, 1984) or use of a maternal insulin infusion (DiGiacomo & Hay, 1990). However, in pregnant women known to have a severely IUGR fetus, maternal ^{13}C-glucose infusions failed to demonstrate fetal gluconeogenesis, despite low fetal glucose concentrations (Marconi et al., 1993).

Placental and fetal energy requirements must be met through placental facilitated diffusion of maternal glucose. The principal transport mechanism appears to involve GLUT1 which is found in abundance on the microvillous side of the syncitiotrophoblast, and at a lesser amount on the basal membrane side (Barros, Yudilevich, Jarvis, Beaumont, & Baldwin, 1995). Human data, from paired maternal and fetal samples obtained during cordocentesis, show that fetal glucose concentration is dependent on gestational age, maternal glucose concentration and, if present, the severity of IUGR (Marconi et al., 1996). In particular, fetal glucose concentrations are lower in IUGR pregnancies than in those where fetal growth is not compromised.

Amino Acid Transport

Amino acid transfer across the placenta is by an active transporter mechanism, with neutral amino acids (alanine, proline, serine and glycine), phenylalanine and branched amino acids and cationic amino acids such as arginine each having a specific transporter system (Cetin, 2003). Fetal concentrations of phenyalanine, leucine and other amino acids are lower in IUGR fetuses (Paolini et al., 2001). Whether this is due to impaired placental transport or altered fetal uptake is unclear, and different mechanisms may exist for specific amino acids.

Fatty Acids

Linoleic acid (n-6) or α-linoleic acid (n-3) are ‘essential’ fatty acids as they are not synthesised by the body, but are needed to form the substrate of the metabolically active long chain polyunsaturated fatty acids (LCPUFA) that include arachadonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fatty acids are essential for the developing fetus; they are an integral part of cell membranes, mediate gene expression, represent a potential energy source (Das, 2006; Hanebutt, Demmelmair, Schiessl, Larque, & Koletzko, 2008), and are required for mammalian brain development (Innis, 2007).

Placental perfusion (Haggarty, Ashton, Joynson, Abramovich, & Page, 1999) and stable iosotope (Larque, Demmelmair, Berger, Hasbargen, & Koletzko, 2003) studies have demonstrated transport of LCPUFAs from maternal to fetal circulation so that both the fetus and newborn have greater plasma concentrations than the mother (Cetin et al., 2002; Innis, 2005). Fetal LCPUFA concentrations parallel the increases in fetal fat deposition that occur in the latter part of gestation; lower levels in IUGR infants may therefore reflect reduced fetal fat deposition (Haggarty, 2002).

Oxygenation

Adequate provision of oxygen to the fetus is also critical for adequate fetal growth and development (Gluckman & Harding, 1997), with relative maternal hypoxia linked to reduced fetal size (Jensen & Moore, 1997), possibly mediated by a reduction in uterine artery diameter and blood flow (Zamudio et al., 1995). However, even in women with no evidence of hypoxaemia, fetal oxygen tension is reduced in fetuses with IUGR (Nicolaides, Economides, & Soothill, 1989; Soothill, Nicolaides, & Campbell, 1987).

1.3.3. Fetal factors influencing growth

Fetal sex (Thomas, Peabody, Turnier, & Clark, 2000) and number (Joseph et al., 2009), congenital infection (especially in countries without widespread and accessible vaccination programmes (Tookey, 2004)), and single gene or chromosomal abnormalities (Gross, 1997) account for the majority of identifiable fetal causes of poor intrauterine growth (Hendrix & Berghella, 2008). In addition, artificially-conceived singleton pregnancies (Helmerhorst, Perquin, Donker, & Keirse, 2004; Jackson, Gibson, Wu, & Croughan, 2004), or those spontaneously conceived by couples with sub-fertility, are also more likely to have fetal growth restriction, suggesting that the underlying cause(s) for reduced fertility itself, rather than assisted reproduction techniques *per se*, may adversely influence fetal growth (Zhu, Obel, Hammer Bech, Olsen, & Basso, 2007).

1.3.3.1. Fetal glucose-insulin axis

Insulin is a major contributor to fetal growth regulation (Fowden & Forhead, 2004). Fetal insulin secretion is detectable from early gestation (Fowden & Hill, 2001) and is largely driven by fetal glucose and amino acids supply. Infants of diabetic mothers with high blood glucose concentrations are exposed to relative fetal hyperglycaemia, and consequently hyperinsulinaemia. This promotes fetal glucose uptake, and ultimately fat deposition with

resultant fetal macrosomia. By comparison, conditions with reduced insulin production or efficacy are associated with fetal growth restriction (Fowden, 1992). Many of the growth promoting effects of insulin are in fact mediated through IGF1, whereas the direct effects of insulin are primarily to promote adipocyte development (Gluckman & Pinal, 2003).

It has been suggested that a sub-optimal *in utero* environment, with consequent fetal growth restriction, promotes fetal metabolic adaptation to optimise nutrient disposal. Human IUGR newborns at 2 days of age have increased insulin sensitivity (Bazaes et al., 2003) and similarly, fetal lambs with experimentally induced IUGR have increased insulin sensitivity and insulin mediated glucose disposal (Limesand, Rozance, Smith, & Hay, 2007).

1.3.3.2. Fetal IGF axis

The insulin-like growth factor (IGF) family of hormones plays a crucial role in fetal tissue accretion and differentiation (Fowden, 2003), and IGF gene expression has been described throughout gestation. No consistent association has been reported between circulating maternal IGF 1 or 2 concentrations and fetal size or birth weight (Murphy, Smith, Giles, & Clifton, 2006).

IGF I expression is mediated by fetal insulin concentrations and by fetal nutritional status and both IGF I gene deletion (Woods, Camacho-Hubner, Barter, Clark, & Savage, 1997) or IGF-1 receptor gene mutations (Abuzzahab et al., 2003) result in growth restriction. IGF II is the dominant isoform during fetal life in humans (Gohlke, Fahnenstich, Dame, & Albers, 2004), sheep (Gluckman & Butler, 1983) and rodents (Daughaday, Parker, Borowsky, Trivedi, & Kapadia, 1982), and expression is mediated by an insulin-independent effect of fetal glucose concentration (Oliver, Harding, Breier, & Gluckman, 1996). IGF II is an important growth factor in early pregnancy; over expression in humans leads to tissue overgrowth syndromes and in animal models, under expression of IGF II results in poor fetal growth (DeChiara, Efstratiadis, & Robertson, 1990). Six IGF binding proteins (IGFBPs) have been characterised to date and are important moderators of IGF function. Of these, IGFBP I is able to bind IGF I and II with higher affinity than either their receptors and thus stop them from exerting their mitogenic effects (Lee, Conover, & Powell, 1993).

Although GH concentrations are high during fetal life, GH receptor expression is limited (Fowden, Li, & Forhead, 1998). In late gestation fetal sheep the pre-partum increase in fetal cortisol concentrations induces expression of hepatic growth hormone receptor and IGF I (Li

et al., 1996). This is essential for the transition from nutrition and insulin driven stimulation of the IGF axis during fetal life to growth hormone stimulation of the IGF axis in postnatal life.

1.3.4. Maternal factors in adverse pregnancy outcomes

Poor maternal health both prior to and during pregnancy is associated with preterm birth (Howarth, Gazis, & James, 2007), pregnancy loss (Stella & Sibai, 2006), and poor fetal growth (Allen, 2001; Haeri, Khoury, Kovilam, & Miodovnik, 2008; Murphy, et al., 2006).

Poor fetal growth is also more likely in pregnancies in younger (Haldre, Rahu, Karro, & Rahu, 2007) and older (Takimoto, Yokoyama, Yoshiike, & Fukuoka, 2005) mothers, and in those pregnancies exposed to toxins through maternal cigarette smoking (Andres & Day, 2000), alcohol consumption (Mills, Graubard, Harley, Rhoads, & Berendes, 1984) or illicit drug use (Naeye, Blanc, Leblanc, & Khatamee, 1973; Zuckerman et al., 1989).

1.3.5. Maternal nutrition in pregnancy

Maternal diet and nutritional plane have an effect on fetal growth and development, but also on the long term health of the offspring, although the nature of these effects reflects differences between species studied, the type of dietary intervention (total energy provided, micronutrient and macronutrient composition) and the timing and duration of exposure (Moore & Davies, 2005).

1.3.5.1. Maternal nutrition and gestation length

Maternal nutrition may also moderate gestation length; women with a low periconceptional BMI (Hendler et al., 2005), as well as those with limited weight gain during pregnancy (Ehrenberg, Dierker, Milluzzi, & Mercer, 2003) are at increased risk of preterm labour and delivery. Women in developing world nations often have inadequate nutrition throughout their life course, including the periconceptional period and pregnancy, whereas women in the developed world may have a calorie dense but nutritionally poor diet, or choose to actively diet during pregnancy. In both cases sub-optimal nutrition has been linked to preterm birth (Beck et al., 2010). Consumption of specific micronutrients (e.g. folic acid or iron; reduced intake linked to reduced gestation length (Gambling & McArdle, 2004; Shaw, Carmichael, Naelson, Selvin, & Schaffer, 2004)) and food types (e.g. liquorice; increased intake linked to reduced gestation length (Strandberg, Jaervenpaa, Vanhanen, & McKeigue, 2001)), have also

been associated with gestation length. The quality of maternal diet, distinct from its calorific content therefore has significant implications for preterm birth rates (Bloomfield, 2011).

Similarly in experimental paradigms of altered maternal nutrition, periconceptional under nutrition in sheep is associated with reduced gestation length (Bloomfield et al., 2003).

1.3.5.2. Maternal nutrition and fetal growth and health of the offspring

The Dutch Winter Famine was a period of severe starvation in the Netherlands during the last months of the Second World War, starting December 1944 and lasting until liberation in May 1945. Prior to the famine women had a reasonable plane of nutrition, thus, using ration cards and maternal and infant medical records it is possible to examine the effect of acute starvation on fetal and maternal outcomes.

Famine experienced during the third trimester of pregnancy resulted in lighter (Smith, 1947) and smaller (Stein, Zybert, van de Bor, & Lumey, 2004) infants. Neither periconceptional or mid trimester famine altered birth weight, although those born after periconceptional famine had a small reduction in head circumference and crown-to-heel length (Stein, et al., 2004). Irrespective of the effects of acute maternal starvation on birth weight, the offspring from these pregnancies have demonstrated adult morbidity that includes impaired glucose tolerance (de Rooij et al., 2006; Ravelli et al., 1998), poor mental health (Hoek et al., 1996), and risk factors for cardiovascular (Roseboom, 2000), and respiratory disease (Lopuhaa et al., 2000), and cancer (Painter et al., 2006; Roseboom, de Rooij, & Painter, 2006).

By contrast, offspring of women *in utero* during the Leningrad siege demonstrated a reduction in birth weight for those exposed late in gestation (Antonov, 1947), but have not demonstrated the same adult sequelae (Stanner et al., 1997; Stanner & Yudkin, 2001). The phenotypic differences between individuals exposed *in utero* to either the Dutch or Leningrad famines may be due to the greater duration of the Leningrad famine, the poorer nutritional plane of women and their children pre- and post-famine or the relatively greater protein deficiency of the Leningrad women.

Various experimental paradigms have also tried to define the effects of under nutrition during pregnancy. In rats calorie restricted to 50% of the feed consumed by pregnant *ad libitum* fed control animals, birth weight, but not litter size or survival, is reduced in offspring exposed to under nutrition during mid to late gestation, but not early gestation (Garofano, Czernichow, & Breant, 1998). In sheep periconceptional under nutrition does not affect birth weight (Todd,

Oliver, Jaquiery, Bloomfield, & Harding, 2009) but is associated with altered late-gestation fetal HPAA function (Bloomfield et al., 2004) and impaired glucose tolerance in post-pubertal offspring (Todd, et al., 2009), similarly, under nutrition during mid- or late-gestation has little impact on birth weight, but does alter later body composition and glucose tolerance (Ford et al., 2007; Gardner et al., 2005). In non-human primates maternal under nutrition alters fetal IGF axis function but not fetal weight (Li et al., 2007).

In contrast, pregnancies of overweight mothers are more likely to be complicated by fetal macrosomia, even if the mother is glucose tolerant (Jensen et al., 2003). There is also an association between late fetal death and maternal obesity (Cnattingius, Bergstrom, Lipworth, & Kramer, 1998). Animal models have demonstrated that in mice maternal obesity is associated with altered insulin-glucose axis and GH axis function, even in 2nd generation offspring (Dunn & Bale, 2009). If post-weaning rats are challenged with a fat and calorie dense diet, those from obese rat dams develop significantly worse hepatic steatosis (Bayol, Simbi, Fowkes, & Stickland, 2010), suggesting a reduced capacity to compensate for postnatal or environmental stressors.

1.3.6. Abnormal fetal growth

Although birth weight is conventionally regarded as a surrogate measure of fetal wellbeing, the data described above illustrate how maternal factors both prior to and during pregnancy may have significant effects on fetal outcomes, even when birth weight is unaffected. In addition, while size at birth is a marker of fetal growth and wellbeing, it is a static measurement taken in isolation. It does not, therefore, represent the varying trajectories of growth prior to delivery, or confer any information about likely causality if growth has deviated from the anticipated percentile.

Small for gestational age (SGA) is usually a descriptive term for an infant with a birth weight below the 10th percentile for gestational age, irrespective of the cause for that low birth weight. *In utero* growth restriction (IUGR), however, describes a pathophysiological process in which fetal growth is constrained so that the full growth potential of the fetus is not achieved. It is therefore possible for a fetus to experience intrauterine growth restriction and yet be of 'normal' birth weight, or conversely be labelled as SGA when growing well *in utero* and along a course determined by genetic potential (Beltrand et al., 2008; Mongelli & Gardosi, 1995; Platz & Newman, 2008). Customised fetal growth percentiles are now available that take into account the effects of maternal ethnicity, parity, height and weight,

and other factors linked to fetal growth (Clausson, Gardosi, Francis, & Cnattingius, 2001; de Jong, Gardosi, Dekker, Colenbrander, & van Geijn, 1998; Gardosi, Mongelli, Wilcox, & Chang, 1995). With better identification of normal and abnormal fetal growth patterns it should be possible to improve the diagnosis of both IUGR, leading to better identification of those at risk of adverse perinatal outcomes (Gardosi, 2004; McCowan, Stewart, Francis, & Gardosi, 2004).

Poor growth in the IUGR fetus is usually asymmetrical with head growth relatively preserved compared to somatic growth (Gardosi, 2004). Small infants in whom growth parameters are proportional are conventionally regarded as either appropriately grown for genetic potential, or as having some other multi-system disorder (for instance, trisomy 13) underlying uniform disruption of growth. This distinction is based on the non-linear characteristics of growth in early and late gestation; between conception and mid second-trimester growth is principally due to rapid cell division associated with organogenesis, but thereafter growth is principally due to maturation of these tissues and cellular hypertrophy rather than cell division. When growth is expressed as a percentage mass gained per week of gestation, gains are significantly greater in early and mid pregnancy than in its later stages, despite the fact that absolute weight gain is clearly greater during the third trimester (Cox & Marton, 2009). Medical dogma suggests that genetic abnormalities or adverse conditions in very early pregnancy alter growth during a phase of rapid cell division and disrupt all organ systems equally, leading to symmetrical growth restriction; whereas acquired pathological processes resulting in altered placental function and therefore fetal malnutrition in later pregnancy result in asymmetrical growth restriction.

Post mortem studies of fetuses and infants demonstrate that such clear distinctions are rarely found (Cox & Marton, 2009). Studies of infants in whom a fetal diagnosis of SGA had been made, based on abdominal circumference measurements $<10^{th}$ percentile, showed that anthropomorphic measurements at birth represented a continuum of body proportionality, and not a bimodal pattern of symmetrical or asymmetrically growth restricted infants as had been anticipated (Todros, Plazzotta, & Pastorin, 1996). Additionally, symmetry or asymmetry of size at birth did not correlate to the presumed aetiology underlying the fetal growth restriction (Todros, et al., 1996). Body proportionality of newborns has also been assessed using ponderal index (PI: weight/length^3) with the inference that if PI is within the population normal range (i.e. symmetry between head and somatic growth), size at birth probably reflects a constitutional not pathological processes (Landmann, Reiss, Misselwitz, & Gortner,

2006). Other investigators however have found that PI at birth is a poor indicator of IUGR (Haggarty, Campbell, Bendomir, Gray, & Abramovich, 2004).

In utero growth restriction in human fetuses is characterised by reduced plasma concentrations of insulin and amino acids (Economides, Proudler, & Nicolaides, 1989), minimal fetal gluconeogenesis (Marconi, et al., 1993) and, compared to the response seen in normally grown fetuses (albeit with other structural malformations), minimal increments in plasma insulin concentration following a glucose challenge (Nicolini, Hubinont, Santolaya, Fisk, & Rodeck, 1990). Placental GLUT1 is not different between pregnancies with an IUGR or normally grown fetus, suggesting that fetal hypoglycaemia in IUGR may be an adaptive response to increase the feto - maternal glucose gradient and enhance glucose transfer across a limited placental interface (Marconi & Paolini, 2008). Beltrand *et al.* propose that the IUGR fetus and newborn infant have heightened insulin sensitivity, and that this adaptation optimises nutrient disposal and fetal growth in an adverse, nutritionally-restricted, uterine environment (Beltrand, et al., 2008). If during postnatal life nutrition is more abundant, this adaptation becomes disadvantageous, favouring increased adiposity and the development of insulin resistance. Others have argued that the insulin resistant phenotype is already present at birth in those born IUGR (Hattersley & Tooke, 1999).

In experimentally induced IUGR, in both rats and sheep (generated using a thermal stress model), growth restriction is accompanied by a similar profile of hypoglycaemia and hypoinsulinaemia (Limesand, et al., 2007; Ogata, Bussey, & Finley, 1986; Owens, Falconer, & Robinson, 1989) and increased insulin sensitivity (Limesand, et al., 2007). Unlike humans IUGR in sheep is accompanied by fetal gluconeogenesis (Limesand, et al., 2007) and up-regulation of key hepatic gluconeogenic enzymes (Thorn et al., 2009). As a normally grown fetal sheep has negligible rates of gluconeogenesis (DiGiacomo & Hay, 1990), this must therefore represent part of a fetal compensatory mechanism to an adverse *in utero* environment. Other experimental paradigms utilising a less severe IUGR phenotype (ovine carunclectomy model: 20% reduction in fetal weight vs. 60% reduction in the thermal stress model) have however found no difference in fetal plasma glucose or insulin concentrations or insulin sensitivity between control and IUGR fetuses (Owens et al., 2007). Interestingly, experimental (Rozance, Limesand, Barry, Brown, & Hay, 2009) and clinical (Nicolini, et al., 1990) attempts to improve fetal growth by restoration of fetal euglycaemia have resulted in fetal morbidity.

Insulin-mediated signalling is also altered in IUGR fetus. In skeletal muscle of fetal IUGR sheep, insulin signalling proteins Akt2, PKCζ and GLUT4 are reduced, which may be compensated for by increased expression of insulin receptor (IR) protein (Muhlhausler et al., 2009). The anabolic actions of insulin (Fowden, Hughes, & Comline, 1989) are also reduced in IUGR. Low insulin concentrations and altered down-stream signalling pathways necessary for protein synthesis (Kimball, 2007) may therefore explain the reduced muscle mass seen in the fetal IUGR lambs. The normal cortisol-induced pre-partum reduction in skeletal muscle IGF I expression may also be prematurely invoked if fetal cortisol is increased as is seen in the IUGR fetus (Li, Forhead, Dauncey, Gilmour, & Fowden, 2002).

Additionally, pancreatic β cell mass is reduced in late fetal life in experimental IUGR sheep (Limesand, Jensen, Hutton, & Hay, 2005) and rats (Garofano, Czernichow, & Breant, 1997), with deficits that in rats persist into adulthood (Garofano, Czernichow, & Breant, 1999). Expression of Pdx1 (pancreatic duodenal homeobox-1), a gene involved in β cell differentiation and function (Melloul, 2004), is also reduced in islet cells of IUGR rat pups (Arantes et al., 2002). Both reduced β cell mass and altered function may have implications for later glucose homeostasis.

1.3.7. Mechanisms regulating the onset of parturition or timing of birth

1.3.7.1. Introduction

Parturition is the process which co-ordinates and controls the expulsion of the fetus and placenta from the uterus. Tight physiological control mechanisms exist so that, under normal circumstances, parturition is not induced until fetal growth and maturation are complete. Despite this, rates of spontaneous preterm labour in human pregnancies continue to rise (Goldenberg, Culhane, Iams, & Romero, 2008), as does the number of preterm deliveries for medical indications (concerns about fetal viability or maternal health if pregnancy continued) (Goldenberg, et al., 2008). Increasing numbers of infants are therefore born in whom the normal processes of growth and organ maturation are incomplete.

Despite species-specific variations, the onset and regulation of parturition is the result of many different signalling pathways evolving in concert, culminating in a pro-inflammatory cascade and delivery of the fetus.

1.3.7.2. Hypothalamic-pituitary-adrenal axis and parturition

Much of our understanding of the mechanisms involved in parturition is derived from chronically catheterised animal experimental models.

In sheep the fetus itself regulates the initiation of parturition through activation of the fetal hypothalamic-pituitary-adrenal axis (HPAA) with a dramatic increase in fetal circulating cortisol concentrations prior to birth (Fowden, et al., 1998; MacIsaac et al., 1985). Experimentally induced disruption of the ovine fetal HPAA leads to significant increases in gestation length (Gluckman, Mallard, & Boshier, 1991; McDonald & Nathanielsz, 1991), whereas directly (Thorburn, Hollingworth, & Hooper, 1991) or indirectly (through maternal periconceptional under nutrition) (Bloomfield, et al., 2003) increasing fetal ACTH or cortisol concentrations reduces gestation length. Fetal cortisol therefore is necessary to both determine gestation length, and to ensure the final maturation of organ systems such as the lung and gut prior to birth (Fowden, et al., 1998). Interestingly, the synthesis and secretion of surfactant protein by the alveolar type II pneumocytes, a hallmark of pulmonary maturity and induced by corticosteroids, may also be important in the initiation of parturition. Surfactant protein injected into the uterine cavity of pregnant mice at d15 (therefore prior to the normal, maturation dependent expression of surfactant) induces a local inflammatory response and preterm delivery; conversely, functional depletion of surfactant protein in amniotic fluid using a blocking antibody results in prolongation of pregnancy (Condon, Jeyasuria, Faust, & Mendelson, 2004).

Ovine fetal cortisol acts on the placental trophoblast causing up-regulation of prostaglandin H synthase type 2 (PGHS-II) leading to increased PGE2 expression (Gyomorey et al., 2000). This in turn increases the activity of placental $P450_{C17}$ and increases the local, placental conversion of pregnenolone to C19 precursors such as dehydroepiandrosterone (DHEA). Placental aromatase enzymes can then convert DHEA to oestrogen. PGE_2 also acts as a positive feedback mechanism, augmenting the effects of ACTH on fetal cortisol production (Young, Deayton, Hollingworth, & Thorburn, 1996). It has been shown through the infusion of radio-labelled androstenedione into the fetal compartment that aromatisation of C19 compounds occurs in the placenta, resulting in increased oestrogen in both maternal and fetal compartments (Mitchell, Lye, Lukash, & Challis, 1986). $P450_{C17}$ regulated change in placental steroidogenesis therefore promotes the conversion of progesterone to oestrogen, leading to a reduction in progesterone concentrations and an increase in oestrogen

concentrations (Mason, France, Magness, Murry, & Rosenfeld, 1989). Oestrogen acts by both increasing the expression of contraction-associated proteins and by increasing endometrial PGHS-II expression, and thereby increasing $PGF_{2\alpha}$ expression (Whittle, Holloway, Lye, Gibb, & Challis, 2000). PGF_2 activity is thought to be pivotal in orchestrating the pro-inflammatory cascade of parturition. Experimental blockade of PGF_2 signalling in sheep and mouse models of preterm parturition leads to suppression of uterine activity (Cirillo et al., 2007; Hirst et al., 2005).

In human and primate pregnancy, the placenta lacks $P450_{C17}$ and therefore is not able to synthesize C19 compounds directly; instead the principal products of the fetal adrenal gland are C19 compounds, particularly dehydroepiandrosterone sulphate (DHEAS) (Challis, Matthews, Gibb, & Lye, 2000). Aromatization of DHEAS occurs in the placenta thus increasing both local and systemic oestrogen concentrations. In primates, systemic oestrogen concentrations do not alter gestation length, whereas increased systemic androstenedione through the action of placental aromatase activity, leads to preterm labour unless co-administered with an aromatase inhibitor which prevents placental conversion of androstenedione to oestrogen (Mecenas et al., 1996; Nathanielsz et al., 1998). Fetal DHEAS synthesis is regulated by both fetal ACTH and by placental corticotrophin-releasing hormone (CRH), a hormone unique to higher-order primates (Power & Schulkin, 2006). It has been suggested that CRH may be the key intermediary between the fetal and maternal compartments at the onset of parturition, playing a similar role to that of PGE_2 in sheep (Challis et al., 2005). CRH concentrations increase dramatically close to term (McLean et al., 1995), and the rate of increase in CRH in mid pregnancy has been likened to a 'placental clock' in which a faster rate of increase predicts women at increased risk of preterm delivery (Sandman et al., 2006).

Although there are many similarities between the role of the fetal HPAA in the control of parturition between humans and other animals, there are also points of difference. For instance, systemic administration of glucocorticoids does not shorten human pregnancy, although it has been shown to increase uterine activity (Yeshaya et al., 1996). However, cordocentesis from human fetuses has shown a small but significant increase in fetal serum cortisol concentrations between 36 weeks gestation and term that precedes the onset of labour (Oh et al., 2006), and human infants with defects in the cortisol synthetic pathway, such as 21-hydroxylase deficiency, have significantly increased rates of post-term delivery compared with infants with intact adrenal function (O'Sullivan, Iyer, Taylor, & Cheetham, 2007).

1.3.7.3. Progesterone withdrawal and parturition

Progesterone is a maternal pro-gestational hormone, with increasing plasma concentrations during pregnancy thought to be crucial in maintaining uterine quiescence.

In sheep, rat and mouse, maternal serum progesterone concentrations fall rapidly at the onset of parturition, with progesterone withdrawal thought to be necessary for parturition (Young, 2001). In sheep, experimental blockade of 3β hydroxysteroid dehydrogenase (3β HSD; a key enzyme in the synthesis of progesterone) in late gestation causes a sudden and permanent reduction in progesterone concentrations that is followed by an increase in PGF_2, a transient drop in maternal and fetal cortisol concentrations, and preterm delivery of the fetus (Silver, 1988).

In humans and higher order primates, parturition occurs in the face of ongoing elevated progesterone concentrations that only fall once the fetus and placenta are expelled (Tulchinsky, Hobel, Yeager, & Marshall, 1972; Walsh, Stanczyk, & Novy, 1984). Withdrawal of progesterone via pharmacological blockade of the progesterone receptor with mifepristone (RU486) is, however, sufficient to terminate pregnancy in women at preterm or term gestations (Frydman et al., 1992; Lievre & Sitruk-Ware, 2009). In Rhesus monkeys, experimentally increasing plasma progesterone concentrations does not prevent the normal onset of parturition at term, although progesterone blockade using epostane (a competitive inhibitor of 3β-HSD) results in preterm delivery (Haluska, Cook, & Novy, 1997). This apparent conundrum may be explained by the concept of 'functional', rather than systemic, progesterone withdrawal.

Progesterone receptors (PR) belong to the family of steroid nuclear receptors (Ellmann et al., 2009). The two principal receptor subtypes are the PRB, which is the full length receptor, and the PRA, which lacks a 164 amino acid sequence at the N terminal. Both subtypes may be present in tissue as hetero- or homo-dimers, and both have equal DNA and ligand binding affinity (Ellmann, et al., 2009). PRA, however, is transcriptionally mute and, once activated by binding to progesterone is able to inhibit the effects of PRB in addition to other steroid receptors such as the ooestrogen and glucocorticoid receptors (Vegeto et al., 1993). In contrast, PRB mediates the effects of progesterone regulated gene expression (Giangrande, Kimbrel, Edwards, & McDonnell, 2000). It has therefore been suggested that altered expression of these PR splice variants may explain the functional progesterone withdrawal of human parturition (Madsen et al., 2004; Mesiano et al., 2002). Given the importance of local

or paracrine effects of steroid hormones, it is also possible that it is the local progesterone concentration or progesterone:oestrogen ratio that is of more significance than the systemic circulating plasma concentrations (Mazor et al., 1994; Romero, Scoccia, Mazor, Wu, & Benveniste, 1988).

1.3.8. Metabolic adaptation to birth

At birth, a swift co-ordinated transition from fetal to *ex-utero* life is critical for the survival of the newborn. While the changes required for cardiovascular and respiratory adaptation are dramatic they will not be discussed here. The metabolic and endocrine adaptation is less immediately visible, but no less critical.

1.3.8.1. Glucose-Insulin axis in the perinatal period

Hepatic glycogen deposition begins during the first trimester and continues throughout pregnancy as a buffer against the hypoglycaemia consequent on birth (Shelly, 1961). During the third trimester glucose is also converted to fat as a further energy depot. Following birth, the transplacental nutrient supply line is terminated. Blood glucose concentrations fall and metabolic homeostatic mechanisms to maintain independent normoglycaemia are initiated. An endocrine stress response predominates, insulin concentrations fall and glucagon concentrations rise (Sperling et al., 1974), the net result of which is initiation of hepatic glycogenolysis and gluconeogenesis, lipolysis and fatty acid oxidation. The provision of ketone bodies is critical for the neonatal brain which is able to use them as an additional, glucose-sparing, fuel source. A key part of this stress response is the fetal catecholamine surge, which is further exaggerated by intra partum stressors such as hypoxia (Padbury, Martinez, Thio, Burnell, & Humme, 1989; Sperling, Ganguli, Leslie, & Landt, 1984). Catecholamines stimulate both hepatic glycogenolysis and gluconeogenesis (Sacca, Vigorito, Cicala, Corso, & Sherwin, 1983). Experimental infusion of catecholamines into fetal sheep replicates the profile of insulin:glucagon seen in the immediate human newborn period (Padbury, et al., 1989).

Postnatal blood glucose concentrations reach their nadir at around an hour of age, then even in the absence of feeding, rise to normal levels by 3 hours of age (Srinivasan, Pildes, Cattamanchi, Voora, & Lilien, 1986). Hepatic glycogen stores are largely depleted by 12 hours of age, so that ongoing glucose requirements must be met either by exogenous supply, or gluconeogenesis. PEPCK (phosphoenolpyruvatecarboxykinase) is the rate limiting enzyme

in gluconeogenesis. Enzyme levels are low in fetal life and at birth (Girard, 1986) but falling insulin concentrations and rising glucagon levels (Sperling, et al., 1974) in the immediate postnatal period stimulate PEPCK activity with a consequent increase in gluconeogenesis.

1.3.8.2. Birth transition for the preterm infant

Preterm birth abbreviates the period during which fetal glycogen and fat stores are usually laid down (Shelly, 1961) and although gluconeogenic substrate is abundant, G6P (the final enzyme necessary for both gluconeogenesis and glycogenolysis) activity levels are low (Hawdon, Ward Platt, & Aynsley-Green, 1992; Hume & Burchell, 1993). The resultant postnatal drop in blood glucose concentrations is greater than that seen in term infants. Limited lipolysis and ketogenesis lead to a blunted counter-regulatory ketogenic response to low blood glucose concentrations (Hawdon, et al., 1992). Immaturity of these counter-regulatory responses to hypoglycaemia may persist beyond the immediate postnatal period, and even past the infants term equivalence date (Hume, McGeechan, & Burchell, 1999). In addition, higher blood concentrations of pro-insulin, insulin and C-peptide, and lower concentrations of glucagon have been reported in preterm as compared to infants born at term (Mitanchez, 2007).

Preterm infants also lose heat far more readily than their term born counterparts. Brown fat laid down during fetal life is the major source of uncoupling protein 1 (UCP1) that is key to effective thermogenesis (Clarke, Heasman, Firth, & Symonds, 1997) even in preterm infants (Chardon et al., 2006).

1.3.8.3. Birth transition for the IUGR infant

Metabolic stress *in utero* is reflected by low umbilical cord blood glucose concentration, and raised concentrations of free fatty acids (FFAs) and lactate (Soothill, et al., 1987), although others report lower concentrations of FFA in the IUGR newborn (Jones, Gercel-Taylor, & Taylor, 1999). Immaturity of postnatal PEPCK or other gluconeogenic pathways has been inferred by the presence of higher concentrations of gluconeogenic substrate in growth restricted rather than appropriately grown infants (Haymond, Karl, & Pagliara, 1974). Others, however, have found similar metabolic and hormonal responses in both growth restricted and appropriately grown infants (Ward Platt & Deshpande, 2005).

1.4. Postnatal consequences of the perinatal environment

Although small size at birth has been implicated as a causal factor in the later development of disease or disease risk, increasing evidence suggests that small size *per se* is instead a proxy for reduced gestation length, *in utero* growth restriction (IUGR) or altered postnatal growth trajectory. Both preterm birth and IUGR may co-exist, and are in themselves associated with altered postnatal growth compared to appropriately grown term-born infants, and this is further modified by postnatal feeding modality and nutrition. Increasing efforts are therefore underway to try and isolate the effect of a given perinatal variable on later outcomes. Studies in the human newborn are however significantly hampered by ethical considerations and logistical constraints; numbers tend to be small and the test populations are relatively heterogeneous in terms of range of gestation, ethnicity and clinical condition. With advances in neonatal intensive care practices, the majority of small or preterm infants are now expected to survive (Mangham, Petrou, Doyle, Draper, & Marlow, 2009; Tucker & McGuire, 2004). However, most adult morbidity is usually not clinically apparent until middle age (Tate, Manfreda, & Cuddy, 1998). As a group, survivors from an era of contemporary perinatal care are relatively young, and consequently any association between adult morbidity and perinatal condition may be hard to ascertain. Preterm birth in particular is often confounded by, amongst others, fetal or maternal co-morbidity, antenatal corticosteroid exposure and socio-economic status. Separation of the effect of reduced gestation length from other potentially confounding factors potentially is managed best through suitable animal paradigms. Sheep and primates have been used, although such studies are limited and tend to relate to short term outcomes, in part due to the high morbidity and mortality of preterm animals (Blanco et al., 2010; Thymann et al., 2009; Verney et al., 2010).

1.4.1. Postnatal consequences of preterm birth

There are undoubtedly huge challenges for infants born extremely preterm, both during the immediate neonatal period and with respect to their ongoing health needs (Costeloe, Hennessy, Gibson, Marlow, & Wilkinson, 2000; Markestad et al., 2005; Samara, Marlow, & Wolke, 2008; Westby Wold et al., 2009; Zwicker & Harris, 2008). Infants born at late-preterm gestations also have significant health needs (Melamed et al., 2009), and it is this late-preterm population that will be discussed in the context of preterm birth.

1.4.1.1. Definition and epidemiology of preterm birth

Provision of appropriate care to pregnant women and the newborn infant requires standardised terminology for gestational age (Engle, 2006; Engle & Committee on Fetus and Newborn, 2004), as gestation-specific patterns of morbidity and mortality will inform the management of pregnancy, birth and postnatal care. Accordingly, preterm refers to a neonate born at or before $36^{6/7}$ following the onset of the last menstrual period (LMP). Term refers to a neonate born $37^{0/7}$- $41^{6/7}$ following onset of LMP, and post term refers to a neonate born after 42 weeks following onset of LMP (Engle, 2006). In addition, the late-preterm infant has been defined as one born between $34^{0/7}$ and $36^{6/7}$ following onset of LMP (Engle, 2006). This classification highlights the immaturity of 'near term' infants, even if birth weight falls within the term normal range. Gestational age dating depends on local resource availability. The principal methods used in clinical practice are based on: (1) recollection of onset of LMP, (2) first trimester ultrasound measurements of the conceptus (Bottomley & Bourne, 2009), and (3) postnatal assessment of maturity (Ballard, Novak, & Driver, 1979).

Recollection of accurate LMP date is highly variable, and is complicated by poor recall or rounding of days to 'preferred' numbers (Martin, 2007; Waller, Spears, Gu, & Cunningham, 2000), variable cycle length and irregular ovulation (Rowland et al., 2002; Tunon, Eik-Nes, & Grottum, 1996).

Attempts have been made to validate ultrasound pregnancy dating by assessing the growth of fetuses conceived through assisted reproduction techniques (ART) when the exact dates of ovum retrieval and implantation are known. Both first and second trimester ultrasound measurement of ART conceived fetuses can calculate gestational age with a high degree of accuracy (Kalish et al., 2004; Sladkevicius et al., 2005). While this may validate the use of early USS dating, especially when dates derived from LMP or USS are discordant, it is possible that fetuses conceived through artificial reproduction techniques have different *in utero* growth patterns than those conceived naturally.

However, first trimester dating implicitly relies on the assumption that early fetal growth is linearly related to gestational age alone (Blaas, Eik-Nes, & Bremnes, 1998; Henriksen, Wilcox, Hedegaard, & Secher, 1995; Sawyer & Jurkovic, 2007). Where early or mid trimester USS dating are discordant with LMP-derived gestational length, medical convention favours USS as a more accurate dating modality; therefore a smaller-than-expected fetus is inferred to have a lesser gestational age and is not thought to be growth

restricted. The validity of this assumption is under investigation. In one large retrospective study involving nearly 30,000 singleton pregnancies with first trimester USS assessment of fetal growth, re-assigning estimated due date when the fetus was smaller than anticipated was associated with double the rate of SGA but no change in the rate of preterm birth (Thorsell, Kaijser, Almstrom, & Andolf, 2008). The authors conclude that contrary to medical dogma, fetal growth restriction is evident as early as the first trimester. Others have shown that where first trimester CRL is less than expected for gestational age there is a 2 fold increase in the relative risk of delivery between 24 and 32 weeks gestation, suggesting early growth perturbation is present in pregnancies destined to deliver preterm (Smith, Smith, McNay, & Fleming, 1998). Additionally, Bottomley *et al.* report a small but significant influence of maternal ethnicity and age on first trimester measures of fetal growth (fetuses of older women were smaller, with growth disparity equivalent to a two day difference in estimation of gestational age; fetuses of Asian mothers had a growth disparity equivalent to 1.5 days gestation) (Bottomley et al., 2009). Thus, even within the first trimester, fetal growth is dependent on maternal factors, and the trajectory along which the fetus progresses towards either fetal growth restriction or preterm delivery may already be set.

Postnatal assessment of gestational age is most usually preformed using the Ballard score technique. Although this technique may not be suitable for infants born below 28 weeks gestation (Donovan et al., 1999), within the 29-35 week gestation range the New Ballard Score gives a gestational age within 2 weeks of that calculated from certain LMP (Sasidharan, Dutta, & Narang, 2009).

As early as the 1960s it was recognised that there was additional morbidity and mortality conferred if infants were both preterm and small for gestational age (Battaglia & Lubchenco, 1967; Lubchenco, Searls, & Brazie, 1972), and although overall morbidity and mortality rates have fallen, this association holds true (Pulver, Guest-Warnick, Stoddard, Byington, & Young, 2009).

Despite continuing advances in obstetric and perinatal care, the rate of preterm birth continues to rise globally (Barros et al., 2005; Slattery & Morrison, 2002), especially in the developing world (Beck, et al., 2010). In the USA the rate has climbed from 9.5% in 1981 to 12.7% in 2005 (Hamilton, Martin, & Ventura, 2005), while in NZ in 2004, 7% of babies were born preterm (New Zealand Health Information Service, 2007). Although it has been suggested that the increase in preterm birth rate is a statistical artefact due to the inclusion of

infants born at the extremes of viability or birth weight who would not previously have figured in birth data (Di Renzo & Roura, 2006), the increase is primarily attributable to the late-preterm population. In the USA (Davidoff et al., 2006) and NZ (New Zealand Health Information Service, 2007) rates of spontaneous and non-medically indicated late-preterm birth continue to rise and account for over 70% of all preterm deliveries (Holland, Refuerzo, Ramin, Saade, & Blackwell, 2009); in England and Wales alone, this late-preterm population accounts for an estimated 66% of the nearly £3 billion annual public sector cost of preterm birth (Mangham, et al., 2009).

While the mechanisms underlying spontaneous preterm birth remains open to debate (Goldenberg, et al., 2008), it is known to be associated with poor maternal health and socioeconomic deprivation (Kramer, Séguin, Lydon, & Goulet, 2000; Moutquin, 2003; Swamy, Ostbye, & Skjaerven, 2008). In NZ thirty percent of pregnant women live in deciles 9 and 10 (most deprived areas), compared to fourteen percent in deciles 1 and 2 (most affluent areas) (New Zealand Health Information Service, 2007). Public policy to address these disparities in living conditions may therefore have additional benefits in addressing rates of preterm birth.

Fetal factors also play a role in determination of gestation length. In embryo transfer experiments where sheep embryos from a short or long gestation breed were implanted into recipient ewes, parturition occurred according to the fetal genotype (Kitts, Anderson, BonDurant, & Stabenfeldt, 1984).

1.4.1.2. Antenatal management of preterm labour

Below 34 weeks gestation, obstetric Practice Guidelines published by the American College of Obstetrics and Gynaecology recommend maternal corticosteroids to improve neonatal outcomes and tocolytics to try and increase gestation length, (American College of Obstetricians and Gynecologists, 2003, 2007). After 34 weeks gestation there are no recommendations for either intervention, possibly attributable to the underestimated consequences of late-preterm birth at the time the guidelines were originally constructed (Engle & Kominiarek, 2008).

1.4.1.3. Early complications of preterm birth

Early mortality in late-preterm infants

All cause mortality rates in infants born at late preterm gestations are significantly higher in both the neonatal period and throughout infancy (Engle, Tomashek, & Wallman, 2007; Kitsommart et al., 2009; Pulver, et al., 2009). For instance, for babies born at 34 weeks the male infant mortality rate was 13.5/1000 compared to 1.4/1000 for those born at 40 weeks, and even at early-term gestation (37-38 weeks) infant mortality is higher than at 39-40 weeks, especially in those infants that are also born small for gestational age (Pulver, et al., 2009). Although infants with lethal congenital conditions are over-represented in the late-preterm group (Tomashek, Shapiro-Mendoza, Davidoff, & Petrini, 2007) exclusion of such infants does not alter the increased mortality rates of late-preterm infants (Pulver, et al., 2009). Others have shown that when looking solely at infant death ascribable to Sudden Unexplained Death in Infancy, rates are twice as high in the late preterm population than in those born at term (Malloy & Freeman, 2000), suggesting perhaps an altered level of arousal, or cardio-respiratory control in this population.

Early morbidity in late-preterm infants

Even if birth weight is within the normal term range, late-preterm infants have excess morbidity compared with those born at term. For instance, in the late preterm infant rates of respiratory distress syndrome (RDS) requiring medical intervention (Escobar, Clark, & Greene, 2006; Kitsommart, et al., 2009; Melamed, et al., 2009; Rubaltelli, Bonafe, Tangucci, Spagnolo, & Dani, 1998; Wang, Dorer, Fleming, & Catlin, 2004), apnoea (Arnon et al., 2001; Melamed, et al., 2009; Merchant, Worwa, Porter, Coleman, & deRegnier, 2001), feeding problems (Meier, Furman, & Degenhardt, 2007; Walker, 2008; Wang, et al., 2004), jaundice (Sarici et al., 2004; Wang, et al., 2004), and temperature instability (Melamed, et al., 2009; Wang, et al., 2004) are more common. As gestational age at birth increases morbidity rates fall.

The incidence and severity of RDS declines with increasing gestational age, even within the range considered term (Madar, Richmond, & Hey, 1999). Emerging evidence indicates that rates of RDS are reduced among infants born at term by elective Caesarean section after antenatal corticosteroid exposure (Stutchfield, Whitaker, & Russell, 2005), and it has therefore been suggested that the late-preterm infant may also benefit from treatment with antenatal corticosteroids (Bonanno & Wapner, 2009). Large multicentre trials run by the

National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network are planned to explore this more fully (Bonanno & Wapner, 2009).

1.4.1.4. Long term consequences of preterm birth

Cardiovascular consequences of preterm birth

Blood pressure and vascular function

The Auckland steroid trial was the first, and largest, randomised controlled trial looking at the short term effects of antenatal steroid exposure on neonatal outcomes and therefore represents a unique cohort for whom detailed perinatal records are available (Liggins & Howie, 1972). From the 30 year follow up of neonatal survivors from that trial Dalziel *et al.* report a graded effect between gestation length and blood pressure, whereby a one week decrement in gestational age was associated with a 0.5 mmHg increase in systolic blood pressure (Dalziel, Parag, Rodgers, & Harding, 2007). When all individuals born preterm were analysed as a group, preterm birth increased systolic blood pressure by 3.5 mmHg. These results were independent of sex, antenatal steroid exposure, adult BMI and birth weight z-score. The majority of infants were born at gestations in excess of 30 weeks, (Dalziel et al., 2005) and as the rate of preterm births below 32 weeks gestation has remained virtually static (Tucker & McGuire, 2004), the characteristics of, and findings from, this birth cohort are likely to be relevant to infants born today. Additionally, antenatal steroid exposure was equivalent between the preterm and the term cohort, making it unlikely that these differences are ascribable to a late steroid effect. Others too have found no differences in blood pressure between children (de Vries et al., 2008) and adults (Finken et al., 2008) born preterm and either exposed, or not, to antenatal corticosteroids

Data from the prospectively followed 1958 British birth cohort have also shown a graded association, whereby for a one week increment in gestational age, blood pressure at age 44-45 years fell by 0.53 mmHg (Cooper, Atherton, & Power, 2009), whereas preterm birth as a dichotomous variable was associated with an increase in mean diastolic blood pressure of 2.59 mmHg. This cohort dates from an era prior to the advent of antenatal steroids or contemporary perinatal practice and comprises 7,847 individuals of whom only 279 were born between 28 and 37 weeks' gestation. It does, however, give an indication that the consequences of preterm birth extend well into middle age.

When other groups have stratified preterm born infants according to birth weight appropriate or small for gestational age (AGA or SGA), blood pressure at age 18 was significantly elevated in all those born preterm, but with the greatest elevation in the preterm SGA cohort (Evensen et al., 2009). Examination of a much larger cohort found the same inverse relationship between gestational age and blood pressure in young adult men, although the additional risk conferred by preterm and SGA, compared to preterm and AGA, was only evident in those born after 33 weeks' gestation (systolic BP≥ 140 mmHg; Odds Ratio 1.33, 95% confidence interval 1.12-1.57) (Johansson et al., 2005).

Infants born preterm are often exposed to different socio-economic influences than those born at term (Jenkins, Gardner, McCall, Casson, & Dolk, 2009; Smith, Draper, Manktelow, Dorling, & Field, 2007), and this inequality may be a powerful confounder of late effects ascribed to preterm birth. Full siblings are usually exposed to the same family-based socio-economic factors during the perinatal period and sibling studies therefore allow the effect of sibling-specific variables, such as birth weight or gestation length, to be compared while minimising the influence of perinatal socio-economic factors (Davey Smith, Leary, Ness, & Lawlor, 2009). Using this approach in a large cohort of young men has identified an inverse relationship between gestation length and adult BP both within sibling pairs, and between non-siblings (Lawlor et al., 2007) that is independent of confounding due to socio-economic environment.

The association between reduced gestation length and increased adult BP is not universal (Kaijser et al., 2008). Correlating perinatal data for a cohort born between 1925 and 1949 with national health record entries for those individuals still alive in 1987 (age 38 to 63 years) found an association between low birth weight, but not prematurity, and ischaemic heart disease. This cohort included a large group of preterm individuals, many of whom were born either at less than 32 weeks gestation or had a birth weight above 2.5 kg. Given the constraints of perinatal care in the early part of the 20th century, the demographic of their preterm population seems unusual; care is therefore needed before making any inference about gestation length and later outcome. A study of preterm birth, postnatal growth and later blood pressure in sheep also failed to show any association between preterm birth or early postnatal growth trajectory and increased blood pressure in early postnatal or adult life (De Matteo, Stacy, Probyn, Brew, et al., 2008). This experimental cohort consisted of both twins and singletons, and it is therefore possible that the effects of multiple gestation, and the differing growth patterns of twins and singletons, might have masked the influence of

preterm birth *per se.* In addition, the adult cohort had a marked female preponderance and, at a year of age (young post-pubertal), may have been studied prior to the expression of cardiovascular differences between preterm and term born animals. Interestingly, although blood pressure was not different between preterm and term lambs, at 8 weeks of age the hearts of lambs born preterm weighed significantly more per kilo body weight than those born at term (De Matteo, Stacy, Probyn, Brew, et al., 2008).

Mechanistically it remains unclear how preterm birth might influence later cardiovascular outcomes. In fetal sheep, mean arterial pressure is 43 mmHg in late gestation increasing to around 75 mmHg soon after birth (De Matteo, Stacy, Probyn, Desai, et al., 2008; Probyn, Stacy, Desai, Ross, & Harding, 2008), presumably due to an increase in systemic vascular resistance once the transition is made to a postnatal circulation. It has been shown in lambs that removal of the low pressure placental circulation at birth results in a significant fall in aortic blood flow (Langille, Brownlee, & Adamson, 1990). Postnatal aortic growth must, therefore, continue in the face of considerably lower blood flow and higher systemic arterial pressures. In preterm infants, this transition by necessity must happen earlier than usual and may lead to disruption of normal vascular development. When aortic cross sectional area and function were studied during adolescence, those born preterm had significantly narrower (Bonamy, Bengtsson, Nagy, De Keyzer, & Norman, 2008), but more pulsatile (Bonamy et al., 2005), aortas, and also higher blood pressure than those born at term. Although this demonstrates structural and functional differences in large vessels caused by preterm birth, it does not explain the possible mechanism behind increases in blood pressure.

One dynamic marker of endothelial function and vascular risk is based on the characteristics of brachial artery flow-mediated vasodilatation (FMD). This technique involves the use of high resolution ultrasound to measure the reflex dilatation of the brachial artery after a standardised period of occlusion, and has been well validated as a marker for coronary artery endothelial dysfunction (Inoue et al., 2008; Redberg et al., 2003). No differences between adolescents born at term or preterm have been demonstrated using this technique (Singhal, Kattenhorn, Cole, Deanfield, & Lucas, 2001). More recent studies based on the 20 year follow-up of a prospectively recruited preterm cohort has confirmed increased blood pressure (Lazdam et al., 2010) and increased carotid intima-medial thickness (IMT) (a measure of structural atherosclerosis (O'Leary et al., 1999) and a robust marker for future cardiovascular risk (Lorenz, Markus, Bots, Rosvall, & Sitzer, 2007)) in individuals born preterm. Additionally, when preterm birth was stratified to those with or without a history of maternal

hypertension during pregnancy, those exposed to maternal hypertension had significantly reduced FMD and pulse wave velocity (indicating increased arterial stiffness), and significantly increased IMT (Lazdam, et al., 2010). Dynamic and anatomical changes in capillary micro-circulation leading to reduced capillary density are another form of vascular remodelling associated with hypertension (Agabiti-Rosei, 2008). Dermal capillary density is reduced in normotensive offspring of hypertensive parents (Antonios et al., 2003) as well as those with clinically detectable hypertension (Antonios, Singer, Markandu, Mortimer, & MacGregor, 1999), suggesting that perhaps microcirculatory changes pre-date overt hypertension. In addition, reduced capillary density in muscle or other metabolically active tissue might have significant metabolic sequelae, perhaps accounting for the link between insulin-glucose axis function and cardiovascular disease (Hattersley & Tooke, 1999). Reduced dermal capillary density has been described in one study of school-age children born preterm (Bonamy, Martin, Jorneskog, & Norman, 2007). Others, however, have found reduced dermal capillary density in low birth weight, but not preterm, school-age children and adults (Irving et al., 2004). The apparent discrepancy between these studies however may reflect the original cohort selection criteria (birth weight rather than gestational age in Irving et al,) or the lower average gestational age in the cohort investigated by Bonamy et al. (29 weeks compared with a mean of 33 weeks gestation) (Bonamy, et al., 2007; Irving, et al., 2004).

Recently it has been proposed that plasma uric acid concentrations be re-evaluated as a causal factor in the evolution of hypertension (Johnson, Feig, Herrera-Acosta, & Kang, 2005). Rodent models of hyperuricaemia develop hypertension that can be treated by reduction of plasma uric acid concentrations (Mazzali et al., 2001). The renal arterioles of these rats develop atheromatous lesions comparable with those seen in hypertensive humans. Treatment with allopurinol, but not other blood pressure lowering agents, prevents atheroma formation (Mazzali et al., 2002). Elevated plasma concentrations of uric acid in adolescents with essential hypertension have been reported (Feig & Johnson, 2003), with improvement in their blood pressure following treatment with allopurinol (Feig, Soletsky, & Johnson, 2008). Prospective studies have demonstrated an association between elevated uric acid concentrations in childhood and elevated adult blood pressure (Alper et al., 2005) and the possible prognostic value of elevated uric acid concentrations for the development of ischaemic heart disease or cerebrovascular disease (Holme, Aastveit, Hammar, Jungner, & Walldius, 2009). In addition, both preterm birth and rapid postnatal growth have been

associated with increased serum uric acid concentrations at age 3 (Park et al., 2009), which, given the body of epidemiological evidence supporting the prevalence of increased blood pressure in those born preterm may provide an insight into possible mechanisms linking the two phenomena, and therefore translate into clinical management strategies.

Cardiac autonomic function

Innervation of the heart by the sympathetic and parasympathetic arms of the autonomic nervous system continues throughout the second and third trimesters of pregnancy (Hildreth, Anderson, & Henderson, 2009). Using measures of heart rate variability this can be appreciated by the emergence of characteristic, gestational age specific, profiles of cardiac autonomic activity (David, Hirsch, Karin, Toledo, & Akselrod, 2007; Van Leeuwen, Geue, Lange, Hatzmann, & Gronemeyer, 2003). Preterm birth disrupts the normal ontogeny of cardiac autonomic development and altered autonomic function is detectable both soon after birth and at term corrected age in those born preterm compared to those born at term (De Rogalski Landrot et al., 2007; Patural et al., 2008; Thiriez et al., 2009). It has been suggested that there is a phase of 'accelerated' autonomic maturation in early childhood so that at age 2-3 years and 6-7 years measures of HRV are not related to gestational age. However, these conclusions are based on a very small number of individuals and therefore need to be interpreted with caution (De Rogalski Landrot, et al., 2007). No long term longitudinal data yet exist with which to correlate patterns of HRV in early life with later cardiovascular outcomes.

Metabolic consequences of preterm birth:

Insulin-glucose axis homeostasis following preterm birth

The majority of individuals with altered glucose-insulin axis function do not present clinically until type 2 diabetes is present in middle age (Hillier & Pedula, 2001). As survivors of contemporary perinatal care practices are only now approaching middle age (Dalziel, et al., 2007), markers of diabetic risk rather than diabetes *per se* are used to explore the association between preterm birth and altered insulin-glucose homeostasis.

At birth, concentrations of glucose and insulin in venous umbilical cord blood are higher in preterm than term infants, independent of birth weight (Martos-Moreno et al., 2009), and in over 80% of extremely preterm infants hyperglycaemia (plasma glucose concentration > 8.3 mmol/L) develops in the first 24 hours of life (Alexandrou et al., 2010). Hyperinsulinaemic euglycaemic clamp (HEC) studies in appropriately grown preterm infants (gestational age

between 32 and 33 weeks) within the first 3 days of life (Farrag et al., 1997) have shown that compared to well, non-diabetic adult women, preterm infants immature insulin-glucose homeostatic mechanisms manifest as increased peripheral insulin sensitivity, but impaired suppression of gluconeogenesis in response to insulin, possibly due to an inability of preterm infants to secrete mature forms of insulin (Mitanchez-Mokhtari et al., 2004). Such studies do not, however, determine whether altered glucose homeostasis is an intrinsic feature of newborn infants when compared to adults, or a phenomenon peculiar to newborn infants born preterm rather than at term.

Using a milk tolerance test in newborn AGA or SGA preterm infants and SGA term born infants, Gray et al. found no association between gestation length and insulin or glucose concentrations in either the fed or fasted state (Gray, Cooper, Cory, Toman, & Crowther, 2002). There was, however, an increase in post-feed insulin but not glucose concentrations in SGA infants, and an association between increased growth velocity between birth and point of testing with increased markers of insulin sensitivity (Gray, et al., 2002). However, gestational age was based on Ballard scoring within 48 hours of birth, and the cohort comprised of preterm infants or those with a birth weight below the 10^{th} percentile and therefore had a preponderance of SGA vs. AGA infants (86 vs. 14) and did not include a term AGA control group.

Using rodents as the experimental paradigm, it has been shown that there is a progressive, age dependent shift in hepatic GLUT subtypes from fetal through to juvenile life (Lane, Flozak, & Simmons, 1996). Similarly in baboons, differences in the expression of skeletal muscle insulin signalling proteins and glucose transporters GLUT-1 and GLUT-4 were reported between those euthanised at d125 (equivalent to 67% of a 185 day term gestation) and term (Blanco, et al., 2010). At d125 expression and phosphorylation of IRβ and IRS-1 were increased whereas expression of GLUT1 and GLUT4 was reduced. The authors speculated that increased expression and activity of the insulin receptor and proximal insulin signalling pathway may reflect the growth promoting actions of insulin and IGF, acting through the IR, and that the reduced expression of GLUT-1 and 4 may explain the progressive development of hyperglycaemia in their preterm baboon paradigm (Blanco, et al., 2010). As none of these animals were kept alive for more than 14 days, the long term significance of these findings is unknown. In contrast, studies in sheep have shown that fasting plasma glucose and insulin concentrations and insulin responses to a glucose infusion

are not different between either preterm or term born lambs studied within 2 days of birth, or juvenile sheep (Cowett et al., 1980).

Insulin-glucose axis homeostasis in individuals born preterm has also been investigated in childhood (Darendeliler et al., 2008; Hofman et al., 2004), and in adulthood (Dalziel, et al., 2007; Rotteveel, van Weissenbruch, Twisk, & Delemarre-Van de Waal, 2008b; Willemsen, Leunissen, Stijnen, & Hokken-Koelega, 2009), with some groups electing to further stratify cohorts according to birth weight categorized as appropriate or small for gestational age.

Results from prepubertal children born preterm compared to those born at term are conflicting. Hofman et al., have shown reduced insulin sensitivity (calculated according to Bergman's minimal model for frequently sampled iv GTTs, modified for children (Cutfield, Bergman, Menon, & Sperling, 1990)) in both SGA and AGA preterm-born children (Hofman, et al., 2004), whereas others have found that SGA at term is associated with increased indices of insulin resistance while preterm birth is not (Darendeliler, et al., 2008). This may reflect differences in the sample populations or reflect the differences in assessment technique; Hofman *et al.* used a dynamic test of insulin-glucose axis function, whereas Darendeliler *et al.* used a single measure of fasting plasma glucose and insulin concentrations to derive HOMA-IR.

By adulthood, preterm birth (birth < 37 weeks gestation) and reduced gestation length (gestation length described as a continuous variable across the entire range of term and preterm gestations) have been associated with reduced glucose tolerance (Dalziel, et al., 2007), reduced insulin sensitivity (Hovi et al., 2007; Rotteveel, et al., 2008b) and type 2 diabetes (Kaijser et al., 2009). Reduced glucose tolerance and insulin sensitivity were independent of relative size at birth, although reduced insulin sensitivity was also associated with increments in weight and height gain during childhood (Rotteveel, et al., 2008b). Type 2 diabetes was also independently associated with SGA although this cohort contained a surprisingly large number of SGA and preterm infants given the era of birth, and findings may therefore need to be interpreted with caution. Importantly, neither glucose tolerance (Dalziel, et al., 2007) nor insulin sensitivity (Finken, et al., 2008) have been associated with antenatal corticosteroid exposure.

In contrast to these findings, Willemsen *et al.* report the results of 305 young adults (age 18-24 years) who underwent assessment of insulin sensitivity using iv GTTs (Willemsen, et al., 2009). This cohort comprised singletons born preterm (< 36 weeks gestation) or at term (36-

43 weeks), with an uneventful neonatal course and were stratified SGA or AGA based on birth length greater than 2SD below the mean. No associations were found between gestation length or preterm birth and insulin sensitivity, whereas increased adult trunk fat mass was strongly associated with reduced insulin sensitivity. Early growth rates in preterm infants between birth and term corrected age, or between birth and 3 months term corrected age had a weak positive association with insulin secretion in adulthood, although this association was lost when the model was adjusted to include variables such as gestation length, size at birth and adult body composition. However, the threshold for defining preterm birth included infants born at 36-37 weeks gestation, usually regarded as late-preterm, in the term cohort. Additionally, both cohorts comprised high numbers of SGA individuals, with an unequal proportion of SGA to AGA between the preterm and term populations; it is possible that this may have confounded the analysis.

Consumption of a test diet (high fat mixed meal) may represent a more physiological assessment of *in vivo* metabolic responses (Hovorka, Chassin, Luzio, Playle, & Owens, 1998; Mari et al., 2002), and may elicit a different pancreatic response than an isolated glucose challenge (Rotteveel, et al., 2008b). Test diet consumption results in increased postprandial insulin and triglyceride concentrations (both recognized early markers of metabolic syndrome (Heine & Dekker, 2002)) in young adults both preterm and SGA at birth compared to those born AGA either preterm or at term (Rotteveel, van Weissenbruch, Twisk, & Delemarre-Van de Waal, 2008a), suggesting heightened metabolic risk in those born early and small for gestation.

Given the conflicting data currently reported in the literature, further long term follow-up of preterm populations, augmented by experimental data, is needed to establish how insulin-glucose axis function evolves over time in those born preterm or at term.

Hypothalamic-pituitary-adrenal axis (HPAA) function following preterm birth

Morning salivary cortisol concentrations have been shown to be greater among children born preterm and without antenatal corticosteroid exposure compared to age and sex matched term controls (Buske-Kirschbaum et al., 2007). Although statistical significance was not reached, there was also a trend towards an attenuated cortisol response, measured as cortisol area under the curve, following dynamic testing of HPAA function using the Trier Social Stress Test for Children.

In the Auckland Steroid 30 year follow-up trial no association was found between fasting morning serum cortisol and preterm birth, gestational age, birth weight or birth weight corrected for gestation (Dalziel, et al., 2007). Others however have found that when both AGA and SGA individuals were included in a group of young adults born preterm or at term, morning cortisol concentrations were not different between those born preterm or at term (Walker, Irving, Andrew, & Belton, 2002). Excretion of urinary cortisol metabolites was, however, reduced in the female preterm AGA but not SGA group leading the authors to speculate on sexual dimorphism in HPAA regulation, and to hypothesize that differences in cortisol metabolism may mediate different mechanisms underlying cardiovascular and metabolic effects of both preterm birth and IUGR (Walker, et al., 2002).

Reproductive consequences of preterm birth

A recent population-based study from Norway linked Medical Birth Registry data on all live singletons born between 1967 and 1988 with the personal identification number assigned at birth to all Norwegians (Swamy, et al., 2008). The records of approximately 580,000 adult survivors of this cohort were assessed for reproductive function (fetal stillbirth or live birth). Men and women born preterm had significantly fewer reproductive outcomes than those born at term, the shorter the gestation of the index case, the less likely they were to have had a registered reproductive outcome (25% women born at 22 – 27 weeks compared to 68% women born at term: 14% of men born 22-27 weeks compared to 50% men born at term). In addition, women, but not men, born preterm had a significantly higher chance of giving birth to a preterm infant themselves. How much of this effect can be attributable to a biological phenomenon, and how much reflects a shared socio-economic environment between the generations remains poorly defined.

Renal consequences of preterm birth

Nephrogenesis is usually complete by 36 weeks gestation. Infants born prior to this therefore have relatively immature kidneys, although limited nephrogenesis continues for up to 40 days after preterm birth and glomerular hypertrophy may be one mechanism through which the preterm kidney compensates for a reduced nephron endowment (Rodriguez et al., 2004). In a population of children and young adults with renal disease, those born preterm, especially if obese, had a significantly poorer outcome, with an accelerated progression towards end stage kidney disease than obese but term-born individuals (Abitbol et al., 2009). Preterm birth was also associated with a significant reduction in renal mass. It is possible that structural differences in renal morphology may alter fluid and electrolyte balance and the renin-

angiotensin-aldosterone pathway, and thus have effects on BP regulation, although this is speculative.

1.4.1.5. Antenatal corticosteroid exposure

It has been hypothesised that the long term consequences ascribed to either preterm birth or fetal growth restriction are mediated through the effects of abnormal fetal corticosteroid exposure; via exogenous administration to the mother, in the case of preterm infants, or via altered feto-maternal HPAA function in the case of growth restricted infants. Within the preterm population varying cumulative dose and type of steroid used, gestational age at exposure and interval between exposure and delivery make for a very heterogeneous group. Additionally, in many population based studies there are likely to be a number of term-born 'control' individuals whose mothers were managed for preterm labour and therefore given corticosteroids, but who did not deliver until term.

Regulation of endogenous fetal corticosteroid exposure

During the majority of gestation, fetal glucocorticoids (cortisol in human and sheep, corticosterone in rats) are derived from the maternal circulation via a materno-fetal diffusion gradient, and are therefore present at lower concentrations in the fetal circulation compared to the maternal (Sun, Yang, & Challis, 1998). The concentration gradient is modified by placental 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), which converts active glucocorticoids into their inactive metabolites in humans (Benediktsson, Calder, Edwards, & Seckl, 1997; Edwards, Benediktsson, Lindsay, & Seckl, 1993), non-human primates (Baggia, Albrecht, & Pepe, 1990), sheep (Yang et al., 1997) and rats (Mark, Augustus, Lewis, Hewitt, & Waddell, 2009). Altered 11βHSD2 activity may be provoked by changes in maternal diet: for instance, in rat dams a low protein diet reduces placental 11βHSD2 activity and is associated with increased blood pressure in the adult offspring, possibly mediated through increased in fetal steroid exposure (Langley-Evans et al., 1996). In sheep, periconceptional under nutrition increases basal fetal cortisol concentrations in late gestation and alter's fetal responses to dynamic tests of HPAA function (Bloomfield, et al., 2004). Similarly pharmacological manipulation of 11βHSD2 alters fetal steroid exposure leading to phenotypic changes in the offspring; enzyme inhibition with carbenoxolone in rat dams reduces birth weight of their offspring, and results in increased blood pressure and impaired glucose tolerance in adult progeny (Langley-Evans, 1997; Lindsay, Lindsay, Waddell, & Seckl, 1996). Additionally, in rat dams reduction of maternal corticosteroid concentrations

are associated with significantly increased β cell mass in the offspring (Blondeau, Lesage, Czernichow, Dupouy, & Breant, 2001). These data illustrate the dynamic nature of maternal regulation of fetal steroid exposure. Due to the potent growth retarding and gene inducing capabilities of these steroids, this separation of the fetus from maternal steroid exposure is thought to be crucial for normal co-ordinated development of the fetal HPAA (Fowden, et al., 1998).

Maturation and increased activation of the fetal HPAA, with consequent rise in fetal production of glucocorticoid, occurs in late gestation, and is integral to the initiation and co-ordination of parturition, and in postnatal physiological function (Challis et al., 2001). Additional modification of fetal glucocorticoid exposure is potentially dependent on 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) activity, also found in the human (Alfaidy, Li, MacIntosh, Yang, & Challis, 2003) and ovine placenta (Yang, et al., 1997). Acting through enzymic reductase and oxidase pathways, it can convert both active glucocorticoids into their inactive form (oxidase), and inactive glucocorticoid metabolites into their active forms (reductase) (Moore, Mellon, Murai, Siiteri, & Miller, 1993). Under normal conditions, oxidase activity predominates. Recent work has shown that in human pregnancy, 11βHSD1 expression and activity are increased in fetal membranes during pregnancy, and further increased with the onset of labour, potentially contributing to the rise in fetal corticosteroids at parturition (Alfaidy, et al., 2003).

Exogenous corticosteroids

Exogenous steroids such as dexamethasone are poorly metabolised by 11βHSD2 (Siebe et al., 1993), and therefore freely cross the placenta (Bayard, Louvet, Ruckebusch, & Boulard, 1972; Smith, Thomford, Mattison, & Slikker, 1988). Although human data have not shown significant long term effects of antenatal corticosteroid exposure, much debate has been generated by data from animal work in which antenatal corticosteroids can provoke altered morphology and function of most organ systems.

Maternal dexamethasone during pregnancy in sheep and rats has been shown to increase blood pressure in their adult offspring, but only when the steroid exposure occurred during specific developmental windows (Dodic, May, Wintour, & Coghlan, 1998; Ortiz, Quan, Zarzar, Weinberg, & Baum, 2003). Exogenous corticosteroid administration to the late gestation ovine fetus also increases fetal blood pressure through increased activation of the fetal renin angiotensin system (RAS) (Forhead, Broughton Pipkin, & Fowden, 2000). In

addition, maternal dexamethasone treatment during the last week of pregnancy in the rat has been shown to have a significant impact on glucose tolerance in adult offspring and increase hepatic expression and activity of phosphoenolpyruvate carboxykinase (PEPCK) and the hepatic glucocorticoid receptor mRNA expression (Nyirenda, Lindsay, Kenyon, Burchell, & Seckl, 1998).

In the marmoset monkey, oral dexamethasone administered over a seven day period, in either early (d42-48; term 144 days) or late (d90-96) gestation, resulted in slight reduction in gestational length, but had no effect on birth weight (Hauser et al., 2007). At 2 years of age blood pressure, kidney morphology and body weight were not different between marmosets exposed or not to antenatal dexamethasone (Bramlage et al., 2009). Other primate studies have demonstrated that exogenous dexamethasone exposure *in utero* can result in differential gene expression in the aorta (Atanasova et al., 2009), or reduced pancreatic beta cell numbers at one year of age (de Vries et al., 2007).

Alteration of fetal corticosteroid exposure, either through exogenous administration of corticosteroids or manipulation of endogenous steroid production clearly has the capacity to alter many different organ systems and metabolic pathways, although the nature of these alterations depends on developmental maturity at exposure, degree of altered exposure and other species-specific variables.

1.4.2. Postnatal consequences of poor fetal growth

The association between small size at birth and adult morbidity was first raised by David Barker and his colleagues in the 1980s (Barker, Osmond, Golding, et al., 1989; Barker, Winter, et al., 1989; Hales, et al., 1991) and has subsequently been demonstrated in other populations (Barker, 1992; Eriksson, Osmond, Kajantie, Forsen, & Barker, 2006; Huxley et al., 2007; McMillen et al., 2001; Newsome et al., 2003). However, as low birth weight may be due to a modest degree of prematurity, *in utero* growth restriction, or both, the association between fetal growth *per se* and later outcomes remains poorly defined.

1.4.2.1. Cardiovascular consequences of IUGR

Blood pressure regulation and ischaemic heart disease

Although the 30-year follow-up of the Auckland steroid trial identified an inverse association between birth weight and adult blood pressure, this association did not persist once birth

weight was corrected for gestation length (Dalziel, et al., 2007); thus small size, if appropriate for gestation length, was not associated with increased blood pressure in adulthood.

Birth cohorts recruited from an era predating routine antenatal dating scans have found strong associations between low birth weight and increased blood pressure and rates of ischaemic heart disease in adult life (Kaijser, et al., 2008; te Velde et al., 2004). While similar results have also been reported in a recent meta-analysis, the era of birth of some included cohorts predates robust confirmation of gestational age (Huxley, Shiell, & Law, 2000). As exemplified by the Auckland steroid trial, unless birth weight is scrutinised within the context of clear knowledge of gestational age, it is plausible that effects ascribed to low birth weight may be due to inclusion of appropriately grown, but modestly preterm individuals.

Twins as a group are smaller at birth than singletons yet do not, as a group, have a higher incidence than singletons of cardiovascular morbidity or mortality (Vagero & Leon, 1994). Twin pair analysis removes the possible confounding effects of prematurity, socioeconomic and environmental factors as each member of the pair has the same gestation length and shares the same early environment. In a large Swedish twin cohort a 500 g disparity in birth weight between same sex dizygotic and monozygotic twins was respectively associated with a 34% and 74% increased risk of hypertension in the lighter twin (Bergvall et al., 2007). This finding in monozygotic twins implies that fetal growth restriction and not genetic factors mediate hypertensive risk.

Long term outcomes of poor fetal growth may be different if co-existing with preterm birth. In prospectively followed 20 year olds from a contemporaneous birth cohort, there was no difference in blood pressure between those born preterm and small or those appropriately grown for gestation (Lazdam, et al., 2010).

Altered structure or biomechanics of vessel walls, such as increased arterial stiffness (Liao et al., 1999) may mediate a link between perinatal events and later cardiovascular outcomes. In vivo measures such as pulse wave velocity (PWV) give an index of arterial stiffness and within the Bogalusa birth cohort small size at birth was associated with increased PWV in adulthood (Mzayek et al., 2009). Similarly, PWV and other markers of arterial stiffness and vascular impedance were increased in children born SGA (birth weight < 10^{th} percentile for gestational age) (Bradley et al., 2010), although as the gestational age SGA children was significantly less than that of the control cohort, it is difficult to ascribe these differences in vascular function solely to SGA. However, in another study of retrospectively recruited 8

year olds, those born preterm and SGA had higher mean arterial pressure and PWV than those born AGA whether preterm or term (Cheung, Wong, Lam, & Tsoi, 2004).

Mechanistically it remains unclear how growth restriction or preterm birth can lead to permanent alteration of vascular biomechanics. Mori *et al.* (Mori, Uchida, Inomo, & Izumi, 2006) have shown that both preterm birth and SGA (defined as: (1) infants free of congenital malformation with a normal chromosomal complement; (2) fetal abdominal circumference $<10^{th}$ percentile; (3) increased umbilical cord Doppler pulsatility index; and (4) birth weight $<10^{th}$ percentile for gestation) are important determinants of vascular wall dynamics in the immediate newborn period as both are associated with markers of increased arterial stiffness (Mori, et al., 2006). It has been shown that with an increase in umbilical artery pulsatility index the fraction of fetal cardiac output directed to the placenta falls (Kiserud, Ebbing, Kessler, & Rasmussen, 2006). As fetal cardiac output is maintained, this infers a recirculation of deoxygenated umbilical blood within the fetus (and may account for increased oxygen extraction and low blood oxygen content in SGA fetuses). Once fetal hypoxia is established, cardiac output is redistributed to favour critical organs (brain and heart) (Cohn, Sacks, Heymann, & Rudolph, 1974; Itskovitz, LaGamma, & Rudolph, 1987). This pattern of redistribution of cardiac output persists even when hypoxia sufficient to produce fetal acidosis is present (Bocking et al., 1988). Altered vascular flow dynamics are known to alter vascular wall function (Shyu, 2009), thus they hypothesize that altered fetal circulation and haemodynamics in IUGR may alter fetal vascular development.

Alternatively, altered renal morphology and function have been proposed as a link between perinatal events and later outcomes. Reduced nephron count and therefore renal filtration surface area and therefore renal pressure-naturesis curve has been implicated in the aetiology of hypertension (Brenner, Garcia, & Anderson, 1988; Keller, Zimmer, Mall, Ritz, & Amann, 2003), although it is uncertain whether reduced nephron count is causal in the evolution of hypertension, or a secondary phenomenon.

Small size at birth is also associated with a reduced nephron count in infants, children and adults (Hughson, Farris, Douglas-Denton, Hoy, & Bertram, 2003; Manalich, Reyes, Herrera, Melendi, & Fundora, 2000), and in experimentally induced IUGR, rats exposed to maternal protein restriction (Woods, Weeks, & Rasch, 2004), uterine artery ligation (Pham et al., 2003) or antenatal steroids (Ortiz, Quan, Weinberg, & Baum, 2001), or sheep exposed to antenatal mid-gestation corticosteroids (Wintour et al., 2003), are small at birth, become

hypertensive in adult life and have a reduced nephron endowment. Similarly, unilateral nephrectomy during renal organogenesis in the fetal sheep (Moritz, Wintour, & Dodic, 2002) or postnatal rat (Woods, Weeks, & Rasch, 2001) leads to elevated blood pressure and altered renal function. However, in humans, acute loss of renal tissue once renal development is complete has minimal effect on either long term renal function (Fehrman-Ekholm, Duner, Brink, Tyden, & Elinder, 2001; Garg et al., 2006), or blood pressure (Boudville et al., 2006). Thus, if fetal growth is compromised during a critical period of renal development, renal structure is permanently altered. If nephron number falls, the renal filtration surface area is reduced thus limiting urinary sodium excretion, this allows an increase in blood volume and, ultimately, an increase in blood pressure (McDonough, 2010). In normotensive term-born adults there is an inverse relationship between birth weight and salt-sensitive regulation of blood pressure; each 1 kg reduction in birth weight was associated with an increase in mean arterial blood pressure (MAP) salt sensitivity of 2 mmHg (salt sensitivity described as the increase in MAP achieved by moving from a low salt to a high salt diet) (de Boer et al., 2008). Individuals with a low birth weight were therefore less able to maintain renal homeostatic mechanisms, manifest as a greater increase in MAP when challenged by increased salt consumption (de Boer, et al., 2008).

Cardiac autonomic function

Evidence of altered cardiac autonomic activity primarily comes from studies of human infants and young children. There is a paucity of animal experimental data addressing the impact of perinatal events on HRV parameters.

A direct relationship between birth weight and resting heart has been demonstrated in late childhood (Abe, Minami, Ohrui, Ishimitsu, & Matsuoka, 2007) and in adulthood (Phillips & Barker, 1997). In the adult cohort, for each additional kilo in birth weight, there was a 2.7 beat per minute reduction in adult heart rate. This relationship was independent of other cardiovascular risk factors including adult body BMI, smoking and sex, and persisted after correction for gestation length; it is noteworthy though that the cohort was born between 1935 and 1943, a time when precise pregnancy dating was not available (Phillips & Barker, 1997).

Fetal adaptation to an unfavourable intrauterine environment includes increased activation of the sympathetic arm of the ANS, resulting in elevated circulating fetal catecholamine concentrations in experimental and human IUGR fetuses (Limesand, Rozance, Zerbe, Hutton,

& Hay, 2006; Okamura et al., 1990). Whether altered autonomic function persists in postnatal life is uncertain.

Newborn term SGA infants (birth weight < 3rd percentile) have a higher HR and reduced measures of HRV compared to AGA infants (birth weight 10-75th percentile) (Spassov et al., 1994). When others have looked at the effects of SGA in the context of a mixed preterm and term population, no difference in HRV parameters or salivary amylase (a marker of sympathetic activation) was found between SGA and AGA infants (Schaffer et al., 2008). Whether the effect of late preterm birth confounds the effect of poor fetal growth is unknown.

Paired assessment of HRV parameters at 1 and 3 months of age in a cohort of term-born AGA and SGA infants showed postnatal maturation of the ANS with reduction in SNS activity between 1 and 3 months of age in all infants (Galland, Taylor, Bolton, & Sayers, 2006). No difference in HR was found between SGA and AGA at baseline. SGA infants had some evidence of increased SNS activity (SDRR/SDΔNN), and ANS provocation tests revealed reduced tilt test evoked tachycardia, which the authors postulate is a marker of delayed maturation of the cardiac ANS. In contrast to these findings, a marked disparity in HRV parameters between low birth weight male and female subjects has been found in adulthood (Jones et al., 2007). In young women, but not men, psychological stress tests invoke increased indices of SNS activity and increased BP that are inversely related to birth weight (Jones, et al., 2007). It is not clear whether birth weight was corrected for gestational length, and therefore how low birth weight relates to either fetal growth restraint or gestation length.

1.4.2.2. Metabolic consequences of IUGR

Human studies

Reduced beta cell mass has been found in some (Van Assche, et al., 1977), but not all studies of infants with low birth weight (Beringue et al., 2002). This discrepancy may reflect the different inclusion criteria for low birth weight between groups (Van Assche et al. used birth weight <5th percentile, whereas Beringue et al. used birth weight <10th percentile), although neither study differentiated fetal growth restriction from the constitutionally small infant.

Blood glucose and insulin concentrations are low, but estimates of insulin sensitivity are increased in newborn infants with IUGR (ponderal index < 10th percentile for gestational age) (Setia et al., 2006), and in two day old term SGA infants compared with AGA infants

(Bazaes, et al., 2003). By 3 years of age, in keeping with Beltrand's proposition (Beltrand, et al., 2008), this profile is reversed and SGA-born children have increased insulin concentration and HOMA-IR, reduced glucose disposition index (a measure of insulin action that reflects whether insulin secretion is appropriate given the individual's insulin sensitivity (Bergman, Ader, Huecking, & Van Citters, 2002)), and fail to demonstrate a compensatory increase in insulin secretion in response to a short IVGTT (Mericq et al., 2005).

In young non-diabetic adult men with normal BMI and normal glucose tolerance, birth weight below the 10th percentile has been associated with abnormalities of skeletal muscle fibre distribution, lipid profile and insulin signalling pathways. Type 2 diabetics and individuals with insulin resistance have a reduction in skeletal muscle type 1 fibres (with higher GLUT 4 content (Gaster, Handberg, Beck-Nielsen, & Schroder, 2000), mediating insulin-stimulated glucose uptake (Henriksen et al., 1990)), and an increase in type 2 fibres, favouring 2*x* rather than 2*a* fibre sub-types (Oberbach et al., 2006; Toft, Bonaa, Lindal, & Jenssen, 1998). Jensen *et al.* report that although the number of type 1 fibres was no different between low birth weight (LBW; defined in this study as birth weight < 10th percentile) and control men, there was a shift in type 2 fibres with increased 2*x* and reduced 2*a* (Jensen, Storgaard, Madsbad, Richter, & Vaag, 2007). LBW men also had reduced GLUT4 and insulin signalling proteins in muscle (reduced p85α, p110β, PKCζ) (Ozanne et al., 2005) and fat (reduced p85α, p110β, IRS1) (Ozanne et al., 2006). Physical inactivity is also known to be a major contributor in the evolution of the metabolic syndrome (Eriksson et al., 1999; Helmrich, Ragland, Leung, & Paffenbarger, 1991). Alibegovic et al. (2010) have shown how experimental bed rest can induce peripheral insulin resistance in adult men; however, those men born with low birth weight developed hepatic insulin resistance and increased rates of basal whole body lipolysis compared to those with a birth weight within normal range (Alibegovic et al., 2010). Individuals born with low birth weight may therefore have a heightened susceptibility for adverse metabolic effects of lifestyle choices in adulthood.

To try and separate the effects of reduced fetal growth from small size at birth, Vielwerth *et al.* prospectively measured third trimester fetal growth velocity (Vielwerth et al., 2008). In young adulthood IUGR (fetal growth velocity < 10th percentile) was associated with an altered cholesterol profile, but no other features of the metabolic syndrome, whereas SGA (birth weight < 10th percentile) was associated with increased adiposity, dyslipidaemia, increased basal insulin concentrations and HOMA-IR (Vielwerth, et al., 2008). However, limiting assessment of fetal growth velocity to the third trimester may not be sensitive enough

to detect infants in whom growth velocity is compromised from much earlier in gestation (Thorsell, et al., 2008). An alternative strategy has been to assess serial growth measurements from fetal and early postnatal life; downward percentile crossing in the third trimester was associated with upward percentile crossing as early as the first 6 weeks of postnatal life, and both poor fetal growth and rapid postnatal growth were associated with increased fat mass at 6 months of age (Ay et al., 2009). Whether these findings have any implications for later health remains to be determined.

Many strands of evidence indicate that poor fetal growth is linked to altered insulin-glucose homeostasis throughout the life course. It is hypothesized that if individuals have a relatively smaller β cell complement at birth they may be unable to adequately increase β cell function in the face of increased metabolic demand, thus increasing their propensity for type 2 diabetes.

Animal studies

IUGR in lambs results in proportionately reduced pancreatic mass but a disproportionately reduced insulin content and the β cell mass compared to control animals (Limesand, et al., 2005), potentially attributable to a reduction in mitotic rates that appears to be specifically restricted to β cells rather than endocrine cells *per se*. Similar findings have been reported in IUGR rodents (Dumortier et al., 2007), although the mechanism underlying the reduction in β cell mass appears to be determined by the experimental protocols used to establish IUGR (Breant, Gesina, & Blondeau, 2006; Dumortier, et al., 2007; Reusens & Remacle, 2006). For instance, bilateral uterine artery ligation in rats produces small pups with normal β cell mass but low pancreatic and duodenal homeobox-1 (PDX-1) expression in the newborn period (Stoffers, Desai, DeLeon, & Simmons, 2003). PDX-1 is known to play a key role in pancreatic development through fetal and postnatal life, and in the regulation of β cell function through its actions as a transcription factor modulating gene expression of, amongst others, insulin and GLUT2 (Boucher, Simoneau, & Edlund, 2009). These animals demonstrate progressive epigenetic modification during fetal and adult life leading to a reduction in β cell mass and PDX-1 expression (Park, Stoffers, Nicholls, & Simmons, 2008), manifest clinically as a decline in glucose tolerance and the development of frank diabetes. Treatment of these animals with exendin-4, a pancreatic beta cell trophic factor, in the neonatal period restores both β cell mass and PDX-1 expression, and prevents the age related decline in glucose tolerance (Stoffers, et al., 2003). Others have shown that the offspring of rat dams fed a low protein diet throughout pregnancy are growth restricted but compared to

control animals have a normal total pancreatic weight, reduced rates of β cell proliferation and increased β cell apoptosis leading to a reduction in the total β cell mass (Petrik et al., 1999). Alternatively, offspring of rat dams fed a low energy diet throughout gestation develop IUGR, have a reduced β cell mass at birth but normal β cell proliferation rates despite a significant reduction in PDX-1 expression in mid gestation (Breant, et al., 2006; Dumortier, et al., 2007). These findings illustrate that different nutritional insults experienced during fetal life may have an adverse impact on pancreatic development, but that the mechanism underlying β cell loss differs according to the nature of the insult.

In one month old IUGR lambs whole body insulin sensitivity and glucose disposition are increased and insulin secretion is reduced compared to control lambs (De Blasio, Gatford, McMillen, Robinson, & Owens, 2007). Young IUGR lambs also have increased growth velocity relative to control lambs and, as has been demonstrated in fetal life, up-regulated IR protein expression in skeletal muscle. Unlike the case in fetal life however, so too was the skeletal muscle protein expression of Akt1, Akt2, GLUT1 and GLUT4 (Muhlhausler, et al., 2009). Increased postnatal expression of these insulin signalling pathways therefore coincides with increased postnatal growth and muscle accretion in IUGR lambs. Increased expression of both insulin-independent (GLUT 1) and insulin dependent (GLUT 4) glucose uptake pathways has also been demonstrated (Muhlhausler, et al., 2009); consistent with this, glucose disposition index in young IUGR lambs is positively correlated with postnatal 'catch-up' growth (De Blasio, Gatford, McMillen, et al., 2007). In adult life, however, IUGR in sheep is associated with reduced insulin secretion and insulin disposition, despite a compensatory increase in beta cell mass (Gatford et al., 2008). In rodents too, a similar trajectory of fetal growth restriction, enhanced insulin sensitivity and increased IR expression in early postnatal life has been described (Ozanne, Wang, Coleman, & Smith, 1996), followed by reduced insulin sensitivity in skeletal muscle, reduced expression of insulin signalling proteins p85α and PKCζ (Ozanne, et al., 2005; Ozanne et al., 2003), GLUT4 and reduced expression of adipocyte p110β (Ozanne, et al., 2005). It has also been shown that IUGR induced by uterine artery ligation in rats is associated with increased hepatic gluconeogenesis, impaired hepatic insulin receptor signalling (reduced IRS2 and Atk-2 phosphorylation) and hepatic insulin resistance prior to the onset of whole body insulin resistance (Vuguin, Raab, Liu, Barzilai, & Simmons, 2004).

Postnatal environment is an important mediator of metabolic function in animals as well as humans. Global nutrient restriction of rat dams during the final week of gestation induces

IUGR in their offspring (Jimenez-Chillaron et al., 2005). Randomization of Control (C) and under-nourished (U) dams to either standard chow between birth and weaning (C), or global nutrient restriction (U) yields offspring subjected to one of CC, CU, UC, UU reflecting maternal nutritional planes during pregnancy and lactation. All pups were fed on standard lab chow post-weaning. UC pups demonstrated accelerated growth during the lactation phase, whereas CU and UU pups demonstrated accelerated growth during the post-weaning phase. At 6 months of age, male animals were studied. UC pups had become obese and glucose intolerant whereas CU animals were leaner and more glucose tolerant than CC animals. UU animals did not develop obesity or glucose intolerance (Jimenez-Chillaron et al., 2006). These data suggest that rapid postnatal weight gain, irrespective of birth weight, is a major contributor to later disease risk. They also suggest that reduction of early postnatal growth ameliorates the effect of low birth weight, and it is the tempo of postnatal growth that determines later disease risk (Jimenez-Chillaron, et al., 2006).

Taken together these data suggest that *in utero* poor fetal growth is associated with adaptive changes that promote efficient utilisation of available nutrient substrate. Once the fetus is independent of such constraint, these 'thrifty' adaptations are mismatched with nutrient rich post-natal life. Rapid early growth and weight gain, facilitated by these *in utero* adaptations, promotes growth and the development of adiposity. This in turn promotes the transition from increased insulin sensitivity to insulin resistance and glucose intolerance.

1.5. Regulation and consequences of postnatal growth

Controversy exists with respect to the interaction between fetal and neonatal growth, the potential hazards or benefits of accelerated or catch-up growth, and the period of infancy or childhood during which growth trajectory is most influential. In addition, different strategies are used to promote weight gain and growth in otherwise potentially vulnerable populations, such as preterm or growth restricted infants. Despite this, it remains unclear as to how best to measure and quantify growth; weight, rates of weight gain, increments in skeletal growth and proxies of body composition such as body mass index (BMI) or ponderal index (PI) are all reported in the literature, as indicated below.

1.5.1. Infant feeding modality and growth

Human infants, like all other mammalian young, are dependent on milk as their sole dietary intake in early life. Significant variation exists in the type, frequency and volume of milk

offered to human young and the duration of milk feeding prior to weaning (Binns, Fraser, Lee, & Scott, 2009). Exclusive breast feeding has been endorsed by the World Health Organisation as the optimal source of infant nutrition for the first 6 months of life (Kramer & Kakuma, 2002). In contemporary industrialised societies many infants are partially, if not fully, formula fed from the outset, and milk feeds are often supplemented with a variety of weaning foods from three to four months of age (Binns, et al., 2009), especially in infants who are formula fed or born to mothers of low educational attainment (Schiess et al., 2010).

Infant weight gain is, in part, dependent on the type of milk provided and disparity in weight gain between exclusively breast or formula fed infants is evident as early as the first week of life (Macdonald, Ross, Grant, & Young, 2003). Well term-born breast fed infants lose a greater proportion of their birth weight and take longer to regain their birth weight than formula fed infants, although the time taken to reach maximal weight loss is the same in each group. After the first week of life, breast fed infants have a similar growth profile to formula fed infants during the first months of life (Nelson, Rogers, Ziegler, & Fomon, 1989), but have a slower rate of weight gain thereafter (Cole, Paul, & Whitehead, 2002; Dewey, Heinig, Nommsen, Peerson, & Lonnerdal, 1992; Hediger, Overpeck, Ruan, & Troendle, 2000; Kramer et al., 2004). At one year of age this reduction in growth velocity is demonstrated by as much as a 650 g weight discrepancy between breast milk fed and formula fed infants (Dewey, 1998). The most pronounced separation in growth profile between formula and breast milk fed infants therefore occurs after the introduction of solid weaning foods. This may reflect differences in weaning practices, or may suggest that factors regulating or mediating infant growth are influenced by early diet, the effects of which persist well after the substitution of a milk-based diet for a solid based diet.

The WHO has published new infant and child (0-5 years) growth standards to replace the widely used CDC 2000 reference charts (World Health Organisation, 2006). To compile the WHO standard, infant growth was assessed from a prospectively followed, culturally and ethnically diverse population of healthy term-born breast fed infants, all from affluent socio-economic environments. Growth of young children was assessed using cross sectional analysis of children from the same population demographic that had been exclusively breast fed for a minimum of 3 months. The standard was promoted as a document describing how children should grow when provided with an optimal environment, the converse being that deviation from this standard, in whatever direction, represents abnormal postnatal growth. Weight-for-age z-scores from a test population of healthy breast fed babies tracked along the

mean WHO z-score from 1-12 months, whereas the same population of infants assessed using the CDC 2000 reference charts appeared to experience growth-faltering from 2 months of age (de Onis, Garza, Onyango, & Borghi, 2007). Recognition of the potential differences in normal growth, as defined by these reference standards, is of importance when interpreting clinical or epidemiological studies.

Many factors may influence the different trajectories of early growth between breast and formula fed infants. Possible explanations include the lower protein, energy and micronutrient content of breast milk compared to formula milk (Butte, Garza, Smith, Wills, & Nichols, 1987; Heinig, Nommsen, Peerson, Lonnerdal, & Dewey, 1993; Heird, 2007), or variation in care-giver responses to infant feeding cues (Hodges, Hughes, Hopkinson, & Fisher, 2008) that may underlie differences in self-regulation of feed intake seen between bottle and breast fed infants, both in infancy (Dewey, Heinig, Nommsen, & Lonnerdal, 1991; Dewey & Lonnerdal, 1986; Sievers, Oldigs, Santer, & Schaub, 2002) and post weaning (Taveras et al., 2004). It has also been speculated that increased bioavailability of the amino acid tryptophan in breast milk may modulate central nervous system concentrations of serotonin, a neurotransmitter involved in regulation of satiety (Luhovyy, Akhavan, & Anderson, 2007).

The type of weaning diet offered to breast fed and formula fed infants has been shown to vary widely in terms of its nutrient composition, although total energy provision is usually comparable (Noble & Emmett, 2006). Importantly, it is known that diet in infancy correlates closely with that consumed through childhood (Northstone & Emmett, 2005; Northstone & Emmett, 2008), which in turn is a factor in the establishment of adult food preferences (Mikkila, et al., 2005).

Circulating insulin and IGF-1 both promote growth in early life, and regulate the development and differentiation of adipocytes (Sato et al., 2008). The higher protein content of formula milk compared to breast milk (Heinig, et al., 1993) leads to a significantly higher total protein consumption during infancy (Alexy, Kersting, Sichert-Hellert, Manz, & Schoch, 1999). There is now evidence that infant feeding modality modulates plasma concentrations of IGF-1, such that breast fed infants have lower levels than formula fed infants in the first months of life independent of infant weight (Chellakooty et al., 2006; Savino et al., 2005; Socha et al., 2005). The converse relationship, however, was found in a cohort of older children; those partially or fully breastfed as infants had higher IGF-1 concentrations than

those formula fed as infants (Martin et al., 2005). IGF-1 concentrations were positively associated with increased height in the breast fed cohort. Others have demonstrated higher post prandial insulin concentrations after a formula milk feed than after a breast feed in six day old infants (Lucas, Boyes, Bloom, & Aynsley-Green, 1981).

The metabolic effects of dietary protein appear to differ depending on whether protein was derived from dairy or meat sources. In 7 year old German children a positive association has been found between total protein intake at 1 year of age and both BMI and body fat percentage (Gunther, Buyken, & Kroke, 2007), especially in those children that had relatively greater dairy protein consumption (Gunther, Remer, Kroke, & Buyken, 2007). In an intervention trial in which total protein consumption was increased in healthy 8 year old boys by increasing either meat or dairy consumption, ingestion of dairy protein, but not meat protein significantly increased serum concentrations of IGF-1 and the ratio of IGF1 to IGFBP3 (Hoppe, Molgaard, Juul, & Michaelsen, 2004), fasting serum insulin concentrations, and HOMA-IR (Hoppe, Molgaard, Vaag, Barkholt, & Michaelsen, 2005). In particular, the whey component of dairy protein appears to increase plasma insulin concentrations, whereas the casein component mediates the IGF1 response (Hoppe, Molgaard, Dalum, Vaag, & Michaelsen, 2009). It is possible, therefore, that the relatively high protein load in formula milk and the difference in whey:casein ratio and amino acid composition (Lien, 2003) alters the dynamics of IGF and insulin axis function and adipocyte differentiation leading to increased early growth rates and a predisposition to later adiposity (Ketelslegers, Maiter, Maes, Underwood, & Thissen, 1996; Koletzko et al., 2005; Parizkova & Rolland-Cachera, 1997; Rolland-Cachera, Deheeger, Akrout, & Bellisle, 1995).

A recent large randomised multicentre trial has looked at the effects of high versus low protein content in formula milk during the first year of life, referenced to a breast fed group of infants (Koletzko, von Kries, Closa, et al., 2009). Protein content of the trial milk represented the maximal and minimal concentration advised by the EU Directive on infant formula milk (European Commission, 1991). Protein content in the low protein formula was equivalent to that found in human breast milk. Infants fed high protein formula milk had significantly greater weight, BMI and weight-for-length z-scores at 2 years of age than the breast fed group, whereas there was no difference between BMI and weight-for-length z-scores between the low protein and breastfed group. Reducing protein intake during the first year of life to emulate the levels found in breast milk therefore results in a growth profile not dissimilar to that of breast fed babies, and has effects on growth that persist for at least a year

after cessation of the intervention. Whether long term health outcomes will be influenced by the protein content of early diet, or altered growth trajectory remains to be determined.

Animal studies examining the effects of increased postnatal protein are not conclusive. While increasing the dietary protein intake of juvenile rats increases serum IGF-1 and reduces serum IGFBP concentrations, and increases weight gain (Filho et al., 1999), such associations are not universal. In piglets, no association was found between early diet (postnatal d7-28), growth and plasma IGF-1 concentrations during the period of dietary intervention (Morise et al., 2009).

Another group have reported different growth rates, and concentrations of IGF-1, insulin and other metabolites, between cohorts of artificially fed male lambs (Greenwood et al., 2002). All lambs were removed from the ewe at birth and fed artificial formula either *ad libitum* to promote maximal weight gain (average daily weight gain 337 g/d), or as a twice daily regimen at a volume intended to restrict growth (average daily weight gain 148 g/d). The *ad libitum* cohort had higher IGF and insulin concentrations than the restricted group both in the first two weeks of life, and from then until they reached 20 kg weaning weight. The highest concentrations of IGF1 and insulin were seen in those animals that were small at birth and fed *ad libitum*. Sub-groups of animals were euthanised at several time points between birth and weaning. The percentage of post mortem fat content was significantly higher in the *ad libitum* group, independent of body weight (Ehrhardt, Greenwood, Bell, & Boisclair, 2003), suggesting that rate or pattern of postnatal growth is an important determinant of later composition and that composition cannot be inferred by absolute body weight alone. Volumes of milk or macronutrient consumption were not, however, reported, and there was no reference ewe-fed group to help tease out the effects of artificial versus breast milk or cumulative macronutrient intake on later body composition.

1.5.1.1. Biologically active constituents of breast milk

The link between infant breast feeding and reduced risk of obesity in later life has been made in many different populations (Arenz, Ruckerl, Koletzko, & von Kries, 2004; Harder, Bergmann, Kallischnigg, & Plagemann, 2005; Koletzko, von Kries, Monasterolo, et al., 2009; von Kries et al., 1999). Intriguingly however, the PROBIT (promotion of breast feeding intervention trial) study represents a large multicentre trial designed to increase breast feeding rates amongst healthy term AGA singleton infants whose mothers intended to breast feed (Kramer et al., 2001). Breast feeding was initiated in all infants, with higher rates in the

intervention group at 3, 6, 9 and 12 months. Despite this, there was no difference in measures of adiposity at 6.5 years of age between the control and intervention group (Kramer et al., 2009). However, rates of breast feeding were higher than anticipated in the control group; the experiment may therefore have lacked the power necessary to detect small differences in childhood adiposity. In addition, all infants were initially breast fed; Plagemann et al. have demonstrated differences in childhood adiposity depending on the type of milk infants were fed during a developmental 'window' limited to the first week of life (Plagemann, Harder, Franke, & Kohlhoff, 2002; Rodekamp, Harder, Kohlhoff, Dudenhausen, & Plagemann, 2005). It is therefore plausible that universal early breast feeding amongst both the control and intervention arms of the experiment confounded later assessment of adiposity between the two groups. The extent to which the association between breast feeding and later adiposity is tempered by breast feeding *per se*, the associated difference in early life growth trajectory, weaning and post-weaning food preferences or exposure to the different macro and micro nutrients present in formula milk compared with breast milk remains uncertain. For instance, unlike formula milk, human breast milk is known to contain energy regulating hormones such as leptin (Casabiell et al., 1997), ghrelin (Kierson, Dimatteo, Locke, Mackley, & Spear, 2006), resistin (Ilcol, Hizli, & Eroz, 2008), adiponectin (Martin et al., 2006) and insulin-like growth factors (Milsom, Blum, & Gunn, 2008) in addition to a host of other biologically active compounds (Chirico, Marzollo, Cortinovis, Fonte, & Gasparoni, 2008; Labbok, Clark, & Goldman, 2004).

It has been suggested that exogenous leptin derived from milk ingestion may have an important role in appetite regulation and satiety. In suckling rodents, endogenous leptin production by the gastric mucosa is low, and exogenous leptin from maternal milk is the principal source of leptin within the upper gastrointestinal tract. Once solid food is introduced endogenous gastric leptin is secreted by gastric mucosal cells in response to feeding (Oliver, Pico, De Matteis, Cinti, & Palou, 2002). Leptin may act either at receptors that are present in the upper GI tract (Morton, Emilsson, Liu, & Cawthorne, 1998), or directly stimulate vagal afferent fibres in the gastric and GI mucosa (Yuan, Attele, Wu, Zhang, & Shi, 1999). Supplementing rat pups with exogenous leptin during lactation reduced feed intake during the suckling phase, but did not alter body weight at weaning (Sanchez et al., 2005). However, by 6 months of age leptin treated animals weighed less and had lower adipose tissue depots than control animals (Pico et al., 2007) and when studied in adulthood, those treated with leptin had lower HOMA-IR, lower insulin rise in response to re-feeding after prolonged fasting,

increased glucose but not insulin AUC in response to a GTT, and an enhanced preference for carbohydrate rich rather than fat rich food (Sanchez et al., 2008). Leptin has been identified in fresh and frozen samples of human milk from lactating women who delivered their babies at term (Lyle, Kincaid, Bryant, Prince, & McGehee, 2001). Comparable concentrations of leptin have been identified in frozen milk samples obtained from mothers 1-4 weeks after preterm birth (23-34 weeks), but not if the milk had been pasteurised (Resto et al., 2001). Gestational age at birth was not associated with leptin concentrations leading the authors to speculate that parturition itself and the initiation of lactation is sufficient to stimulate mammary leptin production. In a prospectively followed cohort of women and their breast fed infants a negative correlation was demonstrated between concentrations of leptin in maternal milk during the first months of lactation and infant BMI in the second year of life (Miralles, Sanchez, Palou, & Pico, 2006). Although bovine leptin has been identified using RIA techniques in commercially available formula milk preparations (Lage et al., 2002), the technical validity of this approach has been questioned (Resto, et al., 2001). Analysis based on Western Blot protein detection has demonstrated no bovine leptin in artificial formula milk preparations (Resto, et al., 2001).

While many infants clearly thrive on a formula milk diet, and go on to become healthy adults, it is possible that on a population basis the unique composition of breast milk and the presence of biologically active components confers biological advantage.

1.5.2. Influence of infant growth rate on later morbidity

Adequacy, or otherwise, of early postnatal and childhood growth is a surprisingly difficult measurement to quantify. Optimal 'target' growth rates may differ between different populations (for instance, infants born preterm or SGA, or born in the developing world) and no clear guidelines exist to define an ideal growth rate that meets the specific short term and longer term health needs of an individual.

Rapid growth during infancy or childhood has been described in terms of 'accelerated' or 'catch-up' growth. Catch-up implies that pathological growth restriction has occurred at some prior point in time and that rapid growth represents physiological compensation or correction and will thus return the child to a pre-morbid or biologically optimal plane of growth. For this reason, the term 'accelerated' growth is preferred by many investigators as it does not imply a prior causal pathology. Whichever term is used, accelerated growth is usually taken to represent upward (weight) percentile crossing of at least one percentile band on a standard

growth chart (e.g. 2nd to 9th percentile) or 0.67 standard deviations (Ong, Ahmed, Emmett, Preece, & Dunger, 2000). Children born SGA commonly demonstrate accelerated weight (Hokken-Koelega et al., 1995) and length (Karlberg & Albertsson-Wikland, 1995) gains during the first 6 to 12 months of life. It may, therefore, be this accelerated postnatal growth, rather than SGA *per* se that mediates the association between SGA and later health outcomes.

The current obesity epidemic is not confined to adults; increasing numbers of children (Ogden, et al., 2006) and even infants (Kim et al., 2006) are recognised as overweight or obese, and therefore at significantly higher risk of remaining overweight as adults (Singh, Mulder, Twisk, van Mechelen, & Chinapaw, 2008). A recent systematic review has found that excessive growth in infancy, a developmental period characterised by rapid growth, may be a key determinant of later adiposity in both AGA and SGA individuals (Ong & Loos, 2006). The separation of good infant weight gain or growth increments from those harmful in the short or longer term has, however, not been made explicit. In addition, the time period in which growth rate is most influential on later size is unclear, as rapid growth in the first months (Ekelund et al., 2007; Taveras et al., 2009), weeks (Chomtho et al., 2008) or even days (Stettler et al., 2005) of life have all been associated with increased risk of overweight or obesity in later childhood or adulthood. Conversely, simple educational strategies reinforcing healthy sleeping and feeding practices in the first year of life without advocating curtailment of nutrition have been shown to reduce weight-for-length at one year of age (Paul et al., 2010), although how this translates into later obesity risk is unknown.

The dynamic of insulin sensitivity and resistance in relation to childhood growth remains intriguing. In one prospectively followed group of 2 year olds, those born SGA had similar body composition and were no different in height and weight from their AGA peers, but were more insulin sensitive. By age 4 years, despite similar weight increments and BMI, SGA children had evidence of insulin resistance and had significantly increased central adiposity (Ibanez, Ong, Dunger, & de Zegher, 2006). Others have shown that rapid weight gain in the first year in SGA infants is associated with increased fasting insulin concentrations (Soto et al., 2003), whereas rapid weight gain between birth and 3 years of age, but not low birth weight *per se,* was associated with increased insulin resistance and BMI at age 8 years (Ong et al., 2004). Insulin resistance in addition to dyslipidaemia and increased adiposity also have been reported in a population of young adults that were either SGA or AGA at birth, but who gained weight rapidly during the first 3 months of life. Others, though, have found that growth acceleration later in childhood is more predictive of increased metabolic risk later on

life (Crowther et al., 2008; Eriksson, et al., 2006; Hemachandra, Howards, Furth, & Klebanoff, 2007). It is, however, possible that age dependent changes in insulin sensitivity may be necessary for normal growth and may therefore have a different significance to insulin resistance in mature adults.

The association between rapid early growth and adverse adult outcome has not universally been reported. Large Finnish cohort studies (The Helsinki Birth Cohort) have shown the converse: that rapid infant growth is strongly associated with enhanced glucose tolerance in adult life (Eriksson, et al., 2006). They also have shown that slower growth between birth and 2 years of age if followed by more rapid increases in BMI or weight gain during childhood is strongly associated with cardiovascular morbidity and mortality in adulthood (Barker, Osmond, Forsen, Kajantie, & Eriksson, 2005). It is important, though, to acknowledge that the Helsinki Birth Cohort comprises individuals born in Helsinki during 1934-44 for whom data were collected with respect to growth but not diet or nutritional intake. Erratic nutrition or relatively sudden changes in nutritional plane in early life as a consequence of food restriction during and following the Soviet-Finnish wars (1939-1940, 1941-1944), or other psychosocial stressors encountered by a war-time population may confound some of their findings (Pesonen et al., 2007).

In the developing world, the association between malnutrition in early life and morbidity and mortality has been widely recognised (Black et al., 2008), whereas there is a relative paucity of information on the relationship between postnatal growth rates and later outcomes in nutritionally deprived infants and children. In Brazil, one large population based study has shown that in both AGA and SGA infants rapid growth in the first 2 years of life is associated with reduced morbidity and mortality rates (Victora, Barros, Horta, & Martorell, 2001), thus endorsing programmes to promote early growth in nutritionally deprived populations. However, as has been described in more affluent nations, rapid growth in infancy and childhood is also associated with components of the metabolic syndrome in young adulthood (Fall et al., 2008; Sachdev et al., 2005). Amongst adults from countries in which malnutrition during childhood was widely prevalent, a relatively rapid transition to an urban 'Western' type diet has been associated with an increased prevalence of obesity, cardiovascular and metabolic dysfunction (Misra et al., 2001; Popkin & Gordon-Larsen, 2004).

There is clearly a complex relationship between diet and postnatal growth. Although breast milk itself may be protective against later metabolic dysfunction (Koletzko, von Kries,

Monasterolo, et al., 2009), it has been found that increased early growth trajectory in breast fed babies also predicted those at risk of later obesity (Monteiro & Victora, 2005) and increased blood pressure aged 6-8 years (Singhal et al., 2007). Recently, a study protocol has been published of a trial that aims to prospectively follow a population based cohort from birth, and look specifically at the impact of perinatal factors and postnatal growth patterns on cardiometabolic outcomes (de Beer, van Eijsden, Vrijkotte, & Gemke, 2009). The impact of infant feeding modality on body composition is discussed below.

1.5.3. Postnatal growth and nutrition in preterm infants

Preterm infants inherently have different nutritional requirements to those of infants born at term. Infants born preterm almost universally demonstrate weight loss followed by poor weight gain in the early newborn period. This, in part, reflects the frequent co-morbidities of preterm birth and the difficulty in establishing enteral nutrition when the gut is immature (Ehrenkranz et al., 1999). This may also be compounded by the clinical practice of prescribing feed at a rate based per kilo body weight of the infant (Klein, 2002); low weight gains mean that nutrition will, initially at least, be provided at a suboptimal rate.

Detailed analysis of size and body composition of human fetuses from 24 to 40 weeks gestation have provided the framework for gestation-specific target growth increments and nutrient accretion rates (Ziegler, O'Donnell, Nelson, & Fomon, 1976). Nutritional support for preterm infants is based on the premise that 'optimal' growth and nutrient provision emulates that of a well AGA baby *in utero* (American Academy of Pediatrics Committee on Nutrition, 1985); new preterm growth standards are, however, under construction (Villar et al., 2010).

Embleton et al. have calculated the daily and total protein and energy intake of infants ≤ 34 weeks gestation during their inpatient care and shown the accrual of significant cumulative deficits in both energy and protein intakes compared to recommended guidelines (Embleton, Pang, & Cooke, 2001), which may explain the postnatal growth restriction commonly found in preterm infants (Henriksen et al., 2009). Recently published recommendations suggest that preterm infants should receive either fortified breast milk or preterm formula milk to meet their specific nutritional requirements (Agostoni et al., 2010). Although fortification of breast milk improves growth rates (Kuschel & Harding, 2004), the higher protein content of preterm formula further promotes weight gain and linear growth in preterm infants (Schanler, Shulman, & Lau, 1999), possibly through increased rates of protein accretion (de Boo,

Cranendonk, Kulik, Harding, & Lafeber, 2005). The overall reduction in morbidity, however, in preterm infants fed human milk rather than formula mean that this remains the preferred feeding modality (Groh-Wargo & Sapsford, 2009; Schanler, Lau, Hurst, & Smith, 2005).

Following discharge from hospital it has been suggested that preterm infants continue to need supplemented formula (Roggero et al., 2008) or human milk (O'Connor et al., 2008) in order to overcome acquired postnatal nutritional deficits and improve growth parameters. However, in a randomised control trial, infants born preterm whose mothers elected to formula-feed were randomised to standard term formula, or a more calorific formula, enriched with protein, calcium and phosphate, for the 12 months following hospital discharge (Koo & Hockman, 2006). Growth was assessed with serial anthropometric measures and DXA body composition scans; those fed standard term formula were heavier and longer, with greater lean and fat mass and greater bone mineral density than those fed enriched formula. A Cochrane Review similarly failed to find long term advantage associated with enriched rather than standard formula milk for post-discharge preterm infants (Henderson, Fahey, & McGuire, 2005). Selectively increasing the protein component of formula milk without increasing energy intake results in a lower fat mass index and higher lean mass index at 6 months CGA compared to infants either receiving human milk or standard term formula (Amesz, Schaafsma, Cranendonk, & Lafeber, 2010). It has been suggested that this pattern of growth represents ‘optimal’ post-discharge nutrition, but the long term impact of this alteration in body composition is unknown.

Rapid postnatal growth in preterm infants has been associated with an adverse cardiometabolic profile in later life. Both reduced flow mediated vasodilatation (Singhal, Cole, Fewtrell, Deanfield, & Lucas, 2004) and increased markers of insulin resistance (Singhal, Fewtrell, Cole, & Lucas, 2003) have been described in adolescents born preterm who had increased growth rates during the first weeks of life. In a separate cohort study of preterm and SGA children, upward weight and height percentile crossing during childhood has been associated with increased blood pressure and insulin resistance in young adulthood (Rotteveel, et al., 2008b), and similarly, in healthy term AGA infants increased weight gain during infancy or childhood has been associated with increased blood pressure at age 6.5 years (Tilling et al., 2011)

Although BMI does not give accurate estimates of body composition, it does give an index of proportionality of growth. An inverse relationship has been described in 6 to 8 year old

Chilean children between gestational age at birth and BMI (Mardones et al., 2008) and, amongst young adults born at less than 32 weeks gestation, a positive relationship has been reported between rapid growth in the first 3 months and fat mass and BMI (Euser et al., 2005), suggesting that both preterm birth *per se* and rapid early weight gain in preterm infants lead to persistent alterations in the relationship between weight gain and linear growth.

It is important to acknowledge that while cardiometabolic function may be improved by slower early growth, there is strong evidence that neurodevelopmental outcomes are improved if early growth is good (Ehrenkranz et al., 2006), especially in males (Lucas, Morley, & Cole, 1998).

The complexity of the short and longer term consequences of parenteral nutritional support for very sick or very preterm infants is beyond the scope of this thesis.

1.5.4. Experimental paradigms of postnatal growth

It is possible to manipulate maternal and postnatal nutrition to try and isolate key stages in which altered growth trajectory impacts on either body composition or adult metabolic or endocrine outcomes. In one such paradigm, ewes were undernourished (U) or fed normal diet (C) from d1-30 of gestation, and thereafter fed a standard diet (Cleal et al., 2007). There was no difference in birth weight between lambs from U or C ewes, but the U lambs grew at a faster rate until weaning at 12 weeks, and were therefore heavier at weaning. Lambs were then randomized to nutritional restriction (to reduce weight to 85% of their calculated target weight based on pre-weaning growth trajectory) or a control diet between 12 and 25 weeks with *ad libitum* feeding thereafter, thus generating CC, CU, UC and UU groups reflecting maternal and post weaning diet exposure. At both 1.5 and 2.5 years of age, poor weight gain during postnatal under-nutrition and increased growth velocity at cessation of nutrient restriction were associated with enhanced glucose tolerance in female but not male sheep (Poore et al., 2007), whereas maternal under-nutrition did not influence the glucose tolerance of either male or female offspring. At 2.5 years of age, UC sheep (therefore those with the greatest disparity between pre- and postnatal nutritional planes) had cardiac hypertrophy and increased vascular reactivity (Cleal, et al., 2007).

Preterm sheep have been proposed as a valuable experimental paradigm to explore the long term effects of moderate preterm birth (De Matteo, Blasch, Stokes, Davis, & Harding, 2010). Different patterns of body proportionality (measured as ponderal index (PI); body weight /

crown rump length[3]) and early growth trajectories have been described; compared to term-born lambs, preterm lambs have a significantly lower birth weight and birth PI, are heavier at term-corrected age but, by 9 weeks CGA, body weight and PI in preterm and term-born lambs is not different. Unlike human infants, though, lambs are lean at birth and accrue fat depots postnatally.

Guinea pigs have been proposed as another useful experimental paradigm (Kind et al., 2005) as, like human infants, their young have well developed adipose stores at birth. Unlike rodents they are viable at preterm gestation (Lyall, Scott, & Burchell, 1997), although as they are a litter-bearing species any experimental outcomes will be confounded by the effects of multiple gestation.

1.5.5. Postnatal growth and body composition

Anthropometric growth data (such as weight, skin fold thickness and height or length) provide the foundation from which growth is clinically assessed. Further manipulation of such data to yield calculations of PI (weight (kg) / height^3 or length^3 (m^3)) and BMI (weight (kg) / height^2 or length^2 (m^2)) describe the relationship between weight and height, and therefore inferred adiposity. Measures such as BMI, though, do not provide information about the distribution of adipose tissue, a significant limitation given the increasing information about the different metabolic risk conferred by upper and lower body fat depots (Boorsma et al., 2008). It has also been shown that for a given BMI there is as much as a twofold variation in actual fat mass, in obese as well as normally grown children (Wells, 2000; Wells et al., 2006). Similarly, assessing newborn growth according to conventional percentile charts has poor correlation with actual fat stores and therefore markers of adequacy, or otherwise, of *in utero* growth (Schmelzle, Quang, Fusch, & Fusch, 2007). Differing patterns of infant and childhood nutrition and growth may well reflect differences in rates of tissue accretion and body composition. Non-anthropometric methods used to assess body composition in infants and children include deuterium labelled water (D2O) and bioelectrical impedance analysis (BIA) measures of total free water, whereas air displacement plethysmography using the PEA POD technique in infants, and dual-energy X-ray absorptiometry (DXA) provide details of total body fat and fat free mass, and for DXA, the regional distribution of that fat (Ellis, 2007; Wells & Fewtrell, 2006).

1.5.5.1. Effect of postnatal diet on body composition

The impact of infant feeding modality on body composition either in infancy or later childhood remains controversial. Breast feeding has been associated with increased lean muscle mass at 1 year (Dewey, Heinig, Nommsen, Peerson, & Lonnerdal, 1993) and a lower fat mass at 4 years (Robinson et al., 2009), whereas following a 'recommended' diet in early life is associated with increased lean muscle mass at age 4 (Robinson, et al., 2009). However, in another longitudinally followed cohort of well, term-born infants, breast feeding was associated with an increased fat mass at 6 months of age compared to infants fed formula milk, but that by age 2 years, body composition was not related to infant feeding modality (Butte, Wong, Hopkinson, Smith, & Ellis, 2000). The biological significance of differences in adiposity in infancy remains unknown.

Primate studies have also demonstrated an interaction between sex and infant diet on growth and body composition. After rearing newborn baboons on formula milk that was either calorie restricted, standard or calorie dense all animals were placed on a standard primate diet at weaning. Calorie restricted animals had a lower weaning weight, whereas calorie supplemented animals had a higher weaning weight (Lewis et al., 1986) and increased fasting and postprandial plasma insulin concentrations in early but not late infancy (Lewis, Jackson, & Mott, 1992). By 5 years of age there was no difference in weight between either those undernourished as infants or over-nourished males and control animals. Sex-specific effects were described in which females, but not males, over-nourished as infants were significantly heavier than control animals, with increased lean body mass and strikingly increased total body fat mass (Lewis et al., 1989).

1.5.5.2. Effect of preterm birth and IUGR on body composition

Yet further differences exist between babies born either preterm or following *in utero* growth restriction, both in terms of body composition at birth, and in the patterns of fat and fat free mass accrued postnatally. Preterm babies are born with relatively few fat stores (Rigo et al., 1998), but as they grow may lay down more visceral than peripheral fat (Uthaya et al., 2005). At time of discharge from NICU preterm infants have downward percentile crossing with respect to weight-for-age, but upward percentile crossing for weight-for-length, suggesting increased early adiposity at the expense of linear growth (Olsen et al., 2009). By one year corrected gestational age, ex-preterm infants have been shown to have a higher fat mass and lower lean mass than normally grown term infants (Cooke, Rawlings, et al., 1999).

Additionally, female infants born preterm tend to have increased fat mass compared to preterm born males (Pieltain, De Curtis, Gerard, & Rigo, 2001). At 1 year CGA preterm males have significantly greater lean mass than preterm born females (Cooke, Rawlings, et al., 1999), especially when fed with protein and energy enriched preterm formula rather than standard term formula for the first 6 months following discharge form NICU (Cooke, McCormick, et al., 1999). Thus gestation length, sex and infant diet all influence tissue accretion and body composition in infancy.

Conversely, at age 4 to 6 (Gianni et al., 2008) and 8 to 12 years (Fewtrell, Lucas, Cole, & Wells, 2004) preterm birth is associated with a reduced total body fat and fat mass index (FMI) respectively. In both studies, though, there is the suggestion of altered regional fat deposition, with an increase in truncal vs. peripheral fat, especially in the subgroup of preterm SGA children (Gianni, et al., 2008). Similar regional differences in fat mass have been found in term SGA children as early as 2 years of age (Ibanez, et al., 2006).

Preterm infants born small for gestational age (SGA) examined at term CGA, compared to term-born SGA infants examined on day 3 of life, have comparable body weights but a significantly higher fat mass (Gianni et al., 2009). This highlights the limitations of weight as a comparative measure between groups. Additionally, in term-born infants adiposity correlates with growth trajectory *in utero*; those with a reduced growth trajectory had less fat mass irrespective of their final birth weight (Verkauskiene et al., 2007), and within a cohort of preterm infants rapid growth in the first 3 months was positively associated with fat mass and BMI at age 19 (Euser, et al., 2005).

1.6. Summary

Perinatal events clearly impact on many aspects of health, often at a time far removed from the initiating insult or exposure. The complex relationship between fetal growth, gestation length, and postnatal diet and growth makes isolation of a single causal or dominant factor almost impossible. The experiments set out in this thesis were therefore designed to try and address these factors in a systematic, randomised manner, using a well established sheep paradigm. Although perinatal events may influence outcomes in all major organ systems, this experiment has focused on outcomes related to growth, body composition and cardiovascular and metabolic function.

Chapter 2. Materials and Methods

2.1. Pregnant ewes

Approval for all animal experimental procedures was obtained from the University of Auckland Animal Ethics Committee.

To generate successive birth cohorts of lambs with a known gestation length, oestrus was synchronised in multiparous Romney ewes using an intravaginal progesterone-containing controlled drug release device (ovine CIDR, Southern Veterinary Supplies, Hamilton, New Zealand) (Hernandez et al., 2009). CIDRs were withdrawn after 14 days. Two days after CIDR withdrawal ewes were put to a proven Poll-Dorset ram for a 3 day period.

Ultrasound scanning at 42 and 56 days after mating was performed to confirm pregnancy and fetal number. Non-pregnant ewes and the majority of those with multiple pregnancies were not included in this study. Differences in fetal and postnatal growth and metabolic function have been ascribed to offspring of multiple pregnancies (Miller, Blache, Jackson, Downie, & Roche, 2010; Poulsen & Vaag, 2006). As size at birth and postnatal growth were key components of this study, only lambs from singleton pregnancies were used in the main project. A small number of ewes with twin pregnancies were retained and the lambs used in pilot studies for method development.

Singleton bearing ewes were randomised antenatally to spontaneous labour at term (term-spont), induction of labour with dexamethasone at preterm (preterm) or term (term-dex) gestations, induction of labour at term using Alizin™ (term-Alizin) or to a separate pilot study protocol for creation of intrauterine growth restricted (IUGR) lambs.

At day 131 of a 147 day pregnancy, ewes returned to the photoperiod controlled feedlot (lights on 0600-1800) at Ngapouri Research Station to acclimatise to indoor conditions and pelleted feed (Camtech, Cambridge, New Zealand). Ewes were cared for in individual pens with open mesh sides so that at all times they had visual and auditory contact with other animals. Feed sufficient to meet the recommended nutritional standards for pregnant or lactating animals was provided daily and water was freely available to all animals at all times. Individual feeding ensured that any welfare or acclimatisation problems were identified and addressed promptly.

2.2. IUGR cohort generation

2.2.1. Preoperative care

Indoor feedlot acclimatisation commenced on d82. Necks and abdominal walls were shorn on d84 and on d90 ewes underwent surgical placement of uterine arterial lines.

2.2.2. Anaesthesia

Following an overnight fast, ewes received a single prophylactic dose of antibiotics (3 ml i.m. Duplocillin LA, Southern Veterinary Supplies, Hamilton, NZ). Propofol 5 mg/kg (Diprivan 10 mg/ml, Southern Veterinary Supplies, Hamilton, New Zealand) was administered by slow i.v. injection to induce anaesthesia. The ewe was then positioned supine, intubated with a size 9 cuffed endotracheal tube (ETT), and converted to maintenance inhalational anaesthesia with a blended 2% isoflurane (Isoflurane, Lunan Better Pharmaceutical Co, ShanDong, China, 3 L/min) and oxygen mixture through a ventilator circuit (Ivent, Auckland, New Zealand). At completion of surgery, isoflurane was discontinued, and the ETT removed. Once the animals were chewing and swallowing they were returned to their pens and allowed to recover and, when fully awake and standing, were offered their usual feed.

2.2.3. Aseptic skin preparation

Wool was closely clipped from the surgical or procedural site with wide lateral margins. For operative procedures (e.g. surgical line placement) the skin was scrubbed with iodine surgical scrub solution then liberally sprayed with ethanolic Hibitane and ethanolic iodine (Southern Veterinary Supplies, Hamilton, New Zealand) solutions. The area adjacent to the operative site was then covered with sterile drapes.

For other invasive procedures (e.g. jugular vein cannulation) the site was clipped and liberally sprayed with ethanolic Hibitane and ethanolic iodine solutions prior to the procedure.

2.2.4. Uterine artery catheterisation

The skin was cleaned (2.2.3), the abdominal cavity opened through a midline incision, a trocar positioned through the incision and exteriorised through a 1 cm incision in the ewe's flank. Two vascular catheters were advanced through the trocar into the abdominal cavity, the trocar removed and 3-way stopcocks attached to the distal end of the catheters.

Each uterine horn was identified, a main uterine artery isolated and cannulated with a vascular catheter through a small incision on the anterior aspect of the vessel wall. Catheters were flushed with heparinised saline (0.9% saline, 10 U/ml sodium heparin, Multiparin, Health Support Ltd., Auckland, New Zealand) and secured at the point of distal artery ligation. The abdominal wound was closed and the wound margins infiltrated with bupivacaine (Marcaine, Southern Veterinary Supplies, Hamilton, NZ).

The distal ends of the catheters were secured in a Ziploc™ (S. C. Johnson & son, Racine, USA) plastic bag over the hind quarters. Each surgically manipulated animal was marked with an additional red 'X' ear tag to assist rapid identification and ensure non-commercial disposal at the end of the experiment.

2.2.5. Placental embolisation

Superose 12 microspheres (Superose 12 bead slurry; 20-50 μm diameter) were suspended in normal saline to make a 1% microsphere suspension (Eremia, de Boo, Bloomfield, Oliver, & Harding, 2007).

Between d93 and d97 inclusive, 1 ml of the microsphere suspension was administered twice daily to each uterine artery catheter (UAC). After administration of the last embolisation dose, UACs were knotted as close to the flank as possible and the distal catheter cut off. The remaining knot was buried subcutaneously in the original trocar exit site, the skin closed with super glue and a prophylactic dose of antibiotic administered (3 ml Duplocillin, (150,000 IU procaine penicillin and 150,000 IU benzathine penicillin per ml; Southern Veterinary Supplies, Hamilton, NZ) i.m.). Ewes were monitored in individual pens for evidence of fetal loss or maternal illness for a further 3 days before being returned to pasture. Thereafter, care was identical to that received by ewes in the main project cohort.

2.3. Induction of labour

Ewes randomised to preterm labour received two doses of 0.25 mg/kg dexamethasone sodium phosphate (Dexa 0.2, 2 mg/ml, Southern Veterinary Supplies, Hamilton, NZ) by i.m. injection. The first dose was administered between 1700 and 1900 hrs of d135 and the second dose between 0800 and 0900 hrs of d136. Labour was reliably induced on the evening of d137, 36 hours after the second injection.

Ewes randomised to the term-dex group received two doses of 0.25 mg/kg dexamethasone sodium phosphate by i.m. injection. The first dose was administered between 1700 and 1900 hrs of d145 and the second dose between 0800 and 0900 hrs of d146. Labour was reliably induced 24 - 36 hours after the second injection, during the day of d147.

Ewes randomised to term-Alizin group received two doses of 10 mg/kg algepristone (Alizin™, 30 mg/ml, Virbac, Auckland, NZ) by subcutaneous injection. The first dose was administered between 1700 and 1900 hrs of d145 and the second dose between 1700 and 1900 hrs of d146. Labour was reliably induced 12 -18 hours after the second injection, during the day of d147.

Ewes randomised to the term-spont group received no pharmacological interventions during pregnancy.

2.4. Lambing protocol

Lambing started in May and continued through to November and, therefore, included the coldest months of the year when temperatures overnight were often around freezing point. Ngapouri Farm feedlot is not a temperature controlled unit and animal pens have an open mesh floor to promote pen hygiene. To reduce the thermal stress exposure of preterm lambs, 'cosy corners' were made; one corner of the pen was lined with a durable rubber mat, and the mesh walls covered with plywood.

Ewes were regularly assessed during labour. Intervention to assist delivery was provided if necessary. All lambs were weighed and measured (crown rump length (CRL), biparietal diameter (BPD), abdominal girth (AG), chest girth (CG), left hind hock-toe length (HTL) and leg length (LL)), blood sampled, tagged and given artificial colostrum (Colostrum powder, Halen Health, Southern Veterinary Supplies; 6 g dissolved in 50 ml warm water) within 2 hours of birth. Three ear tags were placed; one breed group specific coloured tag and two identical lamb-specific numbered tags. Rectal temperature was checked if lambs looked unwell. If <37.5 °C (normal rectal temperature is 39.5 °C) lambs were warmed with wool jackets and heated pads in the 'cosy corners'.

At completion of the project, birth weight was additionally expressed as a z-score based on birth weights of singleton animals only (birth weight - mean birth weight for gestation and sex / SD of mean birth weight for gestation and sex).

Feeding was assessed regularly. When feeding was inadequate (lambs not standing to feed, unable to find the udder or with weight loss of more than 100 g per 24 hours) ewes were hand milked (expressed ewe milk, EM) and the milk given to their lambs by drench gun at regular intervals until independent feeding was established (usually 2-3 days for preterm animals). If ewes had poor milk supply (<50 ml on hand expressing) matched donor preterm or term EM was given for a maximum of 3 days. If lambs were unable to feed independently after this time they were excluded from the trial. No lambs received artificial formula milk.

2.5. Blood sampling protocol

All blood samples were obtained by either direct venepuncture or withdrawal of blood from an indwelling jugular venous line, as indicated in the specific test chapters. All samples were immediately placed in chilled labelled tubes and stored on ice. Plasma was separated from whole blood by centrifuge (3000 rpm for 10 minutes at 4°C) and frozen at -20°C until analysed. All samples taken from indwelling lines had a 3 ml dead-space sample withdrawn first to avoid possible contamination.

During the hyperglycaemic clamp (HGC), blood samples were processed at Ngapouri for whole blood glucose concentrations on a YSI 2300 (Yellow Springs Instruments, Dayton, OH, USA: glucose oxidase in the machine sensor is used to generate a micro current proportional to the blood glucose concentration). All other hormone and metabolite assays were performed at the analytical laboratory at the Liggins Institute, University of Auckland, NZ or, for D_2O measurement, Massey University, NZ.

2.6. Lamb metabolites

Blood was sampled by direct venepuncture from all lambs at birth (prior to the first feed), and between 0730 and 0930 on postnatal days 1-8 and 14. Lambs were not fasted prior to blood sampling as it was not practicable to standardise the time between last feed and venepuncture.

Laboratory analysis for free fatty acid (FFA), insulin, glucose, urea and lactate concentrations were however only performed on samples taken at birth and days 3, 6, 8 and 14 from lambs randomly selected from each birth group to yield 7-10 of each birth group, sex and supplementation status (other than term-Alizin: males n=6, females n=5). Plasma insulin concentration relative to plasma glucose concentration was calculated at each time point.

2.7. Nutrient supplement

To try and promote early postnatal growth a nutrient supplement, analogous to human milk fortifier (HMF) was formulated.

2.7.1. Ewe milk supplement 1

The original supplement (ewe milk supplement 1; EMS1) was created to emulate the composition of HMF and thereby increase daily calories by approximately 22%, daily carbohydrate intake by 45% and total daily protein intake by 63%. The composition of human expressed breast milk (EBM), EMB plus HMF, ewe milk (EM) and EMS1 are shown in Table 2-1.

Supplemental protein (dehydrated whey protein isolate; NZMP Whey Protein Isolate 895, Fonterra New Zealand) and carbohydrate (Maltodextrin powder, Dextrose Equivalence of 18.0, Unitech Industries Limited, Auckland, New Zealand) were reconstituted in the minimum volume of water possible (30-100 ml). The total amount of supplement was dependent on postnatal day and weight of the lamb (rounded to nearest 500 g). Supplement for day 1- 3 was based on birth weight and an estimated intake of 100 ml/kg·day, and was increased to reflect an intake of 120 ml/kg·day after day 4 and 150 ml/kg·day from day 8 onwards (Dr AL Jaquiery, personal communication). The amount of supplemental protein and carbohydrate was recalculated using the lamb weights on days 4, 8 and 11. Individual supplement aliquots were made up three to four days in advance, labelled and refrigerated at 4°C until needed.

To further emulate clinical practice, supplemental vitamin A, C and D were added to the supplement (7 drops per day of Karicare™ Vitadol C, Nutricia Ltd, Auckland, New Zealand).

Supplement was heated to skin temperature in a warm water bath prior to administration. The total daily dose was divided into 2-4 smaller aliquots so that no more than 25 ml was administered at a time. Lambs were fed the supplement through a teat attached to a syringe, starting the first morning after birth and continuing to d14.

	EBM	EBM + HMF	% increase with HMF	EM	EM + EMS1	% increase with EMS1
Energy (kcal)	72	88	22	108	132	22
Protein (g)	1.4	2.2	63	6	9.8	63
Carbohydrate (g)	7.2	10.5	46	5.3	7.7	45
Fat (g)	4.3	4.3	0	7	7	0

Table 2-1: Milk, human milk fortifier and ewe milk supplement-1 composition (per 100 ml)

Expressed breast milk (EBM), human breast milk fortifier (HMF), ewe milk (EM) and ewe milk supplement-1 (EMS1).

A 3 kg lamb on d1 of supplemental feeding would therefore theoretically consume a total daily fluid intake of 100 ml/kg of ewe milk. To achieve an equivalent percentage increase in the total protein, carbohydrate and energy intake to that achieved using HMF, the lamb would need to consume an additional 3 x 3.8 g (11.4 g) protein and 3 x 2.4 g (7.2 g) carbohydrate. Whey protein isolate (WPI) contains 92% protein by weight; 12.4 g of WPI is therefore required to provide 11.4g protein. Maltodextrin powder contains 94% carbohydrate by weight; 7.6 g of Maltodextrin powder is therefore required to provide 7.2 g carbohydrate. Each gram of carbohydrate (CHO) yields 4 kcal and each gram of protein yields 4 kcal.

2.7.2. Ewe milk supplement 2

Supplement 1 did not promote growth in the first two weeks of life, and was therefore re-formulated as ewe milk supplement 2 (EMS2).

The composition of ewe and human milk is not comparable; an equivalent volume of human milk contains more carbohydrate but less fat and protein than ewe milk, and is less energy dense (Table 2-1). The resultant balance of macronutrients is therefore different between human and ewe milk. When constituent macronutrients are expressed relative to the protein content of milk, the relative proportions of fat and carbohydrate are preserved in human milk fortified with HMF (Table 2-2). Ewe milk fortified with EMS1 did not have the same relative proportions of macronutrients as unfortified ewe milk (Table 2-2). To better emulate the relative macronutrient composition of unfortified ewe milk, EMS2 was re-formulated to include fat, and the protein and carbohydrate components were adjusted, and the total energy content increased (Table 2-2; Table 2-3).

	EBM	EBM + HMF	EM	EM + EMS1	EM + EMS2
Protein (reference)	1	1	1	1	1
Fat (relative to protein)	3.3	1.95	1.17	0.71	1.16
Carbohydrate (relative to protein)	5.6	4.77	0.88	0.78	0.89
% Kcal from protein	7.5	10	22	28	20

Table 2-2: Milk and fortifier macronutrient composition expressed relative to the protein component.

*Expressed breast milk (EBM), human breast milk fortifier (HMF), ewe milk (*EM*), ewe milk supplement-1 (EMS1), ewe milk supplement-2 (EMS2).*

	EM	EMS2	EM + EMS2	% increase
Protein (g)	6	2	8	33
Fat (g)	7	2.3	9.3	33
Carbohydrate (g)	5.3	1.8	7.1	33
Energy (Kcal)	108	36	144	33

Table 2-3: Composition of ewe milk and ewe milk supplement-2 (per 100 ml)

*Ewe milk (*EM*), ewe milk supplement 2 (EMS2).*

A more soluble form of whey protein was used for EMS2 (Instantised Whey Protein Isolate 895, Fonterra New Zealand; 92% protein content by weight), carbohydrate was as for EMS1, and fat was in the form of palm oil (Palmolein oil, Palmol20, Davis Trading Co. Ltd, Auckland, NZ; 99.9% fat content by weight). As palm oil contained the fat soluble vitamins A, D, E, K, Vitadol C was not added to EMS2.

To make a stock solution of maximally concentrated EMS2: 220 g WPI, 190 g Maltodextrin and 230 ml palm oil were mixed with warm water to a final volume of 1 L. This mixture was refrigerated for up to 5 days, and warmed prior to use. Supplement administration (total daily volume, ml) was based on lamb weight (kg) x (0.1 x estimated fluid intake (ml/kg·d)); thus, a 5 kg lamb feeding at an estimated: (i) 100 ml/kg·d would receive 50 ml supplement per day; (ii) 120 ml/kg·d would receive 60 ml supplement per day; and (iii) 150 ml/kg·d would receive 75 ml supplement per day. Control lambs received an equivalent volume of warm water per day.

Volumes of supplement or water were based on postnatal age and current weight, and calculated daily. To minimise any possible adverse effects of large volume boluses, the total

daily amount of supplement or water was divided into 4 equal aliquots and administered at regular intervals throughout the day. Supplementation was started the first morning after birth and based on an estimated milk intake of 100 ml/kg·d, increased on day 4 based on an estimated intake of 120 ml/kg·d and increased again on d8 based on an estimated intake of 150 ml/kg·d. Supplementation was administered through a drench gun while the lambs were in the pen with their mothers, and was well tolerated.

2.8. D_2O dilution technique for assessment of milk intake

Deuterium oxide (D_2O) is a stable isotope of water that is rapidly and uniformly distributed in total body water. In the early weeks of life, lambs obtain their fluid and nutritional requirements through milk ingestion. Fluid volume intake can therefore be estimated by measuring total body turnover of water by calculating the rate of D_2O dilution ((Auchtung, Baer, Erdman, Barao, & Dahl, 2002)).

During the first week of life, lambs were weighed and had baseline 5 ml blood samples taken. D_2O (Sigma-Aldrich, St Louis, Mo, USA) was prepared for injection by adding 60 ml D_2O to 40 ml deionised H_2O and 0.9 g NaCl. This solution was then filtered (Acrodisc 25 mm filters, 0.2 µm; Alphatech Systems, Auckland, NZ). A dose of 300 mg D_2O per kg body weight (0.5 ml/body weight (kg)) was drawn into a syringe which was then weighed to the nearest 0.1 g. The D_2O dose followed by a 3 ml saline flush was injected, via a butterfly needle, into the lamb's jugular vein. The time recorded and the D_2O empty syringe weighed to calculate gravimetrically the actual amount of D_2O administered.

Further 5 ml blood samples were collected and the precise sample time recorded at 2 and 6 hours post-injection, and in the mornings of days 1, 2, 3, 4 and 8 following the injection.

2.8.1. D_2O Mass Spectrometry

D_2O concentrations were measured using the mass spectrometry techniques previously reported by our group (Alsweiler, 2010).

2.8.2. D_2O Calculations

The plasma samples for each lamb generated a D2O disappearance curve from which the estimated total fluid intake could be calculated.

Dose of D_2O (mg) administered was calculated as:

Final syringe weight (mg) – start syringe weight (mg)) x 0.6*

*amount of D_2O in dose is 60%

Fluid intake (ml/hr) was calculated as:

$$\frac{\text{Dose of } D_2O \text{ (mg)} \times k}{D_2O \text{ concentration at time 0}}$$

where the constant k is the exponential decay of D_2O over time, calculated from the measured D_2O in the plasma samples.

Fluid intake (ml/hr) was then divided by the weight at the beginning of the experiment to give the volume intake per bodyweight (ml/ kg·hr).

2.9. Milk and macronutrient intake calculations

Fluid intake comprised both milk and either supplement or water (intervention volume). The average daily intake of intervention volume during the period of D2O volume intake estimation was calculated from individual lamb record sheets. The intervention volume was subtracted from the total D2O-derived volume intake to yield a measure of actual milk intake. Average daily macronutrient and energy content derived from both milk and the intervention volume was then calculated. Cumulative energy derived from individual macronutrients was additionally expressed as a percentage of the total daily intake. Milk macronutrient content was based on average composition data from preterm and term ewe milk sampled on postnatal days 2 and 7 (Table 2-4; Dr. A. Jaquiery, personal communication).

	Term-spont (n=14)	Preterm (n=12)
Energy (Kcal)	127 ± 5	121 ± 6
Protein (g)	5.4 ± 0.1	5.2 ± 0.1
Fat (g)	9.9 ± 0.6	9.2 ± 0.6
Carbohydrate (g)	4.2 ± 0.1	4.3 ± 0.1

Table 2-4: Ngapouri ewe milk composition (per 100 ml)

Data are mean ± SEM.

2.10. Assessment of postnatal growth

Lambs were weighed daily (0730-0930) for two weeks with measures of skeletal growth obtained on days 4, 8, 11 and 14. From week 3 until weaning at week 12, lambs were weighed and measured weekly. To maintain accurate and comparable measures, lambs born Sunday-Tuesday had their weekly assessment on a Monday, whereas those born Wednesday-Saturday had their weekly assessment on a Thursday.

Growth velocity (GV) was calculated using the method developed by Patel et al (Patel, Engstrom, Meier, & Kimura, 2005) for the following epochs: early (GV birth to 2 weeks ($GV_{0\text{-}2}$); weaning (GV 2 weeks to weaning ($GV_{2\text{-}W}$); and late (GV weaning to one year ($GV_{W\text{-}Yr}$). In preterm lambs, GV was also calculated from birth to term equivalent age ($GV_{0\text{-}TEA}$) and from TEA-weaning ($GV_{TEA\text{-}W}$).

$$GV\ (g/kg{\cdot}day) = [1000 \times \ln(W_n/W_1)]/(D_n - D_1)]$$

where W_1 and W_n represent body weight (in kg) on the initial day (D_1) and second time point (D_n) respectively, where D represents the day after birth; a factor of 1000 was used to correct units to g/kg·day.

Although this approach has been validated in humans as an accurate method for quantifying average daily weight gains during the period of interest (Patel, et al., 2005), it nevertheless is based on calculations derived from weight measured at two time points only and necessarily can give no information on the pattern of growth between those two time points. As the profile of growth is as of much interest as the overall average rate of growth, relative daily weight gain was calculated during the period of nutrient supplementation ($[W_n\text{-}W_{n\text{-}1}]/ W_{n\text{-}1}$), and fortnightly GV was calculated between birth and weaning.

While growth velocity gives a measure of relative weight gain, it does not show where or how that mass is accrued. Weight for length gives an estimate of weight relative to skeletal size and was calculated at birth, days 4, 8, 11 and 14, at weekly intervals from week 3 until weaning at 12 weeks of age, with final weight for length calculated at the time of adult cardiovascular and metabolic testing.

2.11. Management of animal reproductive status

All lambs were left intact (i.e. males were not castrated and females were not oophorectomised). After weaning, animals were maintained on pasture in single sex flocks.

Juvenile metabolic and cardiovascular assessment was performed prior to reproductive maturity, and therefore prior to the onset of oestrus cycling in the females.

As adult assessments needed to be performed throughout the year, it was not possible to co-ordinate time of testing with the spontaneous oestrous cycle of the ewes. All ewes therefore had a CIDR inserted 3 days prior to the start of testing. The CIDR remained *in situ* for the duration of their assessment.

2.12. Jugular venous catheter placement

Indwelling catheters were inserted into each jugular vein a minimum of 24 hours prior to use. One catheter was an infusion line and the other a dedicated sampling line.

To place the catheters, animals were briefly restrained in a purpose built crush. Skin preparation was as previously described (2.2.3). Two ml of local anaesthetic (lignocaine 2%, Health Supplies Ltd., Auckland, New Zealand) were injected subcutaneously over the jugular vein and a small (6-8 mm) horizontal cut made in the anaesthetised skin with a scalpel blade. A 12 gauge 1" introducer needle was inserted through the cut in the skin and into the jugular vein. A polyvinyl catheter (#sv74 tubing, ID 0.040", OD 0.10", Biocorp Australia, Dandenong, Australia) flushed with heparinised saline (0.9% saline, 10 U/ml sodium heparin, Multiparin, Health Support Ltd., Auckland, New Zealand) was inserted through the introducer to a length of 10 cm. The introducer was removed and a 3-way tap attached to the free end of the catheter via a blunt connector. The catheter was secured at the insertion site with masking tape and superglue, tracked in the wool to the back of the neck and placed in a ziplock bag tied to the wool and secured with a tubigauze 'collar'. Catheter patency was maintained with alternate day flushes of 2 ml heparinised saline. At completion of the tests, the catheter was removed and haemostasis achieved by applying firm pressure to the vascular access site.

2.13. Metabolic tests

Animals returned to individual pens within the feedlot a week prior to the start of their assessment to allow sufficient time to acclimatise to indoor conditions and pellet feeding. Metabolic tests were performed in juvenile (18 weeks of age) and adult (15 months of age) animals.

Juveniles underwent a glucose tolerance test (GTT), hyperglycaemic clamp test (HGC), growth hormone (GH) stimulation challenge, adrenaline stimulation challenge, fasting muscle and liver biopsies, and assessment of heart rate variability (HRV) and blood pressure (BP). Adult sheep underwent GTT and HGC testing with a muscle biopsy at completion of the HGC only, Dual energy X-ray absorptiometry (DXA) scanning for body composition, HRV and BP assessment. BP was assessed in all animals using indwelling arterial catheters.

2.13.1. Glucose tolerance test

Glucose tolerance tests (GTTs) were started in the morning following an overnight fast. Feed buckets were removed at 5 pm of the evening prior to the GTT, and only *ad libitum* access to water permitted until after completion of the test.

A 3 ml baseline blood sample was taken, followed by an age dependent dose of 50% dextrose (Baxter, Health Support Ltd., Auckland, New Zealand) administered by intravenous injection over 30 seconds. Juvenile sheep received 0.5 g/kg (1 ml/kg), whereas adult sheep received 0.25 g/kg (0.5 ml/kg). Three ml blood samples were then taken at 2, 5, 10, 15, 20, 30, 40, 50, 60, 120 and 180 min and processed as described in section 2.5. After the 180 min sample, the catheters were flushed with heparinised saline to maintain patency and the usual daily quota of feed provided. Blood samples were analysed for plasma glucose and insulin concentrations. The test was accepted as being of adequate quality if the peak plasma glucose concentration was at least 10 mmol/l greater than the baseline (time 0) plasma glucose concentration.

The intercept of the glucose curve with the fasting glucose concentration was used to calculate the time taken to restore fasting glucose concentration (Gatford et al., 2004).

Using the trapezoid rule, total insulin (I) and glucose (G) areas under the curve (AUC) were calculated from baseline in two epochs; first phase (first 15 minutes of the GTT; AUC_{15}) and total (baseline to 3 hours or point at which plasma concentrations of insulin or glucose returned to baseline). Relative insulin secretion during either period was calculated as AUC_I / AUC_G.

2.13.2. Hyperglycaemic clamp

Hyperglycaemic clamps were performed to assess insulin sensitivity (Si) and insulin secretory capacity in response to a non-glucose stimulus. Although hyerinsulinaemic,

euglycaemic clamps (HEC) remain the gold standard method for calculating insulin sensitivity, HGCs are technically more reliable, have been shown to yield measures of insulin sensitivity that correlate well with those obtained from an HEC (Mitrakou et al., 1992) and provide additional information about insulin secretory responses.

HGCs were performed at least 24 hours after the glucose tolerance test, using the same indwelling jugular catheters. Food, but not water, was withdrawn at 5 pm of the evening prior to the HGC, and only *ad libitum* access to water permitted until after completion of the test.

5 ml baseline blood samples were taken from the sampling catheter at –20 min and -10 min for insulin and glucose concentrations. Additional 0.2 ml samples were collected at -15, -5 and 0 min. These 0.2 ml samples were used to determine the average baseline glucose concentration, and were processed immediately on a YSI 2300 (Yellow Springs Instruments, Dayton, OH, USA). At 0 min, a bolus infusion of 25% dextrose (Baxter, Health Support Ltd., Auckland, NZ) was commenced at 7.7 ml/min per m^2 of surface area ($Weight^{0.67}$x 0.09 (Berman, 2003)) for 5 min to increase the baseline glucose concentration to 10 mmol/L (DeFronzo, Tobin, & Andres, 1979). The rate of glucose infusion was then titrated against the blood glucose concentrations by means of a computer algorithm (DeFronzo, et al., 1979) to keep the blood glucose concentration at 10 mmol/L. Blood samples were taken every 5 min over a 135 min period. After one hour of variable dextrose infusion, steady state hyperglycaemia was achieved. During this period blood samples were taken every 5 minutes for determination of blood glucose concentrations, and an additional 5 ml of blood was taken every 15 minutes for determination of plasma glucose and insulin concentrations.

At 135 minutes a 100 mg/kg bolus of intravenous arginine (Acros Organics, Geel, Belgium) was given over 30 seconds. Arginine is a potent stimulant of insulin secretion and is used in combination with a hyperglycaemic clamp to maximise insulin secretion. Blood samples for plasma insulin and glucose concentrations were collected immediately prior to and 5, 10, 20 and 30 minutes after the arginine bolus. At 165 minutes the dextrose infusion was stopped and blood sampling ceased.

Hyperglycaemic clamps were only analysed further if the blood glucose coefficient of variation (CV) was ≤ 10% during steady state hyperglycaemia.

Using the trapezoid rule, insulin AUC (AUC_I) was calculated as the steady state AUC_I (AUC above baseline insulin concentration, from beginning to the end of steady state) and the arginine-stimulated AUC_I (insulin secretion in response to an intravenous arginine bolus;

AUC calculated as the area above the mean steady state insulin concentration). The mean glucose intake (mmol/kg·min) and mean plasma insulin concentrations (ng/ml) during the period of steady state were also calculated and from these, insulin sensitivity (Si) was derived (mean glucose intake / mean plasma insulin concentration) (Mitrakou, et al., 1992). In animals with age matched GTT and HGC data glucose disposition index, a measure of the insulin dependent component of glucose tolerance, was calculated (DI = Si·AIR, where AIR is the insulin response in the first ten minutes following a glucose bolus in a GTT (Long et al., 2010)).

2.13.3. Adrenaline stimulation test

Animals were not fasted for the adrenaline stimulation test. Blood sampling (3 ml) and adrenaline administration were via the jugular venous catheters. Blood samples were obtained prior to, and 2.5, 5, 7.5, 10, 15, 20, 30, 45 and 60 minutes after, a rapid intravenous bolus of 0.1 μg/kg adrenaline. Samples were processed as described in section 2.5, and analysed for glucose and free fatty acids. At completion of the test, both jugular catheters were removed.

Using the trapezoid rule, areas under the curve (AUC) were calculated for glucose ($GAUC_{ADR}$) and free fatty acids ($FAUC_{ADR}$) from baseline in two epochs: first 15 minutes of the adrenaline challenge (reflecting the period of most dynamic change, $AUC_{ADR\text{-}15}$) and total (baseline to 1 hour $AUC_{ADR\text{-}TOT}$). Peak fatty acid and glucose concentration from baseline was also calculated.

2.13.4. Growth hormone stimulation test

Juvenile animals underwent a growth hormone (GH) stimulation test at the completion of all other tests. Each dose of GH was made up immediately prior to administration. Freeze dried GH powder was re-suspended in filtered carbonate buffered saline (CBS), and a dose of 0.15 mg/kg given by i.m. injection twice daily (0830, pre-feed, and 1630) for four days. Blood samples (5 ml) were drawn by jugular venepuncture while the animal remained in its pen prior to the first, second, third, fifth and seventh GH injections and on the morning following the last GH injection. Samples were processed as described in section 2.5, and analysed for IGF-1, glucose, free fatty acids, ketone bodies and urea concentrations.

Ad libitum feed (sufficient to allow approximately 10% by weight feed refusal per day) was provided in the 3 days prior to and throughout the GH challenge, and daily feed intake was

recorded. Animals were weighed at the start of the GH challenge and on the day following the last GH injection.

2.13.5. Cardiovascular tests

All juvenile animals had tarsal arterial lines placed. Although it was possible to flush and freely aspirate blood from all arterial lines at the time of surgery, not all arterial lines remained patent at the time of blood pressure assessment. To try and improve the functional longevity of the catheters, the technique of carotid arterial line placement was trialled in adult animals from the 2008 birth cohort.

Juvenile blood pressure (BP) data were only obtained from animals born in 2009. During data analysis it became apparent that the calibration equipment used in the first year of the project was inaccurate. Retrospective re-calibration was performed using pooled calibration reference points from data collected during the second year of the experiment. As it was impossible to corroborate the validity of this approach, ECG but not BP data from 2008-born animals is presented. As a consequence, few juvenile animals have both ECG and BP data.

Preliminary data analysis showed that blood pressure was different in adult animals with carotid line placement compared to those with peripheral tarsal arterial lines, and that there was no improvement in arterial line patency. Hind tarsal arterial cannulation was resumed for all adult 2009-born animals. Adult blood pressure data from 2009-born animals only is therefore presented.

2.14. Arterial line placement

2.14.1. Intravenous anaesthesia

Propofol (Diprivan 10 mg/ml, Southern Veterinary Supplies, Hamilton, New Zealand) intravenous anaesthesia for arterial line placement was trialled unsuccessfully. Depth of anaesthesia was hard to maintain, and the high number of animals with idiosyncratic side effects made it unsuitable for continued usage.

Animals were fasted overnight prior to surgery. A combination of diazepam and ketamine was used for induction and maintenance of anaesthesia, for all arterial line placements and liver biopsies. Diazepam 0.5 mg/kg (Palmlin 5 mg/ml, Southern Veterinary Supplies, Hamilton, New Zealand) was mixed with ketamine 10 mg/kg (Ketamine 100 mg/ml,

Southern Veterinary Supplies, Hamilton, New Zealand) in a single syringe. The drugs adsorb to plastic therefore the mixture was made no more than half an hour prior to use. Of this mixture, half the volume was administered as an intravenous bolus via one of the indwelling jugular lines. The remaining volume was administered in 2 ml aliquots as necessary to maintain anaesthesia. Cumulative doses of up to diazepam 1.5 mg/kg and ketamine 30 mg/kg were required. At completion of surgery, the jugular catheter was flushed with heparinised saline and the animal returned to a metabolic crate. The usual daily feed quota was provided once the animal had recovered.

2.14.2. Arterial catheters

Arterial catheters were made using lengths of clear vinyl tubing (Biocorp Australia, Dandenong, Australia). Arterial lines were made from a 90 cm length of #sv74 tubing (ID 1.0 mm, OD 2.0 mm). Two rings of #sv110 (ID 2.0 mm, OD 3.0 mm) vinyl tubing were placed 10 cm from one end of the tubing and fixed with cyclohexanone (Scientific Supplies, Auckland, New Zealand). These rings formed a cuff around the catheter to enable the catheter to be secured. Catheters were sterilized by gamma irradiation prior to use.

2.14.3. Tarsal arterial line placement

Aseptic skin preparation was used as previously described (2.2.3). A trocar was introduced at the site of the muscle biopsy (2.16.2) and tunnelled subcutaneously down the limb to a 2 cm vertical incision on the lateral aspect of the hock where it exited. The solid introducer was then removed and an arterial catheter (2.14.2) placed through the hollow centre of the trocar. A small incision made in the anterior wall of the tarsal artery, the catheter threaded in, to a length of 10 cm, and anchored to the distal artery. The skin was closed, the distal end of the catheter attached to a 3-way tap and placed in a Ziploc bag secured to wool overlying the spine of the hind quarter. The arterial line was flushed with heparinised saline post operatively and daily thereafter. Prophylactic antibiotics were administered at the time of surgery (1.5 ml Duplocillin i.m (150,000 IU procaine penicillin and 150,000 IU benzathine penicillin per ml; Southern Veterinary Supplies, Hamilton, NZ).

Once BP recordings had been obtained, arterial lines were removed by applying traction to the distal end of the catheter whilst maintaining firm pressure at the arterial access site.

2.14.4. Carotid arterial line placement

Anaesthesia was maintained through an intravascular access device (16G venflon, Schoof, Cambridge, NZ) sited in the hind tarsal vein. Skin preparation of the neck was as described in 2.2.3. A 3 cm vertical incision was made medial to the jugular vein, and the carotid artery exposed by careful blunt dissection of the subcutaneous tissue, and identification and separation of the vagus nerve. A small incision was made in the anterior wall of the artery, a vascular catheter threaded in to a length of 6 cm, anchored to the vessel and flushed with heparinised saline through a three-way tap attached to its distal end. Once the unit was secure the overlying skin was closed with the distal end of the catheter exteriorised through the superior end of the wound. The catheter was placed in a Ziploc bag secured to wool on the back of the neck. Carotid catheters were flushed daily with heparinised saline. Once BP recordings had been obtained, catheters were knotted as close to the skin as possible and excess tubing removed. The internal component of the catheter remained *in situ* until post-mortem examination.

2.15. Physiological data recording system set-up

The PowerLab® (ADInstruments, Dunedin, NZ) physiological data acquisition system comprised one animal bioamp per sheep (ML136), an Octal bridge amp (ML228), the Powerlab 16/30 unit and a designated laptop running LabChart 7 software (ADInstruments, Dunedin, NZ) for both data acquisition and analysis. Four animals had simultaneous electrocardiogram (ECG) and BP recordings. A total of 8 channels of data were recorded, 1 ECG and 1 BP trace per animal, with all data sampled and recorded at a rate of 1 k/s.

Multipoint BP calibration (manual calibration at 0, 50, 100, 150 and 200 mmHg) was performed prior to each experiment, and the accuracy of that calibration checked across a range of points.

Subcutaneous ECG needles (MLA124; Needle electrodes 29 guage 12mm) were sited and the quality of the ECG was checked to ensure that the QRS conformation was recognisable with the R wave signal voltage well differentiated from the voltages achieved by other elements of the ECG signal.

Blood pressure transducers were filled with heparinised saline, all air bubbles removed and attached to the flushed arterial line.

2.15.1. ECG signal analysis

The raw ECG was scrutinised to identify a period of at least 5 minutes that was free from gross movement artefact or other signal interference. A five minute section was analysed using LabChart 7 software to identify all 'R' waves. The validity of each selected 'R' wave was manually verified, and the software signal recognition software manipulated as necessary. ECG traces with poor signal quality, ectopic or missing beats or significant amounts of movement artefact were not analysed. ECGs were then analysed for HR and measures of HRV (SDΔNN [standard deviation of differences between adjacent R-R intervals, ms]; NN50% [percent of difference between adjacent R-R intervals that are greater than 50 ms] and the frequency domain parameters of high frequency [HF 0.15-0.4 Hz], HF expressed relative to the total power of the ECG [HFnu], low frequency [LF; 0.04-0.15 Hz], low frequency expressed relative to the total power of the ECG [LFnu] and the LF/HF ratio) and the data entered manually into JMP v. 8 (SAS Institute Inc., Cary, NC, USA).

2.15.2. Blood pressure signal analysis

For each animal a minimum of three and maximum of five, two minute epochs were selected at 5 – 10 minute intervals throughout the raw data trace. Traces in which there was significant signal damping (taken as pulse pressure < 20 mmHg) or significant amounts of movement artefact were not analysed. LabChart 7 software was used to identify each BP cycle. The validity of each cycle was manually checked, and the average systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP) for each epoch calculated. The overall average SBP, DBP and MAP for all epochs was then calculated and taken as representative for each animal, and the data entered manually into JMP v.8.

2.16. Biopsy tissue collection

For all tissue sampling aseptic technique was used with skin preparation as described in 2.2.3. Haemostasis at all tissue collection sites was achieved with local pressure and apposition of the wound edges with superglue. All wound sites were checked daily and any signs of infection treated with topical wound care and systemic antibiotics (Duplocillin 3 ml i.m.).

2.16.1. Juvenile liver biopsy

Liver biopsy samples were taken from fasted juvenile animals at the time of arterial line placement.

Animals were positioned with their right side uppermost and a small bolster placed under the flank (to assist with positioning and stabilisation of the liver). The inferior margin of the rib cage was identified, and an entry point two rib spaces cephalad and 10 cm ventral from the spine was marked and small stab incision made through the skin. A custom made liver biopsy trocar (Schoof veterinarian supplies, Cambridge, NZ) was inserted vertically through this incision until it was felt to 'pop' through the diaphragm. The pointed introducer was removed, the hollow outer trocar manipulated to direct the tip towards the forequarter, and advanced until the typical resistance of liver tissue was felt. A 5 ml syringe was attached to the end of the trocar, 2-3 cm of continuous negative pressure applied and the trocar removed with the syringe still attached. Pressure on the syringe was then released and the core of liver tissue removed from the trocar, cut into two, snap-frozen and stored at -80˚.

2.16.2. Juvenile muscle biopsy

Muscle biopsies were taken from fasted juvenile animals at the time of arterial line placement.

A 1 cm skin incision was made in the skin of the lateral aspect of the right hind limb overlying vastus lateralis, the subcutaneous tissue blunt dissected and using a pair of Aliss forceps, a small (~1 cm^3) piece of muscle identified and removed. The muscle sample was divided into quarters, snap frozen, placed in two labelled eppendorf tubes and stored at -80°C.

2.16.3. Adult muscle biopsy

In adult animals muscle biopsy samples were obtained at completion of the HGC for analysis of insulin signalling pathway activation.

After completion of the sampling phase of the HGC the dextrose infusion was left running at its last infusion rate. After standard skin preparation 2 ml of lignocaine was injected subcutaneously and then intramuscularly into the right hind vastus lateralis. Biopsy technique was otherwise as previously described 2.16.2.

The dextrose infusion was then discontinued, the catheters flushed with heparinised saline and the animals fed their usual daily feed quota.

2.17. DXA body composition scanning

Dual energy X-ray absorptiometry (DXA) scanning facilities were only available during the latter part of the PhD. Body composition scans were therefore only acquired in adulthood from animals born during 2009.

The scanner (XR-800, Norland Corp., Fort Atkinson, WI, USA) was calibrated daily prior to use. Sheep were anaesthetised using Ketamine and Diazepam as described in 2.14.1 and placed on their right sides with their legs extended on the DXA scanning platform. Scans were performed with a resolution of 6 x 6 mm, and incorporated the area between the thoracic inlet (root of neck) and the rump of the animal (extending inferiorly to the level of the hip joint), and between the spine, the ventral surface of the sheep.

Analysis of DXA scans using Illuminatus 434S160 software (Norland Corp., Fort Atkinson, WI, USA) yielded measures of body fat and lean muscle mass and bone mineral content (BMC). Total measured body mass was calculated as the sum of BMC, lean and fat mass, and the composite elements then expressed as a percentage of that mass (%BMC, %lean, % fat respectively). The ratio of upper body to lower body fat was expressed as a percentage ([upper body % fat mass / lower body % fat mass] · 100).

Animals in which total body fat was below the lower limit of DXA scanning capability were arbitrarily assigned a total fat mass of 0.05 kg; these animals were therefore excluded from regression analyses examining associations between fat mass and other outcomes, and from assessment of differences in regional fat distribution.

Fat mass, relative to measures of linear length or height was calculated from the method described by Fewtrell et al. (Fewtrell, et al., 2004); Fat mass index (FMI) = fat mass (Kg) / CRL $(m)^2$.

In addition, body composition elements within the upper (thoracic inlet to the diaphragm, between the spine and ventral surface of the sheep) or lower (diaphragm to rump, between the spine and ventral surface of the sheep) part of the body were assessed. Using the available software it was not, however, possible to differentiate between specific fat depots (e.g. subcutaneous vs. visceral fat).

2.18. Post mortem protocol

Planned post mortems (PMs) were conducted in a subset of juvenile animals as part of the original supplement development, and in adult animals at completion of all other testing. Both juvenile and adult PMs were conducted with a minimum one week period between any experimental intervention and post-mortem. Tissues were collected for molecular biology (frozen samples) and immunohistochemistry (fixed samples) from all juvenile PMs, and from a subset of adult animals; these data do not form a part of this thesis.

For all post-mortem examinations, following an overnight fast, animals were weighed, killed with a lethal dose of pentobarbitone (20 ml per animal by i.v. injection) and then rapidly exsanguinated. Anthropometric measurements were taken (BPD, CRL, CG, AG, HTL, LL, body length, shoulder and rump height). The pancreas was then rapidly dissected out, weighed and cut longitudinally into 1 cm wide strips. One of these was placed in 4% paraformaldehyde (PFA; extra pure paraformaldehyde, Scharlau, Barcelona, Spain) the other was frozen on dry ice and stored at -80 ºC.

Samples of skeletal muscle were dissected from the vastus lateralis, and samples of liver, omental, perirenal and subcutaneous fat, one whole adrenal gland and a section of the descending abdominal aorta were removed, snap frozen and stored at -80 ^{o}C. The other adrenal gland and a section of descending abdominal aorta were also fixed in PFA. Organs were weighed before sample preparation, and weights were also obtained for the heart, lungs, kidneys, perirenal fat, thymus, spleen and gonads. The bowel was then removed and the eviscerated carcass weighed.

The heart was cut longitudinally from the apex to the aortic root. Mid cavity measurements were made of the thickness of the interventricular septal wall and the left and right ventricular free wall.

All tissues fixed in PFA were kept refrigerated, decanted after 72 hours and stored thereafter in 70% ethanol.

2.19. Laboratory Assays

All laboratory assays were performed by technicians in the analytical laboratories of the Liggins Institute, University of Auckland apart from D_2O measurements which were performed by Massey University, Palmerston North campus.

2.19.1. Metabolite assay

Plasma metabolite concentrations were measured on a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan): glucose by enzymatic colorimetric assay (Roche, Mannheim, Germany); urea by kinetic UV assay (Roche); and free fatty acids (FFA) by enzymatic colorimetric assays (Randox Laboratories Ltd, Ardmore, Crumlin, UK).

2.19.2. Ovine insulin, IGF1 and cortisol assays

Plasma ovine insulin, IGF1 and cortisol concentrations were measured by radioimmunoassay (RIA), IGFBP-blocked RIA (Blum & Breier, 1994) and mass spectrometry (Jaquiery et al., 2006) techniques respectively. These techniques have all been previously used and reported by our group (Jaquiery, Oliver, Bloomfield, & Harding, 2011).

2.20. Statistical analyses

Data were entered into JMP files (SAS Institute Inc., Cary, NC, USA) either manually or imported electronically from Excel spreadsheets (Microsoft Corp., Seattle, WA, USA). Results are presented as the mean with the standard error of the mean (SEM), median with either interquartile range (IQR) or number or percentage (%) as appropriate. Normality of distribution was checked using the Shapiro-Wilk test, and non-parametric data log transformed to approximate a normal distribution where possible. χ^2 tests were used to check that key nominal variables were evenly distributed between groups of sheep.

The effect of preterm birth was examined by ANOVA in a cohort comprising all preterm, term-spont and term-Dex sheep with sex as a covariate. The effect of corticosteroid induction of labour was assessed in un-supplemented term-spont, term-Dex and term-Alizin sheep; as this was a secondary analysis of the effect of birth group, statistical significance was set at a p-value of 0.01.

The effect of early nutrient supplementation was examined in supplemented and un-supplemented term-spont, preterm and term-Dex sheep, with sex and birth group as covariates.

Stepwise regression analysis followed by ANOVA and *post-hoc* testing was used to investigate the extent to which other factors, such as birth weight z-score, weight and age might influence outcomes. Associations between continuous variables were examined using

regression analysis with sex as a covariate. Separate analyses of male and female sheep were performed when there was a significant interaction between sex and a variable of interest.

Longitudinal changes over time were examined using RM ANOVA; where significant differences were found, data were analysed by factorial ANOVA with *post hoc* testing.

In all analyses, unless explicitly indicated, data were analysed in same sex groups.

Post-hoc analysis was performed in all analyses using either the student t-test for paired analyses or Tukey for multiple comparisons.

A p-value of < 0.05 was taken as statistically significant, unless explicitly indicated.

Chapter 3. Generation of experimental groups

3.1. Introduction

This project aimed to test the hypothesis that in sheep, preterm birth and rapid postnatal growth, achieved through the provision of nutrient supplementation during early postnatal life, would influence metabolic, endocrine and cardiovascular outcomes in juvenile and adult life.

Preterm birth was induced with maternal dexamethasone administration (Chapter 2.3). Term-born lambs were born spontaneously (term-spont), born following maternal dexamethasone administration to control for the potential effect of dexaemthasone induction of labour on the outcomes of interest (term-dex), or born following induction of labour with a pregesterone receptor antagonist (Alizin) to control for the potential effect of induction of labour itself on the outcomes of interest (term-Alizin). The term-Alizin group all received water with no supplementation arm, because of the prohibitive cost of Alizin.

This chapter reports the number, characteristics and disposal of animals born during the course of this experiment.

3.2. Methods

Ewes were randomised to a preterm, term-spont, term-dex or term-Alizin upon entry to the feedlot at d131 of gestation. A second randomisation before delivery allocated lambs to nutrient supplementation or water (Chapter 2.7). Ewes in which preterm induction of labour was unsuccessful (failure to lamb within 3 days of administration of the first induction agent) or which delivered unexpected twins were returned to the farm and their offspring not studied.

Total experimental animal numbers refer to the number of animals born into the experiment (both live born and stillborn). Experimental losses refer to any singleton animals born into the experiment but no longer present at the time of adult post-mortem.

Weight and size at birth were only recorded in animals free of congenital malformation.

Where possible, all animals had growth data recorded and were studied at all ages. Animals which were sick or missing were not studied at that time, and experimental failure reduced

numbers of completed tests. Specific issues will be highlighted as necessary in the relevant chapters.

3.3. Statistical analyses

Growth is known to be different between males and females; thus, the sexes were analysed separately.

First, the effects of preterm birth and supplementation were assessed in all term-spont, preterm and term-dex sheep by 2-way ANOVA (independent variables birth group and supplementation status) with the interaction term birth group x supplement. Second, the effects of corticosteroid induction of labour at term were assessed by factorial ANOVA in un-supplemented term-spont, term-dex and term-Alizin sheep. If ANOVA was significant, a Tukey *post hoc* test was performed with significance set at a p-value of <0.05 for the primary analysis and <0.01 for the secondary analysis of birth group. Data from these analyses are presented as the least square mean ± SEM.

Comparisons between nominal variables were made using the χ^2 test.

3.4. Animal numbers and groups

Two hundred and one singleton sheep were generated for this experiment (Figure 3-1). A further 18 twin lambs were unplanned and therefore not studied. Thirty seven additional lambs were used for method development (developing ewe milk supplement, see chapter 2.7) and a further 7 lambs were born following pilot trials of experimentally induced *in utero* growth restriction (IUGR, see chapter 2.2).

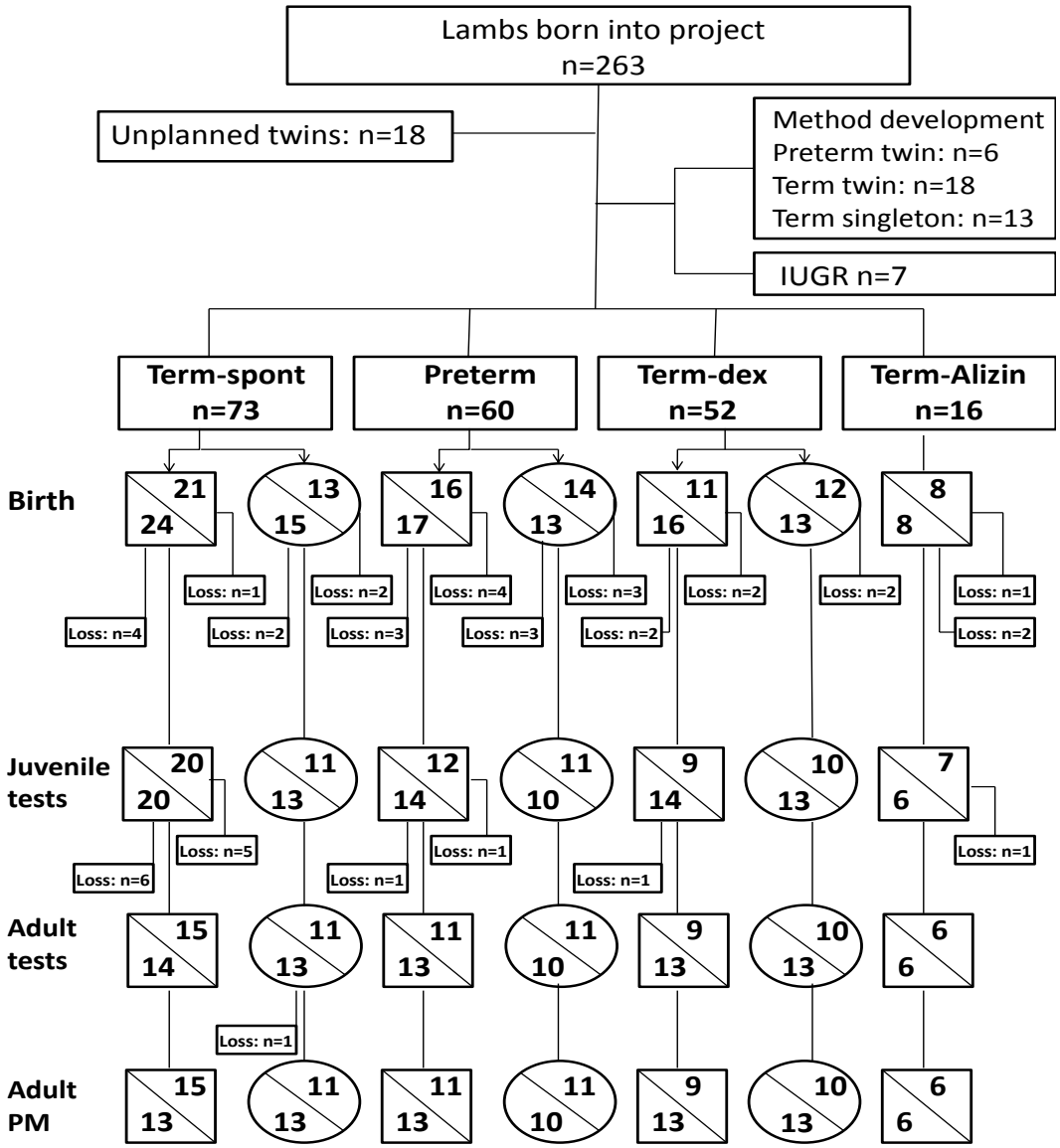

Figure 3-1: Characteristics of experimental groups

Sheep born during the project according to their birth group, supplementation status and sex. Lambs antenatally randomised to water are shown in squares, those randomised to supplement are shown in circles. The top right division of each square or circle denotes the number of female animals; the lower left division denotes the number of male animals. The number of animals lost (Loss: n=) to the experiment from each group is given.

Term-Alizin lambs were generated only from ewes mated during their natural breeding season. Term-spont, Term-dex and preterm lambs were evenly distributed between ewes mated during, or outside of, their natural breeding season (χ^2 p=0.14).

Numbers of lambs allocated to receive supplementation were not different between birth groups of either sex (male, χ^2=0.8; female χ^2=0.5).

Animals were lost from each birth group during the course of the experiment (Figure 3-1). These losses were assessed by the local veterinarian as within the expected range and frequency of incidents expected from animals managed on pasture (Table 3-1). Nine term-spont un-supplemented animals were euthanised after their juvenile assessment to provide control tissue for a separate study based on the supplement method development work (Chapter 2.7.1).

		Term-Spont	Preterm	Term-dex	Term-Alizin
Stillborn		3	1	0	0
Birth injury		1	4	1	0
Congenital malformation [a]		0	0	0	1
Ewe factors:	Lamb crushed	1	0	0	0
	Lactation failure	2	2	1	0
	Udder damage	0	2	1	0
	Rejection	1	0	0	0
Early sepsis (birth-2 weeks) [b]		0	3	0	0
On farm:	Accident [c]	2	0	0	0
	Infection [d]	0	2	4	3
Other:	Juvenile PM	9	0	0	0
Experiment related death [e]		2	1	0	0

Table 3-1: Experimental animal losses

Number of animals lost within each birth group. [a] anal atresia. [b] urinary tract infection, umbilical stump infection. [c] fight injury, accidental drowning. [d] worm infestation, pulpy kidney, respiratory and joint sepsis. [e] liver biopsy induced injury and anaesthetic complications.

3.5. Lamb anthropometric characteristics at birth

Preterm lambs of both sexes were lighter and smaller at birth with a lesser weight for length than term-spont and term-dex lambs (Table 3-2). Birth weight z-scores were not different between preterm and term-spont or term-dex lambs of either sex.

There was no difference in size or weight at birth between lambs randomised antenatally to receive nutrient supplementation or not (Table 3-2).

The size and weight of term-Alizin lambs at birth was not different to that of same sex term-spont or term-dex lambs (Table 3-2).

3.6. Gestational age at birth and corrected postnatal age at juvenile and adult assessment

Preterm lambs were born significantly earlier than term-spont or term-dex lambs (Table 3-3). Corrected postnatal age (CA) at the time of juvenile and adult assessment was not different amongst groups (Table 3-3).

Supplemented sheep were not different from un-supplemented sheep, and term-Alizin sheep were not different from un-supplemented term-spont or term-dex sheep in either gestational age at birth, or CA at juvenile or adult challenges (Table 3-3).

MALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN (n=24)	S (n=14)	UN (n=17)	S (n=13)	UN (n=16)	S (n=13)	UN (n=8)
BWT (Kg)	*** ‡‡‡	6.1 ± 0.2	6.0 ± 0.2	4.8 ± 0.2	5.2 ± 0.2	5.7 ± 0.2	6.0 ± 0.2	6.0 ± 0.3
Birth weight z-score		0.3 ± 0.2	0.1 ± 0.3	-0.2 ± 0.3	0.4 ± 0.3	-0.3 ± 0.3	0.1 ± 0.3	0.2 ± 0.4
CRL (cm)	*** ‡‡‡	52.1 ± 0.6	51.2 ± 0.8	47.9 ± 0.7	48.8 ± 0.8	50.9 ± 0.7	51.6 ± 0.8	52.9 ± 1.0
HL (cm)	*** ‡‡‡	39.8 ± 0.4	39.6 ± 0.5	36.1 ± 0.4	36.6 ± 0.5	38.6 ± 0.4	39.1 ± 0.5	39.6 ± 0.6
BPD (cm)	*** ‡‡‡	6.4 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.3 ± 0.1
W-L (Kg/m)	*** ‡‡‡	6.6 ± 0.1	6.6 ± 0.2	5.6 ± 0.2	6.0 ± 0.2	6.3 ± 0.2	6.6 ± 0.2	6.5 ± 0.2
FEMALE		UN (n=21)	S (n=13)	UN (n=16)	S (n=14)	UN (n=11)	S (n=12)	UN (n=7)
BWT (Kg)	*** ‡‡‡	5.7 ± 0.2	5.6 ± 0.2	4.7 ± 0.2	4.5 ± 0.2	5.6 ± 0.2	5.5 ± 0.2	5.3 ± 0.3
Birth weight z-score		0.2 ± 0.2	-0.1 ± 0.3	0.2 ± 0.3	-0.1 ± 0.3	-0.1 ± 0.3	0.2 ± 0.3	-0.4 ± 0.4
CRL (cm)	*** ‡‡‡	51.4 ± 0.6	50.7 ± 0.8	48.2 ± 0.7	47.3 ± 0.7	50.6 ± 0.8	49.7 ± 0.8	52.4 ± 1.1
HL (cm)	*** ‡‡‡	38.8 ± 0.4	38.1 ± 0.5	36.4 ± 0.5	35.3 ± 0.5	37.7 ± 0.5	38.0 ± 0.5	38.8 ± 0.7
BPD (cm)	***	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.1 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.1 ± 0.1
W-L (Kg/m)	*** ‡‡‡	6.3 ± 0.1	6.2 ± 0.2	5.5 ± 0.2	5.4 ± 0.2	6.3 ± 0.2	6.2 ± 0.2	5.8 ± 0.3

Table 3-2: Birth characteristics according to sex, birth group and supplementation allocation in lambs

*UN = un-supplemented. S=supplemented. BWT= birth weight. CRL= crown rump length. HL=hind limb. BPD=bi-parietal diameter. W-L=weight for length. Data are least square mean ± SEM. ***p<0.001 preterm compared to term-spont. ‡‡‡ p<0.001 preterm compared to term-dex.*

MALE	Term-spont		Preterm		Term-dex		Term-Alizin
	UN	S	UN	S	UN	S	UN
	(Birth; n=24)	(Birth; n=15)	(Birth; n=17)	(Birth; n=13)	(Birth; n=16)	(Birth; n=13)	(Birth; n=8)
	(Juv; n=20)	(Juv; n=13)	(Juv; n=14)	(Juv; n=10)	(Juv; n=14)	(Juv; n=13)	(Juv; n=6)
	(Adult; n=14)	(Adult; n=13)	(Adult; n=13)	(Adult; n=10)	(Adult; n=13)	(Adult; n=13)	(Adult; n=6)
Gestational age (days) *** ‡‡‡	147.8 ± 0.3	146.4 ± 0.4	137.5 ± 0.4	137.4 ± 0.4	147.1 ± 0.3	147.0 ± 0.4	147.0 ± 0.5
CA: juvenile assessment (weeks)	17.7 ± 0.7	17.0 ± 0.8	18.4 ± 0.8	17.2 ± 0.9	16.6 ± 0.7	18.1 ± 0.8	19.8 ±1.0
CA: adult assessment (months)	16.2 ± 0.3	16.0 ± 0.3	15.7 ± 0.4	16.3 ± 0.4	15.8 ± 0.3	15.9 ± 0.3	15.2 ± 0.5
FEMALE	(Birth; n=21)	(Birth; n=13)	(Birth; n=16)	(Birth; n=14)	(Birth; n=11)	(Birth; n=12)	(Birth; n=8)
	(Juv; n=20)	(Juv; n=11)	(Juv; n=12)	(Juv; n=11)	(Juv; n=9)	(Juv; n=10)	(Juv; n=7)
	(Adult; n=15)	(Adult; n=11)	(Adult; n=11)	(Adult; n=11)	(Adult; n=9)	(Adult; n=10)	(Adult; n=6)
Gestational age (days) *** ‡‡‡	147.5 ± 0.4	146.8 ± 0.4	137.4 ± 0.4	137.2 ± 0.5	147.2 ± 0.5	146.8 ± 0.5	146.9 ± 0.6
CA: juvenile assessment (weeks)	19.2 ± 0.7	17.8 ± 0.8	18.1 ± 0.8	18.5 ± 0.8	19.7 ± 0.9	17.6 ± 0.8	19.3 ± 0.9
CA: adult assessment (months)	16.1 ± 0.3	15.7 ± 0.4	15.5 ± 0.4	15.2 ± 0.4	16.0 ± 0.4	15.7 ± 0.4	15.0 ±0.4

Table 3-3: Effect of birth group and supplementation on gestational age at birth and CGA at time of juvenile and adult assessment

*UN=un-supplemented. S=supplemented. Corrected postnatal age (CA) at juvenile (Juv) and adult assessment. Data are least square mean ± SEM. *** p<0.001 preterm compared to term-spont. ‡‡‡ preterm compared to term-dex.*

3.7. Discussion

Numbers of animals were well matched for sex and supplementation status between term-spont, preterm and term-dex sheep. Although it was possible that term-dex and term-Alizin ewes commenced labour prior to exposure to induction agents, both term-dex and term-Alizin lambs were born within a much narrower range of gestation lengths then term-spont, suggesting that induction of labour did occur.

The numbers of term-Alizin sheep were small because of the prohibitive cost of Alizin (a progesterone receptor antagonist). Therefore, Alizin sheep were not randomised to supplementation or water, but all received water. This approach was intended to allow the separation of potential consequences due to induction of labour *per se* from those of antenatal corticosteroid exposure; any effects due to an interaction between Alizin induced labour and early nutritional supplementation would, however, be undetected. The data in this chapter demonstrate that Alizin is a safe and efficacious agent and that it is possible to reliably induce labour in sheep without administering corticosteroids to the ewe. However, experiments with a 3β hydroxysteroid dehydrogenase inhibitor (Epostane; an agent no longer commercially available that results in functional progesterone withdrawal), have demonstrated that following administration to the ewe (d137-139) there is an initial fall in fetal cortisol concentrations, a rise in fetal ACTH followed by an 'overshoot' in fetal plasma cortisol concentrations to a level comparable with a normal pre-partum cortisol surge seen at term. This resulted in delivery of a viable lamb within 36 hours of drug administration (Silver, 1988). Thus, although we did not measure the effect of Alizin on maternal or fetal plasma cortisol concentrations, it is likely that the fetus was still, indirectly, exposed to increased plasma concentrations of corticosteroids.

Preterm lambs were significantly smaller and lighter at birth than either term-spont or term-dex lambs, some still had their eyelids fused and had signs of respiratory distress (increased work of breathing and tachypnoea) and, during the first 2-3 days, most were weaker (struggled to stand and feed) and less able to maintain their own temperature than term-born lambs. Term-dex and Term-Alizin lambs, however, were not different in weight or size from each other or from term-spont lambs, and no obvious differences in lamb behaviour between term-induction and term-spont lambs were noted during the first days of life.

Both glucocorticoids and Alizin were effective at inducing labour within a predictable time-frame with the result that the interval between exposure to induction agents and birth was small. In late gestation fetal cortisol is an important regulator of the reduction in growth rate that occurs in preparation for birth (Fowden, Szemere, Hughes, Gilmour, & Forhead, 1996) and, together with the short interval between administration of induction agents and delivery, a difference in birth weight between same sex term-born lambs exposed to the two different induction agents would be difficult to detect even if one agent slowed growth more than another. The possible effects of antenatal steroid exposure on postnatal growth will be discussed in Chapter 4. Whether steroid exposure during preterm induction of labour slows growth is not known; artificial preterm induction of labour, whatever the drug used, is likely to increase endogenous steroid concentrations, but is required to promote tissue maturation necessary for postnatal life. Preterm Caesarean section to create a steroid naïve group would enable the assessment of preterm birth weight without the confounding influence of antenatal steroid exposure, but would also be associated with significant morbidity and mortality.

Following a single or repeated course of maternal antenatal corticosteroids, reduced birth weight has been reported in lambs born preterm by caesarean section, and therefore delivered prior to the usual decline in growth that occurs close to term (Ikegami et al., 1997; Newnham et al., 1999). Repeated, but not single, courses of maternal antenatal corticosteroids also reduced birth weight in lambs born spontaneously at term (Moss et al., 2001). Steroid treatment in all cases commenced at a much earlier stage in gestation (d104) than in our experiment and required co-treatment of both steroid and control groups with medroxyprogesterone to prevent the onset of labour. One recent study in humans has tried to isolate the effects of antenatal corticosteroid and preterm labour on fetal growth, quantified as size at birth. Infants were born either following an uneventful pregnancy without either steroid exposure or preterm labour (PTL), or after pregnancy complicated by PTL with or without maternal steroid treatment; all infants were born at full term. Infants whose mothers had experienced PTL and had received steroid treatment were smaller at birth compared to infants whose mother had experienced PTL but had not received steroid treatment, or to infants not exposed to PTL or antenatal steroids (Davis et al., 2009). Fetal growth prior to either PTL or steroids was retrospectively assessed from routine second trimester ultrasound scans and was not different amongst groups. The authors speculate that corticosteroids and not PTL *per se*, has a negative impact on fetal growth; however, it is important to note that there are relatively small numbers of infants in each group and data from male and female

infants are combined. Cochrane reviews have also found that although mean birth weight is not altered by single (Roberts & Dalziel, 2006) or repeated (Crowther & Harding, 2007) antenatal courses of corticosteroids, repeated courses have been associated with increased rates of babies born small for gestational age.

By the time of juvenile and adult assessment corrected postnatal age was not different amongst groups; differences in age cannot, therefore, account for any differences in metabolic or cardiovascular parameters ascribed to preterm birth, antenatal corticosteroid exposure, induction of labour or nutrient supplementation.

One limitation of this paradigm is the imposition of preterm birth on a previously normal pregnancy with no known fetal morbidity, which is rarely the case in clinical practice. Determinants of spontaneous preterm birth may be established early in gestation, detectable in the first trimester as reduced maternal serum levels of key trophoblast-specific proteins (Smith et al., 2002), or reduced fetal growth (Bukowski et al., 2008; Mook-Kanamori et al., 2010; Smith, et al., 1998); size at birth may therefore be less in preterm infants relative to the estimated weight of a 'well' gestational-age and sex matched fetus *in utero* (Lackman, Capewell, Richardson, daSilva, & Gagnon, 2001). Outcomes in infants born following spontaneous preterm labour may also be influenced by whatever feto-maternal co-morbidity triggered preterm labour.

We believe that the paradigm of corticosteroid-induced preterm birth is a useful one with which to assess the potential long-term consequences of preterm birth. The majority of women at risk of preterm birth receive corticosteroids to reduce neonatal morbidity and mortality (American College of Obstetricians and Gynecologists, 2007) and preterm lambs born without antenatal corticosteroid exposure develop respiratory distress and have a much higher mortality rate (De Matteo, et al., 2010). Other preterm animal paradigms have different philosophical or logistical limitations; primate studies are limited by significant ethical and financial considerations (Verney, et al., 2010), whereas studies using guinea pigs are confounded by the effects of multiple gestation (Lyall, et al., 1997).

Finally, to reduce respiratory complications in the newborn (Bonanno & Wapner, 2009; Stutchfield, et al., 2005) clinicians are increasingly using maternal corticosteroid treatment prior to elective Caesarean section at term (Royal College of Obstetricians and Gynaecologists, 2010). No human data are available to assess the long-term safety of this practice; the term-dex paradigm may offer an insight into potential long term effects.

Chapter 4. Postnatal growth and body composition

4.1. Introduction

Conventional clinical practice aims to promote growth in small or preterm infants, although is not always effective at maintaining an infant's growth along the desired trajectory (Morgan, Young, McCormick, & McGuire, 2011). Weight gain is not necessarily accompanied by similar increments in longitudinal or head growth, and therefore rapid weight gain may reflect increased adiposity or altered body composition rather than uniform increases in lean, fat and bone mass. The trajectory of postnatal growth to ensure optimal short term and long term health outcomes is unknown and may differ between groups of infants (e.g. infants born IUGR at term compared to well grown but preterm infants) and between males and females (Thureen, 2007). In addition, it is unclear whether exposure to antenatal corticosteroids in the days immediately prior to birth influences postnatal growth.

The aim of this experiment was to investigate the effect of supplementation of newborn lambs on postnatal growth trajectory and body composition, and the potential impact of preterm birth, antenatal corticosteroid exposure and induction of labour at term on these outcomes.

4.2. Methods

Animals included in this chapter were generated as described in Chapter 2.3, with distribution between birth groups, sex and supplement status as laid out in Figure 3-1.

Lambs were weighed and measured at regular intervals until weaning at 12 weeks of age and weighed monthly thereafter (Chapter 2.9). Growth (both weight and measures of linear growth) was assessed as the growth velocity (GV) within discrete epochs: birth to 2 weeks (the period of nutrient supplementation; $GV_{0\text{-}2}$); 2 weeks to weaning ($GV_{2\text{-}W}$), and from weaning to 1 year of age ($GV_{W\text{-}1Yr}$) (Chapter 2.9). The dynamic of weight change within these epochs was also assessed between birth and 2 weeks as daily relative weight gain, and between 2 weeks and weaning as average fortnightly GV.

Milk intake was derived from D_2O dilution curves (Chapter 2.8). Although all lambs received D_2O intravenously and underwent serial blood sampling for dilution curve calculation, laboratory constraints restricted analysis to a subset of preterm and term-spont lambs,

randomly selected from within each birth group. Estimated macronutrient intake was derived from milk composition data obtained from animals belonging to a different experiment undertaken at Ngapouri Research facility (Dr AL Jaquiery, unpublished), but otherwise matched with respect to animal husbandry and mode of lamb delivery.

The growth hormone (GH) response test was performed at completion of all other juvenile metabolic and cardiovascular testing. IGF1 and glucose responses to GH were calculated from their pre-GH baselines as areas under the curve (AUC) and maximal response (baseline subtracted from peak response to GH).

4.3. Statistical analysis

Data were analysed in same sex groups.

Non-parametric data were log transformed prior to further analysis, and comparisons between nominal variables were made using the χ^2 test.

Longitudinal changes were analysed using RM ANOVA; where significant differences were found, data were analysed by factorial ANOVA with Tukey *post-hoc* test.

Parametric data were analysed by factorial ANOVA with Tukey *post hoc* test. First, the effects of preterm birth and supplementation were assessed in all term-spont, preterm and term-dex sheep by 2-way ANOVA (independent variables birth group and supplementation status) with the interaction term birth group x supplement. Second, the effects of corticosteroid induction of labour at term were assessed by factorial ANOVA in un-supplemented term-spont, term-dex and term-Alizin sheep. If ANOVA was significant, a Tukey *post hoc* test was performed with significance set at a p-value of <0.05 for the primary analysis and <0.01 for the secondary analysis of birth group. Data from these analyses are presented as the least square mean ± SEM.

Multivariate regression models were then constructed to assess whether the effects of birth group were modified by the inclusion of other perinatal or postnatal variables (adjusted model: birth group, birth weight (bwt) z-score, supplementation status, weight and age at the time of assessment). Single measures of GV from each main epoch ($GV_{0\text{-}2}$, $GV_{2\text{-}W}$ and in adult sheep $GV_{W\text{-}1Yr}$) were then included in the adjusted model to see whether growth rates further modified the effect of birth group.

Correlations between continuous variables were examined by regression analysis within each birth group, in same sex groups. To allow for the effect of multiple analyses, statistical significance was set at a p-value of 0.001.

4.4. Results

4.4.1. Effect of preterm birth on growth

4.4.1.1. Preterm birth and growth during the first two weeks

For data on size at birth, see Chapter 3.5. Preterm lambs remained smaller and lighter than same sex term-spont and term-dex lambs throughout the first two weeks of life (Figure 4-1, Figure 4-2).

At term equivalent age, preterm lambs weighed more than newborn, same sex term-spont or term-dex lambs (Table 4-1, Table 4-2).

Male preterm lambs had a lower daily relative weight gain (Figure 4-1) than male term-spont or term-dex, especially during the first 3 days of life, and a lower GV in the first two weeks of life (GV_{0-2}, Table 4-1). The maximal relative daily weight gain achieved in preterm males was not different from that of term-spont or term-dex males, but occurred 2 days later (Table 4-1).

In females, daily relative weight gain (Figure 4-2) and GV_{0-2} (Table 4-2) were not different between birth groups. Maximal daily relative weight gain was less in preterm than term-dex lambs (Table 4-2), although the age at which it was achieved was not different between birth groups (Table 4-2).

The rate of increase in crown rump length between birth and 2 weeks (CRL_{0-2}) was less in preterm males than term-spont or term-dex males, but not different between females of any birth group.

The rate of increase in hind limb length (HL_{0-2}) and bi-parietal diameter (BPD_{0-2}) between birth and 2 weeks were not different between birth groups of either sex (Table 4-2).

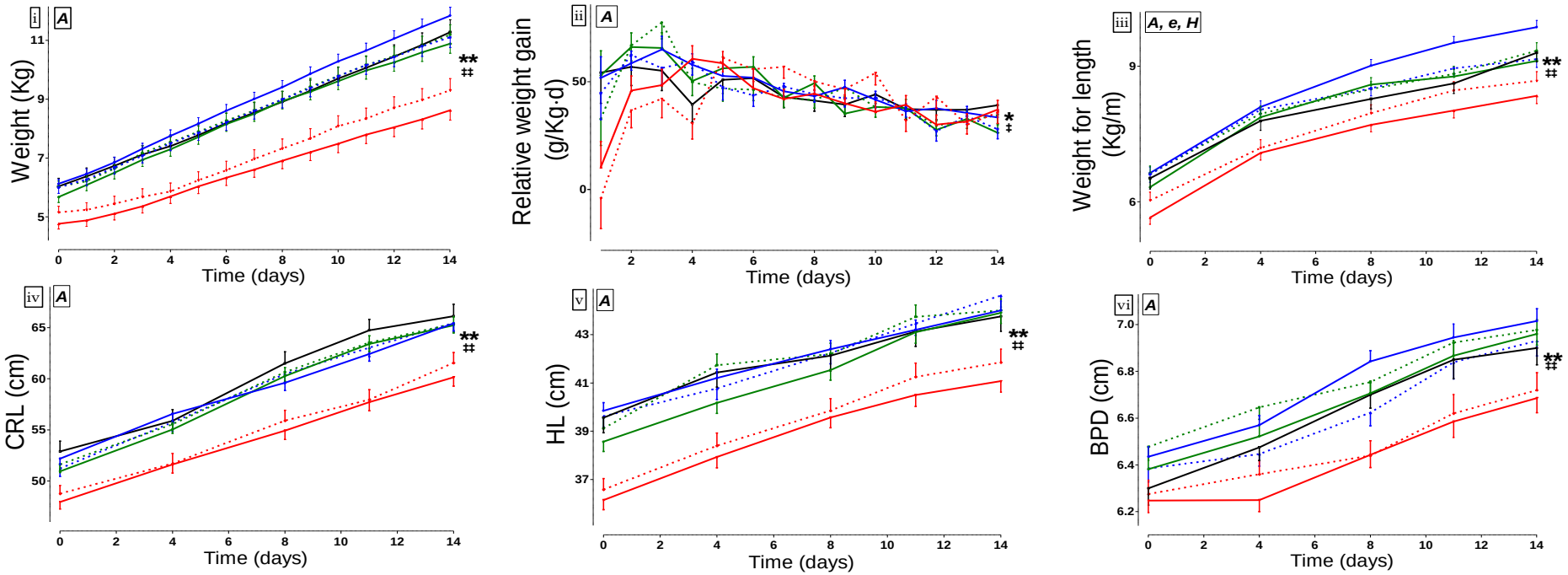

Figure 4-1: Effect of birth group and supplementation on growth during the first two weeks of life in male lambs

*i) weight. ii) relative weight gain. iii) weight-for-length. iv) crown rump length (CRL). v) hind limb length (HL). vi) bi-parietal diameter (BPD). Term-spont, blue; un-supplemented (UN; solid lines) n=20, supplemented (S; interrupted lines) n=13. Preterm, red; UN n=14, S n=10. Term-dex, green; UN n=15, S n=13. Term-Alizin, black, n=8. Data are least square means ± SEM. Letters give effect of RM ANOVA. Symbols refer to post-hoc tests. A, p<0.01 for the comparison between term-spont, preterm and term-dex groups. e, p<0.05 for the effect of birth group x supplement. H, p<0.01 for the comparison between term-Alizin and UN term-spont and term-dex. ** p<0.01, * p<0.05 preterm compared with term-spont. ‡‡ p<0.01, ‡ p<0.05 preterm compared with term-dex.*

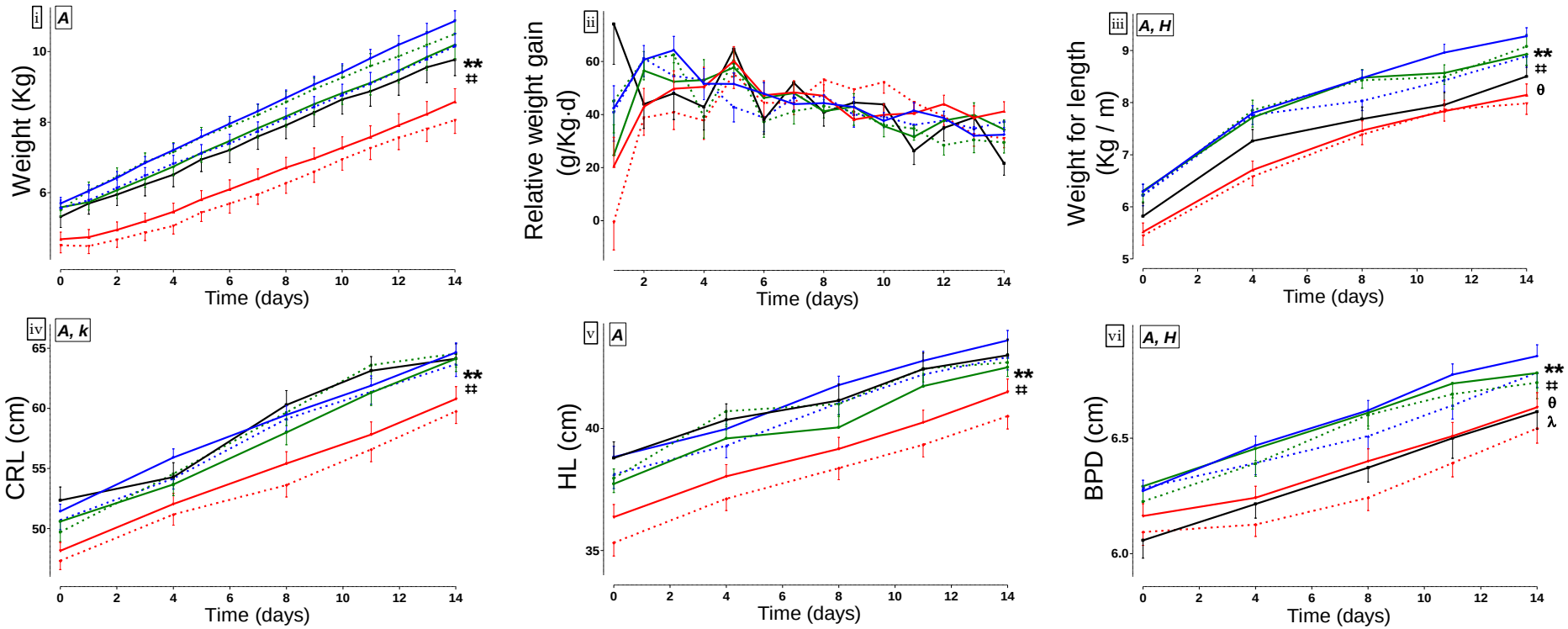

Figure 4-2: Effect of birth group and supplementation on growth during the first two weeks of life in female lambs

*i) weight. ii) relative weight gain. iii) weight-for-length. iv) crown rump length (CRL). v) hind limb length (HL). vi) bi-parietal diameter (BPD). Term-spont, blue; un-supplemented (UN; solid lines) n=20, supplemented (S; interrupted lines) n=13. Preterm, red; UN n=14, S n=10. Term-dex, green; UN n=15, S n=13. Term-Alizin, black, n=8. Data are least square means ± SEM. Letters give effect of RM ANOVA. Symbols refer to post-hoc tests. A, p<0.01 for the comparison between term-spont, preterm and term-dex groups. H, p<0.01 for the comparison between term-Alizin and UN term-spont and term-dex. k, p<0.05 for the comparison between term-Alizin, UN term-spont and UN term-dex over time. ** p<0.01, * p<0.05 preterm compared with term-spont. ‡‡ p<0.01, ‡ p<0.05 preterm compared with term-dex. θ p<0.01 term-Alizin compared to term-spont. λ p<0.01 term-Alizin compared to term-dex.*

MALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN	S	UN	S	UN	S	UN
GV epoch birth to 2 weeks		n=20	n=13	n=14	n=10	n=15	n=13	n=8
GV epoch 2 weeks to weaning		n=19	n=13	n=14	n=10	n=14	n=13	n=6
GV epoch weaning to one year		n=14	n=13	n=13	n=9	n=9	n=11	n=6
Wt-TEA (Kg)	* ‡	6.1 ± 0.2	6.0 ± 0.2	7.5 ± 0.2	8.1 ± 0.3	5.8 ± 0.2	6.0 ± 0.2	6.0 ± 0.3
Max. DRWG (g/Kg·d)		86.7 ± 4.8	88.0 ± 6.0	80.3 ± 5.8	79.6 ± 6.8	90.1 ± 5.6	86.6 ± 6.0	74.7 ± 8.3
Age at max. DRWG (days)	* ‡ †† ■ □	2.4 ± 0.5	3.8 ± 0.6	4.4 ± 0.6	7.0 ± 0.7	2.9 ± 0.6	3.2 ± 0.6	2.4 ± 0.5
GV_{0-2} (g/Kg·d)	* ‡ ψ	47.1 ± 1.3	44.1 ± 1.6	40.9 ± 1.6	40.8 ± 1.8	45.6 ± 1.5	44.5 ± 1.6	44.8 ± 1.9
GV_{2-W} (g/Kg·d)	* ‡ ψ ■ □	14.0 ± 0.4	14.1 ± 0.5	16.7 ± 0.5	17.1 ± 0.6	14.8 ± 0.5	13.5 ± 0.5	14.4 ± 0.9
GV_{W-1Yr} (g/Kg·d)	* ‡ ψ € ■ □	7.9 ± 0.1	7.9 ± 0.1	8.9 ± 0.1	8.8 ± 0.2	8.2 ± 0.2	8.1 ± 0.1	7.6 ± 0.2
Weight at weaning (Kg)	ψ	32 ± 1	30 ± 1	28 ± 1	31 ± 1	31 ± 1	29 ± 1	32 ± 2
W/L at weaning (Kg/m)		14.3 ± 0.3	13.8 ± 0.4	13.4 ± 0.4	14.4 ± 0.4	14.5 ± 0.4	13.9 ± 0.4	14.4 ± 0.3
Weight at 1 year (Kg)		57 ± 2	55 ± 2	58 ± 2	63 ± 2	59 ± 2	57 ± 2	51 ± 3
CRL_{0-2} (cm/d)	* ‡	0.96 ± 0.04	1.0 ± 0.05	0.85 ± 0.05	0.89 ± 0.06	1.00 ± 0.05	0.98 ± 0.05	0.95 ± 0.08
CRL_{2-W} (cm/d)	* ‡	0.39 ± 0.01	0.37 ± 0.01	0.41 ± 0.01	0.43 ± 0.02	0.37 ± 0.01	0.35 ± 0.01	0.35 ± 0.02
CRL at weaning (cm)		93 ± 1	91 ± 1	89 ± 1	92 ± 1	92 ± 1	90 ± 1	91 ± 2
HL_{0-2} (cm/d)		0.31 ± 0.02	0.36 ± 0.02	0.34 ± 0.02	0.37 ± 0.03	0.36 ± 0.02	0.35 ± 0.02	0.30 ± 0.03
HL_{2-W} (cm/d)	‡ #	0.18 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.18 ± 0.02
HL at weaning (cm)		56.6 ± 0.7	55.7 ± 0.8	54.1 ± 0.8	54.5 ± 0.9	54.3 ± 0.8	54.1 ± 0.8	56.8 ± 1.4
BPD_{0-2} (mm/d)		0.41 ± 0.04	0.39 ± 0.04	0.32 ± 0.05	0.31 ± 0.05	0.40 ± 0.04	0.36 ± 0.04	0.43 ± 0.06
BPD_{2-W} (mm/d)		0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.22 ± 0.02
BPD at weaning (cm)	*	8.5 ± 0.1	8.4 ± 0.1	8.1 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	8.4 ± 0.1	8.5 ± 0.1

Table 4-1: Effect of birth group and supplementation on growth rates in male sheep

*UN=un-supplemented. S=supplemented. Wt-TEA=Weight at term equivalent age. Max. DRWG=maximal daily relative weight gain between birth and 2 weeks. Growth velocity (GV) birth to 2 weeks (0-2), two weeks to weaning (2-W), weaning to 1 year (W-1Yr). CRL =Crown rump length. HL=Hind limb length. BPD=Bi-parietal diameter. W/L = weight-for-length. Data are least square means ± SEM. *p<0.05 preterm compared to term-spont. ‡ p<0.05 preterm compared to term-dex. # p<0.05 term-dex compared to term-spont. †† p<0.01 UN compared to S. ψ p<0.05 UN preterm compared to UN term-spont. € p<0.05 UN preterm compared to UN term-dex. ■ p<0.05 S preterm compared to S term-spont. □ p<0.05 S preterm compared to S term-dex.*

FEMALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN	S	UN	S	UN	S	UN
GV epoch birth to 2 weeks		n=20	n=12	n=12	n=12	n=11	n=12	n=7
GV epoch 2 weeks to weaning		n=20	n=11	n=12	n=11	n=9	n=11	n=7
GV epoch weaning to one year		n=15	n=11	n=11	n=11	n=9	n=11	n=7
Wt-TEA (Kg)	* ‡	5.8 ± 0.2	5.6 ± 0.3	7.3 ± 0.3	6.9 ± 0.3	5.6 ± 0.3	5.8 ± 0.3	5.3 ± 0.3
Max. DRWG (g/Kg·d)	‡	80.2 ± 3.5	77.9 ± 4.6	78.3 ± 4.6	67.3 ± 4.4	80.5 ± 4.8	89.6 ± 5.0	101.3 ± 8.1
Age at max. DRWG (days)		4.2 ± 0.7	3.5 ± 0.8	4.7 ± 0.8	6.2 ± 0.8	4.9 ± 0.9	3.0 ± 0.9	3.9 ± 1.3
GV_{0-2} (g/Kg·d)		45.1 ± 1.4	43.5 ± 1.8	43.5 ± 1.8	40.9 ± 1.8	43.0 ± 1.9	42.1 ± 1.9	43.9 ± 2.4
GV_{2-W} (g/Kg·d)	* ‡ ■□	14.3 ± 0.4	14.6 ± 0.5	15.9 ± 0.5	16.8 ± 0.5	14.2 ± 0.5	13.4 ± 0.5	14.9 ± 0.4
GV_{W-1Yr} (g/Kg·d)	* ‡ ■□	7.8 ± 0.2	7.3 ± 0.2	8.3 ± 0.2	8.5 ± 0.2	7.6 ± 0.2	7.6 ± 0.2	7.2 ± 0.3
Weight at weaning (Kg)	*	30 ± 1	30 ± 1	26 ± 1	26 ± 1	28 ± 1	27 ± 1	28 ± 1
W/L at weaning (Kg/m)		14.0 ± 0.3	14.0 ± 0.4	13.3 ± 0.4	13.5 ± 0.4	14.1 ± 0.4	13.6 ± 0.4	13.8 ± 0.4
Weight at 1 year (Kg)	ϕ θ λ	52 ± 2	44 ± 2	48 ± 2	49 ± 2	47 ± 2	49 ± 2	40 ± 2
CRL_{0-2} (cm/d)		0.93 ± 0.05	0.93 ± 0.06	0.90 ± 0.06	0.88 ± 0.06	0.97 ± 0.06	1.00 ± 0.06	0.84 ± 0.08
CRL_{2-W} (cm/d)	‡	0.38 ± 0.01	0.36 ± 0.02	0.38 ± 0.02	0.39 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.33 ± 0.02
CRL at weaning (cm)	*	91 ± 1	90 ± 1	88 ± 1	87 ± 1	89 ± 1	88 ± 1	87 ± 1
HL_{0-2} (cm/d)		0.33 ± 0.02	0.34 ± 0.02	0.36 ± 0.02	0.38 ± 0.02	0.34 ± 0.02	0.29 ± 0.02	0.30 ± 0.03
HL_{2-W} (cm/d)		0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.16 ± 0.01
HL at weaning (cm)	* ‡	54.6 ± 0.6	55.1 ± 0.8	52.3 ± 0.7	51.4 ± 0.8	52.9 ± 0.8	52.8 ± 0.8	54.0 ± 0.8
BPD_{0-2} (mm/d)		0.39 ± 0.03	0.35 ± 0.04	0.33 ± 0.04	0.31 ± 0.03	0.35 ± 0.0	0.34 ± 0.04	0.39 ± 0.04
BPD_{2-W} (mm/d)		0.19 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
BPD at weaning (cm)	*	8.2 ± 0.1	8.1 ± 0.1	7.8 ± 0.1	7.8 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	7.9 ± 0.1

Table 4-2: Effect of birth group and supplementation on growth rates in female sheep

*UN=un-supplemented. S=supplemented. Wt-TEA=Weight at term equivalent age. Max. DRWG=maximal daily relative weight gain between birth and 2 weeks. Growth velocity (GV) birth to 2 weeks (0-2), two weeks to weaning (2-W), weaning to 1 year (W-1Yr). CRL =Crown rump length. HL=Hind limb length. BPD=Bi-parietal diameter. W/L = weight-for-length. Data are least square means ± SEM. *p<0.05 preterm compared to term-spont. ‡p<0.05 preterm compared to term-dex. ϕ p<0.05 S term-spont compared to UN term-spont. ■ p<0.05 S preterm compared to S term-spont. □ p<0.05 S preterm compared to S term-dex. θ p<0.01 term-Alizin compared to term-spont. λ p<0.01 term-Alizin compared to term-dex.*

4.4.1.2. Preterm birth and growth between 2 weeks and weaning

Preterm sheep remained lighter with a smaller BPD and HL than term-spont or term-dex sheep, and a smaller CRL than term-spont sheep (Figure 4-3, Figure 4-4, Table 4-1, Table 4-2).

Weight-for-length tended to be less in preterm males than other sheep (RM ANOVA for group, p=0.06, Figure 4-3), and was less in preterm females than term-spont or term-dex females (Figure 4-4).

Daily relative weight gain declined progressively in all groups between 2 weeks and weaning, but the rate of decline was less in preterm than term-spont or term-dex (Figure 4-3, Figure 4-4). Preterm sheep therefore had a greater GV between 2 weeks and weaning ($GV_{2\text{-}W}$) than term-spont or term-dex sheep (Table 4-1, Table 4-2).

In males, the rate of increase in CRL ($CRL_{2\text{-}W}$) was greater in preterm sheep than term-spont or term-dex, the rate of increase in HL ($HL_{2\text{-}W}$) was greater in preterm and term-spont than term-dex but the rate of increase in BPD ($BPD_{2\text{-}W}$) was not different among birth groups (Table 4-1).

In females $CRL_{2\text{-}W}$ was greater in preterm than term-dex sheep, but there was no difference in $HL_{2\text{-}W}$ or $BPD_{2\text{-}W}$ among birth groups (Table 4-2).

4.4.1.3. Preterm birth and growth between weaning and 1 year of age

Although the overall trajectory of weight gain by RM ANOVA between weaning and 1 year was not different between groups of either sex (Figure 4-5), both male and female preterm sheep had a greater growth velocity between weaning and 1 year ($GV_{W\text{-}1Yr}$) than same sex term-spont or term-dex sheep (Table 4-1, Table 4-2).

4.4.2. Effect of supplementation on growth

4.4.2.1. Supplementation and growth during the first two weeks

Supplementation did not significantly alter increments in weight (absolute or relative to body weight), CRL, HL or BPD during the first 2 weeks of life (Figure 4-1, Figure 4-2). There was a significant effect of birth group x supplementation on weight-for-length ($p<0.05$ on RM ANOVA but not significant on Tukey *post hoc*) in male but not female lambs. Inspection of

the data suggest that between birth and 2 weeks, relative to un-supplemented lambs of the same birth group, supplementation increased weight-for-length in preterm lambs, reduced it in term-spont lambs and had no effect in term-dex lambs.

In males but not females, the age at which of maximal daily relative weight gain was achieved was delayed in supplemented (S) compared to un-supplemented (UN) lambs (Table 4-1, Table 4-2), although the maximal daily relative weight gain itself was no different between UN and S lambs.

Supplementation did not influence the maximal, weight at TEA or rates of increase in CRL, HL or BPD (Table 4-1, Table 4-2) in any lambs.

$GV_{0\text{-}2}$ was less in UN preterm lambs than UN term-spont lambs (Table 4-1).

4.4.2.2. Supplementation and growth between 2 weeks and weaning

Supplementation did not alter CRL, HL or BPD (Figure 4-3, Figure 4-4), or the rate of increase in CRL, HL or BPD ($CRL_{2\text{-}W}$, $HL_{2\text{-}W}$, $BPD_{2\text{-}W}$) between 2 weeks and weaning in either males or females of any birth group (Table 4-1, Table 4-2).

In male sheep, supplementation significantly increased weight-for-length in preterm sheep but reduced weight and weight-for-length in term-spont sheep (Figure 4-3). The effect of supplementation on fortnightly relative weight gain (RWG) was significant on RM ANOVA ($p<0.05$), but not on *post-hoc* testing (Figure 4-3). Inspection of the data suggest that relative to un-supplemented lambs of the same birth group, supplementation reduced fortnightly RWG in term-spont lambs, increased it in preterm lambs but had no effect in term-dex lambs (Figure 4-3).

In female sheep supplementation did not alter weight gain, weight-for-length or fortnightly RWG.

$GV_{2\text{-}W}$ in both sexes was greater in supplemented preterm than supplemented term-spont or term-dex sheep. In males but not females, $GV_{2\text{-}W}$ was greater in un-supplemented preterm than un-supplemented term-spont lambs (Table 4-1, Table 4-2).

4.4.2.3. Supplementation and growth between weaning and 1 year of age

Supplementation did not alter weight gain between weaning and 1 year in males, but did reduce weight gain in female term-spont sheep (Figure 4-5).

$GV_{W\text{-}1Yr}$ was greater in both sexes in S preterm sheep than S term-spont or S term-dex, and in males, but not females, was greater in UN preterm sheep than UN term-spont or UN term-dex sheep (Table 4-1, Table 4-2).

4.4.3. Effect of induction of labour at term on growth

4.4.3.1. Induction of labour at term and growth during the first two weeks

Weight, CRL and HL between birth and 2 weeks of age were not different between term-Alizin and un-supplemented (UN) term-spont or term-dex lambs (Figure 4-1, Figure 4-2).

In males, the effect of birth group on weight-for-length was significant on RM ANOVA ($p<0.01$), but not on *post hoc* test. Inspection of the data suggests that this was due to a lesser weight-for-length in term-Alizin and UN term-dex than UN term-spont. There were no other differences in size or growth rates among term-Alizin, UN term-spont and UN term-dex lambs (Figure 4-1, Table 4-1).

In females, between birth and 2 weeks Term-Alizin had a lesser weight-for-length than UN term-spont and a lesser BPD than UN term-spont and UN term-dex (Figure 4-2). No other growth parameters were different among birth groups (Figure 4-2, Table 4-2).

4.4.3.2. Induction of labour at term and growth between two weeks and weaning

Weight, weight-for-length and HL (Figure 4-3, Figure 4-4), and rates of gain in weight, HL, CRL and BPD (Table 4-1, Table 4-2) between 2 weeks and weaning were not different among term-Alizin, un-supplemented (UN) term-spont and UN term-dex lambs of either sex.

In males, the effect of birth group on CRL and HL was significant on RM ANOVA ($p<0.05$ and $p<0.01$ respectively) but not on *post hoc* test. Inspection of the data suggests that this is due to greater CRL and HL in term-Alizin and UN term-spont compared to UN term-dex sheep (Figure 4-3).

In females, BPD between 2 weeks and weaning was less in term-Alizin than term-spont or term-dex sheep (Figure 4-4).

4.4.3.3. Induction of labour at term and growth between weaning and one year

Weight gain between weaning and 1 year was not different in males, but was significantly less in female term-Alizin than female UN term-spont and term-dex sheep (Figure 4-5).

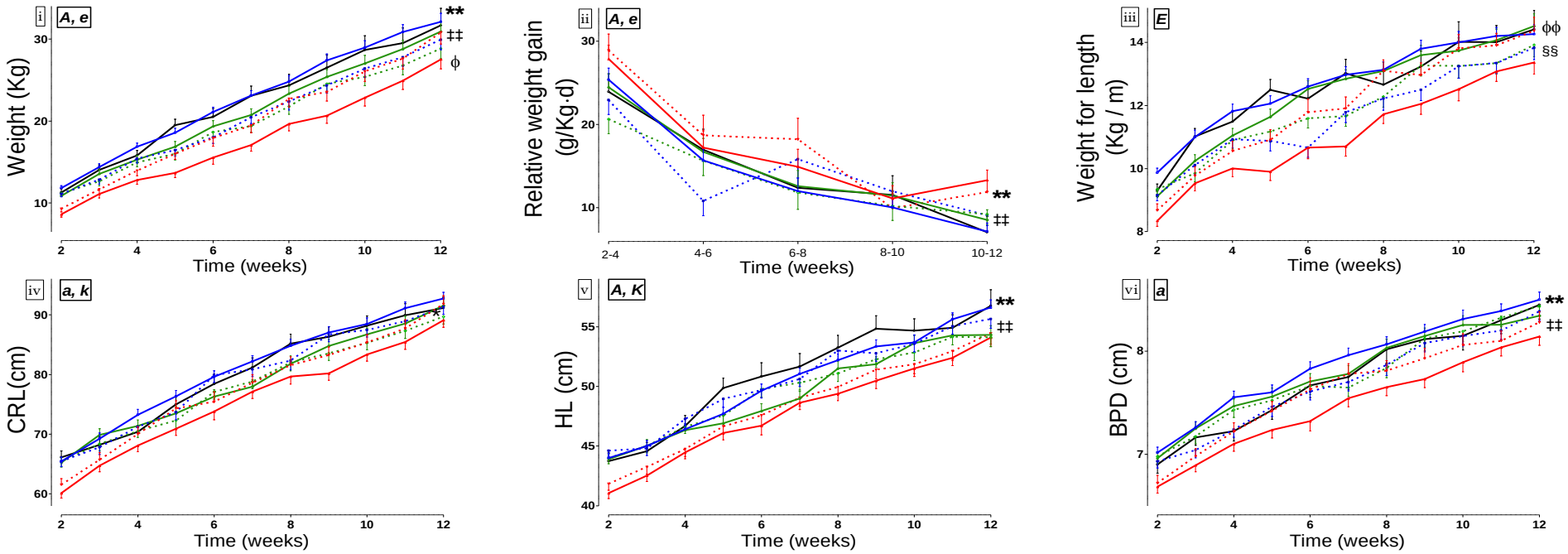

Figure 4-3: Effect of birth group and supplementation on growth between 2 weeks and weaning in male sheep

*i) weight. ii) relative weight gain. iii) weight-for-length. iv) crown rump length (CRL). v) hind limb length (HL). vi) bi-parietal diameter (BPD). Term-spont, blue; un-supplemented (UN; solid line) n=19, supplemented (S; interrupted line) n=13. Preterm, red; UN n=14, S n=10. Term-dex, green; UN n=14, S n=13. Term-Alizin lambs, black n=6. Data are least square means ± SEM. Letters give effect of RM ANOVA. Symbols refer to post-hoc tests. A, p<0.01 a, p<0.05 for the comparison between term-spont, preterm and term-dex groups. E, p<0.01 e, p<0.05 for the effect of birth group x supplement. K, p<0.01 k, p<0.05 for the comparison between term-Alizin and UN term-spont and term-dex over time. ** p<0.01 preterm compared with term-spont. ‡‡ p<0.01 preterm compared with term-dex. ϕϕ p<0.01, ϕ p<0.05 S term-spont compared to UN term-spont. §§ p<0.01 S preterm compared to UN preterm.*

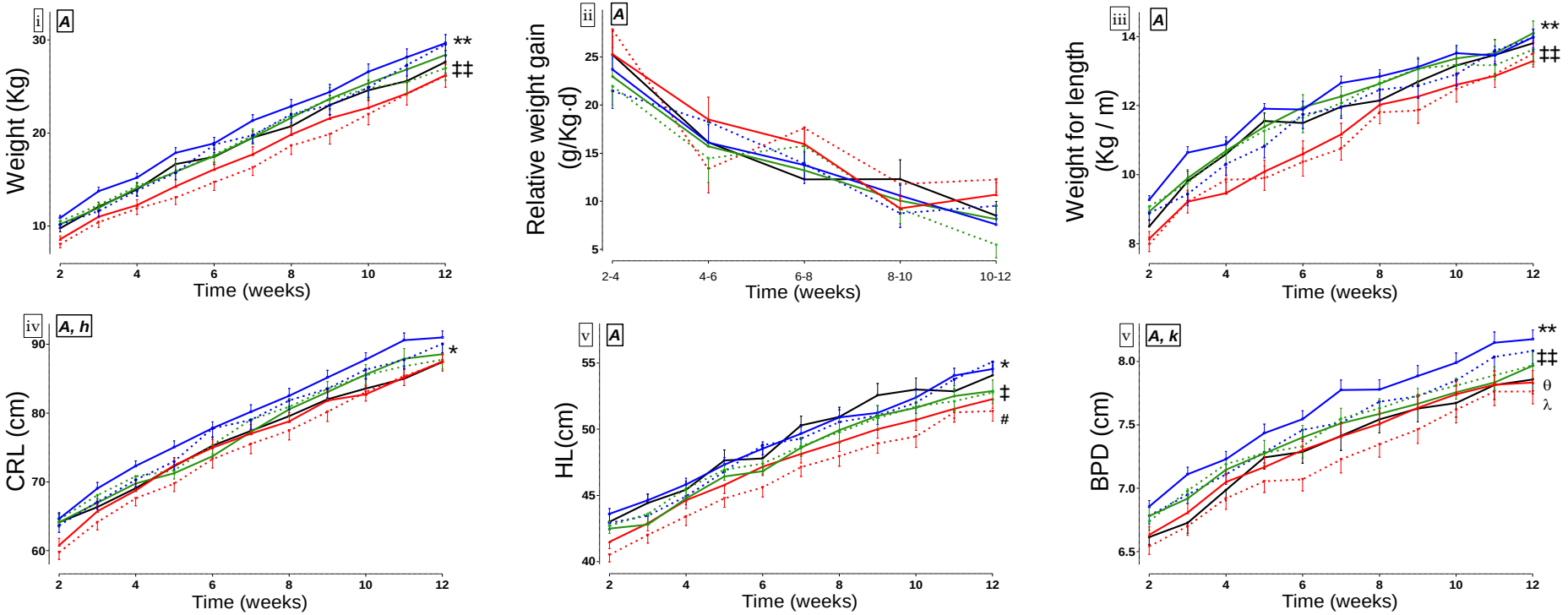

Figure 4-4: Effect of birth group and supplementation on growth between 2 weeks and weaning in female sheep

*i) weight. ii) relative weight gain. iii) weight-for-length. iv) crown rump length (CRL). v) hind limb length (HL). vi) bi-parietal diameter (BPD). Term-spont, blue; un-supplemented (UN; solid line) n=20, supplemented (S; interrupted line) n=11. Preterm, red; UN n=12, S n=11. Term-dex, green; UN n=9, S n=10. Term-Alizin lambs, black n=6. Data are least square means ± SEM. Letters give effect of RM ANOVA. Symbols refer to post-hoc tests. A, p<0.01, a, p<0.05 for the comparison between term-spont, preterm and term-dex groups. h, p<0.05 for the comparison between term-Alizin and UN term-spont and term-dex. k, p<0.05 for the comparison between term-Alizin and UN term-spont and term-dex over time. ** p<0.01, *p<0.05 preterm compared with term-spont. ‡‡ p<0.01 preterm compared with term-dex. # p<0.05 term-dex compared with term-spont. θ p<0.01term-Alizin compared with term-spont. λ p<0.01term-Alizin compared with term-dex.*

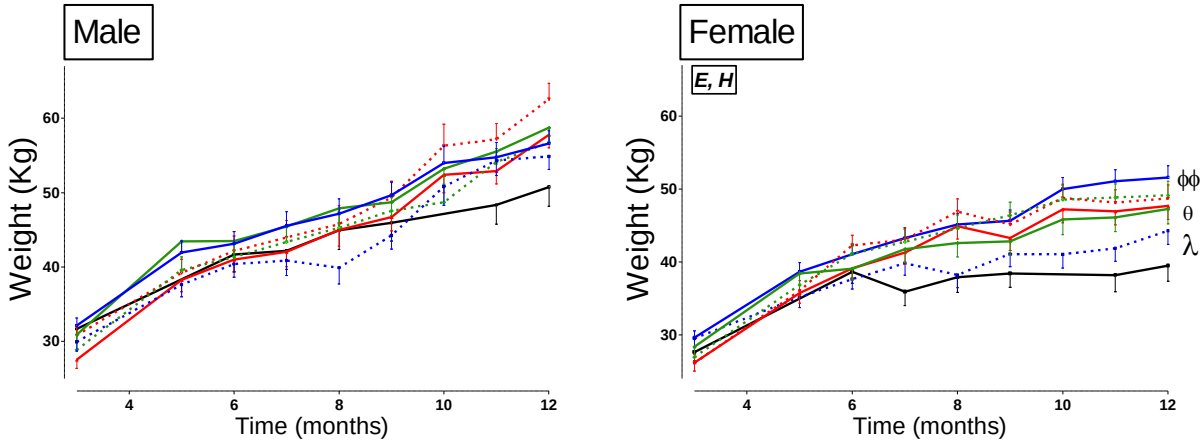

Figure 4-5: Effect of birth group and supplementation on weight between weaning and 1 year

Term-spont; blue, un-supplemented (UN; solid line) male n=14, female n=15; supplemented (S; interrupted line) male n=13, female n=11. Preterm; red, UN male n=13, female n=11; S male n=9, female n=11. Term-dex; green, UN male n=9, female n= 9; S male n=11, female n=10. Term-Alizin; black, male n=6, female n=7. Data are least square means ± SEM. Letters give effect of RM ANOVA. Symbols refer to post-hoc tests. E, p<0.01 for the comparison between S and UN preterm, term-spont and term-dex. H, p<0.01 for the comparison between term-Alizin and UN term-spont and UN term-dex. ϕϕ p<0.01 S term-spont compared to UN term-spont. θ p<0.01 term-Alizin compared to term-spont. λ p<0.01 term-Alizin compared to term-dex.

4.4.4. Correlations between size at birth and growth rates

To allow for the effect of multiple analyses, statistical significance for correlations between size at birth and postnatal growth rates was set at a p-value of 0.001.

Relative small size at birth (low birth weight z-score) was significantly correlated with increased $GV_{0\text{-}2}$ in female term-spont sheep, and trended towards an association in male term-spont sheep (Table 4-3). Small size at birth was not associated with increased early GV in any sheep born after preterm or term induction of labour.

Small size at birth was also significantly associated with increased growth rate between weaning and one year ($GV_{W\text{-}1yr}$) in female term-spont and male term-dex sheep, and trended towards an association in all sheep bar male term-Alizin (Table 4-3).

	MALE				FEMALE			
	Group analysed	R^2	β coefficient (effect per unit increase in birth weight z-score)	p-value	Group analysed	R^2	β coefficient (effect per unit increase in birth weight z-score)	p-value
GV_{0-2}	Term-spont (n=33)	0.3	-2.3 ± 0.7	0.002	Term-spont (n=32)	0.5	-3.8 ± 0.6	<0.0001
GV_{0-2}	Preterm (n=24)	<0.1	0.4 ± 1.6	0.8	Preterm (n=24)	<0.1	1.5 ± 1.2	0.2
GV_{0-2}	Term-dex (n=28)	0.3	-3.1 ± 0.8	0.001	Term-dex (n=21)	<0.1	-1.3 ± 1.9	0.5
GV_{0-2}	Term-Alizin (n=8)	0.2	-2.3 ± 1.9	0.3	Term-Alizin (n=7)	0.7	-7.8 ± 2.5	0.03
GV_{2-W}	Term-spont (n=32)	<0.1	-0.2 ± 0.4	0.6	Term-spont (n=31)	<0.1	-0.4 ± 0.3	0.2
GV_{2-W}	Preterm (n=24)	0.2	-0.9 ± 0.3	0.02	Preterm (n=23)	<0.1	-0.4 ± 0.3	0.3
GV_{2-W}	Term-dex (n=27)	<0.1	-0.2 ± 0.3	0.5	Term-dex (n=19)	<0.1	0.1 ± 0.4	0.8
GV_{2-W}	Term-Alizin (n=6)	<0.1	-0.3 ± 0.1	0.6	Term-Alizin (n=7)	<0.1	-0.2 ± 0.4	0.7
GV_{W-1yr}	Term-spont (n=27)	0.3	-0.3 ± 0.1	0.006	Term-spont (n=26)	0.7	-0.7 ± 0.1	<0.0001
GV_{W-1yr}	Preterm (n=22)	0.3	-0.2 ± 0.1	0.007	Preterm (n=22)	0.2	-0.2 ± 0.1	0.02
GV_{W-1yr}	Term-dex (n=20)	0.6	-0.4 ± 0.1	<0.0001	Term-dex (n=19)	0.4	-0.5 ± 0.1	0.002
GV_{W-1yr}	Term-Alizin (n=6)	0.4	-0.3 ± 0.2	0.2	Term-Alizin (n=7)	0.7	-0.9 ± 0.3	0.02

Table 4-3: Correlations between birth weight z-score and growth velocity in male and female sheep

Growth velocity (GV) birth to 2 weeks (0-2), 2 weeks to weaning (2-W), weaning to 1 year (W-1yr). Data are least square means ± SEM.

4.4.5. Metabolic profile between birth and two weeks of age

4.4.5.1. Effect of preterm birth on lamb plasma metabolite and insulin concentrations

In all sheep, plasma concentrations of glucose, insulin, and urea were low at birth and increased during the first two weeks, whereas plasma free fatty acids (FFA) and lactate were high at birth and fell during the first 2 weeks (Figure 4-6, Figure 4-7).

In both sexes, plasma urea concentrations were lower in preterm sheep than either term-spont or term-dex sheep, but plasma FFA concentrations were not different among birth groups (Figure 4-6, Figure 4-7). The effect of birth group over time on plasma glucose concentrations was significant on RM ANOVA ($p<0.05$ for both male and female), but not on *post hoc* testing. In both sexes this appears to be due to a lower plasma glucose concentration in preterm sheep at birth, but not thereafter, compared with term-spont and term-dex sheep (Figure 4-6, Figure 4-7).

In males, preterm sheep had higher plasma lactate concentrations than either term-spont or term-dex sheep, and a lower plasma insulin concentration and insulin: glucose ratio than term-spont sheep (Figure 4-6).

In females, plasma insulin concentrations, insulin: glucose ratio and plasma lactate concentrations were different between birth groups (significant effect of birth group on RM ANOVA but not on *post hoc* testing) (Figure 4-4). Inspection of the data suggests that the magnitude of these differences is similar to that observed in male sheep.

4.4.5.2. Effect of supplementation on lamb plasma metabolite and insulin concentrations

There was no effect of supplementation *per se* on lamb plasma metabolites or insulin concentrations (Figure 4-6, Figure 4-7). However, in males, but not females, there was an interaction between birth group and supplementation for plasma insulin concentrations and the insulin: glucose ratio (Figure 4-6): UN preterm lambs had a lower plasma insulin concentration and insulin: glucose ratio than UN term-spont or term-dex, whereas S term-dex had a lower insulin: glucose ratio than UN term-dex (Figure 4-6).

4.4.5.3. Effect of induction of labour at term on lamb plasma metabolite and insulin concentrations

There was no difference among term-Alizin, UN term-spont and UN term-dex sheep of either sex in plasma concentrations of glucose, insulin, insulin: glucose ratio, or urea between birth and 2 weeks.

Plasma lactate concentrations in females but not males, were different between birth groups over time (significant on RM ANOVA $p<0.01$, but not on *post hoc* testing). Inspection of the data indicates that this is due to a lower plasma lactate concentration at birth and therefore lesser decline between birth and subsequent time points for term-Alizin sheep compared to UN term-spont and UN term-dex sheep.

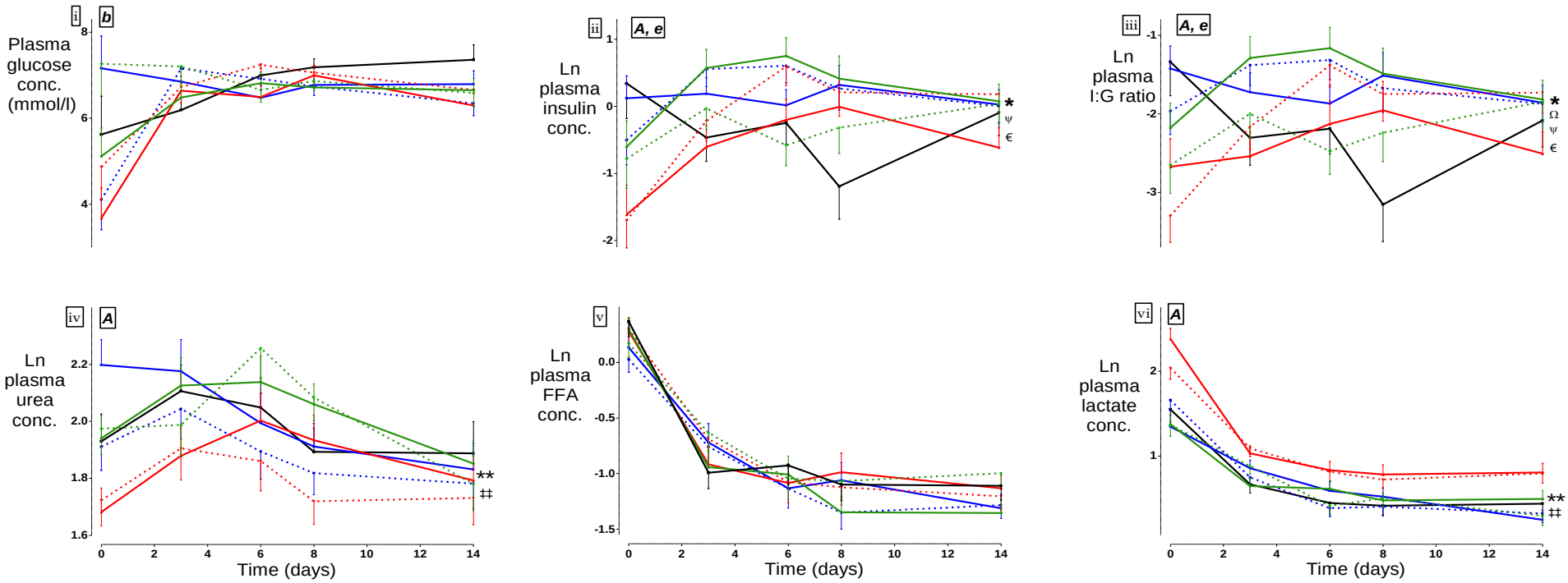

Figure 4-6: Effect of birth group and supplementation on plasma metabolite and insulin concentrations in male lambs during the first two weeks of life

*Plasma concentrations (conc.) of i) glucose (G), ii)insulin (I), iii) I:G, iv) urea, v) free fatty acids (FFA), and vi) lactate. Ln=natural log. Term-spont; blue, un-supplemented (UN; solid lines) n=7, supplemented (S; interrupted lines) n=8. Preterm; red, UN n=6, S n=7. Term-dex; green, UN n=9, S n=7. Alizin; black, n=6. Data are least square means ± SEM. Letters give effect of RM ANOVA. Symbols refer to post-hoc tests. A, p<0.01 for the comparison between term-spont, preterm and term-dex groups. b, p<0.05 for the comparison between term-spont, preterm and term-dex groups over time. e, p<0.05 for the effect of birth group x supplement. ** p<0.01 preterm compared with term-spont. ‡‡ p<0.01 preterm compared with term-dex. ψ p<0.05 UN preterm compared with UN term-spont. € p<0.05 UN preterm compared with UN term-dex. Ω p<0.05 S term-dex compared with UN term-dex.*

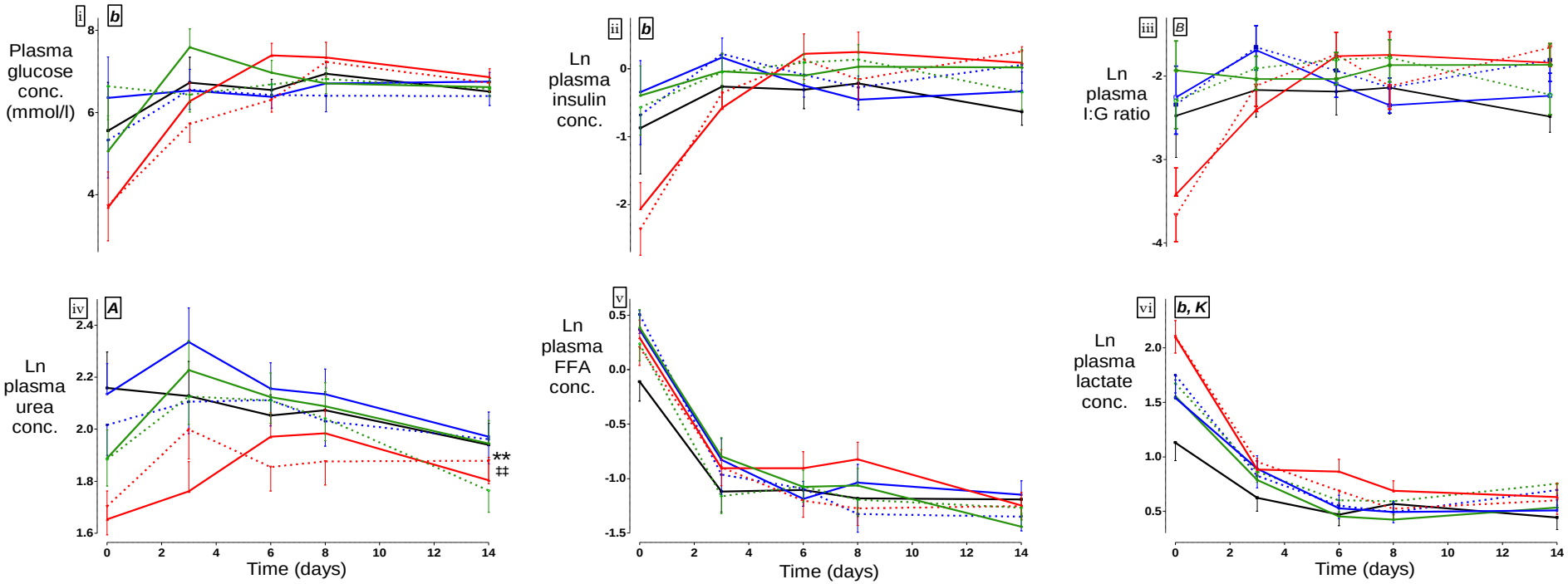

Figure 4-7: Effect of birth group and supplementation on plasma metabolite and insulin concentrations in female lambs during the first two weeks of life

*Plasma concentrations (conc.) of i) glucose (G), ii)insulin (I), iii) I:G, iv) urea, v) free fatty acids (FFA), and vi) lactate. Ln=natural log. Term-spont; blue, un-supplemented (UN; solid lines) n=7, supplemented (S; interrupted lines) n=7. Preterm; red, UN n=8, S n=8. Term-dex; green, UN n=8, S n=9. Term-Alizin; black, n=6. Data are least square means ± SEM. Letters give effect of RM ANOVA. Symbols refer to post-hoc tests. A p<0.01 for the comparison between term-spont, preterm and term-dex groups. B, p<0.01 b, p<0.05 for the comparison between term-spont, preterm and term-dex groups over time. K, p<0.01 for the comparison between term-Alizin and UN term-spont and term-dex over time. ** p<0.01 preterm compared with term-spont. ‡‡ p<0.01 preterm compared with term-dex.*

4.4.6. Milk and macronutrient intake

4.4.6.1. Effect of preterm birth on milk intake and on energy and macronutrient intake

As numbers in individual groups are small and there were no statistically significant effects of sex on measures of milk or nutrient intake, data are shown for combined groups of male and female lambs.

Compared to term-spont lambs, preterm lambs tended to have a greater relative milk intake (p=0.06), and, based on local milk composition data (Table 2-4), had a greater total daily intake of carbohydrate (CHO) and received a greater proportion of their total daily calorie intake from protein and CHO with a lesser proportion of calories derived from fat (Table 4-4).

			Term-spont		Preterm	
			UN (n=7)	S (n=9)	UN (n=6)	S (n=8)
Milk intake (ml/Kg·d)	***TrGp***	***TrS***	126 ± 8	121 ± 7	152 ± 9	126 ± 8
Energy intake (Kcal/Kg·d)		†	160 ± 10	199 ± 9	184 ± 12	198 ± 9
Protein intake (g/Kg·d)		†††	6.8 ± 0.4	9.1 ± 0.4	7.9 ± 0.5	9.1 ± 0.4
% protein energy	**	†††	17.0 ± 0.1	18.2 ± 0.1	17.2 ± 0.1	18.4 ± 0.1
CHO intake (g/Kg·d)	*	†††	5.3 ± 0.4	7.4 ± 0.3	6.6 ± 0.4	7.7 ± 0.3
% CHO energy	***	†††	13.2 ± 0.1 [a]	14.8 ± 0.1 [b]	14.2 ± 0.1 [c]	15.5 ± 0.1 [d]
Fat intake (g/Kg·d)		***TrS***	12.5 ± 0.8	14.9 ± 0.7	14.0 ± 0.9	14.5 ± 0.7
% fat energy	***	†††	70.2 ± 0.1 [a]	67.2 ± 0.1 [b]	68.4 ± 0.1 [c]	65.9 ± 0.1 [d]

Table 4-4: Estimated milk and macronutrient intake

*UN=un-supplemented. S=supplemented. Data are least square mean ± SEM. Symbols in the left hand panel give the effect of birth group or supplementation. Superscript letters in the table give the effect of birth group x supplement interactions; values not linked by the same letter are statistically different from one another (p<0.05). TrGp p<0.1, *p<0.05, **p<0.01, ***p<0.001 preterm compared to term-spont. TrS p<0.1, † p<0.05, ††† p<0.001 S compared to UN.*

4.4.6.2. Effect of supplementation on milk and macronutrient intake

Supplementation increased the total daily intake of protein, fat, CHO and Kcal, increased the proportion of total daily calories derived from protein and CHO, but reduced the proportion of total daily calories derived from fat (Table 4-4).

The effect of birth group x supplement was different for each group of lambs; however, the direction of the effect of supplementation was the same in all lambs (Table 4-4).

4.4.7. Juvenile Growth Hormone response test

4.4.7.1. Effect of preterm birth on a GH response test

Baseline plasma IGF1 and urea concentrations were not different among preterm, term-spont and term-dex groups in either sex. In males, but not females, baseline plasma glucose and free fatty acid (FFA) concentrations were less in preterm than term-spont sheep (Figure 4-8).

There was also no difference between groups, in either sex, in the plasma IGF1, glucose, urea or FFA responses or their areas under the curve to the GH stimulation test (Figure 4-8). Percentage body weight gain and feed intake during the GH stimulation test was not different among birth groups (Table 4-5).

In the adjusted model with $GV_{2\text{-}W}$ as the GV variable, baseline IGF1 concentrations were greater in preterm females than female term-spont or term-dex (adjusted means respectively 199 ± 18, 131 ±11, 135 ± 12, $p<0.05$).

4.4.7.2. Effect of supplementation on a GH response test

Baseline plasma concentrations of IGF1 and glucose were not affected by supplementation in either sex, whereas baseline plasma concentrations of FFA and urea were higher in supplemented than un-supplemented males, but not females (Figure 4-8). In addition, in males, baseline FFA concentrations were greater in supplemented than un-supplemented term-dex sheep, and baseline urea concentrations were greater in supplemented than un-supplemented term-spont sheep.

In males, the interaction between birth group, supplement and time on FFA responses to GH (RM ANOVA <0.01, not significant on *post hoc* testing) appear to be due to the greater decline in FFA concentration in supplemented compared to un-supplemented term-dex and term-spont sheep. There was no other effect of supplementation on plasma IGF1 and glucose responses, either maximal or area under the curve, or on feed intake or percentage weight gain during the GH response test.

In females there was no effect of supplementation on plasma IGF1 and glucose responses, either maximal or area under the curve, in response to GH challenge. However, there was a

lesser decline in plasma urea concentration in supplemented compared to un-supplemented preterm sheep, and a lesser FFA response in supplemented compared to un-supplemented term-dex sheep (Figure 4-8). Supplemented animals consumed less feed each day than un-supplemented animals ($p<0.05$; Table 4-5); however, when feed intake was expressed relative to the animals' weight, there was no longer any effect of supplementation, and there was no effect of supplementation on percentage weight gain.

4.4.7.3. Effect of induction of labour at term on a GH response test

Induction of labour with Alizin did not affect baseline or stimulated plasma IGF1, glucose, urea or FFA concentrations in either sex, and did not alter feed intake or percentage weight change during the GH challenge (Figure 4-8). However, in females but not males, IGF1 AUC was greater in term-Alizin than UN term-spont sheep (Table 4-5). These outcomes did not change in the adjusted analyses.

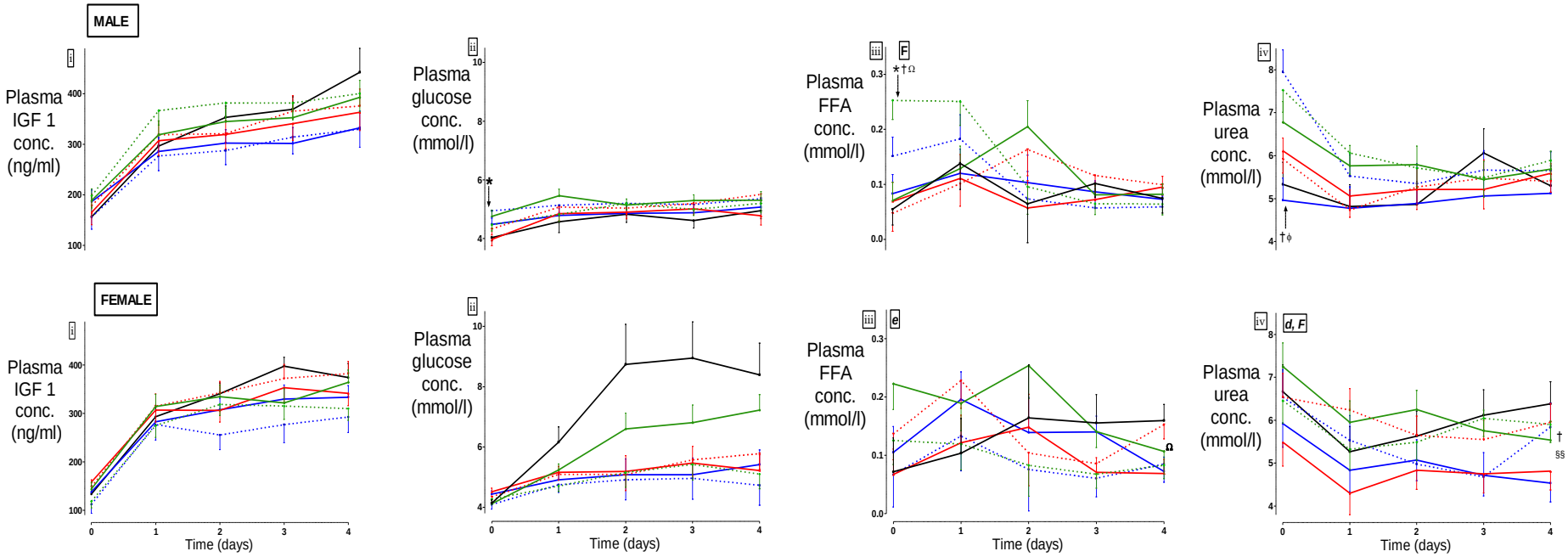

Figure 4-8: Effect of birth group and supplementation on plasma metabolites and IGF1 concentrations following GH treatment

*Plasma concentrations (conc.) of i) IGF1, ii) glucose, iii) free fatty acid (FFA) and iv) urea. Term-spont, blue; un-supplemented (UN; solid line) male n=8, female n=9; supplemented (S; interrupted line) male n=8, female n=5. Preterm, red; UN male n=8, female n=8; S male n=8, female n=8. Term-dex, green; UN male n=8, female n=8; S male n=8, female n=9. Term-Alizin; black, male n=6, female n=6. Data are least square means ± SEM. Letters give effect of RM ANOVA. Arrows indicate the effect of birth group at baseline. Symbols refer to post-hoc tests. d, p<0.05 for the comparison of S and UN over time. e, p<0.05 for the comparison between S and UN term-spont, preterm and term-dex. F, p<0.01 for the comparison between S and UN term-spont, preterm and term-dex over time. * p<0.05 preterm compared with term-spont. † p<0.05 S compared with UN. §§ p<0.01 S preterm compared with UN preterm. Ω p<0.05 S term-dex compared with UN term-dex.*

MALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN (n=8)	S (n=8)	UN (n=8)	S (n=9)	UN (n=9)	S (n=8)	UN (n=6)
IGF1 AUC ($ng \cdot 10^3 \cdot hr/ml$)	θ	9.7 ± 1.7	11.9 ± 1.8	13.6 ± 1.8	13.6 ± 1.7	13.2 ± 1.7	15.3 ± 1.8	16.7 ± 1.6
Maximal increase IGF1 (ng/ml)		156 ± 26	179 ± 28	214 ± 28	210 ± 26	213 ± 26	214 ± 28	320 ± 36
Glucose AUC (mmol·min/L)		38 ± 16	64 ± 15	79 ± 14	70 ± 13	50 ± 13	59 ± 15	57 ± 16
Maximal increase in glucose (mmol/L)		0.7 ± 0.4	1.1 ± 0.4	1.3 ± 0.3	1.5 ± 0.3	1.2 ± 0.3	1.0 ± 0.3	1.0 ± 0.3
% weight increase		10.4 ± 2.6	13.6 ± 2.5	10.9 ± 2.6	8.1 ± 2.9	7.1 ± 2.3	10.2 ± 2.5	21.1 ± 4.1
Total feed intake (Kg/d)		2.04 ± 0.12	1.96 ± 0.10	1.74 ± 0.12	1.95 ± 0.12	1.94 ± 0.10	2.16 ± 0.11	2.30 ± 0.13
Relative feed intake (g/Kg·d)		54 ± 3	60 ± 3	53 ± 3	54 ± 4	58 ± 3	61 ± 3	67 ± 5
FEMALE		UN (n=9)	S (n=5)	UN (n=8)	S (n=8)	UN (n=8)	S (n=9)	UN (n=6)
IGF1 AUC ($ng \cdot 10^3 \cdot hr/ml$)	θ	14.3 ± 1.8	13.3 ± 2.2	14.0 ± 1.8	16.9 ± 1.7	15.4 ± 1.8	15.5 ± 1.6	18.1 ± 1.6
Maximal increase IGF1 (ng/ml)		202 ± 25	179 ± 31	207 ± 27	259 ± 27	236 ± 27	218 ± 25	275 ± 23
Glucose AUC (mmol·min/L)		59 ± 33	64 ± 42	65 ± 33	83 ± 33	183 ± 35	78 ± 31	326 ± 82
Maximal increase in glucose (mmol/L)		1.2 ± 0.6	1.0 ± 0.8	1.3 ± 0.6	1.8 ± 0.6	3.3 ± 0.7	1.7 ± 0.6	5.4 ± 1.3
% weight increase		13.7 ± 2.0	12.3 ± 2.5	11.2 ± 2.6	13.6 ± 2.6	12.4 ± 2.6	13.4 ± 2.5	15.8 ± 3.6
Total feed intake (Kg/d)	†	2.04 ± 0.10	1.77 ± 0.13	2.03 ± 0.14	1.95 ± 0.13	2.13 ± 0.11	1.82 ± 0.11	1.88 ± 0.19
Relative feed intake (g/Kg·d)		60 ± 3	56 ± 3	65 ± 4	58 ± 3	61 ± 3	60 ± 3	67 ± 5

Table 4-5: Effect of birth group and supplementation on responses to GH stimulation test

UN=un-supplemented. S=supplemented. AUC=Area under the curve. Data are least mean squares ± SEM. † $p<0.05$ S compared with UN. θ $p<0.01$ term-Alizin compared to UN term-spont.

4.4.8. Adult body composition

4.4.8.1. Effect of preterm birth on body composition

In males, there was no effect of birth group on % fat or lean mass, % fat: lean ratio or fat mass index (FMI). Preterm sheep had a lower chest: abdomen fat mass ratio than term-spont sheep (Table 4-6).

In females, preterm sheep had a lower % fat mass, fat: lean ratio and FMI than term-dex sheep, with a concomitant increase in lean mass (Table 4-6).

The proportion of animals with fat mass below the minimal detectable threshold was not different between birth groups (χ^2 =0.9) in either sex, but was less in females than males (χ^2 = 0.0004).

In the adjusted multivariate model, the effect of birth group was no longer statistically significant regardless of which, if any, GV variable was included.

4.4.8.2. Effect of supplementation on body composition

In males, supplementation increased the chest: abdomen fat mass ratio, but had no effect on % fat or lean mass, % fat: lean ratio or FMI (Table 4-6).

In females, there were no effects of supplementation *per se* on body composition. Un-supplemented female preterm sheep however had a lower FMI than un-supplemented term-dex sheep (Table 4-6).

4.4.8.3. Effect of induction of labour at term on body composition

Body composition in term-Alizin sheep was no different to that of un-supplemented term-spont or term-dex (Table 4-6). This did not change in the adjusted models. All term-Alizin sheep had a fat mass above the minimal detectable threshold.

4.4.9. Correlations between size at birth, growth rates and body composition

Birth weight z-score, $GV_{0\text{-}2}$, $GV_{2\text{-}W}$ or $GV_{W\text{-}1Yr}$ were not associated with measures of adiposity in male sheep or female term-spont, preterm or term-Alizin sheep. Within the preterm group there were no relationships between $GV_{0\text{-}TEA}$ or $GV_{TEA\text{-}W}$ and any measure of adult body composition.

In female term-dex sheep measures of adiposity were inversely associated with birth weight z-score (% fat mass; $R^2 = 0.4$, β coefficient -4.5 ± 1.4, p=0.007. FMI; $R^2 = 0.4$, β coefficient -2.3 ± 0.7, p=0.007. Fat to lean ratio; $R^2 = 0.4$, β coefficient -5.1 ± 1.8, p=0.02) and positively associated with $GV_{W\text{-}1yr}$ (% fat mass; $R^2 = 0.4$, β coefficient 4.9 ± 1.6, p=0.01. FMI; R^2=0.4, β coefficient 2.3 ± 0.9, p=0.02. Fat to lean ratio; R^2=0.4, β coefficient 6.6 ± 2.2, p=0.01).

4.4.10. Correlation between DXA parameters and anthropometric measurements

Carcass weight and perirenal fat mass had the highest degree of correlation with % fat mass (Table 4-7). Live weight was positively related to % fat mass. Expressing weight relative to length attenuated the strength of this correlation in males, but improved the strength of the association in females (Table 4-7).

Where significant relationships were found between measures of weight-for-length and % fat mass, defining length as CRL + HL, rather than CRL alone, increased the strength and significance of the association (Table 4-7).

MALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN (n=9)	S (n=6)	UN (n=7)	S (n=4)	UN (n=9)	S (n=8)	UN (n=6)
% fat mass		3.3 ± 1.1	1.4 ± 1.3	3.1 ± 1.2	3.4 ± 1.6	2.6 ± 1.1	3.0 ± 1.1	7.0 ± 1.2
% lean mass		94.9 ± 1.1	96.8 ± 1.3	95.3 ± 1.2	94.9 ± 1.6	95.6 ± 1.1	95.2 ± 1.2	91.3 ± 1.2
% fat: % lean		3.6 ± 1.2	1.5 ± 1.5	3.4 ± 1.4	3.7 ± 1.8	2.8 ± 1.2	3.3 ± 1.3	7.7 ± 1.3
C: A fat mass ratio	* †	0.41 ± 0.01	0.46 ± 0.02	0.39 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.39 ± 0.01
FMI (Kg / m^2)	λ	1.6 ± 0.5	0.1 ± 0.6	1.3 ± 0.5	1.7 ± 0.7	1.1 ± 0.5	1.3 ± 0.5	3.6 ± 0.6
% with fat mass < MDT		33	67	29	25	22	50	0
FEMALE		UN (n=10)	S (n=6)	UN (n=8)	S (n=8)	UN (n=7)	S (n=9)	UN (n=6)
% fat mass	‡‡	9.3 ± 1.7	6.3 ± 2.1	5.0 ± 1.9	6.3 ± 1.9	12.3 ± 2.0	11.2 ± 1.7	8.9 ± 2.1
% lean mass	‡‡	89.2 ± 1.7	92.1 ± 2.1	93.5 ± 1.9	92.2 ± 1.9	86.2 ± 2.0	87.2 ± 1.8	89.5 ± 2.1
% fat: % lean	‡‡	10.7 ± 2.1	7.2 ± 2.7	5.6 ± 2.3	7.0 ± 2.3	14.9 ± 2.5	13.3 ± 2.2	10.2 ± 2.7
C: A fat mass ratio		0.39 ± 0.01	0.40 ± 0.01	0.41 ± 0.01	0.39 ± 0.01	0.40 ± 0.01	0.42 ± 0.01	0.37 ± 0.01
FMI (Kg / m^2)	€ ‡‡	4.0 ± 0.8	2.8 ± 1.0	2.0 ± 0.9	2.7 ± 0.9	6.1 ± 0.9	4.8 ± 0.8	4.3 ± 1.1
% with fat mass < MDT		10	17	25	0	0	11	0

Table 4-6: Effect of preterm birth and supplementation on body composition in adult sheep

*UN=un-supplemented. S=supplemented. C:A = chest: abdomen. FMI=fat mass index. MDT=minimal detectable threshold. Data are least square mean ± SEM. * p<0.05 preterm compared to term-spont. ‡‡, p<0.01 preterm compared to term-dex. †, p<0.05 S compared with UN. λ, p<0.01 term-Alizin compared to term-dex. € p<0.05 UN preterm vs UN term-dex.*

	MALE[1] (n=30)			FEMALE[1] (n=49)		
	R^2	β co-efficient (effect per 1% increase in DXA measured fat mass)	p-value	R^2	β co-efficient (effect per 1% increase in DXA measured fat mass)	p-value
Wt-Live (Kg)	0.24	1.61 ± 0.54	<0.01	0.10	0.43 ± 0.19	0.03
Wt-Carcass (Kg)	0.33	1.11 ± 0.3	<0.001	0.23	0.39 ± 0.11	<0.001
PR fat (Kg)	0.22	16.2 ± 5.9	0.01	0.43	20.8 ± 3.4	<0.001
Wt_{CRL} (Kg/m)	0.16	1.13 ± 0.49	0.03	0.12	0.42 ± 0.17	0.02
Wt_{CRL+HL} (Kg/m)	0.17	0.70 ± 0.29	0.02	0.12	0.26 ± 0.10	0.01
Wt_{CRL}^2 (Kg/m^2)	0.07	0.73 ± 0.49	0.1	0.14	0.46 ±0.17	<0.01
Wt_{CRL+HL}^2 (Kg/m^2)	0.10	0.27 ± 0.16	0.09	0.17	0.17 ± 0.06	<0.01
Wt_{CRL}^3 (Kg/m^3)	0.02	0.40 ± 0.51	0.4	0.15	0.50 ± 0.17	<0.01
Wt_{CRL+HL}^3 (Kg/m^3)	0.03	0.09 ± 0.09	0.3	0.19	0.11 ± 0.03	<0.001

[1]: Excludes animals (male n=18, female n=4) with fat mass below the minimum detectable threshold

Table 4-7: Relationships between DXA measured fat mass and anthropometric measurements in adult sheep

Live weight (Wt-Live.) Eviscerated carcass weight (Wt-carcass). Weight relative to crown rump length (CRL; Wt_{CRL}) or CRL + hind limb length (HL; Wt_{CRL+HL}). Weight relative to measures of length squared (Wt_{CRL}^2, Wt_{CRL+HL}^2) or cubed (Wt_{CRL}^3, Wt_{CRL+HL}^3). Perirenal fat mass (PR fat). Data are least square means ± SEM.

4.4.11. Post-mortem carcass characteristics

4.4.11.1. Effect of preterm birth on carcass characteristics

In males, preterm sheep were heavier at PM than term-dex sheep, and term-dex sheep had a lesser total adrenal mass than term-spont sheep (Table 4-8).

In females, un-supplemented preterm sheep had a greater pancreas: live weight ratio than un-supplemented term-dex sheep (Table 4-9).

4.4.11.2. Effect of supplementation on carcass characteristics

In males, supplemented sheep had a lower total pancreatic weight than un-supplemented sheep, and supplemented preterm sheep were heavier than supplemented term-spont sheep (Table 4-8).

In females, supplementation reduced both total and relative liver weight, and supplemented term-spont sheep had a greater relative left ventricular wall thickness than UN term-spont sheep (Table 4-9).

4.4.11.3. Effect of induction of labour at term on carcass characteristics

Male term-Alizin sheep had a greater weight-for-length than term-spont sheep. There were no other differences in carcass characteristics between term-Alizin and un-supplemented term-spont or un-supplemented term-dex sheep of either sex (Table 4-8, Table 4-9).

MALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN (n=14)	S (n=12)	UN (n=13)	S (n=9[1])	UN (n=13)	S (n=13)	UN (n=6)
Live weight (Kg)	‡ ▪	70 ± 2	64 ± 2	69 ± 2	75 ± 3	66 ± 2	66 ± 2	78 ± 3
Carcass weight (Kg)		46 ± 2	42 ± 2	43 ± 2	49 ± 2	43 ± 2	43 ± 2	49 ± 2
Carcass: live weight (%)		65 ± 1	65 ± 1	63 ± 1	65 ± 1	64 ± 1	65 ± 1	63 ± 1
Crown rump length (cm)		114 ± 2	113 ± 2	115 ± 2	117 ± 2	112 ± 2	112 ± 2	112 ± 2
Hind limb length (cm)		70.0 ± 0.8	68.6 ± 0.8	69.1 ± 0.8	70.3 ± 1.0	69.8 ± 0.8	69.5 ± 0.8	70.7 ± 1.1
Weight-for-length (Kg/m)	λ	38.1 ± 1.0	35.5 ± 1.1	37.4 ± 1.0	40.0 ± 1.2	36.5 ± 1.0	36.0 ± 1.0	42.7 ± 1.6
Bi-parietal diameter (cm)		9.9 ± 0.1	9.7 ± 0.1	9.5 ± 0.1	9.9 ± 0.1	9.8 ± 0.1	9.8 ± 0.1	9.8 ± 0.2
Pancreas (g)	†	83.1 ± 3.6	71.4 ± 3.8	77.8 ± 3.6	72.7 ± 4.4	78.1 ± 4.2	75.5 ± 3.6	69.5 ± 5.1
Pancreas: live weight (%)		0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.09 ± 0.01
Liver (g)		1,250 ± 40	1,240 ± 40	1,270 ± 40	1,320 ± 50	1,220 ± 40	1,230 ± 40	1,380 ± 60
Liver: live weight (%)		1.78 ± 0.06	1.95 ± 0.06	1.84 ± 0.06	1.76 ± 0.07	1.84 ± 0.06	1.88 ± 0.06	1.77 ± 0.08
Perirenal fat (g)		167 ± 30	137 ± 30	173 ± 29	263 ± 35	178 ± 31	162 ± 29	300 ± 44
Perirenal fat: live weight (%)		0.22 ± 0.03	0.20 ± 0.04	0.25 ± 0.04	0.34 ± 0.04	0.26 ± 0.04	0.24 ± 0.03	0.38 ± 0.05
Total kidney weight (g)		222 ± 8	222 ± 8	218 ± 8	216 ± 9	212 ± 8	215 ± 8	236 ± 9
Total kidney: live weight (%)		0.32 ± 0.02	0.35 ± 0.02	0.32 ± 0.02	0.29 ± 0.02	0.32 ± 0.02	0.33 ± 0.02	0.30 ± 0.01
Total adrenal weight (g)	#	4.60 ± 0.19	4.69 ± 0.19	4.26 ± 0.19	4.76 ± 0.23	4.16 ± 0.2	3.99 ± 0.19	4.34 ± 0.24
Heart (g)		322 ± 10	306 ± 10	304 ± 10	330 ± 12	304 ± 10	306 ± 10	322 ± 15
Heart: live weight (%)		0.46 ± 0.01	0.48 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.46 ± 0.01	0.47 ± 0.01	0.41 ± 0.02
LV thickness (mm)		11.7 ± 0.7	12.5 ± 0.7	12.9 ± 0.7	14.2 ± 0.8	12.6 ± 0.7	12.0 ± 0.7	12.2 ±1.0
LV: heart weight (mm/g)		0.036±0.001	0.040±0.001	0.042±0.001	0.043 ± 0.001	0.042±0.001	0.039±0.001	0.038±0.001

Table 4-8: Effect of birth group and supplementation on carcass characteristics and organ sizes in male sheep

UN=un-supplemented. S=supplemented. [1]denotes animal with severe macroscopic liver disease due to facial eczema, PM data not recorded. Data are least square means ± SEM. ‡ p<0.05 preterm compared to term-dex. # p<0.05 term-spont compared with term-dex. † S compared with UN. ▪ p<0.05 S preterm compared to S term-spont. λ p<0.01 UN term-dex compared to term-Alizin.

FEMALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN (n=14)	S (n=11[1])	UN (n=11)	S (n=10[1])	UN (n=9)	S (n=10)	UN (n=6)
Live weight (Kg)		63 ± 3	63 ± 3	58 ± 3	62 ± 3	64 ± 3	60 ± 3	63 ± 3
Carcass weight (Kg)		41 ± 2	41 ± 2	37 ± 2	40 ± 2	42 ± 2	40 ± 2	39 ± 2
Carcass: live weight (%)		65 ± 1	64 ± 1	64 ± 1	65 ± 1	65 ± 1	66 ± 1	62 ± 2
Crown rump length (cm)		110 ± 2	111 ± 2	108 ± 2	110 ± 2	106 ±2	107 ± 2	104 ± 2
Hind limb length (cm)		66.9 ± 0.6	66.8 ± 0.7	65.8 ± 0.7	66.4 ± 0.7	65.3 ± 0.8	66.0 ± 0.7	66.9 ± 0.8
Weight-for-length (Kg/m)		35.3 ± 1.2	35.3 ± 1.4	33.3 ± 1.4	35.2 ± 1.4	37.2 ± 1.5	34.8 ± 1.4	36.7 ± 1.7
Bi-parietal diameter (cm)		9.1 ± 0.1	9.0 ± 0.1	8.8 ± 0.1	9.0 ± 0.1	8.9 ± 0.1	9.0 ± 0.1	9.0 ±0.1
Pancreas (g)		69.6 ± 3.1	64.2 ± 3.5	74.5 ± 3.5	66.6 ± 3.7	63.2 ± 3.9	69.6 ± 3.9	70.8 ± 3.3
Pancreas: live weight (%)	€	0.11 ± 0.01	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Liver (g)	†	1,180 ± 40	1,100 ± 40	1,070 ± 40	1,050 ± 50	1,190 ± 50	1,050 ± 50	1,070 ± 60
Liver: live weight (%)	†	1.90 ± 0.08	1.76 ± 0.09	1.92 ± 0.09	1.70 ± 0.09	1.86 ± 0.09	1.75 ± 0.09	1.70 ± 0.09
Perirenal fat (g)		402 ± 58	417 ± 65	301 ± 65	401 ± 68	521 ± 72	349 ± 68	381 ± 92
Perirenal fat: live weight (%)		0.62 ± 0.07	0.64 ± 0.08	0.47 ± 0.08	0.62 ± 0.09	0.79 ± 0.09	0.57 ± 0.09	0.60 ± 0.12
Total kidney weight (g)		195 ± 12	181 ± 14	207 ± 14	185 ± 14	197 ± 15	186 ± 14	197 ± 12
Total kidney: live weight (%)		0.32 ± 0.05	0.29 ± 0.05	0.40 ± 0.05	0.30 ± 0.05	0.31 ± 0.05	0.31 ± 0.05	0.31 ± 0.02
Total adrenal weight (g)		4.38 ± 0.28	4.69 ± 0.32	3.89 ± 0.32	4.33 ± 0.34	4.24 ± 0.35	4.44 ± 0.34	3.97 ± 0.33
Heart (g)		292 ± 10	273 ± 11	274 ± 11	279 ± 12	289 ± 12	273 ± 11	263 ± 13
Heart: live weight (%)		0.47 ± 0.02	0.44 ± 0.02	0.48 ± 0.02	0.45 ± 0.02	0.46 ± 0.02	0.46 ± 0.02	0.42 ± 0.02
LV thickness (mm)		12.5 ± 0.8	14.5 ± 0.8	13.3 ± 0.8	13.7 ± 0.9	12.8 ± 0.9	12.0 ± 0.9	10.8 ± 0.7
LV: heart weight (mm/g)	ϕ	0.043 ± 0.002	0.053 ± 0.002	0.048 ± 0.002	0.049 ± 0.002	0.045 ± 0.002	0.044 ± 0.002	0.041±0.002

Table 4-9: Effect of birth group and supplementation on carcass characteristics and organ sizes in female sheep

UN=un-supplemented. S=supplemented. [1]denotes animal with severe macroscopic liver disease due to facial eczema, PM data not recorded. LV=left ventricle. Data are least square means ± SEM. †, p<0.05 UN compared to S. €, p<0.05 UN preterm compared to UN term-dex. ϕ, p<0.05 S term-spont compared to UN term-spont.

4.5. Discussion

It was hypothesised that provision of additional calories via a small volume calorie dense nutritional supplement, similar to that used clinically to promote growth in preterm or small infants, would promote growth in all lambs. This, however, was not achieved. Not only did supplementation reduce weight gain in some groups of lambs, but the effects on growth and body composition persisted well beyond the period of nutritional intervention. Intriguingly, most of the effect of supplementation on growth was confined to males, and these effects differed according to their gestation: supplemented term-spont males gained less weight and were leaner as adults than un-supplemented term-spont males, whereas supplemented preterm males gained more weight relative to their linear growth than un-supplemented preterm males. In addition, we hypothesised that antenatal exposure to corticosteroids at term would not have significant effects on growth. However, although it did not reach statistical significance, those females that were exposed to antenatal corticosteroids had a 30% increase in adult fat mass compared to term-spont females. This effect was not observed in male term-dex sheep.

Both early nutritional supplementation and corticosteroid exposure at term gestation are routine interventions in clinical practice; however, in neither case are there clinical data on the possible long term implications of these interventions. The data from these experiments suggest that both interventions have long term sequelae that differ between males and females, and between those born preterm or at term. Further research is therefore needed to identify best obstetric practice for the term infant and optimal infant feeding practices.

Effect of preterm birth

Although smaller and lighter at birth with a lesser weight-for-length at birth than term lambs, preterm lambs demonstrated catch-up growth so that by weaning differences in weight and size were diminished, and by adulthood had largely disappeared.

Compared to term-born lambs, similar findings of reduced weight and body proportionality (measured as ponderal index; PI, weight/CRL^3) at birth, but not linear size, have been reported in other preterm lamb paradigms (De Matteo, et al., 2010). Interestingly, De Matteo et al. found that PI subsequently fell in term lambs but remained relatively static in preterm lambs, and speculated that increased PI, and by inference, fat mass, at term is a temporary adaptive phenomenon to promote newborn survival. However, unlike humans, normally

grown term-born lambs are lean at birth and gain fat mass rapidly during postnatal life (Clarke, Buss, Juniper, Lomax, & Symonds, 1997), principally through expansion of the perirenal fat depot. Taken together these data suggest that, similar to human infants (Haggarty, et al., 2004), PI in lambs is not a good measure of adiposity. Additionally, we have found that in adult sheep, weight-for-length is better correlated with DXA measured fat mass than PI. Finally, unlike our paradigm, De Matteo *et al.* studied lambs from twin and triplet pregnancies as well as singletons and used artificial formula in addition to ewe milk to feed preterm lambs during the first days of life. Twin lambs are smaller at birth and grow more slowly than singletons, possibly due to increased milk availability for singleton animals (De Matteo, Stacy, Probyn, Brew, et al., 2008); comparison of groups containing both singletons and lambs from multiple gestation may obscure effects due to gestation length alone.

Milk from both women (Bauer & Gerss, 2011) and ewes (Dr AL Jaquiery, unpublished data) delivering preterm has a different macronutrient composition and balance compared to term-milk. In term-born calves, differences in enteral glucose absorption in the first days of life are found depending on the type of milk feed offered (Steinhoff-Wagner et al., 2011). Although speculative, different composition of maternal preterm-milk compared to formula or maternal term-milk may facilitate nutrient absorption through the immature gut and enhance GI maturation (Sangild, 2006). Postnatal increases in weight-for-length observed in our preterm lambs, unlike those in De Matteo's paradigm, may therefore reflect the nutritional benefits of exclusive ewe milk feeds, as well as increased milk availability for singleton lambs compared to those from multiple gestation.

Preterm lambs weighed more at TEA than term lambs did at birth in both our paradigm and that of De Matteo *et al.*. Increasing fetal cortisol concentrations in late gestation are known to constrain fetal growth prior to parturition (Fowden, et al., 1996); premature liberation from corticosteroid-induced growth constraint may therefore allow faster growth *ex utero* than *in utero.*

Despite being heavier at TEA than term-born lambs, the rate of weight gain and skeletal growth was less in preterm than term lambs during the first two weeks of life, which is consistent with other data on preterm lambs (De Matteo, et al., 2010) and infants (Henriksen, et al., 2009). Newborn preterm lambs have a lower core temperature than newborn term lambs and take longer to achieve a normal core temperature (De Matteo, et al., 2010), and in

human infants, preterm birth has been associated with increased resting energy expenditure (Dechert et al., 1985). Slow growth rates in the early newborn period may therefore in part be due to immature mechanisms for regulating or maintaining energy homeostasis or, especially in human infants born very preterm, may reflect the increased metabolic demand placed by co-morbidities associated with preterm birth (Euser, de Wit, Finken, Rijken, & Wit, 2008).

At birth the fetus must transition from a continuous supply of maternally derived nutrients to the intermittent enteral nutrition of a neonate. The principal enzymes of ovine gluconeogenic pathways are up-regulated during late gestation, although activity in fetal life is negligible (Warnes, Seamark, & Ballard, 1977). At birth, plasma glucose concentrations increase as a result of both glycolysis and gluconeogenesis; lactate, a by-product of glycolysis (Stellingwerff, Leblanc, Hollidge, Heigenhauser, & Spriet, 2006) and anaerobic metabolism, is also a substrate for gluconeogenesis in newborn lambs (Warnes, et al., 1977). Increased plasma lactate concentrations in preterm sheep may therefore be one mechanism through which euglycaemia is maintained. Alternatively it may reflect ongoing tissue hypoxaemia, although persistence of such into the second week of life in the absence of any overt lamb morbidity is unlikely. Increased plasma urea concentrations have been reported at birth in low birth weight term-born lambs, and are thought to represent increased amino acid catabolism necessary to support gluconeogenesis (Greenwood, et al., 2002). Although preterm lambs were of low birth weight, z scores (correcting birth weight for gestation length and sex) were not different from those of term lambs, indicating that there was no fetal growth restriction. As preterm birth was induced in healthy pregnancies, this is what would be expected and may mean that the preterm lambs had adequate carbohydrate stores and did not need to utilise protein as a gluconeogenic substrate. In fact, they had lower plasma urea concentrations than the heavier term lambs. In preterm infants plasma urea concentrations correlate with plasma amino acid concentrations (Blanco et al., 2011); reduced plasma urea concentrations in preterm lambs may therefore reflect increased protein anabolism secondary to the increased protein requirements following preterm birth (Fanaro, Ballardini, & Vigi, 2010). Detailed studies of protein metabolism are needed to fully interpret this finding.

In both our paradigm and that of De Matteo *et al.*, growth rates in preterm lambs increased after TEA (De Matteo, et al., 2010), so that by weaning, differences in weight and size between birth groups had lessened, and were no longer apparent at one year. In preterm infants, poor initial growth is often followed by catch-up growth that is largely completed by puberty; however, this catch-up may be insufficient to return the child to its expected size

(Morgan, et al., 2011). Unlike our preterm lamb paradigm, poor postnatal growth in preterm infants and children may in part be due to a greater degree of immaturity at birth, or to the co-morbidity underlying preterm birth in the first instance. Surprisingly, given the differences in growth rates between animals, the effects of GH stimulation on somatotropic axis function were not different between birth groups. However, no preterm or term-born lambs had evidence of fetal growth restriction; data obtained during the IUGR pilot trial suggests that, in sheep, an adverse *in utero* environment has significant effects on IGF1 responses to GH stimulation (Chapter 7.4.4).

By adulthood, fat mass was less in males than females, and, in females, was less in preterm than term-born sheep; significantly so when compared to term-dex sheep. In humans, preterm birth interrupts the usual deposition of fat stores that occurs during the third trimester (Fanaro, et al., 2010), so that infants are relatively lean at birth (Rigo, et al., 1998). Early weight gain favours adiposity at the expense of skeletal growth (Cooke, Rawlings, et al., 1999; Pieltain, et al., 2001), and although later fat mass is reduced in preterm-born children (Fewtrell, et al., 2004; Gianni, et al., 2008) the distribution tends to favour visceral rather than subcutaneous fat (Gianni, et al., 2008; Uthaya, et al., 2005) with unfavourable implications for later metabolic health (Fox, et al., 2007). Although in our preterm sheep paradigm it was not possible to differentiate subcutaneous and visceral fat on a DXA scan, preterm males had a lesser chest: abdominal fat ratio than term-spont males, suggesting parallels with the clinical scenario. Others have found that although total fat mass was not different between adults born preterm and at term (Hovi, et al., 2007), lean muscle mass was reduced in those born preterm (Sipola-Leppanen et al., 2011). As lean mass is a major determinant of resting energy expenditure (REE) (Eriksson, Forsen, Tuomilehto, Osmond, & Barker, 2002), total REE in adults born preterm was consequently reduced. However, when expressed relative to lean muscle mass, REE was greater in adults born preterm than in those born at term (Sipola-Leppanen, et al., 2011). These data suggest that preterm birth leads to persistent alterations in body composition, and therefore energy homeostasis, which may in turn have implications for health in later life. The loss of effect of birth group on body composition in the adjusted model suggests that the effect of preterm birth is mediated through other factors. Longitudinal studies of the effect of preterm birth or other perinatal factors are complicated by the inter-relationship between gestation length, nutrition, growth and other factors, making it difficult to isolate causal relationships, even in experimental

settings, between individual early life events and later outcomes (Schisterman, Whitcomb, & Bowers, 2011).

Effect of supplementation

Supplementation was intended to emulate the growth promoting effects seen with human breast milk fortification (Kuschel & Harding, 2004) but, contrary to expectations, growth was not enhanced during the period of supplementation.

Supplement was administered to animals as boluses given via drench gun to the back of the oropharynx thus stimulating a swallow reflex. Boluses were given at regular intervals throughout the day without reference to lamb feeding patterns or hunger. In healthy adult humans with access to *ad libitum* food, nasogastric tube administration of a multi-nutrient supplement, but not an equivalent volume of water, reduces appetite, but not sufficiently to compensate for increased supplement-derived calories (Stratton, Stubbs, & Elia, 2008). The cephalic phase responses (Power & Schulkin, 2008) are the anticipatory physiological responses that occur in preparation for food ingestion and modulate feeding behaviours, appetite and the metabolic responses to enteral nutrition in many species, including sheep (Sugino et al., 2002). Thus, the mode of supplement administration bypasses the cephalic phase response; the trend for reduced milk intake in supplemented animals may be one manifestation of disrupted feeding patterns, irrespective of calorie intake. We did not measure either milk or other feed intake beyond the intervention period and therefore do not know the long term impact of supplementation on later feeding behaviours.

Protein is more satiating than either carbohydrate or fat (Yancy, Olsen, Guyton, Bakst, & Westman, 2004; Yu, South, & Huang, 2009) and thus has an important role in appetite regulation. The protein leverage hypothesis (Simpson & Raubenheimer, 2005), which suggests the primacy of a stable protein intake over the intake of energy from other macronutrients, may explain the tendency of supplemented animals to consume less milk. For instance, observational studies of wild monkeys (Felton et al., 2009) showed maintenance of a stable intake of dietary protein, despite significant variation in the range of food types and energy, carbohydrate and fat consumed. Similar findings have been reported in rodents, in which increased energy intake, necessary to achieve adequate protein intake in the low-protein diets, promoted adiposity (Sorensen, Mayntz, Raubenheimer, & Simpson, 2008). In one human intervention study, preterm infants were randomised at hospital discharge to either have maternal milk plus vitamin and mineral supplements, or fortified maternal milk

(75 ml/kg per day of expressed maternal milk, fortified with protein, carbohydrate, vitamins and minerals, and bottle fed to the infant) (Aimone et al., 2009). Milk consumption in the fortified group (assessed using test-weights and a feed diary) was reduced, so that protein and energy intake was not different to the un-fortified group. This suggests that breast-fed infants, even those born preterm, have the ability to self-regulate dietary intake. The impact of this feed intervention on growth is discussed later. Supplementation also delayed the time taken to achieve maximal daily growth rate in males, with the effect especially pronounced in preterm males. Recent studies of breast milk fortifier (BMF) in 'well' enterally fed preterm infants have shown how alterations in the formulation of BMF can alter acid base balance and induce a metabolic acidosis (Rochow et al., 2011), possibly due to an inability of immature preterm kidneys to adequately excrete an increased acid load (Kalhoff & Manz, 2001). They also report a strong correlation between acid-base status and growth rates, in which low base excess and pH correlated with lesser growth rates. While the mechanisms underlying this are unclear, they speculate that relative metabolic acidosis may stimulate respiratory drive and therefore increase the work of breathing and energy expenditure, or reduce the activity of key digestive enzymes, reducing energy uptake. Although we did not measure acid-base status in our lambs, it is possible that the increased protein load, especially in the preterm lambs, similarly altered acid-base status, resulting in a delay in achieving maximal growth rates. The reason for the sexual dimorphism is unclear although De Matteo *et al.* found increased mortality amongst male preterm lambs (De Matteo, et al., 2010), suggesting that in sheep as well as humans (Garg, Abdel-Latif, Bolisetty, Bajuk, & Lui, 2010) preterm males are more vulnerable than females in the early neonatal period.

The most obvious growth effects of supplementation were on measures of weight-for-length (and by inference adiposity) and occurred well after cessation of the intervention period. Effects were especially evident in males, where supplementation appeared to promote weight for length in preterm sheep, but reduce weight for length in term-spont sheep. By adulthood, although not statistically significant, the same profile of supplement-related effects on weight for length were apparent (in males, increased weight-for-length in preterm but reduced weight-for-length in term-spont sheep), and were matched by a similar profile of differences in fat mass. Supplemented term-spont males were twice as likely to have fat mass below the DXA minimum detection threshold (MDT) and had half the measured % fat mass compared to un-supplemented term-spont males, with a very similar profile in supplemented term-spont females, whereas supplementation did not reduce fat mass in preterm animals. Fat mass is

determined by both the number and volume of adipocytes. In humans, final adipocyte numbers are established early in postnatal life (Prins & O'Rahilly, 1997) so that gain and loss of adult fat mass is principally due to changes in adipocyte volume (Spalding et al., 2008). Lambs are born lean, but rapidly increase fat mass postnatally (Clarke, Buss, et al., 1997). Endogenous fatty acid synthesis makes a minimal contribution to lipid deposition in the newborn lambs (Vernon, 1975); development of fat stores is therefore dependent on fat ingestion with increased dietary fat promoting adiposity (Darby, Clarke, Lomax, & Symonds, 1996). In our paradigm, supplementation increased fat as well as protein, carbohydrate and calorie intake, although the ratio of macronutrients relative to ewe milk was preserved. It is possible that different growth patterns or energy requirements of male lambs favoured early adiposity in preterm lambs, whereas additional protein intake impaired weight-for-length in term-spont lambs. The reasons for the disparate, sex specific effects of supplement in preterm and term animals are not clear although sex specific characteristics in preterm infants (Ingemarsson, 2003) and lambs (De Matteo, et al., 2010) have been described.

Modifying the macronutrient balance of formula milk given to healthy term-born infants also has an influence on growth and body composition. Well, term-born infants fed formula with a protein content at the upper limits of that recommended by the EU directive on infant formula milk (European Commission, 1991) had greater gains in weight and weight-for-length at age 2 years than those fed formula with a protein content at the lower end of the recommended range (Grote et al., 2010). Reduced fat, but not carbohydrate content in the high protein formulation ensured that the formulas were isocaloric. In comparison, isocaloric hydrolysate-based formula compared to cow's milk-based formula reduced weight and weight-for-length z-scores, and reduced milk intake in infants from 1.5 to 7.5 months of age (Mennella, Ventura, & Beauchamp, 2011). Hydrolysate-based formula milk also had 35% higher protein content, a lower carbohydrate content but equal fat content. In addition, growth parameters in the hydrolysate-based formula group emulated those of WHO reference standards for breast-fed infants, whereas the cow's milk-based group had increases in weight, weight-for-length and length z-scores that moved away from the breast fed standard. The disparity between the high protein infant feeding paradigms of Grote *et al.* and Menella *et al.* on patterns of infant weight gain may therefore relate to the type of protein, rather than the absolute amount given, or may be a statistical artefact secondary to high drop-out rates (40%) in Grote *et al.*, although drop-out rates were similar between treatment groups.

In preterm infants, early dietary exposure also has persistent effects on body composition. At 10 years of age anthropometric measurements were made of a group of individuals, all born below 35 weeks gestation and randomised to receive formula milk supplemented or not with long chain polyunsaturated fatty acids (LCPUFA) between birth and 9 months (Kennedy et al., 2010). In males, supplementation had no effect on body composition, but in females, those supplemented with LCPUFA were taller, heavier and had greater skin fold thickness, indicating increased adiposity. However, similar to Grote *et al.,* drop-out rates were high, with only ~45% of the original cohort seen at the 10 year follow-up.

Finally, other milk constituents may be important determinants of growth. Skeletal growth was greater following a 12 week trial of post-discharge milk fortification in preterm babies, whereas body composition and bone mineral density were not different from that of un-fortified infants (Aimone, et al., 2009). Infants receiving milk fortification reduced their feed intake so that total protein and energy consumption was equivalent between groups; increased skeletal growth therefore appeared to be due to increased calcium and phosphate. Ewe milk supplement did not contain additional minerals or vitamins, and there remains a lack of consensus on optimal intake of mineral supplementation in infants and children (Abrams, 2011).

Antenatal corticosteroids and postnatal growth

A single course of antenatal corticosteroids at term did not alter postnatal growth or weight gain between birth and adulthood in this study. Similarly, although some human studies have shown a reduction in size at birth following maternal corticosteroids (Roberts & Dalziel, 2006), there does not appear to be a deleterious effect of single or repeat courses of antenatal corticosteroids on postnatal growth (Crowther, McKinlay, Middleton, & Harding, 2011; Peltoniemi et al., 2009; Wapner et al., 2007).

Adult female term-dex sheep appeared to have a greater % fat mass and fat mass index than other females, although not when the model was corrected for other perinatal variables, including birth weight z-score. Subsequent analysis showed that term-dex females uniquely had a strong correlation between relative size at birth and % fat mass (low birth weight correlated with increased adult fat mass). It has been shown in sheep that maternal dexamethasone 2 days prior to term delivery obliterates the normal cold-stress induced fall in leptin mRNA concentrations in the perirenal fat of newborn lambs (Bispham et al., 2002). Plasma leptin concentrations have a positive correlation with perirenal leptin mRNA, and an

inverse relationship between appetite and plasma leptin has been described in young lambs (De Blasio, Blache, Gatford, Robinson, & Owens, 2010). Although speculative, it is possible that if dexamethasone prevents the usual cold-induced fall in leptin, newborn appetite is not stimulated to the same extent as in non-dexamethasone exposed animals. Alternative energy substrate would therefore be required to maintain euglycaemia, and may differ between lambs with relative growth restriction (low birth weight z-score) and normally grown lambs (Greenwood, et al., 2002). Thus differences in newborn size at birth, coupled with antenatal dexamethasone exposure at term 'program' the observed association between birth weight z-score and later adiposity. This association warrants further investigation, especially given the increased clinical use of corticosteroid at term delivery (Bonanno & Wapner, 2009).

The effect of antenatal steroid exposure *per se* on adiposity in humans is uncertain, although BMI is not different between adults born at preterm or term gestation, with or without antenatal corticosteroid exposure (Dalziel, et al., 2005; Finken, et al., 2008). However, BMI does not convey information about fat distribution, and for a given BMI, actual fat mass may vary as much as two-fold (Wells, et al., 2006).

Antenatal corticosteroids may also influence the onset of lactation, and quality of milk produced. Premature lactogenesis is stimulated by maternal corticosteroid treatment in sheep (Henderson, Hartmann, Moss, Doherty, & Newnham, 2008) and, following subsequent parturition at term, milk quality and volume is impaired. This may be due to changes in mammary tissue as, if milk removal does not occur once lactation has been stimulated, mammary tissue undergoes involution (Tatarczuch, Philip, & Lee, 1997). Repeated exposure to antenatal corticosteroids has also been shown to impair pre-weaning weight gain in sheep (Moss, et al., 2001) whereas a single course of antenatal corticosteroids in sheep reduces weight gain in the early neonatal period (days 0-5) (Henderson, et al., 2008). Unlike these studies, corticosteroids in our paradigm were used to initiate parturition in both preterm and term animals; onset of lactogenesis was therefore accompanied by birth and suckling of the lamb, with insufficient time between onset of lactogenesis and suckling for mammary involution to occur. It is therefore possible that maternal steroids enhanced the development of lactogenesis; both ewe milk composition data and lamb fluid intake data from term-dex animals are needed to further investigate this issue.

Size at birth, postnatal growth rates and adiposity

We hypothesised that there would be an association between small size at birth, increased postnatal growth rates and later adiposity. While birth weight z-score was inversely related early growth rates (GV_{0-2}) in term-spont sheep and male term-dex sheep, there was no association either in preterm, term-Alizin or female term-dex sheep. The disconnection between birth weight z-score and early GV in preterm lambs may reflect differences in energy partitioning between tissue maturation and growth compared to lambs born at full term, although why this should be so in the other term-born animals is uncertain. Term-spont, preterm, term-dex and female term-Alizin sheep also had an inverse relationship between birth weight z-score and GV_{W-1Yr} although the strength of the association varied between groups; it is possible that the lack of this association in the male term-Alizin reflects the relatively small effect size in conjunction with low animal numbers. Intriguingly, we found that term-dex females had a unique relationship between birth weight, growth rate and adiposity where small size at birth or rapid growth between weaning and 1 year were associated with increased adiposity in adulthood. Recently published obstetric practice guidelines advocate the use of corticosteroids prior to elective term caesarean section to reduce neonatal respiratory morbidity, although there are no long-term data to adequately assess the safety of such an intervention (Royal College of Obstetricians and Gynaecologists, 2010). Data from our experiments suggest that there might be unanticipated, adverse long term effects on body composition, especially in women who were small at birth or had rapid postnatal growth, which warrants further investigation.

Other paradigms have demonstrated an association between small size at birth and increased adiposity (Gatford, Simmons, De Blasio, Robinson, & Owens, 2010). Although the range of birth weight z-score in our paradigm was not different in term-dex females compared to other groups of sheep, the overall range in birth weight z-scores in all groups was small, making correlations between birth weight z-score and later outcomes difficult. In addition, others have reported strong associations between increased growth rates in childhood and later adiposity, although the time point in which growth rate appears most influential varies. Many of these paradigms have however included much greater experimental manipulation of postnatal growth, or, in human trials, have studied populations exposed to a broader range of weaning practices and post-weaning nutrition. In our paradigm, from 2 weeks of age onwards there were no differences in nutrition or environment between sheep, with little variation in growth rates between groups of sheep. It is possible therefore that the experiment was

underpowered to detect any effect of growth rate on later adiposity; why this relationship should therefore be present in female term-dex sheep alone is unclear.

Induction of labour at term and growth

Early weight gain was not different in term-Alizin compared to other term-born sheep; however, weight gain between 6 and 12 months, especially in females, was less than in other sheep. Unlike other birth groups, term-Alizin lambs were all born towards the end of a single lambing season, and therefore were amongst the youngest animals at the start of their first winter, co-incident with the time of observed growth faltering. Season of birth has a significant impact on growth in pasture fed animals (Benyi, Norris, Karbo, & Kgomo, 2006). Although additional feed was provided over winter, growth may have been adversely affected by the change in nutrition and drop in ambient temperature. By the time of adult DXA scanning, term-Alizin males had a greater weight-for-length than term-dex sheep, and although not statistically significant, a greater % fat mass, whereas in females, catch-up growth in term-Alizin sheep was not accompanied by disproportionate increases in soft tissue or adiposity.

Future experiments need to include a greater number of term-Alizin sheep with induction more evenly distributed throughout the lambing season in order to minimise these potentially confounding effects.

Limitations

The rapid growth and maturation observed in domesticated farm animals may mean that postnatal growth rates in well, term-born lambs are already maximal and therefore dietary changes will have little impact while the lamb is allowed to feed *ad libitum*. Even in preterm lambs, weight, on average, doubled during the first two weeks of life, and increased 10 fold by 1 year of age. By comparison, human infants have a much slower rate of growth; a well human term newborn can expect to double their birth weight by 4 months of age and triple it by 1 year, whereas preterm infants will take even longer to do so (Morgan, et al., 2011).

To generate sufficient animal numbers, ewes were bred over a long period and therefore ambient temperatures, although not recorded, varied considerably during the lambing season. Growth and adiposity are known to be altered by environmental temperature (Darby, et al., 1996); standardising the early life environment in future experiments may allow better isolation of the effects of preterm birth or nutritional intervention.

Milk and macronutrient intake was a derived measure, and assumed that the complete 'dose' of intervention fluid was ingested by all lambs. Placement of a nasogastric feeding tube for each dose would have caused unacceptable stress to a well lamb, whereas gastrostomy tube placement would have ensured compliance, but may have interfered with voluntary feeding, lamb survival or had other undesired effects on gastrointestinal function.

Blood samples for plasma metabolites at birth were drawn prior to the first feed, but samples thereafter were not obtained from fasted lambs. Standardisation of the interval between last feed and blood sampling may allow better characterisation of the effect of the changes in metabolites over the first days of life.

Conclusions

Body composition and growth rates of both preterm and term-born sheep were influenced by nutrition in the immediate postnatal period, the effects of which persisted well beyond the period of nutritional intervention. The extent and direction of that effect was determined by both sex and gestation length, and may reflect both the total macronutrient intake and their relative proportions. Future studies are needed to isolate the long term effects of nutritional content from feeding modality, following both preterm and term birth. The impact of protein type as well as quantity following term birth is especially relevant given the range of artificial formulas commercially available, including casein-dominant formulas (and therefore moving further away from attempting to emulate whey-dominant breast milk) marketed to target 'hungry babies'.

In preterm infants, altered metabolic requirements are coupled with immaturity of homeostatic mechanisms; customised fortification of milk to tailor nutritional intake to metabolic requirements may improve protein and energy intake and allow growth and body composition to emulate that of well, term-born, breast fed infants. However, the optimal growth trajectory and 'ideal' body composition for developing children that maximises both short and long term outcomes awaits clarification.

The possible effects of corticosteroid exposure at term, especially in females, on body composition and increased adiposity is of concern. The long term follow-up of similarly exposed infants needs to be initiated.

Chapter 5. Glucose and insulin homeostasis

5.1. Introduction

Since the first reports of an association between small size at birth and impaired glucose tolerance in adult life (Hales, et al., 1991), there has been much research into the effect of perinatal events on later glucose homeostasis. Despite this, there remains uncertainty, with conflicting data on the effects of preterm birth, *in utero* growth restriction (IUGR), antenatal corticosteroid exposure and postnatal growth rates (which may be a proxy for preterm birth or IUGR).

Preterm birth has been linked to reduced insulin sensitivity in childhood (Hofman, et al., 2004), and to insulin resistance (Dalziel, et al., 2007) (Hovi, et al., 2007) and type 2 diabetes in adulthood (Kaijser, et al., 2009). These findings are not universal; others have found no association between preterm birth and insulin sensitivity in either childhood (Darendeliler, et al., 2008) or adulthood (Willemsen, et al., 2009). It has been suggested that antenatal corticosteroid exposure either mediates, or compounds, the metabolic effects of preterm birth (Moss, et al., 2001). In one population of adults born preterm, antenatal corticosteroid exposure did not affect measures of insulin resistance (Finken, et al., 2008), whereas Dalziel *et al.* found evidence of increased insulin secretion in adults whose mothers were randomised to betamethasone rather than placebo during the Auckland antenatal corticosteroid trial (Dalziel, et al., 2005).

Growth rates in early postnatal life or later in childhood are also linked to insulin-glucose axis function, and although the most influential phase of growth on later outcomes in unknown, it appears to be modified by gestational age and size at birth (see chapter 1.9.2).

Rates of preterm birth continue to rise globally. If preterm birth results in permanent impairment of glucose homeostasis, these individuals are likely to be at a heightened risk for developing type 2 diabetes. As type 2 diabetes is also increasingly prevalent in both paediatric and adult populations, early identification of perinatal risk factors may allow targeted screening or intervention strategies and thus reduce the progression of insulin resistance to frank diabetes.

This experiment aimed to identify the effect of preterm birth, antenatal corticosteroid exposure, nutritional supplementation, relative size at birth, and postnatal growth rates on measures of glucose tolerance, insulin secretion and sensitivity in pre-pubertal and adult sheep.

5.2. Methods

Intravenous glucose tolerance tests (GTT) and hyperglycaemic clamps (HGC) were performed in juvenile and adult animals (2.13) and plasma glucose and insulin concentrations measured as described in Chapter 2.

For the GTTs, the intercept of the glucose curve with the fasting glucose concentration was used to calculate the time taken to restore fasting glucose concentration. Areas under the curve (AUC) for insulin and glucose were calculated for the first 15 minutes following a bolus of dextrose (AUC_{15}), and for the total AUC (AUC_T) (interval between baseline and time at which plasma glucose returned to baseline or, if plasma glucose remained above baseline, AUC was calculated for the entire 3 hour period).

For the HGCs, steady state glucose requirements and insulin responses were calculated, and insulin AUC for the 30 minutes following arginine administration (AUC_{Arg}) (Chapter 2.10). Although all animals underwent HGC testing at both ages, due to laboratory constraints juvenile results are limited to term-spont and preterm sheep whereas adult data are available on all groups of sheep.

Insulin sensitivity (Si) was derived from the HGC, and glucose disposition index (DI) from age matched GTTs and HGCs (2.13).

5.3. Statistical analysis

Non-parametric data were log transformed to approximate a normal distribution prior to further analysis.

Preliminary analysis indicated that effects of birth group and supplementation on glucose tolerance and insulin sensitivity and secretion were different between the sexes. Data were therefore analysed in same sex groups.

First, the effects of preterm birth and supplementation were assessed in all term-spont, preterm and term-dex sheep by 2-way ANOVA (independent variables birth group and

supplementation status) with the interaction term birth group x supplement. Second, the effects of corticosteroid induction of labour at term were assessed by factorial ANOVA in un-supplemented term-spont, term-dex and term-Alizin sheep. If ANOVA was significant, a Tukey *post hoc* test was performed with significance set at a p-value of <0.05 for the primary analysis and <0.01 for the secondary analysis of birth group. Data from these analyses are presented as the least square mean ± SEM.

Longitudinal changes in plasma concentrations of glucose or insulin were examined using RM ANOVA; where significant differences were found data were analysed by factorial ANOVA with Tukey post-hoc test.

Comparisons between nominal variables were made using the χ^2 test.

Multivariate regression models were then constructed to assess whether the effects of birth group were modified by the inclusion of other perinatal or postnatal variables (adjusted model: birth group, birth weight (bwt) z-score, supplementation status, weight and age at the time of assessment) or by inclusion of measures of growth velocity to the adjusted model ($GV_{0\text{-}2}$, $GV_{2\text{-}W}$ and in adult sheep $GV_{W\text{-}1Yr}$).

Correlations between continuous variables were examined by regression analysis within each birth group, in same sex groups. To allow for the effect of multiple analyses, statistical significance was set at a p-value of 0.001.

5.4. Results

5.4.1. Glucose tolerance in juvenile sheep

5.4.1.1. Preterm birth and glucose tolerance

Fasting glucose concentrations were not different between groups in either sex (Table 5-1). There was no effect of birth group on fasting insulin concentrations or fasting insulin: glucose ratio in males, whereas in females, both fasting insulin concentrations and fasting insulin: glucose ratio were greater in preterm than term-dex sheep (Table 5-1).

During a glucose tolerance test (GTT) plasma concentrations of glucose and insulin, insulin: glucose ratios, and total AUC (AUC_T) for glucose and insulin were not different between birth groups in either sex (Figure 5-1, Table 5-1). $AUC_{15\text{-}G}$ was not different between birth

groups in males, but in females was significantly affected by birth group (Table 5-1). Although *post hoc* tests did not identify the level of this effect, inspection of the data suggests that term-spont sheep had a greater $AUC_{15\text{-}G}$ than either preterm or term-dex sheep.

There was no difference between birth groups in the number of animals with plasma glucose above or below baseline at 3 hours (male χ^2=0.4, female χ^2=0.3).

In male sheep in an adjusted model with $GV_{0\text{-}2}$ as the growth variable, preterm sheep had a lesser Log total insulin AUC ($AUC_{T-I)}$ compared to term-dex sheep (adjusted means: AUC_{T-I}; 4.6 ± 0.1 vs. 5.1 ± 0.6 respectively, p<0.05) and tended to have a lesser Log insulin:glucose ratio for total AUC, also compared to term-dex sheep (p=0.06).

In females, the adjusted model lessened the effect of preterm birth (fasting plasma insulin p=0.09, fasting insulin: glucose ratio p=0.06), whereas it increased the effect of birth group on glucose response in the 15 minutes following dextrose administration (AUC_{15G} in term-spont sheep greater than in either term-dex or preterm sheep; adjusted means 195 ± 3 mmol·min/l vs. 183 ± 3 mmol·min/l vs. 181 ± 3 mmol·min/l respectively, p<0.05). This was unchanged by inclusion of $GV_{0\text{-}2}$ in the model.

Inclusion of $GV_{2\text{-}W}$ in the regression model did not alter the findings in either sex.

5.4.1.2. Nutritional supplementation and glucose tolerance

In male sheep, supplementation did not alter plasma glucose or insulin concentrations, the insulin: glucose ratio or other GTT outcomes, in either the fasted state or following the glucose bolus (Figure 5-1, Table 5-1).

In females however, in the first 15 minutes after dextrose administration, supplemented sheep had a lesser rise in glucose than un-supplemented sheep; Figure 5-1, VII. There was also a significant birth group x supplement x time interaction for plasma insulin concentrations and for the insulin: glucose ratio. The post-hoc test was not statistically significant, but inspection of the data suggests that supplementation decreased plasma insulin responses in term females but increased plasma insulin responses in preterm females (Figure 5-1, VIII, IX). This effect appeared to persist over the course of the entire GTT, with supplement similarly appearing to decrease plasma insulin responses in term females but increase plasma insulin responses in preterm females (p=0.06 for the interaction effect, Figure 5-1, V). There was no effect of supplementation on any other GTT outcomes in females (Figure 5-1, Table 5-1).

There was no effect of supplement on the number of animals with plasma glucose concentrations below or above baseline at 3 hours (male χ^2=0.4, female χ^2=0.3).

5.4.1.3. Induction of labour at term and glucose tolerance

Male and female term-Alizin sheep had plasma glucose and insulin responses to a GTT that were not different to those of un-supplemented term-spont or term-dex sheep (Figure 5-1, Table 5-1) in either the simple or the adjusted analysis.

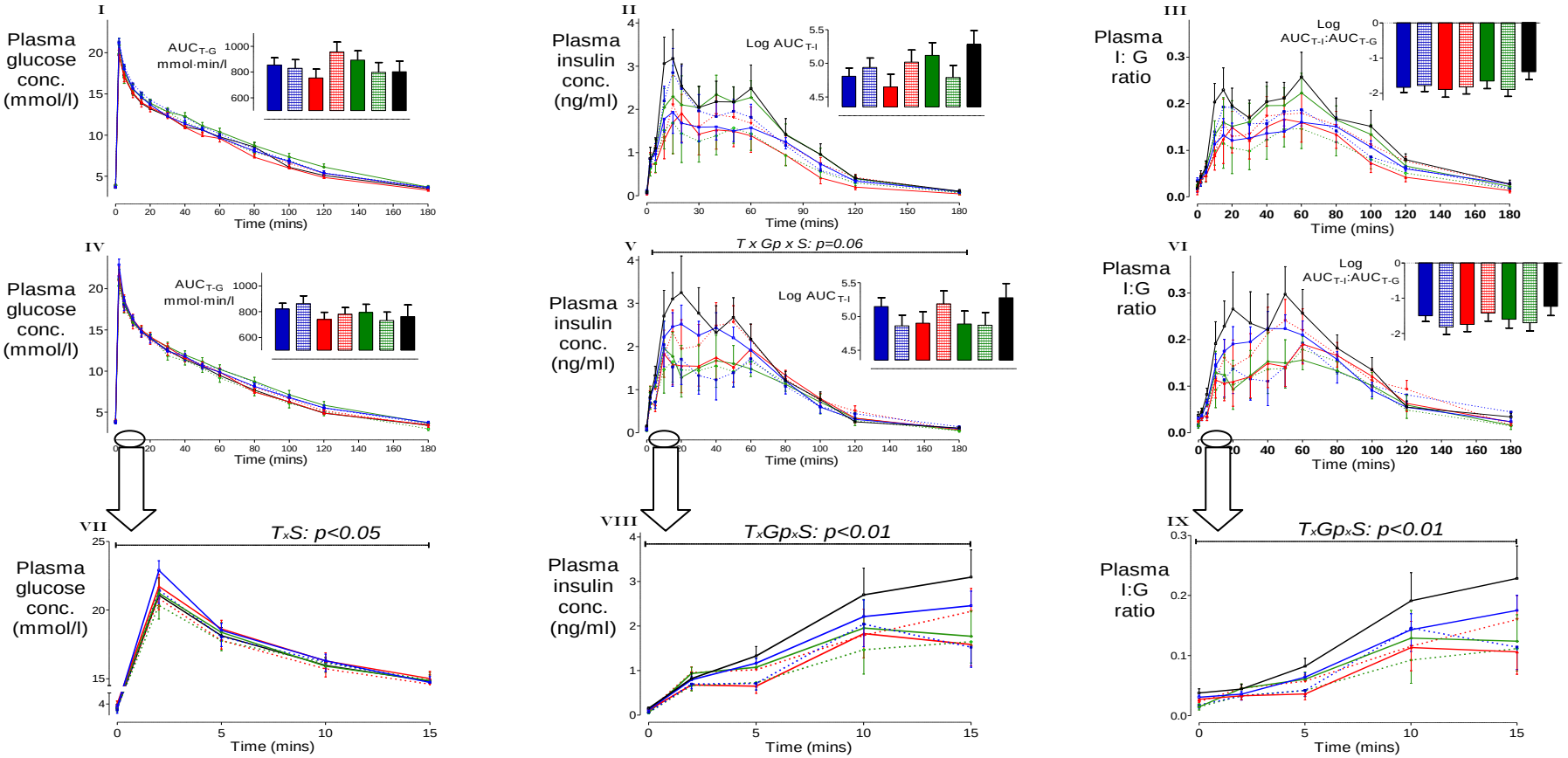

Figure 5-1: Plasma glucose and insulin concentrations during a glucose tolerance test in juvenile sheep

I-III male sheep (0-180 minutes). IV-VI female sheep (0-180 minutes). VII-IX female sheep (0-15 minutes). AUC_T=total area under the curve. G=glucose. I=insulin. Conc.=concentration. T=time. Gp=birth group. UN=un-supplemented; solid lines and bars. S=supplement; interrupted line and hatched bars. Term-spont, blue; UN male n=10, female n=10; S male n=9, female n=10. Preterm, red; UN male n=10, female n=8; S male n=10, female n=9. Term-dex, green; UN male n=10, female n=9; S male n=9, female n=9. Term-Alizin (A), black; male n=6, female n=6. Letters and p values above the graphs give the effect of RM ANOVA. Data are least square mean ± SEM.

MALE	Term-spont		Preterm		Term-dex		Term-Alizin
	UN (n=16)	S (n=12)	UN (n=8)	S (n=9)	UN (n=8)	S (n=8)	UN (n=5)
Baseline $_G$ (mmol/l)	3.7 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.6 ± 0.2
Log Baseline $_I$	-2.5 ± 0.2	-2.9 ± 0.2	-3.1 ± 0.3	-2.7 ± 0.3	-2.5 ± 0.3	-2.6 ± 0.3	-2.8 ± 0.4
Log Baseline $_I$: Baseline $_G$	-3.8 ± 0.2	-4.3 ± 0.2	-4.5 ± 0.3	-4.0 ± 0.3	-3.9 ± 0.3	-4.0 ± 0.3	-4.2 ± 0.4
$AUC_{15\text{-}G}$ (mmol·min/l)	190 ± 7	189 ± 9	172 ± 10	197 ± 10	185 ± 9	172 ± 12	175 ± 12
Log $AUC_{15\text{-}I}$	2.7 ± 0.1	2.7 ± 0.1	2.5 ± 0.2	2.7 ± 0.2	2.9 ± 0.2	2.7 ± 0.2	3.0 ± 0.2
Log $AUC_{15\text{-}I}$: $AUC_{15\text{-}G}$	-2.5 ± 0.1	-2.6 ± 0.2	-2.6 ± 0.2	-2.5 ± 0.2	-2.3 ± 0.2	-2.4 ± 0.2	-2.9 ± 0.2
G time to baseline (mins)	164 ± 6	168 ± 7	145 ± 8	166 ± 8	165 ± 7	161 ± 9	157 ± 10
FEMALE	UN (n=15)	S (n=7)	UN (n=7)	S (n=6)	UN (n=6)	S (n=6)	UN (n=5)
Baseline $_G$	3.9 ± 0.1	3.7 ± 0.2	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.2	3.8 ± 0.2	3.8 ± 0.2
Log Baseline $_I$ ‡	-2.3 ± 0.2	-3.0 ± 0.2	-2.3 ± 0.3	-2.5 ± 0.3	-3.1 ± 0.3	-3.0 ± 0.4	-2.0 ± 0.3
Log Baseline$_I$: Baseline$_G$ ‡	-3.7 ± 0.2	-4.2 ± 0.2	-3.7 ± 0.2	-3.9 ± 0.2	-4.4 ± 0.2	-4.3 ± 0.2	-3.4 ± 0.3
$AUC_{15\text{-}G}$ (mmol·min/l) ***Gp***	197 ± 5	194 ± 7	184 ± 6	178 ± 6	184 ± 7	178 ± 7	174 ± 9
Log $AUC_{15\text{-}I}$	3.0 ± 0.1	2.6 ± 0.2	2.7 ± 0.2	2.9 ± 0.2	3.0 ± 0.2	2.7 ± 0.2	3.2 ± 0.2
Log $AUC_{15\text{-}I}$: $AUC_{15\text{-}G}$	-2.3 ± 021	-2.6 ± 0.2	-2.5 ± 0.2	-2.2 ± 0.3	2.2 ± 0.3	2.5 ± 0.2	-3.0 ± 0.2
G time to baseline (mins)	159 ± 6	167 ± 8	161 ± 8	156 ± 7	158 ± 8	141 ± 9	153 ± 12

Table 5-1: Juvenile glucose tolerance test results

UN=un-supplemented. S=supplemented. G=glucose. I=insulin. AUC_{15}=area under the curve in first 15 minutes. Data are least square means ± SEM. ‡ p<0.05 preterm compared to term-dex. Gp, p<0.05 for effect of birth group.

5.4.1.4. Correlations between size at birth, growth rates, weight and glucose tolerance

In males there were no correlations between birth weight z-score, juvenile weight or $GV_{2\text{-}W}$ and measures of glucose tolerance. Rapid growth during the first two weeks of life however tended to correlate with an increase in the fasting insulin: glucose ratio in term-spont sheep, and an increase in the time taken to return plasma glucose concentrations to baseline values in preterm and term dex sheep (for each 10 g / Kg·d increase in $GV_{0\text{-}2}$: fasting I:G ratio in term-spont sheep; $R^2 = 0.3$, β coefficient 0.9 ± 0.3, p = 0.004. Time take for plasma glucose concentrations to return to baseline in preterm sheep; R^2=0.5, β coefficient 24 ± 7, p = 0.007, in term-dex sheep $R^2 = 0.6$, β coefficient 30 ± 7, p = 0.001).

In females, increased size at birth in preterm sheep correlated with increased glucose AUC during the first 15 minutes of a GTT (per unit increase in birth weight z-score; R^2=0.5, β coefficient 9 ± 2, p=0.001). Increased juvenile weight also correlated with increased glucose AUC during the first 15 minutes of a GTT in preterm and term-dex sheep only (per kilo increase in juvenile weight: preterm; R^2=0.4, β coefficient 1.8 ± 0.5, p=0.003, term-dex; R^2=0.6, β coefficient 1.9 ± 0.5, p=0.002). Neither $GV_{0\text{-}2}$ nor $GV_{2\text{-}W}$ was correlated with any GTT outcomes in any sheep.

In the preterm group, neither $GV_{0\text{-}TEA}$ nor $GV_{TEA\text{-}W}$ correlated with any measures of glucose tolerance in either sex.

5.4.2. Insulin secretion and sensitivity in juvenile sheep

5.4.2.1. Preterm birth and insulin sensitivity and secretion

Steady state hyperglycaemia was achieved in all animals (Figure 5-2)

Neither plasma insulin concentrations during the HGC, nor insulin sensitivity (Si) or glucose disposition index (DI) were different between birth groups, in either sex (Figure 5-2, Table 5-2). In the adjusted model, preterm males had a greater insulin AUC following arginine than term-spont males (adjusted means 3.8 ± 0.2 vs. 3.2 ± 0.1, p<0.05); however, most of this effect appeared to be attributable to the response seen in supplemented preterm animals. There was no effect of preterm birth in females in the adjusted model. Inclusion of either $GV_{0\text{-}2}$ or $GV_{2\text{-}W}$ in the adjusted model did not alter these findings in either sex.

5.4.2.2. Nutritional supplementation and insulin sensitivity and secretion

In male sheep there was no effect of supplementation on plasma glucose or insulin concentrations during the HGC, or on Si, DI or any other HGC outcome measures and no interaction between birth group and supplementation (Table 5-2).

In females, supplemented animals tended to require a lesser glucose infusion rate to maintain steady state hyperglycaemia ($p=0.06$, Table 5-2). During steady state hyperglycaemia there was a trend for a supplement x birth group effect; supplemented term-spont, but not preterm sheep had a lesser plasma insulin response ($p=0.07$ for interaction effect, Figure 5-2) and therefore lower average plasma insulin concentration than un-supplemented sheep ($p=0.07$ for interaction effect, Table 5-2).

Following arginine, plasma insulin concentrations increased in all animals. The magnitude of this increase was less in supplemented than un-supplemented term-spont, but greater in supplemented than un-supplemented preterm sheep ($p<0.05$ for the effect of supplement in both preterm and term-spont sheep, Figure 5-2). Peak insulin concentration and insulin AUC following arginine were also significantly affected by the interaction between birth group and supplementation (for the interaction effect birth group x supplement; peak insulin concentration after arginine, $p=0.02$, insulin AUC after arginine, $p=0.01$). Although post-hoc tests were not statistically significant, inspection of the data suggests that insulin responses were lesser in supplemented than un-supplemented term-spont, but greater in supplemented than un-supplemented preterm sheep (Table 5-2).

Insulin sensitivity tended to be greater in supplemented than un-supplemented term-spont and lesser in supplemented than un-supplemented preterm sheep ($p=0.07$ for the birth group x supplement interaction effect, Table 5-2) whereas DI was not altered by supplementation in either group.

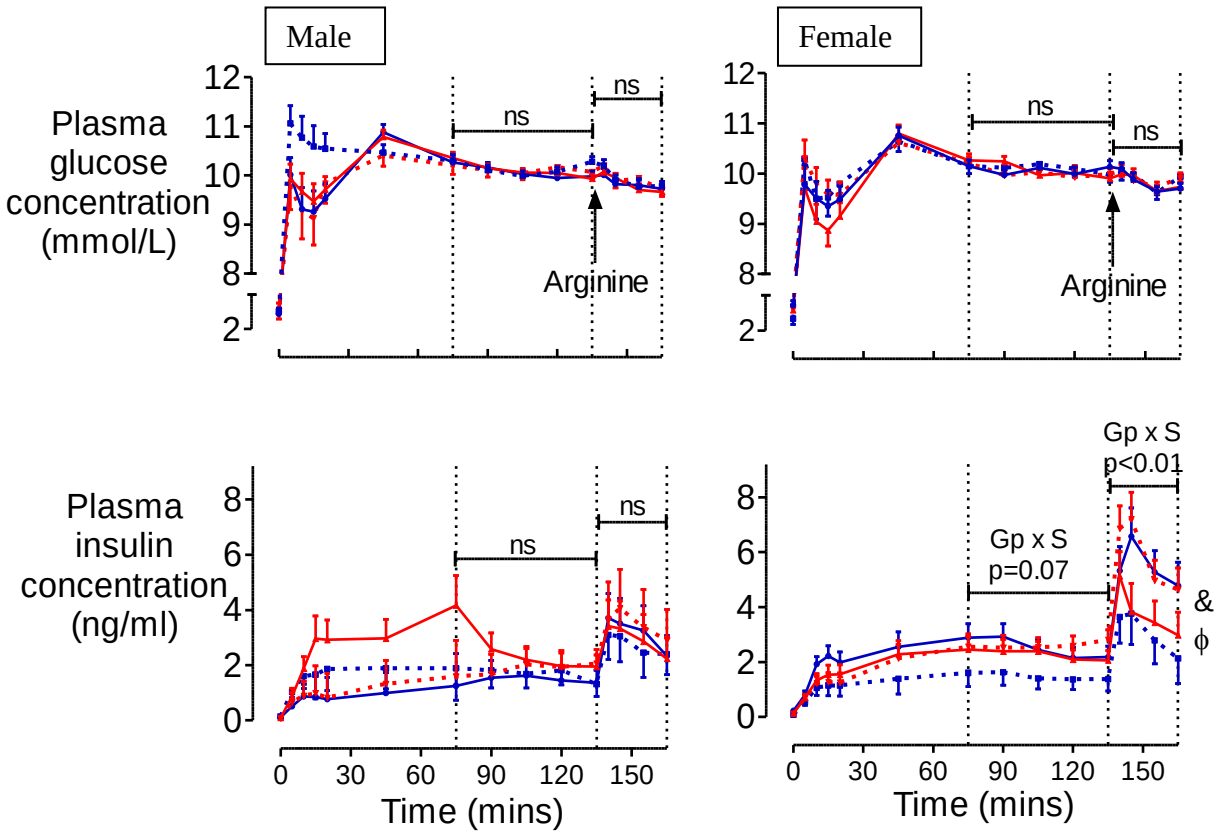

Figure 5-2: Insulin and glucose responses during a juvenile hyperglycaemic clamp

S=supplement. Term-spont: blue; un-supplemented (UN; continuous line) male n=7, female n=7, supplemented (interrupted line) male n=7, female n=7. Preterm: red; UN male n=8, female n=8; S male n=5(t0-135) n=3(t135-165), female n=8. Data are least square mean ± SEM. p values show effect of RM ANOVA for the interaction between birth group and supplementation (Gp x S). Symbols to the right of the graph refer to post-hoc testing of insulin responses to arginine. ϕ p<0.05 UN term-spont vs. S term-spont. & p<0.05 UN preterm vs. S preterm.

5.4.2.3. Correlations between size at birth, juvenile weight and juvenile insulin sensitivity.

Birth weight z-score, $GV_{0\text{-}2}$ and $GV_{2\text{-}W}$ were not correlated with Si or DI, in any group of sheep, in either sex.

In females, but not males, increased juvenile weight correlated with reduced insulin sensitivity in preterm sheep only (R^2=0.6, β coefficient -1.7 ± 0.4, p<0.001).

$GV_{0\text{-}TEA}$ and $GV_{TEA\text{-}W}$ did not correlate with Si or DI in preterm sheep of either sex.

	MALE					FEMALE				
		Term-spont		Preterm			Term-spont		Preterm	
	Effect	UN (n=7[6])	S (n=7[6])	UN (n=8[4])	S (n=5[5])	Effect	UN (n=7[5])	S (n=7[6])	UN (n=8[5])	S (n=8[4])
SS: G rate	***ns***	26 ±3	28 ± 3	29 ± 3	30 ± 4	***TrS***	40 ± 4	31 ± 4	40 ± 3	35 ± 3
SS_I (ng/ml)	***ns***	2.3 ± 0.3	1.7 ± 0.6	2.6 ± 0.6	1.9 ± 0.9	***TrGp$_x$S***	2.5 ± 0.4	1.5 ± 0.4	2.3 ± 0.4	2.6 ± 0.4
Log Si	***ns***	3.0 ± 0.3	3.1 ± 0.3	2.7 ± 0.2	3.1 ± 0.4	***TrGp$_x$S***	2.8 ± 0.2	3.1 ± 0.2	3.0 ± 0.1	2.7 ± 0.1
Log DI	***ns***	4.7 ± 0.2	5.4 ± 0.2	4.9 ± 0.3	4.9 ± 0.3	***ns***	5.3 ± 0.3	5.0 ± 0.3	5.1 ± 0.3	4.7 ± 0.4
Log Arg_I	***ns***	1.2 ± 0.3	0.9 ± 0.3	1.2 ± 0.3	1.1 ± 0.5	***Gp$_x$S***	1.8 ± 0.2	1.3 ± 0.2	1.6 ± 0.2	1.9 ± 0.2
Log AUC Arg_I	***ns***	3.5 ± 0.4	3.1 ± 0.4	3.4 ± 0.4	3.3 ± 0.6	***Gp$_x$S***	4.3 ± 0.3	3.3 ± 0.3	3.6 ± 0.3	4.3 ± 0.3

Table 5-2: Juvenile hyperglycaemic clamp

[n]=number of animals with age matched HGC and GTT for DI calculation. UN=un-supplemented. S=supplemented. G=glucose. I=insulin. SS: G rate=steady state hyperglycaemia G infusion rate. SS_I=insulin secretion during hyperglycaemic steady state. Si=insulin sensitivity. DI=disposition index. ArgI=peak insulin response following arginine. AUC ArgI= insulin area under the curve following arginine administration. TrGpxS p<0.1 birth group x supplement. TrS p<0.1 UN vs. S. GpxS p<0.05 birth group x supplement. ns=0.1. Data are least square means ± SEM.

5.4.3. Glucose tolerance in adult sheep

5.4.3.1. Preterm birth and glucose tolerance

In males there was no effect of birth group on plasma glucose and insulin concentrations at baseline or during the GTT, or on any measures of glucose tolerance (Figure 5-3, Table 5-3).

In females, preterm sheep had a lesser plasma glucose AUC during the first 15 minutes of the GTT (AUC_{15G}) than term-spont sheep (Table 5-3). There were no other differences between birth groups on plasma glucose and insulin concentrations during a GTT (Figure 5-3), or on any measures of glucose tolerance (Table 5-3).

The number of animals with or without plasma glucose concentrations below baseline at 3 hours was not different between birth groups (male χ^2=0.8, female χ^2=0.9).

In the adjusted model, in both sexes, the effect of birth group was unchanged, with or without inclusion of measures of growth velocity.

5.4.3.2. Nutritional supplementation and glucose tolerance

Supplementation did not significantly alter plasma glucose or insulin concentrations at baseline or during a GTT in either sex (Figure 5-3), and did not alter measures of glucose tolerance (Table 5-3).

There was no significant interaction between birth group and supplementation on GTT outcomes in either sex, although in females there was a trend for an interaction between birth group and supplement on fasting plasma glucose concentrations (Table 5-3). Examination of the data suggests that this reflects increased fasting plasma glucose concentrations in supplemented, compared to un-supplemented, preterm sheep only.

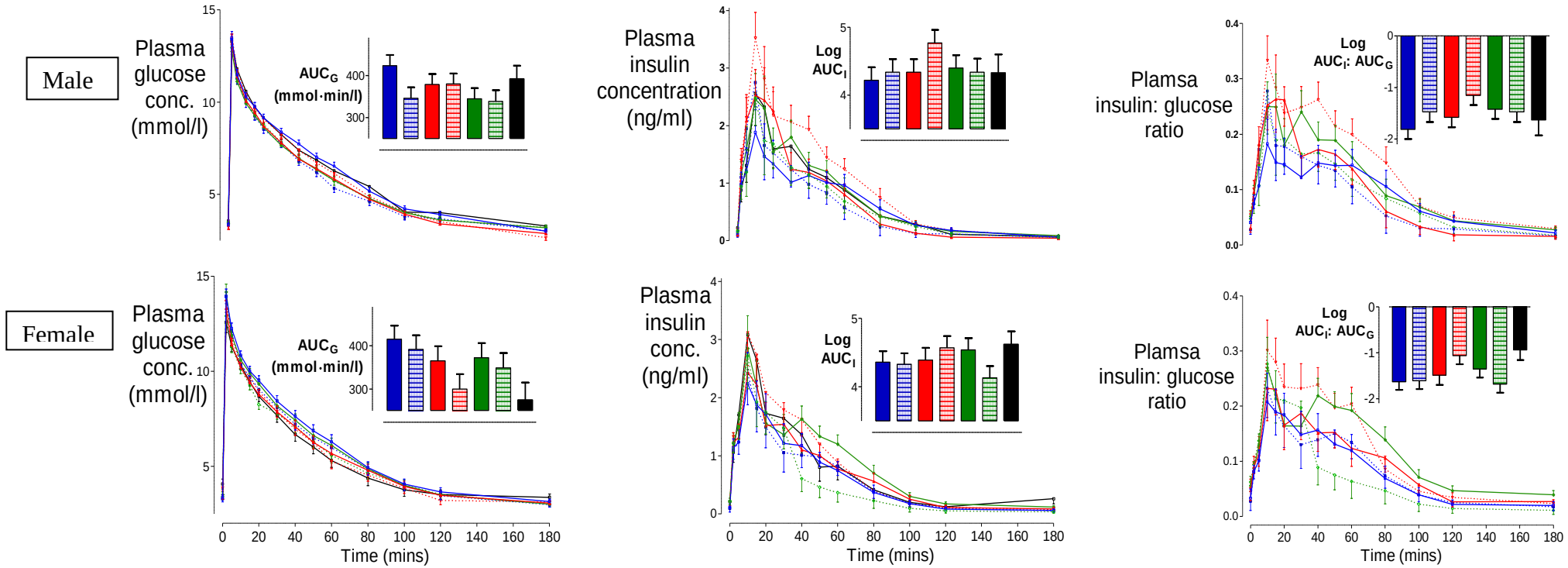

Figure 5-3: Adult glucose and insulin responses during a glucose tolerance test

Area under the curve=AUC. Glucose=G. Insulin=I. Conc.=concentration. Term-spont, blue; un-supplemented (UN; solid lines and bars) male n=10, female n=10; supplemented (S; interrupted lines and hatched bars) male n=9, female n=10. Preterm, red; UN male n=10, female n=8; S male n=10, female n=9. Term-dex, green; UN male n=10, female n=9; S male n=9, female n=9. Term-Alizin, black; male n=6, female n=6. Data are least square means ± SEM.

MALE	Term-spont		Preterm		Term-dex		Term-Alizin
	UN (n=10 [8])	S (n=9 [8])	UN (n=10 [9])	S (n=10 [10])	UN (n=10 [8])	S (n=9 [8])	UN (n=6)
Baseline $_{G}$	3.3 ± 0.1	3.4 ± 0.1	3.2 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	3.6 ±0.1
Log Baseline $_{I}$	-2.3 ± 0.2	-2.6 ± 0.3	-2.4 ± 0.2	-1.8 ±0.2	-2.0 ± 0.2	-2.0 ± 0.3	-1.6 ± 0.3
Log Baseline $_{I}$: Baseline $_{G}$	-3.5 ± 0.2	-3.8 ± 0.2	-3.6 ± 0.2	-3.0 ± 0.2	-3.2 ± 0.2	-3.2 ± 0.2	-2.9 ± 0.3
AUC $_{15\text{-}G}$	109 ± 4	104 ± 4	108 ± 4	110 ± 4	101 ± 4	102 ± 4	108 ± 4
Log AUC $_{15\text{-}I}$	2.8 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	3.4 ± 0.2	3.1 ± 0.2	3.0 ± 0.2	2.9 ± 0.2
Log AUC $_{15\text{-}I}$: AUC $_{15\text{-}G}$	0.026 ± 0.002	0.031 ± 0.002	0.028 ± 0.002	0.031 ± 0.002	0.030 ± 0.002	0.029 ± 0.002	0.027 ± 0.002
G time to baseline [a]	138 ± 10	123 ± 10	134 ± 9	130 ± 9	132 ± 10	122 ± 10	135 ± 14
FEMALE	Term-spont		Preterm		Term-dex		Term-Alizin
	UN (n=10 [9])	S (n=10 [10])	UN (n=8 [8])	S (n=9 [9])	UN (n=9 [9])	S (n=9 [9])	UN (n=6)
Baseline $_{G}$ ***TrGp x S***	3.3 ± 0.1	3.3 ± 0.1	3.3 ± 0.2	3.9 ± 0.1	3.5 ± 0.1	3.4 ± 0.1	4.1 ± 0.3
Log Baseline $_{I}$	-2.3 ± 0.3	-2.7 ± 0.3	-2.1 ± 0.3	-2.0 ± 0.3	-2.1 ± 0.3	-2.5 ± 0.3	-2.4 ± 0.4
Log Baseline $_{I}$: Baseline $_{G}$	-3.5 ± 0.3	-3.9 ± 0.3	-3.3 ± 0.3	-3.3 ± 0.3	-3.3 ± 0.3	-3.7 ± 0.3	-3.8 ± 0.4
$AUC_{15\,G}$ * θ	116 ± 4	113 ± 4	107 ± 4	103 ± 4	112 ± 4	104 ± 4	95 ± 4
Log $AUC_{15\text{-}I}$	3.0 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	3.3 ± 0.2	3.2 ± 0.2	3.1 ± 0.2	3.3 ± 0.2
Log $AUC_{15\text{-}I}$: $AUC_{15\text{-}G}$ θ	0.026 ± 0.002	0.027 ± 0.02	0.027 ± 0.002	0.033 ± 0.002	0.029 ± 0.002	0.030 ± 0.002	0.035 ± 0.002
G time to baseline [a]	134 ± 12	134 ± 11	130 ± 12	97 ± 12	128 ± 12	123 ± 12	96 ± 15

Table 5-3: Adult glucose tolerance test results

*UN=un-supplemented. S=supplemented. Glucose=G. Insulin=I. [a] =includes only those animals in which plasma glucose was below baseline within 3 hours [n]. Area under the curve in first 15 minutes=AUC_{15}. * p<0.05 preterm compared to term-spont. θ p<0.01 term-Alizin compared to term-spont. Data are least square means ± SEM. TrGp x S p<0.06 for the interaction birth group x supplement.*

5.4.3.3. Induction of labour at term and glucose tolerance

In males there was no effect of induction of labour at term on plasma insulin and glucose concentrations at baseline or during a GTT, or on any measures of glucose tolerance (Table 5-3).

In females, term-Alizin sheep had a greater glucose AUC and insulin AUC: glucose AUC during first fifteen minutes of the GTT than term-spont females.

5.4.3.4. Correlations between size at birth, growth rates, adult weight and glucose tolerance

Birth weight z-score, $GV_{2\text{-}W}$, $GV_{W\text{-}1Yr}$ and fat mass did not correlate with any GTT outcomes in any sheep.

Adult weight was positively correlated with glucose AUC in the first 15 minutes of the GTT in term-spont males and preterm sheep of both sexes (for every 1 Kg increase in adult weight: term-spont male; R^2=0.5, β coefficient 0.8 ± 0.2, p=0.001, preterm male; R^2=0.5, β coefficient 0.8 ± 0.2, p<0.001, preterm female; R^2=0.5, β coefficient 1.2 ± 0.5, p=0.002).

$GV_{0\text{-}2}$ tended to correlate with glucose AUC during the first 15 minutes of the GTT ($AUC_{15\text{-}G}$) in male term-Alizin sheep only (for every 10 g /Kg·d in $GV_{0\text{-}2}$; R^2=0.9, β coefficient 1.1 ± 0.2, p=0.004).

$GV_{0\text{-}TEA}$ and $GV_{TEA\text{-}W}$ in preterm sheep were not correlated with GTT outcomes in either sex.

5.4.4. Insulin sensitivity and secretion in adult sheep

5.4.4.1. Preterm birth and insulin sensitivity and secretion

Preterm birth did not affect plasma insulin and glucose concentrations during a HGC (Figure 5-4), or measures of insulin sensitivity or secretion (Table 5-4). The effect of preterm birth was unchanged in the adjusted model, with or without inclusion of measures of growth velocity.

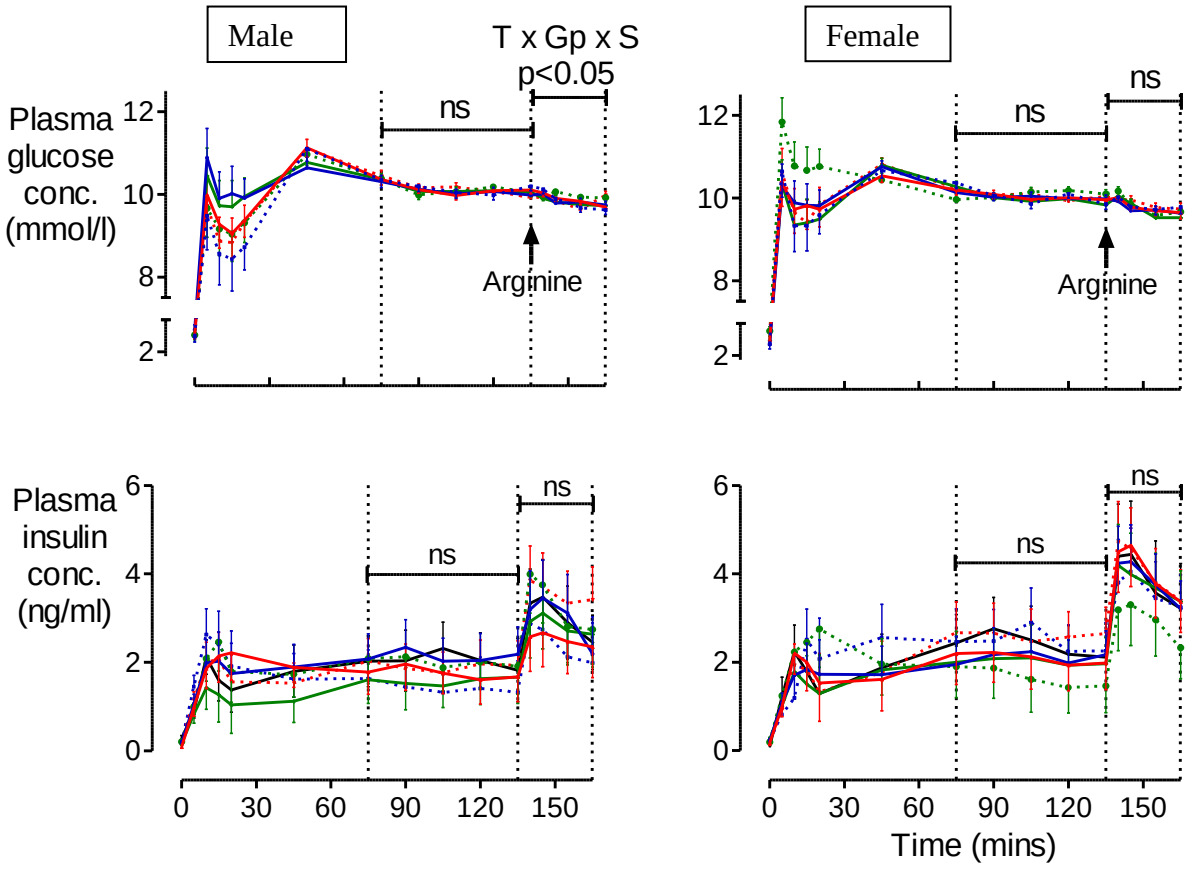

Figure 5-4: Insulin and glucose responses during an adult hyperglycaemic clamp

T·Gp·S=interaction term for birth group by supplement over time. Conc.=concentration. Term-spont: blue; un-supplemented (UN; continuous line) male n=8, female n=11, supplemented (S; interrupted line) male n=6, female n=8. Preterm: red; UN male n=10, female n=9, S male n=10, female n=9. Term-dex: green; UN male n=9, female n=9, S male n=8, female n=9. Term-Alizin: black; male n=6, female n=4. Data are least square means ± SEM.

5.4.4.2. Nutritional supplementation and insulin sensitivity and secretion

Supplementation did not affect plasma insulin and glucose concentrations at baseline or during a HGC (Figure 5-4). In males, there was an interaction between birth group and supplementation over time on plasma glucose concentrations following arginine administration (Figure 5-4); this was not significant on *post hoc* testing. Supplementation did not affect measures of insulin sensitivity or secretion in either sex (Table 5-4).

5.4.4.3. Induction of labour at term and insulin sensitivity and secretion

Induction of labour at term did not affect plasma insulin or glucose concentrations at baseline or during a HGC (Figure 5-4), or measures of insulin sensitivity or secretion in either sex (Table 5-4).

5.4.5. Relationships between size at birth, growth rates, adult weight and fat mass and insulin sensitivity and secretion

Birth weight z-score, growth velocity ($GV_{0\text{-}2}$, $GV_{2\text{-}W}$ or $GV_{W\text{-}1Yr}$), and adult fat mass were not correlated with Si or DI in any sheep.

Adult weight tended towards a negative correlation with Si in male term-spont sheep only (for every 1 Kg increase in adult weight; R^2=0.4, β coefficient -0.6 ± 0.2, p=0.009).

In preterm sheep, neither $GV_{0\text{-}TEA}$ nor $GV_{TEA\text{-}W}$ was correlated with DI or Si in either sex.

MALE	Term-spont		Preterm		Term-dex		Term-Alizin
	UN (n=8 [8])	S (n=6 [6])	UN (n=10 [10])	S (n=10 [10])	UN (n=9 [9])	S (n=8 [8])	UN (n=6 [6])
SS_G infusion rate (mmol/Kg·min)	26 ± 3	27 ± 3	23 ± 2	20 ± 3	21 ± 3	23 ± 3	24 ± 4
SS_I (ng/ml)	2.1 ± 0.6	1.4 ± 0.7	1.7 ± 0.5	1.9 ± 0.5	1.6 ± 0.5	2.0 ± 0.6	2.0 ± 0.6
Log Si	2.7 ± 0.2	3.0 ± 0.2	2.9 ± 0.2	2.5 ± 0.2	2.8 ± 0.2	2.6 ± 0.2	2.5 ± 0.2
Log DI	4.9 ± 0.2	5.7 ± 0.3	5.4 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	5.1 ± 0.2	4.8 ± 0.3
Log Arg_I	1.2 ± 0.2	1.1 ± 0.2	0.8 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.3 ± 0.2	1.2 ± 0.2
Log AUC Arg_I	3.2 ± 0.2	3.2 ± 0.2	3.0 ± 0.2	3.6 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.3 ± 0.2
FEMALE	UN (n=11 [10])	S (n=8 [8])	UN (n=9 [8])	S (n=9 [9])	UN (n=9 [9])	S (n=9 [9])	UN (n=4 [4])
SS_G infusion rate (mmol/Kg·min)	26 ± 2	26 ± 3	28 ± 3	29 ± 3	27 ± 3	25 ± 3	25 ± 5.1
SS_I (ng/ml)	2.1 ± 0.6	2.5 ± 0.7	2.1 ± 0.6	2.6 ± 0.6	2.0 ± 0.6	1.7 ± 0.6	2.4 ± 0.7
Log Si	2.6 ± 0.2	2.7 ± 0.2	2.8 ± 0.2	2.7 ± 0.2	2.7 ± 0.2	3.0 ± 0.2	2.4 ± 0.3
Log DI	5.1 ± 0.2	5.1 ± 0.2	5.3 ± 0.2	5.3 ± 0.2	5.3 ± 0.2	5.5 ± 0.2	5.2 ± 0.4
Log Arg_I	1.4 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.0 ± 0.2	4.8 ± 1.2
Log AUC Arg_I	3.6 ± 0.2	3.5 ± 0.2	3.8 ± 0.2	3.6 ± 0.2	3.8 ± 0.2	3.4 ± 0.2	3.7 ± 0.4

Table 5-4: Adult hyperglycaemic clamp results

[n]=number of animals with age matched HGC and GTT for DI calculation. UN=un-supplemented. S=supplemented. G=glucose. I=insulin. SS=steady state hyperglycaemia. Si=insulin sensitivity. DI=disposition index. Arg_I =peak insulin response following arginine. AUC Arg=insulin area under the curve following arginine administration. Data are least square means ± SEM.

5.4.6. Correlation between juvenile and adult insulin sensitivity

Juvenile DI and Si both predicted their respective adult values. As there was no effect either of sex or birth group, data from all animals are presented together (Figure 5-5).

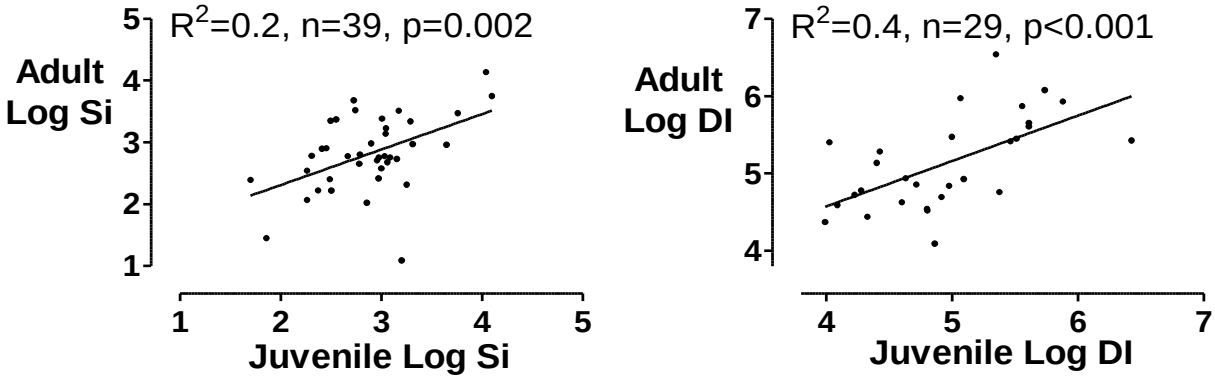

Figure 5-5: Correlation between juvenile and adult Si and DI

Si=insulin sensitivity. DI=disposition index. The p-value for each regression is shown on the specific graph.

5.5. Discussion

It was hypothesised that preterm birth would result in changes in glucose-insulin axis function that would be manifest as impaired glucose tolerance, especially in adult sheep. However, preterm birth *per se* had a minimal impact on glucose homeostasis. Early nutrient supplementation, however, had a significant impact on insulin secretion in response to both dextrose and arginine, although the nature of that impact depended on the gestational age at birth of the sheep, their sex and the age at which they were studied. In juvenile female term-spont sheep, supplementation resulted in an approximately 50% reduction in dextrose-stimulated and arginine-stimulated insulin secretion, whereas in sheep born preterm supplementation led to an approximately 50% increase in arginine-stimulated insulin secretion. In adult sheep, although the effect did not achieve statistical significance, the same pattern of altered insulin secretion in response to dextrose and arginine was found, but was observed in males and not females.

Early nutritional milieu therefore appears to have persistent effects on organ function that differ according to both the sex and the gestational age at which the nutritional intervention occurred. Many different nutrient supplements are used clinically, in addition to the many

different formulations of artificial milk that are commercially available. It is therefore possible that current strategies of nutritional support for preterm and term infants may have late effects that reflect the type of nutritional support offered rather than the gestational age at birth *per se.* Further research is therefore needed to explore the impact that early nutrition has on long term metabolic outcomes.

Preterm birth, nutritional supplementation and glucose homeostasis

During a glucose tolerance test (GTT) in both juvenile and young adult sheep there was no effect of birth group on the initial rise and subsequent fall in plasma glucose concentration, or on the insulin response provoked by hyperglycaemia. Similarly, measures of insulin sensitivity and glucose disposition were not significantly altered by preterm birth.

Data from human studies are conflicting, with some investigators finding no effect of preterm birth on later insulin sensitivity (Willemsen, et al., 2009), whereas others report associations between preterm birth and insulin resistance in children (Hofman, et al., 2004) and young adults (Dalziel, et al., 2007; Hovi, et al., 2007; Rotteveel, et al., 2008b).

It has been suggested that the disparity between these studies relates to the differences in methodology and data interrogation, and that differences ascribed to preterm birth relate more to postnatal growth patterns than to reduced gestation length *per se*. Willemsen *et al.* found that after adjusting for the effect of postnatal growth (by inclusion of adult height z-score in a multivariate regression analysis) truncal fat mass but not preterm birth or gestation length was a powerful determinant of insulin sensitivity (Willemsen, et al., 2009). In contrast, Rotteveel *et al.* found that as a group, adults born preterm were more insulin resistant than those born at term, and that although this relationship was attenuated by adjusting for the effect of adult height, weight or BMI, the relationship was not abolished (Rotteveel, et al., 2008b). Interestingly, when the analysis was limited to those born preterm, increments in height and weight during childhood were associated with increased insulin resistance. Similarly, individuals born preterm who did not experience postnatal growth restraint (birth weight and/or length above -2SD at birth, and above -2SD at term corrected age) were more insulin resistant than those with postnatal growth restraint (birth weight and/or length at birth above -2SD, and below -2SD at term corrected age), although differences between the two groups disappeared after adjusting for the effect of adult BMI. Others have shown that early dietary manipulation to promote growth in preterm infants improved developmental

outcomes (Lucas et al., 1990), but at the expense of increased risk of insulin resistance (Singhal, et al., 2003) in those that grew fastest.

Unlike human studies in which individuals born preterm tend to be smaller and lighter than those born at term, in our experimental paradigm, weight at the time of metabolic testing was not different between preterm and any group of term-born sheep. In our paradigm, the lack of effect of birth cohort on glucose tolerance may be a valid finding, that is, preterm birth is not associated with disturbed glucose-insulin axis function in later life. Alternatively, adequate postnatal growth *per se* may be more important than gestation length alone; in our paradigm preterm birth did not compromise juvenile or adult weight and this may therefore explain the lack of effect of birth group on insulin-glucose outcomes. Similar to the findings of Rotteveel *et al.*, increased weight within the preterm cohort was associated with reduced insulin sensitivity in juvenile females, and with reduced glucose clearance during the first 15 minutes of an adult GTT in both sexes, whereas in male term-spont sheep, increased weight correlated with reduced insulin sensitivity in juvenile and adults, and with reduced glucose clearance during the first 15 minutes of an adult GTT.

Therefore, although weight is correlated with insulin sensitivity and glucose tolerance, there does not appear to be a deleterious effect of preterm birth on insulin sensitivity or glucose tolerance. Whether this would remain true if postnatal growth failed to return those born preterm to their expected size is unknown.

Alternatively, differences between our paradigm and reports in the literature of insulin resistance following between preterm birth may be attributable to the early nutritional environment of preterm lambs compared to preterm infants. Within the first days of life preterm lambs were able to feed independently and therefore, like term-born lambs, exclusively consumed ewe milk and self regulated the timing and quantity of milk ingested. Preterm infants, especially those born very preterm, often receive either parenteral nutrition or dextrose solutions but may remain without enteral nutrition during the first days of life (Hans, Pylipow, Long, Thureen, & Georgieff, 2009). Establishment of enteral feeding in the newborn period is crucial for transition from fetal (and therefore continuous parenteral nutrition via the placenta) to postnatal (intermittent bolus enteral nutrition). Delay in initiating enteral feeds has been shown to attenuate insulin secretion in response to an intravenous dextrose load in piglets (Gentz, Persson, Kellum, Bengtsson, & Thorell, 1971); whether this has a similar effect in humans, and if so, what the long term consequences may

be is unknown. Enteral feeds given to preterm infants usually consist of a pre-determined volume of milk given at regimented intervals (Hans, et al., 2009). Imposition of artificial feeding regimes that do not allow the preterm infant to self-regulate feed intake may have implications for later self-regulation (Li, Fein, & Grummer-Strawn, 2010), feeding behaviours (Koletzko, von Kries, Monasterolo, et al., 2009) and weight gain (Li, Fein, & Grummer-Strawn, 2008), which in turn may contribute to the development of altered glucose-insulin homeostasis.

Glucose tolerance in later life may also reflect the type of milk consumed during infancy. Many preterm, even late preterm, infants do not receive breast milk as their first enteral feed (Hans, et al., 2009), and are less likely to receive breast milk as their sole or main milk source than term infants (Bonet et al., 2011; Merewood, Brooks, Bauchner, MacAuley, & Mehta, 2006; Wooldridge & Hall, 2003). Breast milk from mothers who deliver preterm has a different macronutrient composition to that from mothers who deliver at term (Bauer & Gerss, 2011) and is often fortified with additional protein, carbohydrate, fat, multivitamins and minerals (Hans, et al., 2009; Kuschel & Harding, 2004). It is therefore possible that effects ascribed to preterm birth reflect differences in early nutrition between preterm and term infants rather than shortened gestation length *per se.*

Effect of nutritional supplementation on glucose homeostasis

Nutritional supplementation predominantly altered glucose homeostasis in females, so that in juvenile term-spont females supplementation tended to increase insulin sensitivity but reduce insulin secretion in response to both glucose and arginine, whereas the converse was true in supplemented preterm females. By young adulthood these effects had largely disappeared.

In humans insulin sensitivity is influenced by sex (Murphy et al., 2004; Vital, Larrieta, & Hiriart, 2006) with females at a higher risk of insulin resistance and diabetes than males. In sheep, perinatal events also result in sex specific patterns of glucose tolerance, although the direction and extent depend on the nature of the original insult (Poore, et al., 2007; Todd, et al., 2009). It is possibly not surprising, therefore, that the effects of supplementation on glucose homeostasis were most apparent in females. The temporal changes in insulin responses may represent supplement-induced alteration in beta call number or function that ‘recovers’ or compensates with time. With aging, other studies have identified a progressive decline in beta cell function with commensurate loss of glycaemic control (Cowie et al.,

2009), possibly mediated by a shortened beta-cell lifespan (Manesso, Toffolo, Butler, Butler, & Cobelli, 2011). Our animals were studied in early adulthood, and therefore were perhaps too young for age related decompensation in beta-cell function to be apparent.

All preterm and term animals in our paradigm exclusively received ewe milk within the first hours of life and supplementation (in addition to ewe milk) after 24 hours. Supplemented sheep in all groups received more calories, protein and fat in the newborn period than un-supplemented sheep, yet the effect of supplementation differs between preterm and term-born animals. The enteroinsular axis describes the signalling pathways that exist between the gut and the pancreatic islets that regulate nutrient-stimulated insulin secretion via hormones known as 'incretins' such as gastric inhibitory peptide (GIP) and glucagon-like peptides (GLP) (Miyawaki et al., 1999; Scrocchi et al., 1996). These potentiate insulin secretion such that glucose administered enterally provokes a substantially greater insulin response than an equivalent dose of glucose administered by intravenous infusion (Unger & Eisentraut, 1969), and this augmented insulin secretion can be further modified by the background nutritional milieu (Ahren, Winzell, & Pacini, 2008). GIP and GLP activity is abnormal in both type 2 diabetes (Nauck, 2011; Toft-Nielsen et al., 2001) and in normo-glycaemic first degree relatives of type 2 diabetics (who are therefore at increased risk of becoming diabetic themselves) (Meier et al., 2001).

Supplementation may have induced persistent changes in enteroinsular axis or incretin hormone function, especially as GLP has been implicated in the pancreatic β cell remodelling that occurs in the newborn period (Stoffers, 2004). Infants fed formula milk have higher postprandial plasma insulin and GIP concentrations than breast fed infants, in both the newborn period (Lucas, et al., 1981) and later in infancy (Salmenpera et al., 1988) although it is unknown whether this reflects the different macronutrient or calorie composition between milks, or other constituents of milk. Additionally, preterm infants have increased pre- and postprandial GLP 1 and 2 concentrations compared to term infants or children, with a positive correlation between calorie intake and GLP 1 and 2 concentrations (Padidela, Patterson, Sharief, Ghatei, & Hussain, 2009). If early dietary exposure alters beta-cell and pancreatic development, this might explain the recent observation that humans fed formula milk rather than breast milk as infants have higher rates of type 2 diabetes and markers of insulin resistance in adulthood (Owen, Martin, Whincup, Smith, & Cook, 2006). A change in incretin hormones in response to dietary manipulation is also reported in animal paradigms: GIP in lambs increases in response to fat ingestion (Martin & Faulkner, 1994), whereas in rat

pups, milk protein increases plasma GLP-2 concentrations and promotes gut maturation (Izumi et al., 2009).

'Fuel-mediated teratogenesis' is a construct in which the infants of diabetic mothers are exposed to increased levels of mixed nutrients, especially glucose; the resultant increase in insulin exposure is postulated to have adverse immediate and long-term consequences for the health of the offspring. Clinical studies have shown that infants of diabetic mothers have increased rates of developing diabetes themselves (Simeoni & Barker, 2009). Although the diabetic fetal environment may play an important part in mediating this relationship, the altered glucose and insulin content of breast milk from diabetic mothers may also be important in mediating this association (Jovanovic-Peterson, Fuhrmann, Hedden, Walker, & Peterson, 1989). Amongst term-born offspring of diabetic women, a dose-dependent effect has been described between infants' consumption of maternal milk and banked non-diabetic breast milk and risk of overweight and impaired glucose tolerance at age 2 years; those infants in the tertile with greatest diabetic milk consumption for the first week of life had a significantly greater risk of adverse metabolic profile than those in the lowest tertile (Plagemann, et al., 2002). This effect, however, was restricted to the first week of life as neither volume of diabetic milk in the late neonatal period (weeks 2-4), or total duration of breast feeding had an impact on later risk of overweight or impaired glucose tolerance at age 2 (Rodekamp, et al., 2005). It is hypothesized that the altered macronutrient exposure during a brief postnatal window has prolonged effects on metabolic function, but the importance or persistence of these changes over time is unknown. These data are congruent with our observation of reduced glucose and arginine-stimulated insulin secretion in supplemented (and therefore exposed to increased carbohydrate load in addition to maternal milk) juvenile term-spont sheep.

Taken together these data suggest that the first weeks after birth have an important role in determining metabolic function, even at a time far removed. Entero-insular insular axis function may be modified by preterm birth and by dietary intake in the newborn period. Although speculative, observed divergence of insulin secretory responses to glucose and protein in juvenile animals may reflect persistent changes in enteroinsular axis function, β cell function or insulin signalling pathways arising as a consequence of increased protein or calorie load in mature or immature animals. How the changes conferred by perinatal nutrition impact on the normal age associated decline in beta cell function is uncertain.

Effect of growth and adiposity on glucose homeostasis

The nutritional intervention in this experiment was intended to accelerate early weight gain, and therefore amplify any effects of early growth on later glucose homeostasis. However, despite these expectations, supplementation in lambs did not promote growth, and we did not find that growth velocity from any of the epochs studied correlated significantly with measures of glucose tolerance or insulin sensitivity.

In humans, wide variations in diet, eating and exercise patterns are evident even in young children (Craig, McNeill, Macdiarmid, Masson, & Holmes, 2010; Huh et al., 2011; Isacco et al., 2010), all of which have implications for growth and adiposity and result in a wide range of body size. Increased weight or BMI (Ong, et al., 2004), especially if preceded by low birth weight (Barker, Osmond, Kajantie, & Eriksson, 2009) has a deleterious effect on glucose homeostasis, although trying to isolate discrete epochs during which growth rates have a dominant effect on later outcomes has yielded conflicting results (Eriksson, 2011; Levy-Marchal et al., 2010). Experimental animals were maintained in 2 single-sex flocks, and although we did not investigate feeding behaviours, pasture-fed sheep are gregarious and move together as a feeding flock (Pillot et al., 2010), making it likely that sheep in our paradigm were exposed to homogeneous feed availability, and similar levels of physical activity. Unlike humans, there was little variation in growth velocity either within each cohort, or between cohorts. It is therefore possible that the experiment was underpowered to detect any correlations between growth velocity *per se* and measures of glucose homeostasis. Overall, data suggest that future diabetic risk is less if growth tracks along the anticipated curve and does not rise through percentiles.

In addition, the relationships between glucose tolerance, insulin sensitivity, growth and adiposity may alter with age, and with different patient populations (for instance, those born preterm or growth restricted compared to those born at full term or of normal birth weight). Prospective follow-up studies in normal weight children have shown a positive correlation between insulin resistance in childhood and fat mass in adolescence (Labayen et al., 2011) suggesting that insulin resistance predates adiposity and is not necessarily a consequence of it. Conversely, at 2 years of age, children born SGA are more insulin sensitive than those born AGA, but by 4 years of age, although weight and BMI are not different, have developed increased central adiposity and are insulin resistant compared to those born AGA (Ibanez, et al., 2006). Finally, insulin resistant adults have lesser weight gain over several years of

follow-up than those that are more insulin sensitive (Swinburn et al., 1991). Although we found that insulin sensitivity was correlated in juvenile animals and adult animals, future studies need to include assessment of insulin sensitivity and body composition in the newborn period, and at an older age than presented here in order to clarify longitudinal changes in insulin sensitivity, adiposity and growth, especially within groups at risk of altered postnatal growth trajectories.

Size at birth and glucose homeostasis

In juvenile preterm females, those with a relatively low birth weight (low birth weight z-score) had reduced clearance of glucose following a dextrose bolus, but this effect was transitory and no longer apparent in adulthood. No other effect of relative size at birth on measures of glucose tolerance or insulin sensitivity was found, suggesting that in this cohort at least, small size at birth is not a major determinant of later glucose homeostasis.

These findings are contrary to those of many human and animal studies in which fetal growth restriction and consequent small size at birth initially is associated with increased insulin sensitivity (Bazaes, et al., 2003; De Blasio, Gatford, McMillen, et al., 2007; Setia, et al., 2006). Later in life, this profile is reversed and those small at birth become relatively insulin resistant (Gatford, et al., 2008; Hales, et al., 1991; Ibanez, et al., 2006). In the current study, lambs were born from well nourished, healthy, multiparous ewes and consequently had a narrow range of birth weight z-score, which was therefore treated as a continuous variable. A greater range in size at birth in other studies has allowed birth weight to be treated as a dichotomous variable (small or appropriate for gestational age in human studies, or selected on the basis of a predetermined low birth weight in experimentally derived IUGR). These differences in statistical methodology may explain the apparent absence of correlation between birth weight z-score and later outcomes. To further explore the effect of relative size at birth on later outcomes would require a greater range of birth weight z-scores in each experimental group than exists in the present experiment.

Effect of antenatal corticosteroids on glucose homeostasis

Antenatal corticosteroids at term also had no effect on either glucose tolerance or insulin sensitivity in juvenile or adult sheep. Intriguingly, unlike in term-spont sheep, there was no

correlation between weight and any measures of glucose or insulin axis function following term corticosteroid exposure in sheep of either sex.

Antenatal corticosteroid exposure has been associated with insulin resistance in adult humans although the effect size was small (Dalziel, et al., 2005). In sheep, maternal betamethasone at d104 of gestation is associated with increased insulin responses to a GTT at 6 months and 1 year of age (Moss, et al., 2001) and, although the effect of antenatal corticosteroids on insulin secretion appeared to lessen with age (Sloboda et al., 2005) was associated with an increase in hepatic glucose-6-phosphatase (G-6-Pase; a key regulatory enzyme in both gluconeogenesis and glycogenolysis) in 3 year old animals. Continuous maternal infusions of corticosteroids between 26-28d gestation in sheep also impairs glucose homeostasis in adulthood. However, in both experiments corticosteroid exposure was at a much earlier stage of gestation when effects on the developing pancreas may be different to those on a more mature organ (Sangild, 2006).

Induction of labour on glucose homeostasis

Induction of labour *per se* had minimal effect on any glucose or insulin responses to a GTT or HGC at either age. Although adult female term-Alizin had lower first phase glucose but higher insulin: glucose responses during a GTT than term-spont sheep, no other outcome measures from the GTT at either age were different between term-Alizin and un-supplemented term-spont or term-dex sheep. Repetition of the experiment with greater animal numbers would determine if this was a valid result or statistical type 1 error consequent on small animal numbers.

Limitations of the study

This experiment was designed on the premise that additional nutrients in the newborn period would have similar effects to that of breast milk fortifier for human infants and accelerate early weight gain and growth. This would enable us to examine in a standardised animal paradigm the association found by Singhal *et al.* between rapid early growth in preterm infants and markers for later insulin resistance (Singhal, et al., 2003). Supplementation, however, did not promote growth, and had other, unanticipated consequences on growth.

Graphical representation of the data suggested that supplementation had more effects on glucose and insulin homeostasis than were statistically significant. It is possible that animal

numbers within each of the intervention arms, especially in adulthood, were insufficient to detect differences, and therefore the study was underpowered.

Reducing the gestational age at birth of our preterm lambs, or increasing the age at which glucose homeostasis was tested may unmask differences ascribable to preterm birth.

Conclusions

We hypothesised that preterm birth would alter later glucose tolerance and insulin sensitivity; we have not found this to be so. However, although these data relate to sheep, and not humans, it is reassuring for the majority of preterm infants who are born at late-preterm gestations that in this experimental paradigm preterm birth does not have a deleterious effect on later glucose homeostasis. Further studies of the effect of preterm birth on glucose homeostasis need to clearly identify the late-preterm group, and to clarify or standardise feed modality so that the effects of gestation length can be separated from potential confounding factors. In addition, sufficient numbers of subjects need to be recruited to ensure that statistical power is adequate to detect sex-specific effects.

Finally, only two weeks of supplementing maternal milk with a product comparable to human breast milk fortifier has a significant effect on glucose and insulin axis function months after cessation of the intervention. These data highlight the need for more detailed studies of feeding modality on later outcomes in human infants. In addition they reinforce the concept that for any given intervention, effects on preterm and term infants may differ substantially.

Chapter 6. Cardiovascular outcomes

6.1. Introduction

Small size at birth has been linked with an adverse cardio-metabolic profile in later life (Risnes et al., 2011). Whether this association is mediated through the effects of fetal growth restriction, preterm birth or postnatal growth trajectory remains unclear. While survivors of preterm birth in a contemporary perinatal setting are still too young for most cardiovascular disease to be manifest (Tate, et al., 1998), they may represent a particularly vulnerable population (Koupil, Leon, & Lithell, 2005).

To improve neonatal outcomes, practice guidelines published by the American College of Obstetrics and Gynaecology recommend the administration of maternal corticosteroids to women at risk of delivery below 34 weeks gestation (American College of Obstetricians and Gynecologists, 2003, 2007). Whether antenatal corticosteroid exposure has an impact on later cardiovascular function is uncertain.

Independent predictors of adverse cardiovascular outcomes in adulthood include elevated blood pressure (BP), an elevated resting heart rate (HR), and heart rate variability (HRV) parameters that reflect either an increase in cardiac sympathetic function (LFnu), sympatho-vagal balance (LF/HF) or reduced cardiac parasympathetic function (SDΔNN, NN50%; Chapter 1, pg 18)

This experiment aimed to address the effect of preterm birth, antenatal corticosteroid exposure and postnatal growth trajectory on BP and autonomic indices of cardiovascular function, and responses to an adrenergic stimulus. Associations between cardiovascular outcomes and measures of size or body composition and baseline plasma cortisol concentrations were also investigated.

6.2. Methods

In both juvenile and adult sheep arterial BP was recorded and electrocardiograms (ECGs) were performed to yield measures of HR and HRV (2.15.1).

Although adrenaline stimulation tests (2.13.3) were performed on all juvenile animals, only those conducted in preterm and term-spont sheep were analysed due to laboratory constraints.

Blood sampling for morning cortisol concentrations was performed in 8-10 sheep of each birth group, sex and supplement status, randomly selected from within each group.

Associations between DXA derived measures of fat mass and cardiovascular outcomes in adulthood were studied in animals with a fat mass above the minimum detection threshold of the DXA scanner.

The effect of gestational age at birth across the normal gestational age range was assessed in term-spont animals.

6.3. Statistical analysis

Non-parametric data were log transformed prior to further analysis.

Males and females were analysed separately as growth and cardiovascular outcomes are known to be different between the sexes.

Parametric data were analysed by factorial ANOVA with Tukey *post hoc* test. First, the effects of preterm birth and supplementation on outcomes of interest were assessed in term-spont, preterm and term-dex sheep. Second, the effects of corticosteroid induction of labour at term were assessed in un-supplemented term-spont, term-dex and term-Alizin sheep. Comparisons between nominal variables were made using the χ^2 test. Statistical significance was set at a p-value of <0.05 for *post hoc* tests of the primary analyses and at a p value of <0.01 for secondary analysis of birth group unless otherwise indicated.

Multivariate regression models were then constructed to assess whether the effects of birth group were modified by the inclusion of other perinatal or postnatal variables (adjusted model: birth group, birth weight (bwt) z-score, supplementation status, weight and age at the time of assessment, with the interaction term 'birth group' crossed with each of the other included variables). Single measures of GV from each main epoch ($GV_{0\text{-}2}$, $GV_{2\text{-}W}$ and in adult sheep $GV_{W\text{-}1Yr}$) were then included in the adjusted model to see whether growth rates further modified the effect of birth group. Additionally for adult sheep, the adjusted model contained either weight or weight-for-length or % fat mass, as detailed in each section.

Correlations between continuous variables were examined by regression analysis within each birth group, in same sex animals. Statistical significance was set at a p-value of 0.001 to allow for the effect of multiple comparisons.

6.4. Cardiovascular results

For details of animal characteristics at the time of juvenile and adult studies, see Chapter 3.

6.4.1. Juvenile cardiovascular challenges

6.4.1.1. Effect of preterm birth on juvenile HRV and BP

In males, heart rate and blood pressure were not different amongst groups. Male preterm sheep had a higher LFnu than term-spont and term-dex sheep (Table 6-1). Log LF/HF (p=0.07) tended to be greater, and Log NN50% (p=0.07) tended to be less, in preterm compared to term-spont or term-dex sheep (Table 6-1). In females, preterm sheep had an increased resting heart rate compared to term-spont or term-dex sheep (Table 6-2) but there were no other effects of birth group on HRV or BP.

In male sheep the effect of birth group on HRV was stronger in the adjusted analyses (LFnu, preterm vs. term-spont and term-dex p=0.01. For the overall effect of birth group: Log LF/HF p=0.06; Log NN50% p=0.06; Log SDΔNN p=0.08). After addition of $GV_{0\text{-}2}$ to the model, the effect of preterm birth on LFnu and Log NN50% was unchanged, and the effect of preterm birth on Log LF/HF ($p<0.05$ compared to term-spont) was strengthened. Inclusion of $GV_{2\text{-}W}$ to the adjusted model weakened the effect of birth group on HRV parameters such that there were no longer any statistically significant effects or trends.

The effect of birth group on HRV parameters in females was not changed in the adjusted model, with or without inclusion of $GV_{0\text{-}2}$ or $GV_{2\text{-}W}$.

MALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN (HRV: n=9) (BP: n=5)	S (HRV: n=10) (BP: n=4)	UN (HRV: n=7) (BP: n=6)	S (HRV: n=7) (BP: n=3)	UN (HRV: n=8) (BP: n=5)	S (HRV: n=9) (BP: n=4)	UN (HRV: n=5) (BP: n=5)
Heart rate (bpm)		96 ± 7	106 ± 7	122 ± 8	115 ± 8	105 ± 8	114 ± 7	111 ± 10
Log SDΔNN		3.6 ± 0.3	3.3 ± 0.3	2.8 ± 0.3	2.9 ± 0.3	3.3 ± 0.3	3.5 ± 0.3	3.2 ± 0.4
Log NN50%	***Tr-Gp***	2.1 ± 0.6	1.1 ± 0.6	0.1 ± 0.7	0.3 ± 0.7	1.6 ± 0.7	1.6 ± 0.6	2.5 ± 0.9
HF (nu)	***Gp x S***	28 ± 3	20 ± 3	22 ± 4	24 ± 4	19 ± 3	29 ± 3	20 ± 4
LF (nu)	*** ‡**	30 ± 7	42 ± 7	59 ± 8	58 ± 8	40 ± 8	35 ± 7	52 ± 11
LF/HF	***Tr-Gp***	-0.1 ± 0.3	0.7 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	0.6 ± 0.3	0.0 ± 0.3	0.9 ± 0.4
SBP (mmHg)		113 ± 5	104 ± 6	109 ± 5	111 ± 7	122 ± 5	118 ± 6	112 ± 6
DBP (mmHg)		64 ± 4	61 ± 5	63 ± 4	65 ± 5	64 ± 4	64 ± 5	58 ± 4
MAP (mmHg)		79 ± 5	76 ± 5	80 ± 4	80 ± 6	82 ± 5	83 ± 5	75 ± 4

Table 6-1: Effect of birth group and supplementation on HRV and BP in juvenile male sheep

*UN=un-supplemented. S=supplemented. Systolic (SBP) diastolic (DBP) and mean (MAP) arterial blood pressure. Data are least square means ± SEM. * p<0.05 preterm compared to term spont. ‡ p<0.05 preterm compared to term-dex. Tr-Gp p=0.07 for the effect of birth group. Gp x S p<0.05 for the effect of the interaction between birth group and supplementation.*

FEMALE		Term-spont		Preterm		Term-dex		Term-Alizin
		UN (HRV: n=5) (BP: n=2)	S (HRV: n=9) (BP: n=5)	UN (HRV: n=8) (BP: n=6)	S (HRV: n=7) (BP: n=3)	UN (HRV: n=4) (BP: n=3)	St (HRV: n=8) (BP: n=6)	UN (HRV: n=5) (BP: n=)
Heart rate (bpm)	* ‡	107 ± 5	101 ± 7	123 ± 5	122 ± 6	111 ± 8	96 ± 5	86 ± 5
Log SDΔNN		3.5 ± 0.3	3.9 ± 0.4	3.3 ± 0.3	3.3 ± 0.3	3.0 ± 0.5	3.3 ± 0.3	4.0 ± 0.3
Log NN50%		2.1 ± 0.7	1.9 ± 0.9	1.8 ± 0.7	1.3 ± 0.7	0.8 ± 0.9	1.8 ± 0.7	2.9 ± 0.7
HF (nu)		20 ± 3	23 ± 4	24 ± 3	21 ± 3	22 ± 4	26 ± 3	23 ± 3
LF (nu)		35 ± 8	32 ± 10	45 ± 8	38 ± 9	56 ± 11	36 ± 8	37 ± 8
LF/HF		0.4 ± 0.3	0.1 ± 0.4	0.5 ± 0.3	0.6 ± 0.3	0.8 ± 0.4	0.3 ± 0.3	0.4 ± 0.2
SBP (mmHg)		108 ± 5	107 ± 8	112 ± 4	108 ± 6	111 ± 6	118 ± 4	103 ± 3
DBP (mmHg)		57 ± 2	56 ± 4	64 ± 2	59 ± 3	62 ± 3	59 ± 2	58 ± 3
MAP (mmHg)		74 ± 3	73 ± 4	81 ± 2	76 ± 3	79 ± 3	77 ± 2	73 ± 2

Table 6-2: Effect of birth group and supplementation on HRV and BP in juvenile female sheep

*UN=un-supplemented. S=supplemented. Systolic (SBP) diastolic (DBP) and mean (MAP) arterial blood pressure. Data are least square means ± SEM. * p<0.05 preterm compared to term spont. ‡ p<0.05 preterm compared to term-dex.*

6.4.1.2. Effect of nutrient supplementation on juvenile HRV, BP

Nutrient supplementation *per se* did not alter HRV or BP outcomes (Table 6-1, Table 6-2).

There was a significant interaction between birth group and supplementation on HFnu in male sheep (birth group x supplement effect, p=0.01), with supplementation associated with reduced HFnu in term-spont lambs, but increased HFnu in term-dex, with no effect in preterm lambs (Table 6-1).

6.4.1.3. Effect of induction of labour at term on juvenile HRV, BP

Term-Alizin sheep were not different with respect to any HRV or BP parameters from term-spont or term-dex sheep (Table 6-1, Table 6-2). The effect of birth group was not changed in the adjusted model, with or without inclusion of measures of GV.

6.4.2. Metabolic responses to an adrenergic stimulus in juvenile sheep

6.4.2.1. Preterm birth and the metabolic response to an adrenergic stimulus

Preterm male sheep had a lower glucose, but not free fatty acid (FFA), area under the curve ($GAUC_{ADR\text{-}Tot}$) in response to an adrenergic stimulus than term-spont males ($p<0.05$, Table 6-3), whereas preterm females trended towards a lower FFA AUC than term-spont females ($FAUC_{ADR\text{-}Tot}$, p=0.06).

In the adjusted model, preterm male sheep had a lower glucose area under the curve in the first 15 minutes ($GAUC_{ADR\text{-}15,}$ p=0.02), and overall ($GAUC_{ADR\text{-}TOT}$, p=0.01) after an adrenaline bolus, and tended to have a lower increment in peak glucose concentration (Δ glucose, p=0.06) than term-spont male sheep. The effect of preterm birth in female sheep was not altered in the adjusted model.

MALE		Term-spont		Preterm	
		UN (n=9)	S (n=8)	UN (n=9)	S (n=8)
$FAUC_{ADR-15}$	*TrG x S*	2.0 ± 0.3	1.0 ± 0.4	1.1 ± 0.3	1.4 ± 0.3
$FAUC_{ADR-Tot}$	*Gp x S*	2.9 ± 0.5	1.5 ± 0.6	1.3 ± 0.5	2.1 ± 0.6
Δ FFA	*TrG x S*	0.21 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.14 ± 0.02
$GAUC_{ADR-15}$	*TrG x S*	11.9 ± 1.1	8.5 ± 1.4	7.4 ± 1.2	8.7 ± 1.4
$GAUC_{ADR-Tot}$	*	31.2 ± 2.3	25.5 ± 2.9	22.9 ± 2.6	22.4 ± 2.9
Δ glucose	*TrG x S*	1.1 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
FEMALE		Term-spont		Preterm	
		UN (n=8)	S (n=7)	UN (n=8)	S (n=8)
$FAUC_{ADR-15}$		1.8 ± 0.5	3.1 ± 0.5	1.7 ± 0.5	1.6 ± 0.5
$FAUC_{ADR-Tot}$	*Tr**	2.7 ± 0.6	4.4 ± 0.7	2.4 ± 0.6	2.0 ± 0.6
Δ FFA		0.19 ± 0.04	0.30 ± 0.04	0.18 ± 0.04	0.16 ± 0.04
$GAUC_{ADR-15}$	*TrG x S*	7.3 ± 0.9	10.2 ± 1.1	9.5 ± 1.0	8.9 ± 1.1
$GAUC_{ADR-Tot}$		22.3 ± 4.8	35.5 ± 5.8	31.8 ± 5.4	29.6 ± 5.8
Δ glucose		0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1

Table 6-3: Effect of preterm birth and supplementation on the free fatty acid and glucose responses to an adrenergic stimulus

*UN=un-supplemented. S=supplemented. Maximal increase in plasma free fatty acid (Δ FFA) or glucose (Δ glucose) concentrations. Areas under the curve (AUC) for free fatty acid and glucose during the first 15 minutes ($FAUC_{ADR-15}$, $GAUC_{ADR-15}$), or throughout the test ($FAUC_{ADR-TOT}$, $GAUC_{ADR-TOT}$) following an adrenergic stimulus. * p<0.05 preterm compared to term-spont. Tr* p<0.1 preterm compared to term-spont. Gp x S p<0.05, TrGp x S p<0.1 for the interaction between birth group and supplement. Data are least square means ± SEM.*

6.4.2.2. Nutrient supplementation and the metabolic response to an adrenergic stimulus

Nutrient supplementation *per se* did not affect the metabolic response to an adrenergic stimulus.

In male sheep there were trends towards an interaction between birth group and supplement on the FFA and glucose responses to an adrenergic stimulus (ΔFFA, p=0.07; $FAUC_{ADR-15}$, p=0.06; $FAUC_{ADR-Tot}$, p=0.05; Δ glucose, p=0.06; $GAUC_{ADR-15}$, p=0.08) (Table 6-3); in term spont males FFA and glucose responses tended to be less in supplemented than un-supplemented sheep whereas in preterm males FFA and glucose responses tended to be greater in supplemented than un-supplemented sheep (Table 6-3).

In female sheep there was a trend towards an interaction between birth group and supplement on $GAUC_{ADR-15}$ (p=0.09); inspection of the data suggests glucose responses to adrenaline were increased in supplemented term-spont sheep, but reduced in supplemented preterm sheep (Table 6-3).

Thus, the effect of supplementation on the metabolic response to adrenergic stimulus appeared to be sexually dimorphic, with the appearance of more pronounced effects in term-spont than preterm sheep (Table 6-3).

6.4.3. Correlations between size at birth, growth rates and weight at the time of cardiovascular assessment and HRV and BP in juvenile sheep

Birth weight z-score, $GV_{0\text{-}2,}$ $GV_{2\text{-}W}$ or weight and age at the time of cardiovascular assessment were not correlated with HRV or BP in male or female sheep from any birth group. Within the preterm group $GV_{0\text{-}TEA}$ was not associated with either HRV or BP, whereas $GV_{TEA\text{-}W}$ was inversely associated with blood pressure but not HRV parameters. As there was no interaction between sex and GV, data from both sexes are presented together (Table 6-4).

	R^2	β coefficient (effect on BP per 1 g /Kg·d increase in $GV_{0\text{-}TEA}$)	p-value	R^2	β coefficient (effect on BP per 1 g /Kg·d increase in $GV_{TEA\text{-}W}$)	p-value
SBP (mmHg)	<0.1	0.30 ± 0.26	0.3	0.5	-3.77 ± 0.93	0.001
DBP (mmHg)	0.2	0.28 ± 0.15	0.08	0.3	-1.73 ± 0.68	<0.05
MAP (mmHg)	0.2	0.30 ± 0.17	0.08	0.5	-2.36 ± 0.66	<0.01

Table 6-4: Correlations between growth and blood pressure in juvenile lambs born preterm

Systolic (SBP), diastolic (DBP) and mean (MAP) arterial blood pressure in juvenile sheep born preterm (n=18). Growth velocity=GV. $_{0\text{-}TEA}$=birth to term equivalent age (TEA). $_{TEA\text{-}W}$=TEA to weaning.

6.4.4. Correlations between the metabolic response to an adrenergic stimulus and HRV or BP in juvenile sheep

In male and female sheep, both the fatty acid and glycaemic response to an adrenergic stimulus were positively related to indices of parasympathetic function (Log SDΔNN, Log NN50%, HFnu) but inversely related to indices of sympathetic function and sympatho-vagal balance (HR, LFnu, LF/HF) (Table 6-5). There were no associations between the metabolic responses to an adrenergic stimulus and blood pressure.

6.4.5. Correlations between insulin sensitivity, glucose disposition and HRV

Ten preterm female sheep had ECG data and known insulin sensitivity (Si) or glucose disposition index (DI) (Chapter 5.4.2). Both Si and DI were positively correlated with indices of sympathetic activity (LFnu, Log LF/HF), and negatively correlated with indices of parasympathetic activity (Log SDΔNN, Log NN50%) (Figure 6-1). There were insufficient numbers of animals in other birth groups with Si or DI and HRV data to assess correlations that may exist between them (preterm male n=2, term-spont female n=4, term-spont male n=5).

	$FAUC_{ADR-15;}$			Δ FFA			$GAUC_{ADR-15}$			Δ glucose		
	R^2	β coefficient (effect per 1 mmol/L·min increase)	p-value	R^2	β coefficient (effect per 0.1 mmol/L increase)	p-value	R^2	β coefficient (effect per 1 mmol/L·min increase)	p-value	R^2	β coefficient (effect per 1 mmol/L increase)	p-value
MALE (n=26)												
HR (bpm)	0.2	-12 ± 4	0.01	0.2	-13 ± 5	0.01	0.2	-3 ± 1	0.01	0.2	-31 ± 12	0.01
Log SDΔNN	<0.1	0.2 ± 0.1	0.2	<0.1	0.2 ± 0.2	0.2	02	0.08 ± 0.03	<0.05	0.1	0.9 ± 0.5	0.07
Log NN50%	<0.1	0.3 ± 0.3	0.4	<0.1	3.4 ± 3.5	0.4	0.2	0.2 ± 0.1	<0.01	0.2	2.1± 0.8	<0.05
LF (nu)	<0.1	-6 ± 4	0.2	0.1	-8 ± 4	0.07	0.3	-3 ± 1	<0.01	0.2	-28 ± 12	<0.05
LF/HF	0.1	-0.27 ± 0.15	0.07	0.2	-3.85 ± 1.78	<0.05	0.3	-0.12 ± 0.04	<0.01	0.2	-1.29 ± 0.51	<0.05
FEMALE (n=21)												
HR (bpm)	0.3	-7 ± 2	<0.01	0.3	-8 ± 3	0.01	0.1	-2 ± 1	0.2	0.2	-33 ± 15	<0.05
Log SDΔNN	0.2	0.4 ± 0.2	<0.05	0.3	0.5 ± 0.2	<0.01	0.1	0.1 ± 0.1	0.1	0.2	2 ± 1	0.07
Log NN50%	0.1	0.6 ± 0.3	0.08	0.2	7.4 ± 3.8	0.08	<0.1	0.1 ± 0.1	0.3	<0.1	1.7 ± 1.5	0.3
LF (nu)	0.1	-6 ± 4	0.1	0.1	-8 ± 4	0.08	<0.1	-2 ± 2	0.2	0.1	-32 ± 21	0.1
LF/HF	0.1	-0.31 ± 0.16	0.09	0.2	-4.23 ± 1.89	<0.05	<0.1	-0.09 ± 0.07	0.2	<0.1	-1.23 ± 0.89	0.2

Table 6-5: Relationship between metabolic responses to an adrenergic stimulus and HRV parameters in juvenile sheep

Heart rate (HR). Free fatty acid (FFA) and glucose areas under the curve from baseline to 15 minutes ($FAUC_{ADR-15}$; $GAUC_{ADR-15}$), and the change in FFA and glucose from baseline to peak concentration (Δ FFA, Δ glucose) following an adrenergic challenge. Data are least square mean ± SEM.

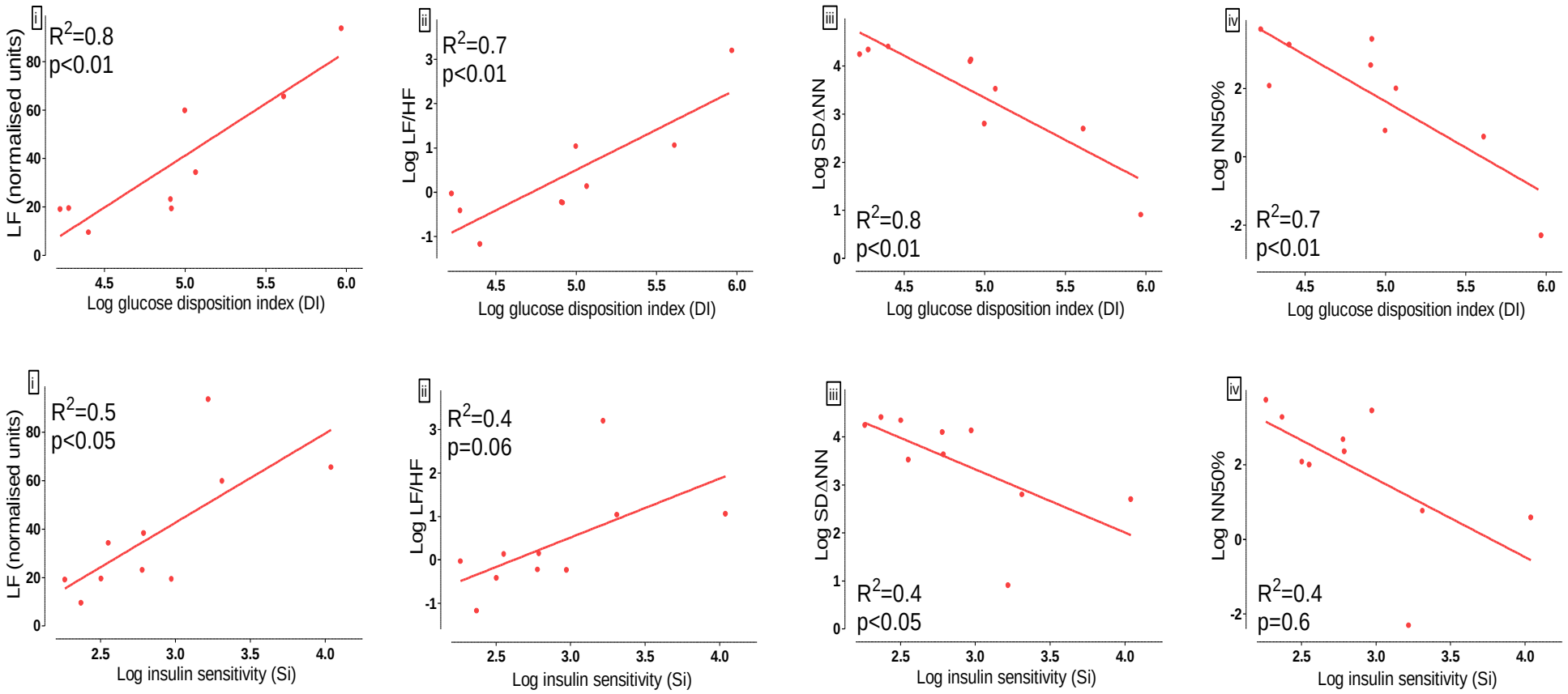

Figure 6-1: Relationship between insulin sensitivity and glucose disposal index and HRV parameters in juvenile female sheep born preterm

i) LF (normalised units), ii) LF/HF, iii) SDΔNN, iv) NN50% and glucose disposition index (DI; top series, n=9), and insulin sensitivity (Si; lower series, n=10). R^2 and p-values are shown with each regression plot.

6.4.6. Adult cardiovascular challenges

6.4.6.1. Effects of preterm birth on HRV and BP in adult sheep

Preterm birth alone did not significantly influence HRV or BP parameters in either sex (Table 6-6).

When adjusted for perinatal variables and current weight-for-length, male preterm sheep had greater indices of sympathetic function and reduced indices of parasympathetic function (Log SDΔNN) compared to male term-spont sheep, and a trend towards reduced Log NN50% in preterm sheep (Table 6-7). There remained no effect of preterm birth in females in the adjusted model (Table 6-7).

Inclusion of $GV_{0\text{-}2}$ or $GV_{W\text{-}1Yr}$ in the model attenuated the effect of preterm birth in males, whereas $GV_{W\text{-}1Yr}$ strengthened the effect in females (Table 6-7). Stepwise regression analysis, however, showed that while GV might modify the effect of preterm birth, it does not account for the effects of preterm birth *per se.*

There was no effect of preterm birth on BP in the adjusted model in either sex; inclusions of GV parameters to the model did not change this.

6.4.6.2. Effect of nutrient supplementation on HRV and BP in adult sheep

Supplemented male sheep had a lower systolic blood pressure than un-supplemented male sheep (Table 6-6). There were no other effects of supplementation, or interactions between birth group and supplement on HRV or BP in sheep of either sex.

		Term-spont		Preterm		Term-dex		Term-Alizin
MALE		UN (HRV: n=13) (BP: n=6)	S (HRV: n=10) (BP: n=4)	UN (HRV: n=12) (BP: n=6)	S (HRV: n=10) (BP: n=4)	UN (HRV: n=11) (BP: n=8)	S (HRV: n=13) (BP: n=7)	UN (HRV: n=5) (BP: n=5)
Heart rate (bpm)		107 ± 6	96 ± 6	118 ± 6	101 ± 6	109 ± 6	113 ± 6	107 ± 7
Log SDΔNN		3.3 ± 0.2	3.6 ± 0.2	2.8 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	3.3 ± 0.2	3.4 ± 0.3
Log NN50%	***TrS***	1.1 ± 0.5	2.2 ± 0.6	0.0 ± 0.6	1.1 ± 0.6	1.1 ± 0.6	1.3 ± 0.5	2.1 ± 0.7
Log HF (nu)		3.2 ± 0.1	3.2 ± 0.3	2.9 ± 0.2	2.9 ± 0.2	3.0 ± 0.2	3.2 ± 0.1	3.1 ± 0.2
LF (nu)	***TrGp***	51 ± 6	45 ± 7	67 ± 6	59 ± 7	53 ± 6	54 ± 6	55 ± 8
LF/HF		1.4 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.2
SBP (mmHg)	†	146 ± 6	126 ± 7	138 ± 6	125 ± 7	134 ± 5	135 ± 5	149 ± 9
DBP (mmHg)		65 ± 3	61 ± 4	72 ± 3	67 ± 4	67 ± 3	68 ± 3	71 ± 4
MAP (mmHg)		89 ± 3	79 ± 4	94 ± 3	87 ± 4	89 ± 3	91 ± 3	94 ± 4
FEMALE		UN (HRV: n=12) (BP: n=8)	S (HRV: n=10) (BP: n=6)	UN (HRV: n=8) (BP: n=6)	S (HRV: n=9) (BP: n=5)	UN (HRV: n=7) (BP: n=5)	S (HRV: n=9) (BP: n=5)	UN (HRV: n=5) (BP: n=5)
Heart rate (bpm)	***TrGp***	112 ± 4	108 ± 5	103 ± 5	103 ± 5	91 ± 6	105 ± 5	105 ± 7
Log SDΔNN		3.1 ± 0.2	3.2 ± 0.2	3.4 ± 0.3	3.5 ± 0.3	3.6 ± 0.3	3.5 ± 0.3	3.0 ± 0.3
Log NN50%		1.2 ± 0.6	1.0 ± 0.6	1.5 ± 0.7	1.6 ± 0.6	2.3 ± 0.7	1.8 ± 0.6	0.8 ± 0.7
Log HF (nu)		3.1 ± 0.2	2.9 ± 0.2	3.1 ± 0.2	2.9 ± 0.2	3.1 ± 0.2	2.8 ± 0.2	2.8 ± 0.2
LF (nu)		59 ± 7	58 ± 7	52 ± 9	54 ± 8	47 ± 9	48 ± 8	59 ± 10
LF/HF		1.6 ± 0.1	1.7 ± 0.1	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	1.7 ± 0.2
SBP (mmHg)		135 ± 5	130 ± 6	136 ± 6	135 ± 7	149 ± 7	142 ± 7	132 ± 6
DBP (mmHg)		67 ± 3	64 ± 4	63 ± 4	68 ± 4	69 ± 4	62 ± 4	69 ± 3
MAP (mmHg)		88 ± 3	84 ± 4	85 ± 4	90 ± 4	92 ± 4	85 ± 4	90 ± 3

Table 6-6: Effect of birth group and supplementation on HRV and BP in adult female sheep

UN=un-supplemented. S=supplemented. Systolic (SBP), diastolic (DBP) and mean (MAP) arterial blood pressure. Data are least square means ± SEM. † p<0.05 S compared to UN. TrS p<0.1 for the overall effect of supplementation. TrG p<0.1 for the overall effect of birth group.

		MALE					FEMALE		
		Term-spont (n=23)	Preterm (n=22)	Term-dex (n=24)			Term-spont (n=22)	Preterm (n=17)	Term-dex (n=16)
Model 1					Model 1				
LFnu	*	48 ± 5	65 ± 5	52 ± 5	LFnu		57 ± 5	49 ± 6	42 ± 7
Log LF/HF	Gp	1.4 ± 0.2	1.8 ± 0.1	1.5 ± 0.1	Log LF/HF		1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
Log SDΔNN	*	3.4 ± 0.2	2.8 ± 0.2	3.3 ± 0.2	Log SDΔNN		3.2 ± 0.2	3.5 ± 0.2	3.7 ± 0.2
Log NN50%	TrGp	1.7 ± 0.4	0.2 ± 0.5	1.3 ± 0.5	Log NN50%		1.2 ± 0.4	1.7 ± 0.5	2.2 ± 0.5
Model 2					Model 2				
LFnu		48 ± 5	64 ± 6	51 ± 5	LFnu		60 ± 6	49 ± 7	42 ± 7
Log LF/HF		1.5 ± 0.1	1.8 ± 0.1	1.4 ± 0.1	Log LF/HF		1.7 ± 0.1	1.7 ± 0.2	0.5 ± 0.1
Log SDΔNN		3.4 ± 0.2	2.9 ± 0.2	3.3 ± 0.2	Log SDΔNN		3.2 ± 0.2	3.5 ± 0.2	3.7 ± 0.2
Log NN50%		1.6 ± 0.5	0.3 ± 0.5	1.3 ± 0.5	Log NN50%		1.1 ± 0.5	1.8 ± 0.6	2.3 ± 0.5
Model 3					Model 3				
LFnu TrGp		49 ± 5	71 ± 8	49 ± 5	LFnu TrGp		54 ± 6	60 ± 22	44 ± 8
Log LF/HF	‡	1.5 ± 0.1	2.0 ± 0.2	1.4 ± 0.1	Log LF/HF		1.6 ± 0.1	2.0 ± 0.4	1.5 ± 0.1
Log SDΔNN	*	3.5 ± 0.2	2.6 ± 0.3	3.4 ± 0.2	Log SDΔNN		3.3 ± 0.2	2.6 ± 0.7	3.7 ± 0.2
Log NN50%	TrGp	1.8 ± 0.5	-0.4 ± 0.8	1.5 ± 0.5	Log NN50%	TrGp	1.5 ± 0.4	-1.5 ± 1.6	2.3 ± 0.5
Model 4					Model 4				
LFnu		52 ± 6	58 ± 11	54 ± 8	LFnu	‡	59 ± 6	77 ±13	32 ± 8
Log LF/HF		1.4 ± 0.1	1.7 ± 0.2	1.5 ± 0.2	Log LF/HF		1.6 ± 0.1	1.8 ± 0.3	1.3 ± 0.1
Log SDΔNN		3.3 ± 0.2	2.9 ± 0.4	3.2 ± 0.3	Log SDΔNN	‡	3.1 ± 0.2	2.6 ± 0.4	3.8 ± 0.3
Log NN50%		1.4 ± 0.5	0.6 ± 0.9	1.2 ± 0.7	Log NN50%	TrGp	1.0 ± 0.5	0.1 ± 1.0	2.6 ± 0.6

Table 6-7: Effect of preterm birth, adjusted for perinatal and postnatal factors, on HRV

*Model 1: Effect of preterm birth adjusted for birth weight z-score, supplementation status, weight and age at time of assessment (adjusted model). Model 2: Adjusted model + growth velocity (GV) between birth and 2 weeks. Model 3: Adjusted model + GV between 2 weeks and weaning. Model 4: Adjusted model + GV between weaning and one year. Data are least square means ± SEM. * p<0.05 preterm compared to term-spont. ‡ p<0.05 preterm compared to term-dex. For the overall effect of birth group; Gp p<0.05, TrGp p<0.1.*

6.4.6.3. Effects of induction of labour at term on HRV and BP in adult sheep

Term-Alizin sheep were not different to either un-supplemented term-spont or term-dex sheep with respect to any HRV or BP parameters (Table 6-6). There remained no effect of birth group in the adjusted model, with or without inclusion of measures of GV.

6.4.7. Effect of gestational age at birth on HRV and BP in term-spont adult sheep

In adult males, in the un-adjusted model there was an inverse relationship between gestational age at birth and DPB (fall in DBP of 2 ± 1 mmHg for every day increase in gestational age at birth; n=10, R^2=0.4, p<0.05). However, in the adjusted model, there was no longer any effect of gestational age on BP or HRV.

In females although there was no effect of gestation age on HRV in the un-adjusted model, gestation length in the adjusted model was positively related to DBP and MAP, inversely related to indices of sympathetic function (Table 6-8) and not related to measures of parasympathetic function.

	MALE			FEMALE		
	BP, n=10; HRV, n=23			BP, n=14; HRV, n=22		
	R^2	β coefficient (effect per 1 day increase in GA)	p-value	R^2	β coefficient (effect per 1 day increase in GA)	p-value
SBP	0.7	-4.3 ± 11.4	0.7	0.5	4.2 ± 5.2	0.4
DBP	0.8	-2.3 ± 2.3	0.4	0.7	4.6 ± 1.6	0.03
MAP	0.8	-3.3 ± 1.9	0.2	0.7	4.1 ± 1.8	0.06
LFnu	0.4	2.3 ± 2.9	0.4	0.7	-4.3 ± 1.6	0.01
Log LF/HF	0.2	-0.1 ± 0.1	0.8	0.8	-0.06 ± 0.03	0.04

Table 6-8: Effect of gestational age at birth, adjusted for perinatal and postnatal factors, on HRV and BP in adult term-spont sheep

GA = gestational age at birth. Systolic (SBP), diastolic (DBP) and mean (MAP) arterial blood pressure. Data are least square means ± SEM.

6.4.8. Adult baseline cortisol and cortisone measurements

6.4.8.1. Effect of birth group on plasma cortisol & cortisone concentrations

Preterm birth did not influence morning plasma concentrations of cortisol or cortisone or the ratio of cortisol: cortisone in either sex (Table 6-9). In males this remained unchanged in the adjusted models. In females, after adjustment for the effect of perinatal variables and % fat mass, term-dex sheep had higher plasma cortisol and cortisone concentrations than preterm

sheep ($p<0.05$ for both), but the ratio of cortisol: cortisone was not different between birth groups (Table 6-9).

6.4.8.2. Effect of supplementation on plasma cortisol & cortisone concentrations

Nutrient supplementation did not influence morning plasma cortisol or cortisone concentrations, or the ratio of cortisol: cortisone in either sex, nor was there any interaction between birth group and supplementation (Table 6-9).

6.4.8.3. Effect of induction of labour at term on plasma cortisol & cortisone concentrations

Induction of labour at term did not influence morning plasma concentrations of cortisol or cortisone, or the ratio of cortisol: cortisone in either sex (Table 6-9); this remained unchanged in the adjusted models, with or without inclusion of measures of GV.

MALE	Term-spont		Preterm		Term-dex		Term-Alizin
	UN (n=9)	S (n=9)	UN (n=11)	S (n=10)	UN (n=10)	S (n=9)	UN (n=6)
Cortisol (ng/ml)	1.8 ± 0.2	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.2	1.6 ± 0.2	1.9 ± 0.2	1.9 ± 0.1
Cortisone (ng/ml)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Cortisol: cortisone	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
FEMALE	Term-spont		Preterm		Term-dex		Term-Alizin
	UN (n=10)	S (n=10)	UN (n=9)	S (n=9)	UN (n=9)	S (n=9)	UN (n=6)
Cortisol (ng/ml)	2.1 ± 0.2	2.0 ± 0.2	1.6 ± 0.2	1.9 ± 0.2	2.2 ± 0.2	2.1 ± 0.2	2.0 ± 0.2
Cortisone (ng/ml)	0.8 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
Cortisol: cortisone	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.2 ± 0.1

Table 6-9: Effect of birth group and supplementation on morning cortisol and cortisone concentrations in adult sheep

UN=un-supplemented. S=supplemented. Data are least square means ± SEM.

6.4.9. Correlations between size, growth rates, body composition and HRV and BP

In term spont females (n=22) heart rate was positively correlated with birth weight z-score (R^2=0.4, β=11 ± 3, p=0.001) but negatively correlated with GV_{0-2} (R^2=0.4, β=-1.8 ± 0.5, p=0.001), whereas adult weight and weight-for-length tended to correlate with LFnu and LF/HF; (Weight: LF/HF R^2=0.3 β=0.04 ± 0.01, p=0.007. Weight-for-length: LFnu R^2=0.3, β=4.1 ± 1.4, p=0.009; LF/HF R^2=0.3, β=0.09 ± 0.03, p=0.006).

Adult weight also tended towards an inverse correlation with SBP in preterm females (SBP, n=11, R^2=0.6, β=-1.1 ± 0.3, p=0.005) whereas adult fat mass was positively correlated with systolic blood pressure in male term-spont sheep (n=10, R^2=0.8, β=9.1 ± 1.8, p=0.001).

In male term-Alizin sheep GV_{W-1yr} was inversely correlated with indices of sympathetic activity (LFnu; R^2=0.9, β= -22 ± 3, p=0.002; Log LF/HF; R^2=0.9, β=0.61 ± 0.06, p<0.001) and tended to correlate with parasympathetic indices (n=6: Log SDΔNN; R^2=0.9, β=0.7 ± 0.1, p<0.008) but was not correlated with heart rate, BP or HRV parameters in any other group of sheep.

Birth weight z-score, adult weight and weight-for-length, $GV_{0-2,}$ GV_{2-W} and GV_{W-1yr} did not correlate with heart rate, BP or HRV parameters in any other groups of sheep.

Within the preterm group, neither GV_{0-TEA} nor GV_{TEA-W} was correlated with blood pressure or HRV in either sex.

6.4.10. Correlations between plasma cortisol and HRV and BP

There were non-significant trends for inverse correlations between the Log cortisol: cortisone ratio and BP in preterm males (n=9, DBP R^2=0.5, β= -31 ± 11, p=0.03; MAP R^2=0.5, β=28 ± 10, p=0.03) and term-spont females (n=9: SBP R^2=0.5, β= -63 ± 24, p=0.03; DBP R^2=0.6, β= -22 ± 7, p=0.02; MAP R^2=0.7, β= -27 ± 7, p=0.006), and with HRV parameters in term-spont males (n=16, LFnu, R^2=0.3, β=54 ± 24, p<0.05; LF/HF, R^2=0.3, β= -4.8 ± 2.2, p<0.05).

6.4.11. Correlations between fasting insulin: glucose ratio, insulin sensitivity and HRV and BP in adult sheep

In adult males fasting insulin: glucose ratio tended towards a positive correlation with sympathetic indices (n=15: LFnu R^2=0.4, β=16 ± 6, p=0.01; LF/HF R^2=0.4, β=0.3 ± 0.1, p=0.01) and an inversely correlation with parasympathetic indices (n=15: SDΔNN R^2=0.5, β= -0.6 ± 0.2, p=0.005; NN50% R^2=0.4, β= -1.5 ± 0.5, p=0.008) in term-dex sheep only. Insulin sensitivity (Si) was not correlated with HRV parameters in any sheep.

In adult females fasting insulin: glucose ratio was not correlated with any HRV parameters whereas insulin sensitivity was inversely correlated with parasympathetic indices in preterm sheep only (n=13: SDΔNN R^2=0.7, β= -1.1 ± 0.2, p<0.001; NN50% R^2=0.7, β= -2.5 ± 0.5, p<0.001). Neither fasting insulin: glucose ratio nor insulin sensitivity were correlated with BP in any sheep.

6.5. Discussion

This work has demonstrated that not only preterm birth, but also reduced gestation length across the normal term continuum, is associated with persistent changes in cardiac autonomic function. In both instances reduced gestation length was associated with an increase in cardiac sympathetic drive and a reduction in parasympathetic drive. However, we found that the effect of preterm birth was confined to males, whereas the effect of gestational age at term was confined to females.

An increasing body of literature indicates that preterm birth is associated with adverse cardiovascular outcomes in adult life, although the pathophysiological mechanisms are not understood. To date the cardiac autonomic nervous system has not been investigated as a possible mediator for these findings; these data suggest that further research needs to include measures of cardiac autonomic function. Additionally, there is an increasing trend for obstetric delivery prior to 40 weeks gestation without medical indication, as there traditionally has not been an appreciation of late effects arising from this practice. These data suggest that this practice may not be as benign as currently thought, and that those born at ‘early-term’ may have persistent changes in cardiac autonomic function that increase their risk of adverse later cardiovascular outcomes. Future research needs to include not only those

born preterm, but also those born across the term continuum to clarify the effect of gestational age on later cardiovascular outcomes.

Preterm birth and cardiovascular function

In preterm males, but not females, there was an increase in cardiac sympathetic drive and trend towards reduced parasympathetic activity in juvenile life, with persistence of this autonomic imbalance at least into young adulthood. Although the effects of preterm birth were difficult to isolate from the effects of growth (preterm lambs grow at a different rate to term-born animals), stepwise regression analyses confirm that GV and birth group are not co-linear, and that preterm birth has the dominant effect on HRV parameters. Maturation of the cardiac ANS occurs during late gestation in both humans and sheep (Booth et al., 2011; Hildreth, et al., 2009; Van Leeuwen, Lange, Bettermann, Gronemeyer, & Hatzmann, 1999; Wakatsuki et al., 1992). Preterm birth in lambs causes transient cardio-respiratory instability and autonomic over activity that is no longer present at TEA (Patural et al., 2010). In humans, preterm infants show a trend for increased sympathetic activity with reduced parasympathetic activity at birth (Patural, et al., 2008) and at TEA but not later in childhood (De Rogalski Landrot, et al., 2007), when compared to those born at term. Indirect markers of sympathetic activity (urinary catecholamines) and heart rate both at rest and in response to a mental stress test are also increased in 9 year old children born preterm (Johansson et al., 2007). Preterm birth in humans has therefore also been linked to altered autonomic function both before and after TEA.

Future studies need to incorporate longitudinal characterisation of HRV maturation from preterm and term birth through to adulthood, and to include dynamic tests of autonomic cardiovascular function. The apparent sexual dichotomy of HRV in our paradigm may explain both the sex-specific patterns of cardiovascular risk and the cardiac autonomic responses of both high performance athletes and individuals with cardiovascular disease (Mitoff et al., 2011; Scott et al., 2007).

Preterm birth does not influence blood pressure, either in our paradigm or that of De Matteo *et al.* in which blood pressure was assessed in preterm sheep at 4 and 8 weeks post term corrected age, and at a year of age (De Matteo, Stacy, Probyn, Brew, et al., 2008). In humans increased blood pressure in children (Relton, Pearce, & O'Sullivan, 2008) and adults (Crump, Winkleby, Sundquist, & Sundquist, 2011; Dalziel, et al., 2007; Johansson, et al., 2005) born

preterm has been reported, although not all studies have found this association (Johansson, et al., 2007).

Thus, in juvenile and young adult sheep, effects of preterm birth are manifest as altered cardiac autonomic function but not altered blood pressure. Changes in cardiac autonomic activity have been found in normotensive individuals at risk of hypertension (Davrath & Goren, 2003) and, in longitudinal studies, have been shown to precede hypertension (Lucini, et al., 2002). Further studies are needed to determine the consequences of preterm birth in an aging animal when the effects of altered cardiovascular homeostasis are likely to be more pronounced.

Gestational age and cardiovascular function

Treatment of gestational age at birth as a dichotomous variable (preterm or term) does not reflect the full continuum of gestation dependent maturation, present even across what is regarded as full term (37-41 weeks gestation) (Fleischman, Oinuma, & Clark, 2010). Within the adult term-spont group, increased gestational age in females was associated with a decline in cardiac sympathetic activity, but an increase in diastolic blood pressure. Although in males the effect of gestational age was not significant, the strength of the association and magnitude of effect size was very similar to that seen in females, but in the opposite direction. Whether gestation age truly does not have a statistically significant effect on HRV in adult males, or whether this is in fact a type 2 error due to insufficient animal numbers is uncertain. Increments in gestational age at birth across the range of full term have also been associated with decrements in resting adult blood pressure in males (Yang, Bergvall, Cnattingius, & Kramer, 2010). When cardiovascular responses to a psychological stress test were assessed, gestational age was positively related to SBP in males, but negatively related in females (Feldt et al., 2007). Thus, sexual dimorphism may be manifest as both sex-specific patterns of baseline cardiovascular function, but also as different cardiovascular responses to stressors.

We were unable to test the effect of a graded association between preterm gestational age and outcome as, *a priori*, our preterm sheep were born on a designated day and are therefore are a homogeneous population with respect to gestational age. In studies incorporating the range of preterm and term gestation, increments in gestational age have also been associated with decrements in blood pressure in children (Relton, et al., 2008) and adults (Crump, et al., 2011; Dalziel, et al., 2007).

Metabolic responses to an adrenergic stimuli

In addition to increased basal sympathetic activity, preterm males had attenuated fatty acid and glucose mobilisation in response to exogenous adrenaline. Early nutritional supplementation and sex altered this dynamic, with nutritional supplementation increasing the FFA and glucose mobilisation in male preterm animals, but reducing it in term animals. Furthermore, animals with the highest basal sympathetic and lowest parasympathetic activity had an attenuated metabolic response to adrenaline, independent of birth group.

Catecholamines are released into the circulation as a result of sympathetic nervous system activity. One site of action is the β-adrenergic receptor (β-AR) located in adipocyte cell membranes; β-AR activation stimulates adipocyte hormone sensitive lipase (HSL) activity. HSL is the rate limiting enzyme in the conversion of intracellular triglyceride fat depots to FFA and glycerol. Experimentally induced IUGR in sheep increases fetal circulating catecholamine concentrations (Limesand, et al., 2006) and results in down-regulation of β-AR mRNA and protein expression in perirenal fat in both fetal and early postnatal life (Chen et al., 2010). Exogenous administration of adrenaline to these animals (d21) results in attenuated fatty acid mobilisation, suggesting that, in adipose tissue at least, there is functional desensitisation of the adrenergic system (Chen, et al., 2010). Preterm birth and increased sympathetic drive in males might also cause functional desensitisation of the adrenergic system explaining the attenuated metabolic responses to adrenaline.

Fat deposition in lambs during the first weeks of life is dependent on nutrition (Darby, et al., 1996); supplementation in males appeared to promote increments in weight for length (and by inference adiposity) in preterm lambs, but to reduce it in term-spont lambs (4.4.2.2). Differences in fat mobilisation between supplemented and un-supplemented animals may be a consequence of supplement-induced alteration in early growth patterns.

Thus in males, increased basal sympathetic function appears to cause functional desensitisation of the adrenergic system, the expression of which is further modified by early diet, possibly through a gestational age dependent effect of diet on developing fat mass.

Effect of antenatal corticosteroid exposure on HRV and BP

This study has shown that exposure to antenatal corticosteroid, when separated from the effects of preterm birth does not cause perturbations in blood pressure or HRV in juvenile or adult life.

Antenatal corticosteroid exposure accelerates not only lung maturation, but the maturation of many other organ systems throughout the body (Liggins, 1976). These systemic effects of antenatal corticosteroid exposure have been proposed as the principal mediator of the late cardiovascular effects ascribed to preterm birth (Seckl, 2004). While maternal corticosteroids have been associated with increased blood pressure in adolescents born preterm (Doyle, Ford, Davis, & Callanan, 2000), most follow-up studies have not found an association between exposure to either single or repeat doses of antenatal corticosteroid and postnatal heart rate, heart rate variability, blood pressure or echocardiographic markers of cardiovascular function in neonates (Mildenhall, Battin, Bevan, Kuschel, & Harding, 2009; Schaffer et al., 2010), children (Dalziel, Liang, Parag, Rodgers, & Harding, 2004) or adults (Dalziel, et al., 2007; Finken, et al., 2008).

However, corticosteroid treatment of women at risk of preterm labour has been shown to reduce fetal HR and HRV in the first 24-48 hours following steroid administration before recovery to pre-steroid levels (Schneider et al., 2010), with some evidence that betamethasone has a more marked effect than other corticosteroids (Senat et al., 1998). Similar autonomic developmental or regulatory processes are likely to be present in the ovine fetus as administration of dexamethasone to a pregnant ewe (127-133 days gestation) causes a transient drop in fetal heart rate and beat to beat variability, followed by an 'overshoot' of both heart rate and variability (Bennet, Kozuma, McGarrigle, & Hanson, 1999). In a preterm lamb paradigm (delivered at 118-125 days gestation), preterm non-steroid exposed lambs failed to generate the sympathoexcitatory response (increase in heart rate and blood pressure) seen at birth in term lambs. Maternal dexamethasone prior to delivery, however, led to maturation of the sympathetic system such that preterm dexamethasone exposed lambs had an increase in sympathetic activity comparable to that seen in term lambs (Segar, Lumbers, Nuyt, Smith, & Robillard, 1998).

Despite the apparent lack of effect in human follow up studies, many experimental paradigms exist in which antenatal corticosteroids are administered at a time 'analogous' to that experienced in the clinical setting and are reported to affect long-term cardiovascular outcomes. In sheep, maternal corticosteroids in mid gestation (d80, a key stage in nephrogenesis; any interventions to prevent preterm labour in the ewe are not reported) result in increased blood pressure, an increased hypertensive response to Angiotensin II (AngII), reduced indices of parasympathetic function and a trend towards increased sympatho-vagal tone in the adult offspring (Shaltout et al., 2010); all of these effects were attenuated by the

administration of Candesartan (an angiotensin 2 type 1 receptor blocker). As Candesartan did not alter blood pressure in the control animals, the authors speculate that these data may indicate altered activity in the renin angiotensin pathway, with steroid exposure increasing the effects of AngII on vascular tone, but also exerting an inhibitory effect on cardiac vagal activity (Shaltout, et al., 2010). Impaired pressure mediated arterial vasoconstriction has also been found in adult female sheep exposed to betamethasone at d80 of pregnancy, suggesting that corticosteroids lead to persistent changes in vascular tone (Eckman et al., 2010). In contrast to this, Bian *et al.* report that in rats, antenatal corticosteroid treatment of the dam results in a persistent reduction in noradrenalin concentrations and turnover (markers for cardiac sympathetic nervous system development) in cardiac muscle of her offspring (Bian, Seidler, & Slotkin, 1993).

However, inferring maturational or developmental equivalence between sheep, rodent or humans at a given percentage of gestation length is of limited value as *in utero* development progresses at a pace to match the degree of independence required in newborn postnatal life. The effect of maternal steroids administered at 0.75 of gestation (suggested to mimic exposure equivalent to that experienced by a viable human fetus at risk of preterm birth) is therefore likely to differ according to the animal paradigm utilised, and to differ between animal paradigms and the human clinical scenario.

As steroids in our paradigm were administered when organogenesis is largely complete, it is possible that differences in outcomes between our and other sheep paradigms reflects the effect of exogenous steroid on organogenesis versus tissue maturation. Our data offer experimental evidence to support to the increasing body of literature from human follow up studies in which no adverse cardiovascular sequelae have been found in those exposed to antenatal corticosteroids prior to preterm birth (Dalziel, et al., 2004; Dalziel, et al., 2007; Finken, et al., 2008; Mildenhall, et al., 2009; Schaffer, et al., 2010).

Size at birth and cardiovascular outcomes

The effect of size at birth on later outcomes may be tested by expressing birth weight relative of the average weight of an appropriate gestational age and sex matched population (birth weight z-score). Although preterm infants are often also growth restricted, this approach enables low birth weight to be assessed in the context of fetal growth restriction, reduced gestation length, or both.

In our paradigm of singleton lambs born to healthy ewes, birth weight z-score did not predict cardiovascular outcomes in either juvenile or adult life, in any group of sheep. In human IUGR (Okamura, et al., 1990) as well as experimental IUGR (Limesand, et al., 2006), fetal adaptation to an unfavourable intrauterine environment includes increased activation of the sympathetic arm of the ANS, resulting in elevated circulating fetal catecholamine concentrations. As previously discussed, desensitisation of the autonomic nervous system in response to increased sympathetic activity has been reported in postnatal IUGR sheep (Chen, et al., 2010), and may therefore also result in altered HRV parameters. However, our population of animals was derived from well nourished, healthy, singleton-bearing ewes, and had a relatively tight cluster of birth weight z-scores; a true assessment of HRV or BP outcomes related to fetal growth restriction would need to be tested in a more appropriate experimental cohort.

In humans, sex-specific effects of birth weight have been reported in childhood and in adulthood; smaller term-born females have increased resting and stress induced indices of sympathetic activation at age 7-9 years, whereas smaller males have increased systemic vascular resistance (Jones et al., 2008). Additionally, low birth weight in adult females, but not males, was associated with increased indices of sympathetic activation and reduced parasympathetic activation that were not explained by differences in gestation length (Jones, et al., 2007), but were significantly influenced by stage of the menstrual cycle at time of assessment. Such differences would be hard to detect in our paradigm as, to correct for the effects of differences in stage of the breeding cycle, all females had a CIDR™ inserted to prevent ovulation.

Postnatal growth and cardiovascular outcomes

The effect of growth rate on HRV and BP parameters appears to have sex and birth group specific associations. For instance, between weaning and one year of age, increased growth rates in male term-Alizin sheep were associated with reduced sympathetic but increased parasympathetic activity, whereas in male term-spont sheep, the opposite effect was found (rapid growth associated with increased sympathetic but reduced parasympathetic activity). In juvenile preterm lambs growth velocity between birth and term equivalent age (TEA) was not related to BP, whereas rapid growth between TEA and weaning was associated with lower blood pressure; by adulthood, however, there was no association between $GV_{0\text{-}TEA}$ or $GV_{TEA\text{-}W}$ and blood pressure. Growth rates are the measurable end point of complex inter-related metabolic, endocrine and physiological processes that reflect differences arising from

sex, and the pre and postnatal environment. Arbitrary separation of growth rates into discrete epochs attempts to tease out the effect of growth in a standardised way; however, these epochs do not reflect the continuum of growth or changes in relative growth rates that might also modify cardiovascular function. Our data suggest that early life events (for instance preterm birth or induction of labour) modify the relationships between early growth rate and later cardiovascular function. As there were relatively small numbers in each group, apparent differences between these relationships may also reflect sample size and inadequate statistical power.

In humans, rapid early growth in both preterm and term infants has been associated with impaired vascular function and increased blood pressure later in life (Law et al., 2002; Singhal, et al., 2004; Singhal, et al., 2007). Larger numbers in these studies however allowed growth to be assessed as a dichotomous variable (rates above or below the population median), or as a relative change in z-score; separation of growth rate into 'faster' or 'slower' growth strengthened the association with later outcomes.

Effect of nutritional supplementation on cardiovascular outcomes

Nutritional supplementation was not associated with growth promotion or with measures of HRV in any sheep, but was associated with lower SBP in adult male sheep. Blood pressure data were only obtained in a small number of term-spont and preterm animals, from which most of the effect of supplementation appears to derive, whereas the larger term-dex group does not show an effect of supplementation. It is possible, therefore, that either the reduction in BP amongst supplemented animals is a statistical artefact consequent on small animal numbers, or that corticosteroid exposure at term alters the effect of supplementation on later blood pressure regulation.

To avoid potentially confounding effects of maternal milk versus artificial formula, all animals received ewe milk, with supplement as an additional, not replacement, source of nutrition. Human infants who are breast fed have a lower resting heart rate at 4 months of age than those fed formula milk, irrespective of size at birth and postnatal growth rates (Butte, Smith, & Garza, 1991); whether the effects of breast milk relate to breast feeding *per se*, to the lower protein content in human milk or to the presence of other components in breast milk itself is uncertain. Further studies of infant nutrition and feeding mode are needed to clarify the effects of early diet on cardiovascular function.

Correlation between HPAA activity and cardiovascular outcomes

Although we found no association between preterm birth or antenatal steroid exposure and morning plasma cortisol concentrations or the cortisol: cortisone ratio, other investigators have found increased morning plasma cortisol concentrations in children (Buske-Kirschbaum, et al., 2007) but not adults (Dalziel, et al., 2007) born preterm.

Many clinical and biochemical similarities exist between the Metabolic Syndrome and Cushings Syndrome. As Cushings Syndrome is characterised by cortisol excess, either from endogenous production or exogenous administration of corticosteroids, it has been postulated that the metabolic and cardiovascular components of the Metabolic Syndrome may too result from relative excess of cortisol (Anagnostis, et al., 2009). Thus, if changes in perinatal environment result in altered HPAA activity in later life, this may predispose individuals to increased metabolic and cardiovascular risk.

The diurnal fluctuations in cortisol make interpretation of isolated results difficult, even if standardised to time of day as in our experiment. A recent large prospective cohort study has demonstrated that a reduction in daily cortisol variation, but not morning salivary cortisol concentrations, was associated with cardiovascular mortality during follow-up of community based adults (Kumari, Shipley, Stafford, & Kivimaki, 2011), whereas in a population of adults with type 2 diabetes, morning plasma cortisol concentrations were positively associated with ischaemic heart disease but not blood pressure.

The cortoisol: cortisone ratio, however, was significantly correlated with HRV and BP outcomes, although the size and direction of the effect varied by sex, and between groups. Biologically active cortisol is converted by 11β-hydroxysteroid dehydrogenase to the biologically inactive cortisone, and reduced conversion of cortisol to cortisone, with consequent renal mineralocorticoid effects, has been proposed as a mechanism underlying hypertension in individuals with diabetes, renal failure (Homma et al., 2001) and Cushing Syndrome (Stewart, Walker, Holder, O'Halloran, & Shackleton, 1995). The mechanism underlying the association between cortisol: cortisone and HRV parameters is less clear, although in humans an association has been described between plasma cortisol concentrations and muscle sympathetic nerve activity, the direction of which is birth weight dependent; those with a low birth weight have an inverse relationship whereas those of normal birth weight had a positive relationship (Weitz, Wellhoener, Heindl, Fehm, & Dodt, 2005).

Collectively these data suggest that there are associations between sympathetic or autonomic responses and the adrenocortical system, the balance of which may be perturbed by perinatal events. As there was no apparent effect of preterm birth on morning plasma cortisol concentrations, this may indicate that, in sheep, preterm birth does not influence later HPAA function. However, cortisol data were derived from the mean value of samples taken by venepuncture on consecutive mornings, prior to the morning feed. Small changes in basal cortisol concentrations may therefore be obscured by differences in animal responses to handling, or to the precise time of day at which the sample was drawn. Better characterisation of the diurnal variation in plasma cortisol concentrations, or the response to a dynamic challenge of the hypothalamic pituitary adrenal axis (HPAA) may clarify the effect of preterm birth or other perinatal events on HPAA activity and its relationship with cardiovascular function.

Insulin and cardiovascular outcomes

This experiment has identified sex and birth group-specific correlations between measures of glucose-insulin axis function and HRV (for instance, increased fasting insulin: glucose positively correlated with sympathetic indices and negatively correlated with parasympathetic indices in term-dex males). The direction of effect is different between groups, again suggesting that early life events set a trajectory along which later physiological or pathological development occurs.

Although insulin is known to have many functions within the cardiovascular system (Muniyappa, et al., 2007), any effects on heart rate variability are uncertain. Reports from human subjects suggest that an acute increase in plasma insulin concentrations is associated with an increase in cardiac parasympathetic activity (Stockhorst, Huenig, Ziegler, & Scherbaum, 2011), whereas prolonged hyperinsulinaemia during a hyperinsulinaemic euglycaemic clamp (HEC) increases muscle sympathetic activity and reduces cardiac parasympathetic activity (Van De Borne, Hausberg, Hoffman, Mark, & Anderson, 1999). These differences may reflect the different paradigms utilised, or may represent different physiological responses to acute versus chronic hyperinsulinism; if this is in fact the case, although speculative, altered cardiac autoniomic responses in pathological states of hyperinsulinaemia may explain the heightened cardiovascular risk in conditions such as type 2 diabetes.

Although the correlations identified in this study are intriguing, further exploration of the interaction between autonomic nervous system activity, glucose homeostasis and the cardiovascular effects of insulin was not possible within the parameters of this experiment.

Limitations of the study

The inclusion of a preterm, non-corticosteroid exposed group potentially would have been advantageous to isolate the effect of preterm birth from that of antenatal glucocorticoid exposure. It is, however, probable that without corticosteroids to enhance lung maturation preterm lambs would not survive the immediate newborn period (De Matteo, et al., 2010). Treatment of the ewe with low dose corticosteroid prior to preterm induction of labour, as described in other studies of preterm lambs, clearly still has systemic effects on the lamb (as manifest by the reduction in pulmonary disease). Thus, using other agents to induce parturition, but giving corticosteroids to hasten lung maturity, probably also does not represent a paradigm of non-corticosteroid exposed preterm birth (De Matteo, Stacy, Probyn, Brew, et al., 2008). Preterm animals were only modestly preterm and did not require ventilator support; this mirrors the clinical setting as the vast majority of preterm birth falls within the late preterm range. Animals born at a lesser gestation are likely to require more intensive care to keep them alive. The necessary disruption of normal ewe-lamb bonding would make it unlikely that such animals could return to their mothers and be reared to maturity on a farm.

First HRV and BP measurements were obtained at approximately 4 months of age; we therefore lack data on the longitudinal changes in cardiovascular development during early postnatal life. Such data would strengthen our understanding of the functional ontogeny of the cardiac autonomic nervous system, especially with respect to differences that may exist prior to or after TEA. Due to logistical constraints we were unable to perform any dynamic tests of cardiovascular function; this is something that future experiments would aim to address. We also have limited numbers of animals with both BP and HRV data. The problems encountered during the method development necessary for arterial line placement have been resolved, which is likely to increase the yield of good quality BP data obtained in future experiments.

Our results include many correlations between continuous variables. By reducing the level of statistical significance to a p-value of 0.001 for such analyses, we may be under reporting important associations. However, we believe that such stringency is required to prevent over-

interpretation of the data as, at a p-value of 0.05, 5% of analyses will, by chance, be statistically significant.

Conclusions

Preterm birth is associated with altered cardiac autonomic control, independent of antenatal corticosteroid exposure or relative size at birth, which favours an increase in sympathetic and reduction in parasympathetic activity, and is manifest in both juvenile and adult life. Preterm lambs grow at a different rate to term-born lambs and it is therefore not possible to fully separate the effects of preterm birth from growth; however, these data show that preterm birth, and not postnatal growth rate, has the dominant effect on later cardiovascular outcomes. In addition, associations between gestational age at birth and adult autonomic indices suggest that cardiac autonomic maturation continues throughout gestation, up to, and including the range of spontaneous term birth. Whether similar changes exist in humans remains unknown.

Autonomic dysfunction is one mechanism through which preterm birth may exert effects on cardiovascular function throughout the life course. Follow-up studies of preterm infants need to include assessment of cardiac autonomic function as a method of identifying cardiovascular risk prior to the onset of overt cardiac pathology.

Chapter 7. IUGR pilot study

7.1. Introduction

Small size at birth has been linked to many adverse health outcomes (Barker, 1992; Mzayek, et al., 2009; Newsome, et al., 2003). One cause of small size is *in utero* growth restriction (IUGR). Human epidemiological studies or experimental trials are often confounded by the difficulty in separating infants exposed to pathological IUGR from those that are constitutionally small (Platz & Newman, 2008), and by the difficulty in separating the impact of IUGR from that of other confounding factors such as socio-economic class (Beard et al., 2009).

Placental embolisation is a well established method for generating IUGR lambs (Bloomfield, Bauer, van Zijl, Gluckman, & Harding, 2002). Fetal vascular catheters are usually placed and fetal blood gases used to monitor the response to embolisation. The embolisation regime is tailored such that there is evidence of fetal metabolic stress without excessive embolisation and fetal demise. The effect of poor fetal growth due to placental insufficiency can thus be separated from other confounding factors.

The aims of this study were two fold. Firstly we wanted to test the feasibility of generating IUGR lambs without fetal blood sampling. Secondly, it was hypothesized that IUGR lambs would have a phase of accelerated postnatal growth. It was intended therefore that we could compare the long term effects of early growth acceleration achieved through spontaneous 'catch-up' growth in IUGR lambs against that achieved in preterm or term lambs with early nutritional supplementation, referenced against a population of normally grown and nourished preterm and term-born lambs.

7.2. Methods

As no fetal monitoring of these pilot animals was undertaken, it was impossible to know antenatally whether sufficient embolisation had occurred to have a deleterious effect on fetal physiology and growth. To minimise the risk of fetal loss through excessive embolisation, it was decided *a priori* that a 15% reduction in anticipated birth weight (referenced against the

birth weight of a large number of animals previously born at Ngapouri station) was an acceptable threshold for prospectively defining IUGR in this population.

This technique ultimately proved to be unsuccessful as the majority of pregnancies ended with either fetal loss (and therefore presumed excessive embolisation, infection or other complication), or a live lamb of normal size and weight (and therefore presumed inadequate embolisation).

Detailed analysis of the pregnancy outcomes from operated ewes will form part of a separate thesis, and therefore will not be discussed here.

All lambs received water as the control postnatal intervention and were treated in an identical fashion to lambs from the main project cohort. All IUGR lambs underwent the same juvenile postnatal tests as other lambs; results, however, are only available for growth outcomes, milk intake, glucose tolerance tests (GTT) and growth hormone (GH) stimulation tests. No adult tests were performed.

7.3. Statistical analysis

Small animal numbers mean that analyses presented in this section are exploratory and represent preliminary findings from IUGR animals compared to normally grown, un-supplemented term-spont animals.

To account for the small numbers and uneven sex distribution, data were analysed in mixed sex groups, using the terms birth group (IUGR or term-spont) sex and the interaction term birth group x sex.

Non-parametric data were log transformed to approximate a normal distribution. Longitudinal changes were examined using RM ANOVA and parametric data were analysed by factorial ANOVA with *post hoc* Student t-test. Statistical significance was set at a p-value of <0.05.

7.4. Results

7.4.1. Birth characteristics and growth in IUGR sheep

Of the lambs born to ewes that underwent placental embolisation, only 7 had a birth weight below 5 kg. One ewe had significant shearing injuries to her udder and therefore was unable

to feed her lamb; birth data only from the lamb was retained, and both ewe and lamb were excluded from the project. No other losses of IUGR lambs occurred during the project.

IUGR lambs were smaller, lighter with a lesser weight for length at birth than un-supplemented (UN) term-spont lambs (Figure 7-1), and remained so until weaning (Figure 7-1, Figure 7-2).

Relative daily weight gain during the first two weeks of life (Figure 7-1), and GV_{0-2} and GV_{2-W} were greater in IUGR than UN term-spont lambs (Table 7-1).

Rates of increase in CRL, HL and BPD were not different in IUGR lambs compared to UN term-spont lambs either during the first two weeks of life (CRL_{0-2}, HL_{0-2}, BPD_{0-2}) or between 2 weeks and weaning (CRL_{2-W}, HL_{2-W} and BPD_{2-W}) (Table 7-1).

Age and weight at the time of juvenile metabolic testing were not different between groups (Table 7-1).

	Term-spont	IUGR	Significance level (p-value)		
	n=40	n=6 (7)	Group	sex	Group x sex
Birth weight z-score	0.2 ± 0.2	-2.1 ± 0.5	<0.0001	0.8	0.6
Gestation day	147.7 ± 0.3	149.2 ± 0.9	0.1	<0.05	0.1
Maximal GV (g/Kg·d)	83 ± 3	93 ± 10	0.3	0.7	0.3
GV_{0-2} (g/Kg·d)	46 ± 1	52 ± 2	<0.05	0.9	0.5
CRL_{0-2} (cm/d)	0.94 ± 0.03	1.04 ± 0.08	0.3	0.9	0.7
HL_{0-2} (cm/d)	0.32 ± 0.01	0.34 ± 0.04	0.6	0.2	0.5
BPD_{0-2} (mm/d)	0.04 ± 0.01	0.04 ±0.01	0.8	0.7	0.9
GV_{2-W} (g/Kg·d)	14.2 ± 0.3	16.1 ± 0.9	<0.05	0.8	0.9
CRL_{2-W} (cm/d)	0.36 ± 0.03	0.37 ± 0.02	0.4	0.7	0.4
HL_{2-W} (cm/d)	0.17 ± 0.01	0.20 ± 0.01	0.1	0.09	0.7
BPD_{2-W} (mm/d)	0.20 ± 0.01	0.21 ± 0.02	0.6	0.3	0.9
Age at juvenile testing (weeks)	18.4 ±0.5	19.8 ±1.2	0.3	0.3	0.9
Weight at juvenile testing (Kg)	35.8 ± 1.0	31.7 ± 2.8	0.2	0.6	0.8

Table 7-1: Growth velocity in IUGR lambs

GV=growth velocity. CRL=crown rump length. HL=hind limb length. BPD=bi-parietal diameter. $_{0-2}$ *=birth to 2 weeks.* $_{2-W}$ *= 2 weeks to weaning. 5 IUGR females have birth data, but only 4 have data from subsequent time points. Data are least square means ±SEM.*

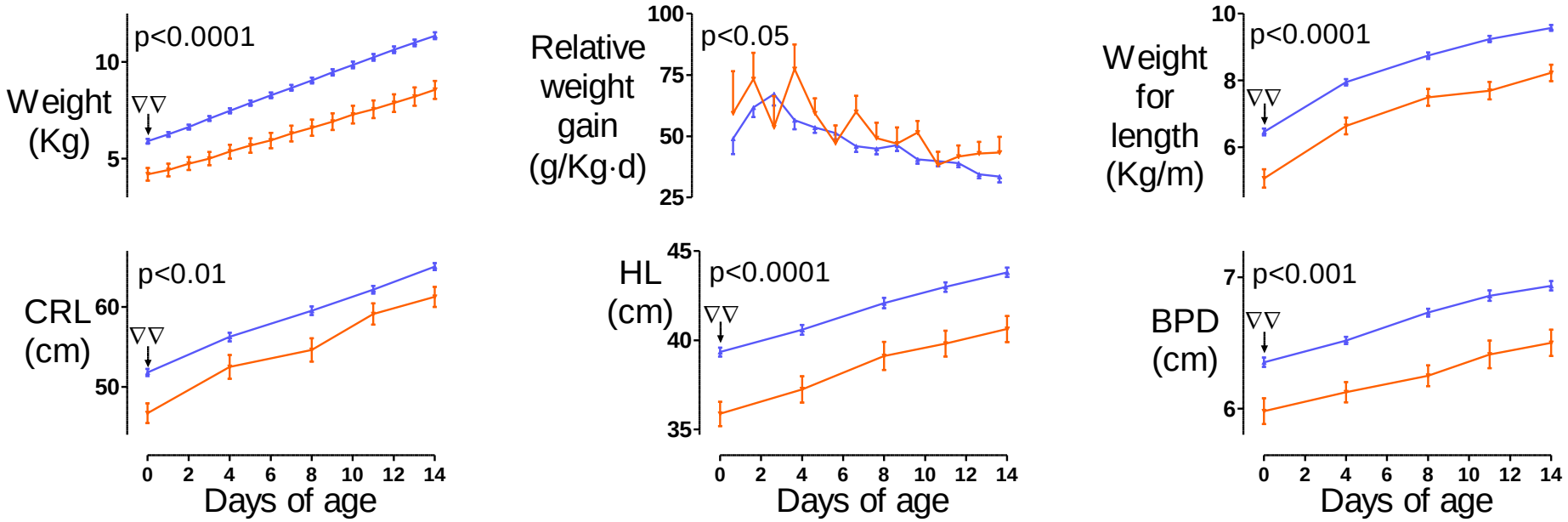

Figure 7-1: Growth between birth and two weeks in IUGR and term-spont lambs

CRL=crown rump length. HL=hind limb length. BPD=bi-parietal diameter. Un-supplemented (UN) term-spont n=40. IUGR n=7 for birth data, 6 thereafter. Data are least square mean ± SEM. p-values represent the overall effect of birth group assessed using RM ANOVA between birth and 2 weeks. Arrows and symbol indicate the effect of group at birth. ∇∇ p<0.01 IUGR compared with term-spont.

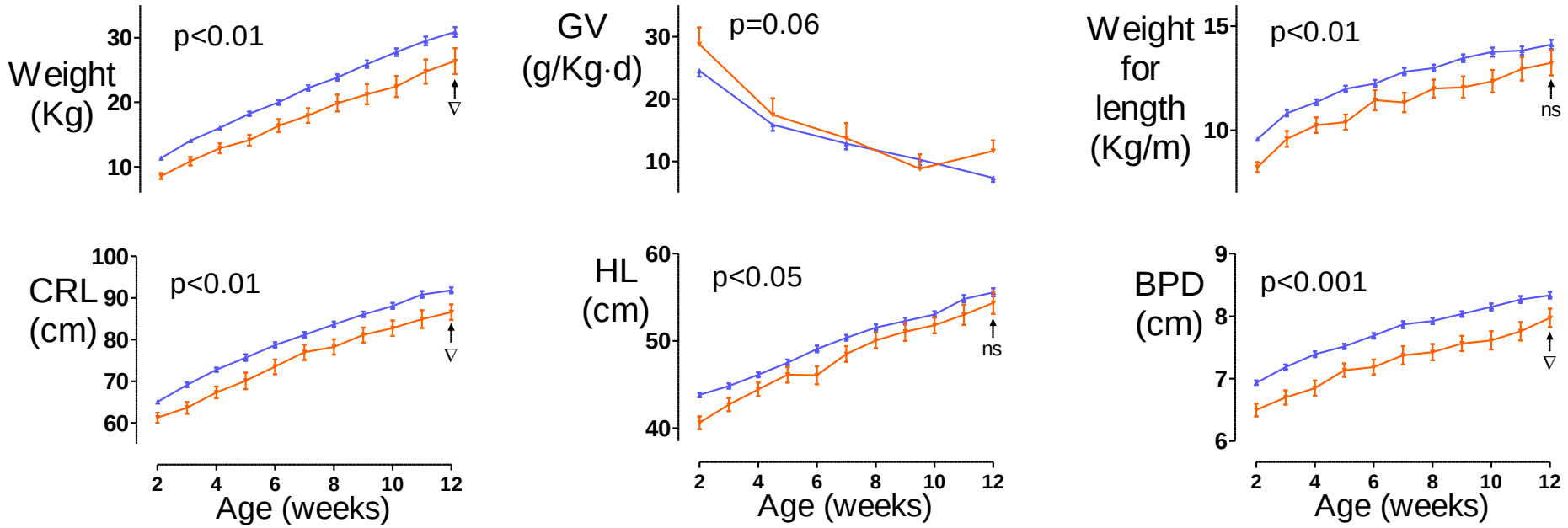

Figure 7-2: Growth between two weeks to weaning in IUGR and term-spont lambs

GV=growth velocity. CRL=crown rump length. HL=hind limb length. BPD=bi-parietal diameter. Un-supplemented (UN) term-spont n=20. IUGR n=6. Data are least square mean ± SEM. p-values represent the overall effect of birth group assessed using RM ANOVA between 2 weeks and weaning. Point of weaning is shown by the arrow. Symbols indicate the effect of birth group at weaning. ∇ p<0.05 IUGR compared with term-spont.

7.4.2. Milk intake in IUGR lambs

Average daily milk intake during the first week of life was approximately 30% greater in IUGR than UN term-spont lambs (166 ± 11 vs. 126 ± 10 ml /Kg·d, p=0.02). Estimated total energy (211 ± 14 vs. 161 ± 12 Kcal/Kg·d) and resultant macronutrient intake were therefore proportionately increased in IUGR compared to UN term-spont lambs.

7.4.3. Plasma metabolites and insulin concentration in IUGR lambs

Plasma insulin, glucose, lactate, urea and free fatty acid (FFA) concentrations between birth and 2 weeks of age were not different between groups (Figure 7-3).

7.4.4. GH stimulation test

Baseline plasma glucose concentrations were lower and urea concentrations were higher in IUGR compared to UN term-spont sheep at the start of the juvenile GH stimulation test (Table 7-2).

Plasma IGF1 concentrations rose more in IUGR than UN term-spont sheep during the GH stimulation test, resulting in a greater maximal IGF1 increment and AUC Figure 7-4, Table 7-2).

Although plasma urea concentrations fell during the GH stimulation test they remained higher in IUGR than UN term-spont sheep (Figure 7-4).

The change in plasma glucose and FFA concentrations during the GH test was not different between groups (Figure 7-4).

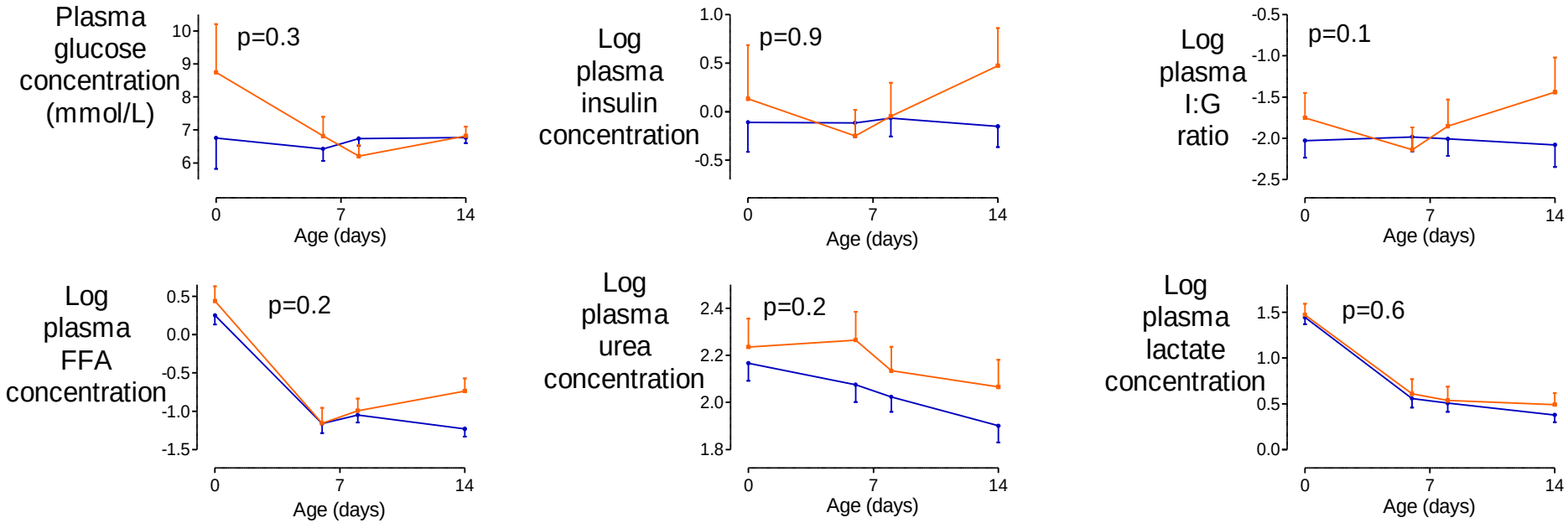

Figure 7-3: Plasma insulin and metabolite concentrations during the first two weeks of life

I=insulin. G=glucose. FFA=free fatty acids. Un-supplemented term-spont; blue, n=14. IUGR; orange, n=6. Data are least square mean ± SEM. p-values represent the effect of birth group assessed using RM ANOVA.

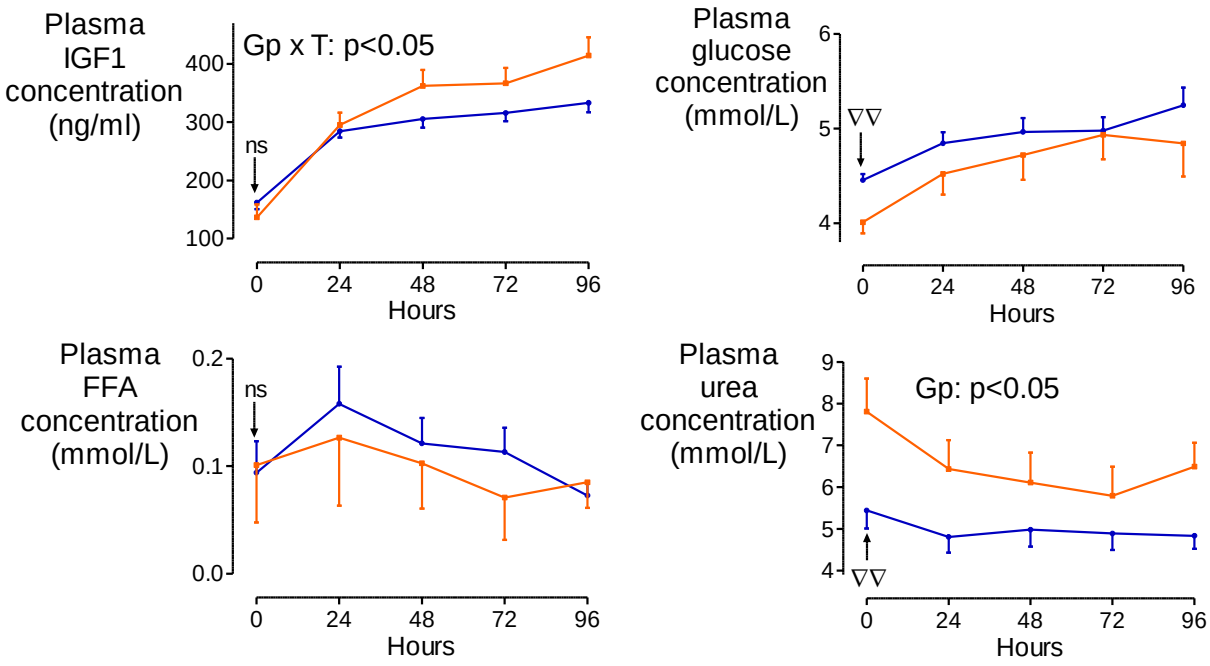

Figure 7-4: Plasma IGF1 and metabolites during a GH stimulation test

FFA=free fatty acids. Gp=birth group. T=time. Un-supplemented term-spont (UN); blue, n=17. IUGR; orange, n=5. Data are least square mean ± SEM. Significant p values for the effect of group or group x time are shown with the relevant graph. Arrow and symbol indicate effect of birth group at baseline. ∇∇ p<0.01 IUGR compared with term-spont.

	UN term-spont (n=17)	IUGR (n=5)	Significance for birth group (p-value)
Max increase in glucose (mmol/L)	1.0 ± 0.2	1.0 ± 0.3	0.9
Glucose AUC (mmol·min/L)	50 ±6	64 ± 11	0.2
Max increase in IGF1 (ng/ml)	179 ± 12	282 ± 23	<0.01
IGF1 AUC (ng·10^3·hr/ml)	12.0 ± 1.0	18.4 ± 1.8	<0.01

Table 7-2: Plasma concentrations of IGF1 and metabolites during a GH stimulation test

UN=un-supplemented. FFA=free fatty acids. AUC=area under the curve. Data are least square mean ± SEM.

7.4.5. Glucose tolerance in IUGR sheep

There was no difference between IUGR and UN term-spont sheep in plasma insulin and glucose concentrations during a glucose tolerance test (GTT) (Figure 7-5) in fasting insulin: glucose ratio, the total or first phase AUC or time taken for plasma glucose concentrations to fall below baseline values (Table 7-3). There was no difference between IUGR and un-supplemented term spont in the proportion of animals with plasma glucose concentration below baseline at 3 hours (IUGR 5/6, term-spont 22/32; χ^2=0.3).

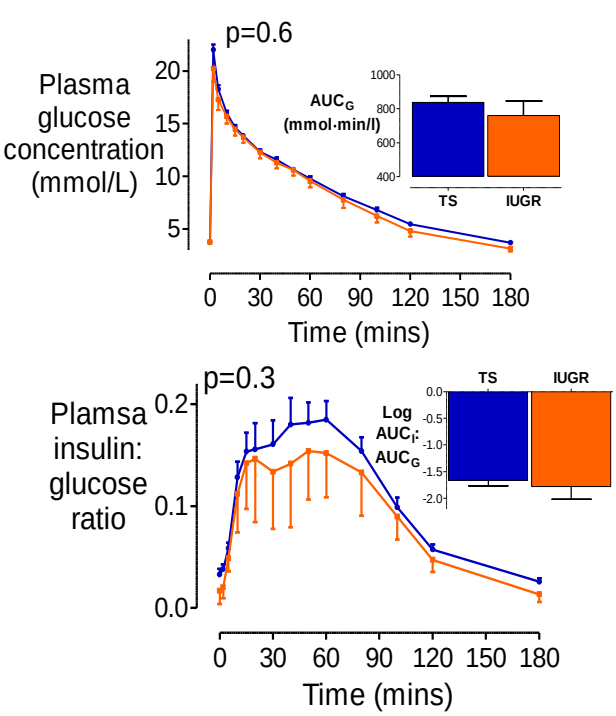

Figure 7-5: Plasma insulin and glucose during a glucose tolerance test

G=glucose. I=insulin. AUC=area under the curve. Un-supplemented term-spont (TS); dark blue (line = plasma I or G concentration, bar = total AUC for G or I), n=32. IUGR; orange, n=6.

	Term-spont (n=32)	IUGR (n=6)	Significance for group (p-value)
Glucose AUC_{15} (mmol·min/L)	194 ± 4	182 ± 10	0.3
Log insulin AUC_{15}	2.8 ± 0.1	2.7 ± 0.2	0.7
Log insulin AUC_{15}: glucose AUC_{15}	-2.4 ± 0.1	-2.5 ± 0.3	0.8
Time for G < baseline (mins)	161 ± 4	156 ± 11	0.6

Table 7-3: Insulin and glucose responses to a glucose challenge in IUGR sheep

G=glucose. AUC=area under the curve. $_{15}$=AUC between baseline and 15 minutes. Data are least square mean ± SEM.

7.5. Discussion

These preliminary findings indicate that *in utero* growth restriction in lambs is followed by accelerated postnatal growth and increased milk consumption so that by age 4-5 months, differences in weight between IUGR and UN term-spont sheep are no longer significant. The increased IGF1 responses to exogenous GH stimulation in IUGR sheep suggest that although IUGR sheep have attained the same weight as control animals, there are differences in somatotropic axis function.

Placental embolisation as a paradigm for IUGR

Experimental IUGR can be achieved using many different paradigms, each with its own advantages and limitations; in sheep, maternal hyperthermia (Wallace, Regnault, Limesand, Hay, & Anthony, 2005), pre-pregnancy carunclectomy (McMillen, et al., 2001), and single uterine artery ligation (Supramaniam, Jenkin, Loose, Wallace, & Miller, 2006) have all been described. Placental embolisation is known to produce a similar profile of fetal hypoxaemia, hypoglycaemia and growth restriction (Eremia, et al., 2007) to that seen in human IUGR fetuses (Economides, et al., 1989). However, the small number of animals generated using a standardised embolisation regime rather than one tailored to fetal response suggests that placental capacitance varies widely between animals. Injection of the same volume of microspheres into the uterine arteries of different ewes resulted in outcomes ranging from fetal death to no appreciable perturbation in fetal growth. This approach therefore has limited use in future studies of IUGR; dosing schedules need to be titrated to fetal response so that growth is reduced without fetal demise.

Postnatal nutrition following IUGR

Estimated milk intake in IUGR lambs during the first week of life was approximately 30% higher than in term-spont lambs. Little is known about milk composition in either humans or experimental paradigms of IUGR. Increased milk intake may therefore be regarded either as a mechanism for increasing nutrient and energy intake to above that of normally grown term lambs (assuming milk composition is comparable) or as a compensatory mechanism to achieve equivalent nutrient and energy intake to that of control lambs (assumes reduced nutrient or energy content in milk).

Rat dams that have undergone uterine artery ligation to generate IUGR pups have evidence of premature lactogenesis followed by disrupted lactation (altered maternal mammary mRNA expression of lactation related genes, a reduction in milk lactose, increased milk

sodium/potassium ratio, but no change in protein or fat content) (O'Dowd, Kent, Moseley, & Wlodek, 2008). In addition, the amount of milk consumed by suckling IUGR pups (measured as pup test weighs before and after feeding) was less than that consumed by control pups. This may be an indication of reduced milk secretory capacity, or it is possible that IUGR pups are less robust, with weaker muscle tone than control animals and therefore are less able to suckle effectively. This might also explain why the usual age related increase in milk intake was delayed in IUGR pups; as suckling promotes lactation, weaker suckling might impair lactation. Other ovine IUGR paradigms have, however, found increased feed duration in IUGR compared to control lambs, although milk intake *per se* or milk composition were not measured (De Blasio, Gatford, Robinson, & Owens, 2007).

Milk composition data from ewes of IUGR lambs is needed to better understand these findings.

Postnatal growth following IUGR

Inclusion in the IUGR cohort was based on a newborn weight of at least 15% less than anticipated for a spontaneously term-born animal. This weight reduction, however, was not achieved by a commensurate decrease in soft tissue and skeletal growth; IUGR lambs had a significantly lesser weight for length, suggesting that soft tissue growth was affected to a greater degree than skeletal tissue. The ability of simple anthropometric measures to discern changes in organ growth or body proportionality following fetal growth restriction remains contentious. In humans, ponderal index (PI; weight / height or length^3) has been proposed by some as a useful tool to adequacy, or otherwise of fetal growth; a low PI indicative of disproportionately poor soft tissue growth and therefore IUGR (Landmann, et al., 2006). Others, however, have found that in human newborns neither BMI or PI are robust measures of either IUGR or fatness at birth (Haggarty, et al., 2004). Similarly, in other ovine paradigms of IUGR, although weight is reduced compared to control animals, both in late gestation (Thorn, et al., 2009) and at term (De Blasio, et al., 2010), body proportionality is preserved, although not the proportionality of internal organs, especially the brain: liver or brain: body weight ratio (McMillen, et al., 2001; Thorn, et al., 2009).

Similar to findings in humans (Ong, et al., 2000; Rogers, 2003) increased relative early growth rates and increased fat mass with reduced lean muscle mass have been found in low birth weight lambs (De Blasio, Gatford, Robinson, et al., 2007), especially if allowed to feed *ad libitum* (Greenwood, Hunt, Hermanson, & Bell, 1998). Therefore, if lambs move from a

nutrient restricted environment *in utero* to one of freely available food, although catch-up growth is achieved, it is at the expense of altered body composition. Future studies are planned that incorporate regular DXA body composition scans throughout postnatal life to track age related changes in adiposity.

Effects of GH stimulation

The GH-IGF1 axis is the principal regulator of postnatal growth (Lupu, Terwilliger, Lee, Segre, & Efstratiadis, 2001), with IGF1 secreted by hepatocytes in response to GH stimulation, mediating many of the growth promoting effects of GH. Pre-GH plasma IGF1 concentrations were not different between groups yet exogenous GH provoked a greater rise in IGF1 in IUGR than control sheep. In both human (Economides, Nicolaides, Linton, Perry, & Chard, 1988) and experimental (Ross et al., 2000) fetal growth restriction there is an increase in fetal plasma cortisol concentrations. In sheep, it has been demonstrated that in late gestation increased fetal cortisol concentrations cause a down regulation in muscle IGF1 mRNA expression (Li, et al., 2002), but an increase in hepatic GH receptor and IGF1 mRNA expression (Li, et al., 1996). Increased fetal cortisol concentrations in IUGR may therefore result in excessive or early reduction muscle IGF1 mediated muscle fibre proliferation and maturation, with possible adverse consequences for lean mass in postnatal life (Bauer, Gedrange, Bauer, & Walter, 2006; Greenwood, Hunt, Hermanson, & Bell, 2000), but increased activity of the hepatic endocrine GH-IGF1 axis. Due to the pulsatile nature of endogenous GH secretion and age dependent changes in GH-IGF1 axis function, reports of differences in baseline IGF1 between IUGR and normally grown individuals vary. Most investigators however report reduced plasma IGF1 concentrations at birth (Giudice et al., 1995), although birth IGF1 concentrations do not predict later growth trajectory (Leger, Noel, Limal, & Czernichow, 1996). Later in childhood, IGF1 concentrations tend to be lower in those born IUGR, especially if they also have poor postnatal growth. In addition, both a reduction in total GH secretion (Boguszewski, Rosberg, & Albertsson-Wikland, 1995) and alteration in the proportions of circulating GH isoforms has been found in SGA children, especially those who remain short (Boguszewski et al., 1997). Others however have found no difference in GH-IGF1 axis between short children born either SAG or AGA (Volkl et al., 2004). Most of these data however are derived from baseline measurements of IGF1. Provocation tests such as the GH stimulation test reported here amplify differences in GH-IGF1 axis, making it easier to appreciate altered axis function following IUGR, which may not be appreciable under normal physiological conditions.

Furthermore, early nutritional environment may also cause permanent alteration to GH-IGF1 axis function. Pituitary GH mRNA expression in adult rats is significantly altered by the diet fed to their dam during lactation. Compared to dams fed standard chow, offspring of those fed isocaloric but protein restricted diets had reduced GH mRNA expression, whereas offspring of those fed an energy deficient diet had increased GH mRNA expression (de Moura et al., 2007). Altered early macronutrient or calorie intake in IUGR lambs, either as a consequence of increased milk intake, or altered milk composition, may therefore have additional implications for later GH-IGF1 axis function.

Finally, lambs in our paradigm exhibited 'catch-up' growth so that by the time of the GH stimulation test were no different in weight to control lambs. The majority of SGA or IUGR children also have rapid early postnatal growth so that by later childhood, height and weight are similar to those born with normal birth weight (Karlberg & Albertsson-Wikland, 1995). Of those that do not achieve catch-up growth, many have mutations in IGF1 or IGF1R that alter IGF1 signalling pathways (Klammt, Pfaffle, Werner, & Kiess, 2008). Altered bioavailability of IGF1, secondary to IGF binding protein concentrations (Bannink, van Doorn, Mulder, & Hokken-Koelega, 2007), may also explain differences in postnatal growth following IUGR.

Metabolic consequences of IUGR

Although GTT outcome measures were not different between IUGR and control sheep, graphical representation shows a very similar profile of plasma insulin and glucose concentrations to that found by De Blasio *et al.* (De Blasio, Gatford, McMillen, et al., 2007). In their study, however, insulin secretion was significantly less in IUGR than control animals, possibly explained by the younger age at which animals were studied and the age related changes in insulin secretion and sensitivity that occur following IUGR (Gatford, et al., 2008).

Limitations of the study

Only 6 animals (2 male, 4 female) were included in the IUGR group and therefore results need to be interpreted with caution; these data are presented as preliminary findings only. We did not have a sham operated control groups of ewes and lambs; however, the experiment tested the effect of IUGR against lambs from healthy animals. By minimising any interventions in the control group, the paradigm may be more akin to the clinical situation than if ewes had undergone the stress of anaesthesia and sham surgery. Additionally, there is evidence to suggest that 'control' interventions may themselves have deleterious effects on

maternal and fetal physiology that continue to influence the offspring well into postnatal life (Franko, Forhead, & Fowden, 2010).

We did not measure plasma IGF1 concentrations at birth or in postnatal life, and we did not collect tissue with which to examine differences on a molecular level between IUGR and control sheep. This will be addressed in future studies of IUGR.

Conclusions

IUGR in lambs is associated with accelerated postnatal growth and evidence of increased IGF1 response to GH stimulation in juvenile life. Whether these differ between the sexes, or are modified by preterm birth, postnatal nutrition or increasing age remains to be determined.

Chapter 8. Thesis conclusions

The experiments described in this thesis were designed to try to separate the effects of preterm birth, antenatal corticosteroid exposure, fetal growth and postnatal nutrition on postnatal growth and later indices of cardiovascular and metabolic function. Importantly, the experiments described here allowed the effects of sexual dimorphism to become apparent as, unlike many other long-term animal studies, our sheep were studied with reproductive function intact. We have demonstrated that any of the perinatal events described above can have effects on postnatal cardiovascular and metabolic function that persist into adulthood and that differ according to sex and to the combination of pre- and postnatal factors.

Gestation length, in sheep, affects indices of cardiac autonomic function in juvenile life that persist to adulthood. In adult males, preterm birth resulted in increased sympathetic activity and reduced parasympathetic activity, whereas in adult females decrements in gestational age across the term continuum were associated with increasing cardiac sympathetic activity. These data raise intriguing questions around the long-term implications to health of birth at less than 40 weeks gestation. Firstly, human data have shown that changes in heart rate variability precede overt cardiovascular disease (Dekker, et al., 2000; Tsuji, et al., 1996). In particular, the pattern of altered sympathovagal balance with relative increases in sympathetic and / or reduced parasympathetic activity appears to predate hypertension and other cardiovascular and metabolic morbidity (Dekker, et al., 2000; Flaa, et al., 2008; Kaufman, et al., 2007; Licht, et al., 2010). Secondly, human data from both prospectively followed birth cohorts and epidemiological studies have shown an increase in both blood pressure (Dalziel, et al., 2007) and the need for anti-hypertensive medication (Crump, et al., 2011) in young adults born preterm, although the underlying pathophysiological basis of this remains unknown. Thirdly, rates of preterm birth continue to climb in NZ and globally (Australian and New Zealand Neonatal Network, 2009; Beck, et al., 2010), with an especially marked increase in the number of infants delivered at late preterm and early-term gestations without medical indication (Holland, et al., 2009; Murthy, Grobman, Lee, & Holl, 2011). Innervation of the heart by the cardiac autonomic nervous system continues throughout the last weeks of pregnancy (Hildreth, et al., 2009) so that with increasing gestational age there is a reduction in the relative contribution of sympathetic activity to overall cardiac autonomic activity (David, et al., 2007). Disruption to the ontogeny of cardiac innervation may, therefore,

underlie the changes in later autonomic activity, although reasons for the sexual dimorphism remain unclear. Advances in perinatal medicine have meant that we are now starting to see survivors of preterm birth enter middle age but, as a group, they are still too young for most cardiovascular morbidity to be manifest (Tate, et al., 1998). The increased rate of hypertension in relatively young adults born preterm raises the suspicion that this population might be at increased risk of other cardiovascular morbidity that becomes manifest with advancing age. In addition, it is now recognised that infants born across the term continuum are not a homogeneous population, and that there are gestational age specific patterns of morbidity and mortality beyond the newborn period (MacKay, Smith, Dobbie, & Pell, 2010; Reddy et al., 2011). If reduced gestation length in humans, even across what is regarded as 'term', also results in altered cardiac autonomic function this may explain some of the observed morbidity and may also have significant implications for long-term cardiovascular health. To date there are no data on cardiac autonomic function in adults born preterm, or on the adult cardiovascular outcomes of infants born at early-term gestations; these are areas of research that urgently need to be addressed.

Gestation length in our paradigm did not, however, appear to alter either glucose tolerance or measures of insulin sensitivity. This was somewhat surprising as there is increasing evidence from human studies that preterm birth is associated with reduced insulin sensitivity in both childhood (Hofman, et al., 2004) and adulthood (Pilgaard et al., 2010). It has been proposed that preterm birth, especially very preterm birth, may disrupt pancreatic organogenesis (Green, et al., 2010) and may therefore reduce the pool of β cell progenitors or alter pancreatic microvasculature; over time these changes may limit the capacity of the pancreas to maintain an adequate β cell mass adequate to meet the metabolic needs of the individual (Hill, 2011). As preterm lambs in our paradigm were born only 10 days earlier than their term-spont counterparts, pancreatic maturity at birth may have been relatively greater than, for example, that of an infant born at < 32 weeks' gestation (the preterm population investigated by Hofman et al). Furthermore, preterm lambs were relatively independent from birth, were able to tolerate full enteral feeds of maternal milk and did not require parenteral nutrition or other supportive interventions that are commonly necessary in preterm infants (Hans, et al., 2009). Given the effects of nutrient supplementation on glucose-insulin axis function (discussed later), it is possible that at least part of the effect of preterm birth on this axis reported in human studies relates to differences in nutritional milieu between preterm and term-born infants.

Nutritional supplementation of newborn lambs was intended to promote their early growth in a manner similar to that seen when preterm or small infants are given human milk fortifier (Kuschel & Harding, 2004). It was hypothesised that the growth response to supplementation would be similar in all cohorts of lambs. Instead, the effects of supplementation on growth were confined to males and persisted well after the cessation of supplementation. It is important to recognise that lambs in this experimental paradigm had free access to the ewe at all times and were able to feed *ad libitum*; nutrient supplementation was therefore in addition to, not instead of, maternal milk. This was confirmed by milk intake studies which showed no difference in milk intake between supplemented and un-supplemented animals.

Nutritional supplementation did promote weight gain in preterm males, but not females, with increased weight-for-length between two weeks and weaning. This was not, however, associated with later changes in direct measures of body composition, although results from DXA scanning were only available for a small number of preterm males. In contrast, supplementation of term-spont males resulted in reduced pre-weaning weight gain and weight-for-length. This unanticipated effect on growth and body proportionality appeared to be persistent; although not statistically significant, supplemented term-spont males were leaner in adulthood than those not supplemented, though with similar body mass.

Both mode of feeding and composition of the feed may be important in determining long-term outcomes. Preterm infants typically receive parenteral fluids and nutrition during the first days of life with little or no enteral feeding before progressing to milk feeds at a time comparable with the last trimester of pregnancy (Hans, et al., 2009). In addition, milk is initially given by gastric gavage at a volume and feed interval pre-determined according to medical indications. It is usually only after term equivalent age and discharge from hospital that infant feeding regimes begin to correspond to infant appetite and feeding cues. Whether bypassing the cephalic phase of feeding or using regular fixed volume feed regimes have later effects on infant appetite and self regulation of food intake is unknown (Li, et al., 2010; Power & Schulkin, 2008).

In comparison to preterm infants, those born at term are usually fed either breast milk or formula milk, with enteral feeds commencing in the immediate newborn period. Nevertheless, even amongst term infants there are important differences in early growth trajectory and body proportionality between those fed breast milk and those fed formula (Kramer, et al., 2004), and these may relate to differences in milk composition (Heinig, et

al., 1993; Heird, 2007; Koletzko, von Kries, Closa, et al., 2009; Mennella, et al., 2011), or differences in regulation of feeding and appetite (Hodges, et al., 2008; Li, et al., 2010; Sievers, et al., 2002). This is especially relevant as there are increasing numbers of artificial formulas available to parents, many of which have a composition substantially different from that of human milk (Joeckel & Phillips, 2009). Preterm infants commonly also experience altered feed composition compared to term infants. Although maternal milk remains the preferred milk for preterm infants, it is frequently supplemented with either breast milk fortifiers or preterm formula (more energy dense than and of a different macronutrient composition from standard term formula) both prior to term-equivalent age and following hospital discharge (Hans, et al., 2009; Kuschel & Harding, 2004).

Differences in nutrition prior to term and following discharge have been associated with changes in growth and body proportionality (Aimone, et al., 2009; Kennedy, et al., 2010), although the long-term health implications of this are unknown.. Much of the existing data regarding early nutrition and growth trajectories are based on nutritional practices no longer relevant to the contemporary standards of neonatal intensive care (Lucas, et al., 1998; Singhal, Cole, & Lucas, 2001; Singhal, et al., 2003). Our data demonstrate the importance of ensuring that growth outcomes beyond the period of intervention are investigated, and that assessments of growth include measures other than simple anthropometric parameters as these do not necessarily convey information about body composition (Wells, 2000). For instance, infants born preterm have a different pattern of fat mass distribution by term-corrected age than newborn term infants (Uthaya, et al., 2005). Where possible direct measures of body composition should also characterise the fat distribution pattern as visceral and subcutaneous adipose tissue have different metabolic sequelae (Fox, et al., 2007).

In addition to the effects of supplementation on growth and direct measurements of body composition we found that there was also a sex-specific effect on the adrenaline-stimulated release of free fatty acids (FFA): supplemented term-spont males tended to have a lesser adrenalin-stimulated FFA response than those not supplemented; the converse relationship was true in preterm males. In females, although term-spont sheep tended to have a greater FFA response than preterm sheep, most of this effect was due to the increased FFA response in those term-spont females that had received nutritional supplementation. These data suggest that supplementation alters fat mobilisation responses, but whether this reflects changes in fat mass or adipocyte function, or both, is uncertain. Thus, a brief period of nutritional supplementation in the newborn period results in changes in patterns of growth and in the

development and function of fat mass that persist beyond the period of intervention itself. Importantly, the effect of supplementation differed by sex, and by gestational age at birth, suggesting that it would be inappropriate to draw inferences from one population of infants, and, by extrapolation, apply them to another population of infants. Although we cannot explain the reason for the differences in response to supplementation between the sexes, other studies have also shown sex-specific effects of early nutritional environment (Lucas, et al., 1998). Given the burgeoning population of obese children and adults the association between early diet and later body size and composition warrants further investigation. Importantly, there is a need for follow-up beyond infancy of those infants already enrolled in trials of newborn nutrition (Grote, et al., 2010); and for ensuring that future studies are adequately powered to detect sex-specific outcomes.

It is intriguing that although neither preterm birth nor antenatal steroid exposure at term had any impact on later glucose-insulin axis function, a brief period of nutritional supplementation did. Post-weaning, supplemented term-spont females had a lesser glucose and arginine-stimulated insulin secretion than un-supplemented females, but tended towards increased insulin sensitivity; whereas supplemented preterm females had greater arginine-stimulated insulin secretion but tended towards reduced insulin sensitivity. By adulthood, this effect was no longer present in females but, although not statistically significant, a similar pattern of insulin responses had emerged in the male sheep. Plagemann et al. have described a brief window in early postnatal life during which time ingestion of ‘diabetic milk’ (milk from diabetic mothers), compared to expressed breast milk from non-diabetic mothers, was associated with increased risk of overweight and impaired glucose tolerance in early childhood (Plagemann, et al., 2002). Whether these changes in body mass and glucose tolerance persisted, worsened or resolved with time is as yet unknown. Exposure to ‘diabetic milk’ outside of this relatively small window was not, however, associated with the same sequelae (Rodekamp, et al., 2005). It is plausible that early nutrition influences enteroinsular axis function during a key phase of pancreatic maturation. Supplementation may therefore elicit different responses in preterm and term-born sheep by imposing the same increase in carbohydrate and protein load at different stages of pancreatic and gastrointestinal maturation. Alternatively, insulin, or other hormones stimulated by nutritional environment and acting on the appetite regulatory centres of the hypothalamus (Rother, et al., 2008), may have different, possibly persistent, effects on appetite regulation and nutrient partitioning in

preterm compared to term-born animals. Early diet, distinct from early growth rate, may therefore have far-reaching implications for later metabolic health.

The term-dex cohort was developed to try to separate the effects of preterm birth from those of antenatal corticosteroid exposure. While antenatal corticosteroid exposure at term had no effect on cardiac autonomic function or glucose-insulin axis function, it did influence adult body composition in females; term-dex females were fatter than either term-spont or preterm sheep, although only the comparison with preterm sheep was statistically significant. There was also a highly significant, negative, association between birth weight z-score and measures of adult adiposity confined to term-dex females. These findings have potentially significant clinical implications. A recent randomised controlled trial of antenatal corticosteroids prior to elective caesarean section at early-term gestation showed a significant reduction in short term respiratory morbidity (Stutchfield, et al., 2005), prompting the Royal College of Obstetricians and Gynaecologists to endorse the use of corticosteroids for all elective caesarean sections prior to 38^{6} weeks gestation (Royal College of Obstetricians and Gynaecologists, 2010). To date, there have been no studies examining potential long-term consequences of this recommendation.

Our finding of increased adiposity after prenatal corticosteroid exposure in healthy, free ranging sheep reared in an optimal nutritional environment makes it imperative that the long-term outcomes for infants similarly exposed to corticosteroids at term are investigated, preferably using the original cohort described by Stutchfield et al. (Stutchfield, et al., 2005) as further prospective clinical trials of this sort are unlikely to be undertaken. Once again, it is plausible that corticosteroids have different long-term effects when exposure is at preterm rather than term gestations. It is reassuring that in children and adults born at both preterm and term gestations there have been no reported differences in cardiovascular and metabolic outcomes between those exposed, or not, to corticosteroids at preterm gestations (Dalziel, et al., 2007; de Vries, et al., 2008; Finken, et al., 2008). Any follow-up studies of infants exposed to corticosteroids at term also need to address the additional impact that steroid exposure might have on infants already disadvantaged by a poor *in utero* environment, manifest as low birth weight.

Many other animal experiments have involved administration of corticosteroids early in gestation to try to mimic the clinical scenario of preterm antenatal corticosteroid exposure, and have identified deleterious effects on later metabolic and cardiovascular function (de

Vries, et al., 2007; Dodic, Abouantoun, O'Connor, Wintour, & Moritz, 2002; Sloboda, et al., 2005). However, many of these studies have used corticosteroid doses far in excess of those used clinically, with exposure starting at a proportionally much earlier point in gestation, and therefore are of limited use for exploring the potential long-term effects.

In order to distinguish the effects of preterm birth from those of the many associated factors that might influence later outcomes, animal studies are essential, but have proven challenging to conduct and interpret. Although other preterm lamb experiments have been reported (De Matteo, et al., 2010), they have had a lower survival rate than that seen in our paradigm and describe a more heterogeneous population that includes lambs from multiple gestation pregnancy and lambs fed cow's milk formula to support early nutrition. Other preterm animal paradigms use litter bearing species, such as pigs (Oste et al., 2010) and guinea pigs (Lyall, et al., 1997). Twinning is known to alter later endocrine function and fat mass in adult sheep (Bloomfield, Oliver, & Harding, 2007; Hancock, Oliver, McLean, Jaquiery, & Bloomfield, 2011), and we have shown that early nutrition has persistent effects of growth and metabolic function. In litter-bearing species littermates may be affected differently by their *in utero* position, (with its consequent implications for fetal nutrient availability and growth (Wise, Roberts, & Christenson, 1997)), the effects of hormones or other blood borne factors arising from adjacent fetuses (Mori, Matsuda, Tsukahara, & Kawata, 2010), the postnatal division of maternal milk between offspring (Rodel, Prager, Stefanski, von Holst, & Hudson, 2008), or the presence of littermate siblings themselves (Nicolas, Martinez-Gomez, Hudson, & Bautista, 2011). We therefore suggest that maintaining as homogeneous a population as possible (for instance, limiting the study group to singleton animals and minimising nutritional variation) is advantageous, especially when assessing effects temporally distant from any perinatal intervention. Preterm primates have also been studied (Blanco, et al., 2010), but such work is hampered by its prohibitive cost and complex ethical considerations (Quigley, 2007).

From a pragmatic standpoint, animals delivered at preterm gestations are unlikely to survive without antenatal corticosteroid exposure (De Matteo, et al., 2010), and it is therefore not practicable to have a preterm but steroid naïve group. In addition, nearly 90% of infants born below 34 weeks gestation in Australia and NZ have been exposed to antenatal corticosteroids (Australian and New Zealand Neonatal Network, 2009). However, in our experiments preterm birth was induced in a healthy ewe that otherwise would not have delivered early. This approach has the benefit of allowing some separation of the effects of the materno-fetal

co-morbidity that sets the trajectory for preterm birth (Smith, et al., 1998) from the effects of reduced gestation length itself, but has the disadvantage that it differs from the clinical scenario where some of the effects of preterm birth may be due to the processes that led to preterm birth in the first place. In particular, there is evidence to suggest that there are higher rates of fetal growth restriction in infants born preterm so that that late effects of preterm birth may relate to poor fetal growth as well as reduced gestation length (Cooke, 2007). The approach described here in sheep is, however, a useful experimental paradigm with which to generate hypotheses addressing the long-term impact of human preterm birth or antenatal corticosteroid exposure.

Poor fetal growth and low birth weight have been linked to adverse cardiovascular and metabolic outcomes in humans (Barker, 1992; Huxley, et al., 2007; Newsome, et al., 2003) and in many different experimental settings (McMillen, et al., 2001; Ozanne, et al., 2005). It is becoming clear that low birth weight is a manifestation of many different pathophysiological processes, and therefore it is not surprising that a recent study showed how the range of postnatal consequences was dependent on the cause of IUGR rather than birth weight *per se* (Nusken et al., 2011). Although we did not find a strong effect of birth weight z-score on later outcomes within the preterm and term-spont, term-dex or term-Alizin sheep, this may well be a reflection of the clustering of z-scores around zero. This in itself was not unexpected given that all ewes selected for the study were themselves healthy, appropriately grown, well nourished at mating and throughout pregnancy, with mode of delivery imposed on an otherwise normal pregnancy that was unlikely to end in spontaneous preterm labour. We have demonstrated in our IUGR paradigm that it is possible to generate viable, growth restricted lambs through the technique of placental embolisation. Although animal numbers were small, we found a greater IGF1 response following GH stimulation in IUGR compared to term-spont sheep. Previously, work in our group has demonstrated low fetal plasma IGF1 concentrations following placental embolisation and IUGR (Eremia, et al., 2007). However, the fetus however remains highly sensitive to the effects of IGF1, as exogenous supplementation increased fetal growth rate without deleterious effects on fetal acid base balance or on other fetal metabolic or endocrine parameters (Eremia, et al., 2007). These data suggest that changes in the GH-IGF1 axis are present in the IUGR fetus and persist at least into juvenile life. Further study of this group will continue but is beyond the scope of this thesis.

In conclusion, the studies described in this thesis show that in sheep, reduced gestation length affects later cardiac autonomic function; that antenatal corticosteroid exposure at term affects later body composition, and that the early postnatal nutritional environment has effects on growth, body proportionality and glucose-insulin axis function that persist into adult life. Additionally, these late effects of the perinatal environment are sex-specific. These experiments therefore demonstrate how relatively minor changes in the perinatal environment, such as small reductions in gestation length or early nutritional interventions analogous to those used in routine contemporary clinical practice, may have significant long-term effects. While there has been major progress in reducing short term morbidity and mortality in infants born preterm, there are relatively few data from human studies to inform strategies that both meet the immediate health needs of the infant and also minimise any future long-term morbidity. Changes in the way we care for newborn infants therefore need to be accompanied by appropriate long-term follow-up studies to ensure that effects that are not immediately visible or anticipated are not 'missed'. This is most likely to be successful if such studies are informed by appropriate animal experiments such as those described in this thesis.

References

Abbott, D. H., Barnett, D. K., Colman, R. J., Yamamoto, M. E., & Schultz-Darken, N. J. (2003). Aspects of common marmoset basic biology and life history important for biomedical research. *Comp Med, 53*(4), 339-350.

Abe, C., Minami, J., Ohrui, M., Ishimitsu, T., & Matsuoka, H. (2007). Lower birth weight is associated with higher resting heart rate during boyhood. *Hypertens Res, 30*(10), 945-950.

Abel, E. D., Peroni, O., Kim, J. K., Kim, Y. B., Boss, O., Hadro, E., et al. (2001). Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature, 409*(6821), 729-733.

Abitbol, C. L., Chandar, J., Rodriguez, M. M., Berho, M., Seeherunvong, W., Freundlich, M., et al. (2009). Obesity and preterm birth: additive risks in the progression of kidney disease in children. *Pediatr Nephrol, 24*(7), 1363-1370.

Abrams, S. A. (2011). What are the risks and benefits to increasing dietary bone minerals and vitamin D intake in infants and small children? *Annu Rev Nutr, 31*, 285-297.

Abuzzahab, M. J., Schneider, A., Goddard, A., Grigorescu, F., Lautier, C., Keller, E., et al. (2003). IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med, 349*(23), 2211-2222.

Accili, D. (2004). Lilly lecture 2003: the struggle for mastery in insulin action: from triumvirate to republic. *Diabetes, 53*(7), 1633-1642.

Ackermann, A. M., & Gannon, M. (2007). Molecular regulation of pancreatic beta-cell mass development, maintenance, and expansion. *J Mol Endocrinol, 38*(1-2), 193-206.

Agabiti-Rosei, E. (2008). From macro- to microcirculation: benefits in hypertension and diabetes. *J Hypertens Suppl, 26*(3), S15-19.

Agostoni, C., Buonocore, G., Carnielli, V. P., De Curtis, M., Darmaun, D., Decsi, T., et al. (2010). Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr, 50*(1), 85-91.

Ahren, B. (2000). Autonomic regulation of islet hormone secretion-implications for health and disease. *Diabetologia, 43*(4), 393-410.

Ahren, B., Winzell, M. S., & Pacini, G. (2008). The augmenting effects of insulin secterion by oral versus intravenous glucose is exaggerated by high-fat diet in mice. *J Endocrinol, 197*(1), 181-187.

Aimone, A., Rovet, J., Ward, W., Jefferies, A., Campbell, D. M., Asztalos, E., et al. (2009). Growth and body composition of human milk-fed premature infants provided with extra energy and nutrients early after hospital discharge: 1-year follow-up. *J Pediatr Gastroenterol Nutr, 49*(4), 456-466.

Alexandrou, G., Skiold, B., Karlen, J., Tessma, M. K., Norman, M., Aden, U., et al. (2010). Early hyperglycemia is a risk factor for death and white matter reduction in preterm infants. *Pediatrics, 125*(3), e584-591.

Alexy, U., Kersting, M., Sichert-Hellert, W., Manz, F., & Schoch, G. (1999). Macronutrient intake of 3- to 36-month-old German infants and children: results of the DONALD Study. Dortmund Nutritional and Anthropometric Longitudinally Designed Study. *Ann Nutr Metab, 43*(1), 14-22.

Alfaidy, N., Li, W., MacIntosh, T., Yang, K., & Challis, J. (2003). Late gestation increase in 11beta-hydroxysteroid dehydrogenase 1 expression in human fetal membranes: a novel intrauterine source of cortisol. *J Clin Endocrinol Metab, 88*(10), 5033-5038.

Alibegovic, A. C., Hojbjerre, L., Sonne, M. P., van Hall, G., Alsted, T. J., Kiens, B., et al. (2010). Increased rate of whole body lipolysis before and after 9 days of bed rest in healthy young men born with low birth weight. *Am J Physiol Endocrinol Metab, 298*(3), E555-564.

Allen, L. H. (2001). Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. *J Nutr, 131*(2S-2), 581S-589S.

Alper, A. B., Jr., Chen, W., Yau, L., Srinivasan, S. R., Berenson, G. S., & Hamm, L. L. (2005). Childhood uric acid predicts adult blood pressure: the Bogalusa Heart Study. *Hypertension, 45*(1), 34-38.

Alsweiler, J. M. (2010). *The effects and management of neonatal hyperglycaemia.* University of Auckland, Auckland.

American Academy of Pediatrics Committee on Nutrition. (1985). American Academy of Pediatrics Committee on Nutrition: Nutritional needs of low-birth-weight infants. *Pediatrics, 75*(5), 976-986.

American College of Obstetricians and Gynecologists. (2003). Clinical management guidelines for Obstetrician-Gynecologists: Management of preterm labor. *Obstetrics & Gynecology, 101*(5, Part 1), 1039-1047.

American College of Obstetricians and Gynecologists. (2007). Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Obstetrics & Gynecology, 109*(1), 189-190.

Amesz, E. M., Schaafsma, A., Cranendonk, A., & Lafeber, H. N. (2010). Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. *J Pediatr Gastroenterol Nutr, 50*(2), 200-207.

Anagnostis, P., Athyros, V. G., Tziomalos, K., Karagiannis, A., & Mikhailidis, D. P. (2009). The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *J Clin Endocrinol Metab, 94*(8), 2692-2701.

Andres, R. L., & Day, M. C. (2000). Perinatal complications associated with maternal tobacco use. *Semin Neonatol, 5*(3), 231-241.

Antonios, T. F., Rattray, F. M., Singer, D. R., Markandu, N. D., Mortimer, P. S., & MacGregor, G. A. (2003). Rarefaction of skin capillaries in normotensive offspring of individuals with essential hypertension. *Heart, 89*(2), 175-178.

Antonios, T. F., Singer, D. R., Markandu, N. D., Mortimer, P. S., & MacGregor, G. A. (1999). Structural skin capillary rarefaction in essential hypertension. *Hypertension, 33*(4), 998-1001.

Antonov, A. N. (1947). Children born during the siege of Leningrad in 1942. *J Pediatr, 30*(3), 250-259.

Arakaki, R., & Welles, B. (2010). Ketoconazole enantiomer for the treatment of diabetes mellitus. *Expert Opin Investig Drugs, 19*(2), 185-194.

Arantes, V. C., Teixeira, V. P., Reis, M. A., Latorraca, M. Q., Leite, A. R., Carneiro, E. M., et al. (2002). Expression of PDX-1 is reduced in pancreatic islets from pups of rat dams fed a low protein diet during gestation and lactation. *J Nutr, 132*(10), 3030-3035.

Arenz, S., Ruckerl, R., Koletzko, B., & von Kries, R. (2004). Breast-feeding and childhood obesity--a systematic review. *Int J Obes Relat Metab Disord, 28*(10), 1247-1256.

Arnon, S., Dolfin, T., Litmanovitz, I., Regev, R., Bauer, S., & Fejgin, M. (2001). Preterm labour at 34--36 weeks of gestation: should it be arrested? *Paediatr Perinat Epidemiol, 15*(3), 252-256.

Arslanian, S., & Suprasongsin, C. (1996). Differences in the in vivo insulin secretion and sensitivity of healthy black versus white adolescents. *J Pediatr, 129*(3), 440-443.

Arslanian, S. A., Suprasongsin, C., & Janosky, J. E. (1997). Insulin secretion and sensitivity in black versus white prepubertal healthy children. *J Clin Endocrinol Metab, 82*, 1923-1927.

Atanasova, S., Wieland, E., Schlumbohm, C., Korecka, M., Shaw, L., von Ahsen, N., et al. (2009). Prenatal dexamethasone exposure in the common marmoset monkey enhances gene expression of antioxidant enzymes in the aorta of adult offspring. *Stress, 12*(3), 215-224.

Auchtung, T. L., Baer, D. J., Erdman, R. A., Barao, S. M., & Dahl, G. E. (2002). Relation of growth hormone response to growth hormone-releasing hormone to estimation of milk production via deuterium oxide dilution in beef cattle. *J Anim Sci, 80*(5), 1270-1274.

Australian and New Zealand Neonatal Network. (2009). *Report of the Australian and New Zealand Neonatal Network 2006*.

Ay, L., Van Houten, V. A., Steegers, E. A., Hofman, A., Witteman, J. C., Jaddoe, V. W., et al. (2009). Fetal and postnatal growth and body composition at 6 months of age. *J Clin Endocrinol Metab, 94*(6), 2023-2030.

Bacha, F., Saad, R., Gungor, N., Janosky, J., & Arslanian, S. A. (2003). Obesity, regional fat distribution, and syndrome X in obese black versus white adolescents: race differential in diabetogenic and atherogenic risk factors. *J Clin Endocrinol Metab, 88*(6), 2534-2540.

Baggia, S., Albrecht, E. D., & Pepe, G. J. (1990). Regulation of 11 beta-hydroxysteroid dehydrogenase activity in the baboon placenta by estrogen. *Endocrinology, 126*(5), 2742-2748.

Bailey, J. R., Fitzgerald, D. M., & Applegate, R. J. (1996). Effects of constant cardiac autonomic nerve stimulation on heart rate variability. *Am J Physiol Heart Circ Physiol, 270*(6 Pt 2), H2081-2087.

Ballard, J. L., Novak, K. K., & Driver, M. (1979). A simplified score for assessment of fetal maturation of newly born infants. *J Pediatr, 95*(5 Pt 1), 769-774.

Bannink, E. M., van Doorn, J., Mulder, P. G., & Hokken-Koelega, A. C. (2007). Free/dissociable insulin-like growth factor (IGF)-I, not total IGF-I, correlates with growth response during growth hormone treatment in children born small for gestational age. *J Clin Endocrinol Metab, 92*(8), 2992-3000.

Barker, D. J. (1992). *Fetal and Infant Origins of Adult Disease*. London: British Medical Journal Publishing Group.

Barker, D. J., & Osmond, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet, 1*(8489), 1077-1081.

Barker, D. J., Osmond, C., Forsen, T. J., Kajantie, E., & Eriksson, J. G. (2005). Trajectories of growth among children who have coronary events as adults. *N Engl J Med, 353*(17), 1802-1809.

Barker, D. J., Osmond, C., Golding, J., Kuh, D., & Wadsworth, M. E. (1989). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ, 298*(6673), 564-567.

Barker, D. J., Osmond, C., Kajantie, E., & Eriksson, J. G. (2009). Growth and chronic disease: findings in the Helsinki Birth Cohort. *Ann Hum Biol, 36*(5), 445-458.

Barker, D. J., Osmond, C., & Law, C. M. (1989). The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. *J Epidemiol Community Health, 43*(3), 237-240.

Barker, D. J., Winter, P. D., Osmond, C., Margetts, B., & Simmonds, S. J. (1989). Weight in infancy and death from ischaemic heart disease. *Lancet, 2*(8663), 577-580.

Barraclough, C. A. (1961). Production of anovulatory, sterile rats by single injections of testosterone propionate. *Endocrinology, 68*, 62-67.

Barros, F. C., Victora, C. G., Barros, A. J., Santos, I. S., Albernaz, E., Matijasevich, A., et al. (2005). The challenge of reducing neonatal mortality in middle-income countries: findings from three Brazilian birth cohorts in 1982, 1993, and 2004. *Lancet, 365*(9462), 847-854.

Barros, L. F., Yudilevich, D. L., Jarvis, S. M., Beaumont, N., & Baldwin, S. A. (1995). Quantitation and immunolocalization of glucose transporters in the human placenta. *Placenta, 16*(7), 623-633.

Battaglia, F. C., & Lubchenco, L. O. (1967). A practical classification of newborn infants by weight and gestational age. *J Pediatr, 71*(2), 159-163.

Bauer, J., & Gerss, J. (2011). Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. *Clin Nutr, 30*(2), 215-220.

Bauer, M. K., Harding, J. E., Bassett, N. S., Breier, B. H., Oliver, M. H., Gallaher, B. H., et al. (1998). Fetal growth and placental function. *Mol Cell Endocrinol, 140*(1-2), 115-120.

Bauer, R., Gedrange, T., Bauer, K., & Walter, B. (2006). Intrauterine growth restriction induces increased capillary density and accelerated type I fiber maturation in newborn pig skeletal muscles. *J Perinat Med, 34*(3), 235-242.

Bayard, F., Louvet, J. P., Ruckebusch, Y., & Boulard, C. (1972). Transplacental passage of dexamethasone in sheep. *J Endocrinol, 54*(2), 349-350.

Bayol, S. A., Simbi, B. H., Fowkes, R. C., & Stickland, N. C. (2010). A maternal "junk food" diet in pregnancy and lactation promotes nonalcoholic fatty liver disease in rat offspring. *Endocrinology, 151*(4), 1451-1461.

Bazaes, R. A., Salazar, T. E., Pittaluga, E., Pena, V., Alegria, A., Iniguez, G., et al. (2003). Glucose and lipid metabolism in small for gestational age infants at 48 hours of age. *Pediatrics, 111*(4 Pt 1), 804-809.

Beard, J. R., Lincoln, D., Donoghue, D., Taylor, D., Summerhayes, R., Dunn, T. M., et al. (2009). Socioeconomic and maternal determinants of small-for-gestational age births: patterns of increasing disparity. *Acta Obstet Gynecol Scand, 88*(5), 575-583.

Beck, S., Wojdyla, D., Say, L., Betran, A. P., Merialdi, M., Requejo, J. H., et al. (2010). The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. *Bull World Health Organ, 88*(1), 31-38.

Beere, P. A., Glagov, S., & Zarins, C. K. (1992). Experimental atherosclerosis at the carotid bifurcation of the cynomolgus monkey. Localization, compensatory enlargement, and the sparing effect of lowered heart rate. *Arterioscler Thromb, 12*(11), 1245-1253.

Begum, N., Ragolia, L., Rienzie, J., McCarthy, M., & Duddy, N. (1998). Regulation of mitogen-activated protein kinase phosphatase-1 induction by insulin in vascular smooth muscle cells. Evaluation of the role of the nitric oxide signaling pathway and potential defects in hypertension. *J Biol Chem, 273*(39), 25164-25170.

Bell, A. W., Kennaugh, J. M., Battaglia, F. C., Makowski, E. L., & Meschia, G. (1986). Metabolic and circulatory studies of fetal lamb at midgestation. *Am J Physiol, 250*(5 Pt 1), E538-544.

Beltrand, J., Verkauskiene, R., Nicolescu, R., Sibony, O., Gaucherand, P., Chevenne, D., et al. (2008). Adaptive changes in neonatal hormonal and metabolic profiles induced by fetal growth restriction. *J Clin Endocrinol Metab, 93*(10), 4027-4032.

Belza, A., Toubro, S., Stender, S., & Astrup, A. (2009). Effect of diet-induced energy deficit and body fat reduction on high-sensitive CRP and other inflammatory markers in obese subjects. *Int J Obes (Lond), 33*(4), 456-464.

Benediktsson, R., Calder, A. A., Edwards, C. R., & Seckl, J. R. (1997). Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. *Clin Endocrinol (Oxf), 46*(2), 161-166.

Bennet, L., Kozuma, S., McGarrigle, H. H., & Hanson, M. A. (1999). Temporal changes in fetal cardiovascular, behavioural, metabolic and endocrine responses to maternally administered dexamethasone in the late gestation fetal sheep. *BJOG, 106*(4), 331-339.

Benyi, K., Norris, D., Karbo, N., & Kgomo, K. A. (2006). Effects of genetic and environmental factors on pre-weaning and post-weaning growth in West African crossbred sheep. *Trop Anim Health Prod, 38*(7-8), 547-554.

Berenson, G. S., Srinivasan, S. R., Bao, W., Newman, W. P., 3rd, Tracy, R. E., & Wattigney, W. A. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med, 338*(23), 1650-1656.

Berggren, P. O., & Larsson, O. (1994). Ca2+ and pancreatic B-cell function. *Biochem Soc Trans, 22*(1), 12-18.

Bergman, R. N., Ader, M., Huecking, K., & Van Citters, G. (2002). Accurate assessment of beta-cell function: the hyperbolic correction. *Diabetes, 51* (S1), S212-220.

Bergvall, N., Iliadou, A., Johansson, S., de Faire, U., Kramer, M. S., Pawitan, Y., et al. (2007). Genetic and shared environmental factors do not confound the association between birth weight and hypertension: a study among Swedish twins. *Circulation, 115*(23), 2931-2938.

Beringue, F., Blondeau, B., Castellotti, M. C., Breant, B., Czernichow, P., & Polak, M. (2002). Endocrine pancreas development in growth-retarded human fetuses. *Diabetes, 51*(2), 385-391.

Berman, A. (2003). Effects of body surface area estimates on predicted energy requirements and heat stress. *J Dairy Sci, 86*(11), 3605-3610.

Bian, X., Seidler, F. J., & Slotkin, T. A. (1993). Fetal dexamethasone exposure interferes with establishment of cardiac noradrenergic innervation and sympathetic activity. *Teratology, 47*(2), 109-117.

Biddinger, S. B., Hernandez-Ono, A., Rask-Madsen, C., Haas, J. T., Aleman, J. O., Suzuki, R., et al. (2008). Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metab, 7*(2), 125-134.

Binns, C. W., Fraser, M. L., Lee, A. H., & Scott, J. (2009). Defining exclusive breastfeeding in Australia. *J Paediatr Child Health, 45*(4), 174-180.

Bispham, J., Budge, H., Mostyn, A., Dandrea, J., Clarke, L., Keisler, D. H., et al. (2002). Ambient temperature, maternal dexamethasone, and postnatal ontogeny of leptin in the neonatal lamb. *Pediatr Res, 52*(1), 85-90.

Bjorntorp, P. (1990). "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis, 10*(4), 493-496.

Blaas, H. G., Eik-Nes, S. H., & Bremnes, J. B. (1998). The growth of the human embryo. A longitudinal biometric assessment from 7 to 12 weeks of gestation. *Ultrasound Obstet Gynecol, 12*(5), 346-354.

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., de Onis, M., Ezzati, M., et al. (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet, 371*(9608), 243-260.

Blanco, C. L., Gong, A. K., Green, B. K., Falck, A., Schoolfield, J., & Liechty, E. A. (2011). Early changes in plasma amino acid concentrations during aggressive nutritional therapy in extremely low birth weight infants. *J Pediatr, 158*(4), 543-548

Blanco, C. L., Liang, H., Joya-Galeana, J., DeFronzo, R. A., McCurnin, D., & Musi, N. (2010). The ontogeny of insulin signaling in the preterm baboon model. *Endocrinology, 151*(5), 1990-1997.

Blondeau, B., Lesage, J., Czernichow, P., Dupouy, J. P., & Breant, B. (2001). Glucocorticoids impair fetal beta-cell development in rats. *Am J Physiol Endocrinol Metab, 281*(3), E592-599.

Bloomfield, F. H. (2011). How is maternal nutrition related to preterm birth? *Annu Rev Nutr, 31*(9), 1-27.

Bloomfield, F. H., Bauer, M. K., van Zijl, P. L., Gluckman, P. D., & Harding, J. E. (2002). Amniotic IGF-I supplements improve gut growth but reduce circulating IGF-I in growth-restricted fetal sheep. *Am J Physiol Endocrinol Metab, 282*(2), E259-269.

Bloomfield, F. H., Oliver, M. H., & Harding, J. E. (2007). Effects of twinning, birth size, and postnatal growth on glucose tolerance and hypothalamic-pituitary-adrenal function in postpubertal sheep. *Am J Physiol Endocrinol Metab, 292*(1), E231-237.

Bloomfield, F. H., Oliver, M. H., Hawkins, P., Campbell, M., Phillips, D. J., Gluckman, P. D., et al. (2003). A periconceptional nutritional origin for noninfectious preterm birth. *Science, 300*(5619), 606.

Bloomfield, F. H., Oliver, M. H., Hawkins, P., Holloway, A. C., Campbell, M., Gluckman, P. D., et al. (2004). Periconceptional undernutrition in sheep accelerates maturation of the fetal hypothalamic-pituitary-adrenal axis in late gestation. *Endocrinology, 145*(9), 4278-4285.

Bluher, M. (2010). The distinction of metabolically 'healthy' from 'unhealthy' obese individuals. *Curr Opin Lipidol, 21*(1), 38-43.

Blum, W. F., & Breier, B. H. (1994). Radioimmunoassays for IGFs and IGFBPs. *Growth Regul, 4*(Suppl 1), 11-19.

Bocking, A. D., Gagnon, R., White, S. E., Homan, J., Milne, K. M., & Richardson, B. S. (1988). Circulatory responses to prolonged hypoxemia in fetal sheep. *Am J Obstet Gynecol, 159*(6), 1418-1424.

Boden, G., & Shulman, G. I. (2002). Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin Invest, 32 Suppl 3*, 14-23.

Boguszewski, C. L., Jansson, C., Boguszewski, M. C., Rosberg, S., Carlsson, B., Albertsson-Wikland, K., et al. (1997). Increased proportion of circulating non-22-kilodalton growth hormone isoforms in short children: a possible mechanism for growth failure. *J Clin Endocrinol Metab, 82*(9), 2944-2949.

Boguszewski, M., Rosberg, S., & Albertsson-Wikland, K. (1995). Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. *J Clin Endocrinol Metab, 80*(9), 2599-2606.

Bonamy, A.-K. E., Bengtsson, J., Nagy, Z., De Keyzer, H., & Norman, M. (2008). Preterm birth and maternal smoking in pregnancy are strong risk factors for aortic narrowing in adolescence. *Acta Pædiatrica, 97*(8), 1080-1085.

Bonamy, A. K., Bendito, A., Martin, H., Andolf, E., Sedin, G., & Norman, M. (2005). Preterm birth contributes to increased vascular resistance and higher blood pressure in adolescent girls. *Pediatr Res, 58*(5), 845-849.

Bonamy, A. K., Martin, H., Jorneskog, G., & Norman, M. (2007). Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm. *J Intern Med, 262*(6), 635-642.

Bonanno, C., & Wapner, R. J. (2009). Antenatal corticosteroid treatment: what's happened since Drs Liggins and Howie? *Am J Obstet Gynecol, 200*(4), 448-457.

Bonet, M., Blondel, B., Agostino, R., Combier, E., Maier, R. F., Cuttini, M., et al. (2011). Variations in breastfeeding rates for very preterm infants between regions and neonatal units in Europe: results from the MOSAIC cohort. *Arch Dis Child Fetal Neonatal Ed, 96*(6), F450-452.

Boorsma, W., Snijder, M. B., Nijpels, G., Guidone, C., Favuzzi, A. M., Mingrone, G., et al. (2008). Body composition, insulin sensitivity, and cardiovascular disease profile in healthy Europeans. *Obesity (Silver Spring), 16*(12), 2696-2701.

Booth, L. C., Bennet, L., Guild, S. J., Barrett, C. J., May, C. N., Gunn, A. J., et al. (2011). Maturation-related changes in the pattern of renal sympathetic nerve activity from fetal life to adulthood. *Exp Physiol, 96*(2), 85-93.

Bottomley, C., & Bourne, T. (2009). Dating and growth in the first trimester. *Best Pract Res Clin Obstet Gynaecol, 23*(4), 439-452.

Bottomley, C., Daemen, A., Mukri, F., Papageorghiou, A. T., Kirk, E., Pexsters, A., et al. (2009). Assessing first trimester growth: the influence of ethnic background and maternal age. *Hum Reprod, 24*(2), 284-290.

Boucher, M. J., Simoneau, M., & Edlund, H. (2009). The homeodomain-interacting protein kinase 2 regulates insulin promoter factor-1/pancreatic duodenal homeobox-1 transcriptional activity. *Endocrinology, 150*(1), 87-97.

Boudville, N., Prasad, G. V., Knoll, G., Muirhead, N., Thiessen-Philbrook, H., Yang, R. C., et al. (2006). Meta-analysis: risk for hypertension in living kidney donors. *Ann Intern Med, 145*(3), 185-196.

Bouret, S. G., Draper, S. J., & Simerly, R. B. (2004). Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. *J Neurosci, 24*(11), 2797-2805.

Bozzetti, P., Ferrari, M. M., Marconi, A. M., Ferrazzi, E., Pardi, G., Makowski, E. L., et al. (1988). The relationship of maternal and fetal glucose concentrations in the human from midgestation until term. *Metabolism, 37*(4), 358-363.

Bradley, T. J., Potts, J. E., Lee, S. K., Potts, M. T., De Souza, A. M., & Sandor, G. G. (2010). Early changes in the biophysical properties of the aorta in pre-adolescent children born small for gestational age. *J Pediatr, 156*(3), 388-392.

Bramlage, C. P., Schlumbohm, C., Pryce, C. R., Mirza, S., Schnell, C., Amann, K., et al. (2009). Prenatal dexamethasone exposure does not alter blood pressure and nephron number in the young adult marmoset monkey. *Hypertension, 54*(5), 1115-1122.

Breant, B., Gesina, E., & Blondeau, B. (2006). Nutrition, glucocorticoids and pancreas development. *Horm Res, 65 Suppl 3*, 98-104.

Brenner, B. M., Garcia, D. L., & Anderson, S. (1988). Glomeruli and blood pressure. Less of one, more the other? *Am J Hypertens, 1*(4 Pt 1), 335-347.

Briaud, I., Dickson, L. M., Lingohr, M. K., McCuaig, J. F., Lawrence, J. C., & Rhodes, C. J. (2005). Insulin receptor substrate-2 proteasomal degradation mediated by a mammalian target of rapamycin (mTOR)-induced negative feedback down-regulates protein kinase B-mediated signaling pathway in beta-cells. *J Biol Chem, 280*(3), 2282-2293.

Brons, C., Jacobsen, S., Nilsson, E., Ronn, T., Jensen, C. B., Storgaard, H., et al. (2010). Deoxyribonucleic acid methylation and gene expression of PPARGC1A in human muscle is influenced by high-fat overfeeding in a birth-weight-dependent manner. *J Clin Endocrinol Metab, 95*(6), 3048-3056.

Brown, M. S., & Goldstein, J. L. (2008). Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab, 7*(2), 95-96.

Bukowski, R., Uchida, T., Smith, G. C., Malone, F. D., Ball, R. H., Nyberg, D. A., et al. (2008). Individualized norms of optimal fetal growth: fetal growth potential. *Obstet Gynecol, 111*(5), 1065-1076.

Burchell, A., Allan, B. B., & Hume, R. (1994). Glucose-6-phosphatase proteins of the endoplasmic reticulum. *Mol Membr Biol, 11*(4), 217-227.

Buske-Kirschbaum, A., Krieger, S., Wilkes, C., Rauh, W., Weiss, S., & Hellhammer, D. H. (2007). Hypothalamic-pituitary-adrenal axis function and the cellular immune response in former preterm children. *J Clin Endocrinol Metab, 92*(9), 3429-3435.

Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A., & Butler, P. C. (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes, 52*(1), 102-110.

Butte, N. F., Garza, C., Smith, E. O., Wills, C., & Nichols, B. L. (1987). Macro- and trace-mineral intakes of exclusively breast-fed infants. *Am J Clin Nutr, 45*(1), 42-48.

Butte, N. F., Smith, E. O., & Garza, C. (1991). Heart rates of breast-fed and formula-fed infants. *J Pediatr Gastroenterol Nutr, 13*(4), 391-396.

Butte, N. F., Wong, W. W., Hopkinson, J. M., Smith, E. O., & Ellis, K. J. (2000). Infant feeding mode affects early growth and body composition. *Pediatrics, 106*(6), 1355-1366.

Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J., et al. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med, 11*(2), 183-190.

Casabiell, X., Pineiro, V., Tome, M. A., Peino, R., Dieguez, C., & Casanueva, F. F. (1997). Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. *J Clin Endocrinol Metab, 82*(12), 4270-4273.

Cetin, I. (2003). Placental transport of amino acids in normal and growth-restricted pregnancies. *Eur J Obstet Gynecol Reprod Biol, 110* (Suppl 1), S50-54.

Cetin, I., Giovannini, N., Alvino, G., Agostoni, C., Riva, E., Giovannini, M., et al. (2002). Intrauterine growth restriction is associated with changes in polyunsaturated fatty acid fetal-maternal relationships. *Pediatr Res, 52*(5), 750-755.

Challis, J. R., Bloomfield, F. H., Bocking, A. D., Casciani, V., Chisaka, H., Connor, K., et al. (2005). Fetal signals and parturition. *J Obstet Gynaecol Res, 31*(6), 492-499.

Challis, J. R., Sloboda, D., Matthews, S. G., Holloway, A., Alfaidy, N., Patel, F. A., et al. (2001). The fetal placental hypothalamic-pituitary-adrenal (HPA) axis, parturition and post natal health. *Mol Cell Endocrinol, 185*(1-2), 135-144.

Challis, J. R. G., Matthews, S. G., Gibb, W., & Lye, S. J. (2000). Endocrine and paracrine regulation of birth at term and preterm. *Endocr Rev, 21*(5), 514-550.

Chardon, K., Cardot, V., Leke, A., Delanaud, S., Bach, V., Dewasmes, G., et al. (2006). Thermoregulatory control of feeding and sleep in premature infants. *Obesity (Silver Spring), 14*(9), 1535-1542.

Chellakooty, M., Juul, A., Boisen, K. A., Damgaard, I. N., Kai, C. M., Schmidt, I. M., et al. (2006). A prospective study of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-3 in 942 healthy infants: associations with birth weight, gender, growth velocity, and breastfeeding. *J Clin Endocrinol Metab, 91*(3), 820-826.

Chen, X., Fahy, A. L., Green, A. S., Anderson, M. J., Rhoads, R. P., & Limesand, S. W. (2010). Beta2-adrenergic receptor desensitization in perirenal adipose tissue in fetuses and lambs with placental insufficiency-induced intrauterine growth restriction. *J Physiol, 588*(18), 3539-3549.

Cheung, Y. F., Wong, K. Y., Lam, B. C., & Tsoi, N. S. (2004). Relation of arterial stiffness with gestational age and birth weight. *Arch Dis Child, 89*(3), 217-221.

Chirico, G., Marzollo, R., Cortinovis, S., Fonte, C., & Gasparoni, A. (2008). Antiinfective properties of human milk. *J Nutr, 138*(9), 1801S-1806S.

Chomtho, S., Wells, J. C., Williams, J. E., Davies, P. S., Lucas, A., & Fewtrell, M. S. (2008). Infant growth and later body composition: evidence from the 4-component model. *Am J Clin Nutr, 87*(6), 1776-1784.

Cirillo, R., Tos, E. G., Page, P., Missotten, M., Quattropani, A., Scheer, A., et al. (2007). Arrest of preterm labor in rat and mouse by an oral and selective nonprostanoid antagonist of the prostaglandin F2alpha receptor (FP). *Am J Obstet Gynecol, 197*(1), 54 e51-59.

Clarke, L., Buss, D. S., Juniper, D. T., Lomax, M. A., & Symonds, M. E. (1997). Adipose tissue development during early postnatal life in ewe-reared lambs. *Exp Physiol, 82*(6), 1015-1027.

Clarke, L., Heasman, L., Firth, K., & Symonds, M. E. (1997). Influence of route of delivery and ambient temperature on thermoregulation in newborn lambs. *Am J Physiol Regul Integr Comp Physiol, 272*(6 Pt 2), R1931-1939.

Clausson, B., Gardosi, J., Francis, A., & Cnattingius, S. (2001). Perinatal outcome in SGA births defined by customised versus population-based birthweight standards. *BJOG, 108*(8), 830-834.

Cleal, J. K., Poore, K. R., Boullin, J. P., Khan, O., Chau, R., Hambidge, O., et al. (2007). Mismatched pre- and postnatal nutrition leads to cardiovascular dysfunction and altered renal function in adulthood. *Proc Natl Acad Sci U S A, 104*(22), 9529-9533.

Cline, G. W., Petersen, K. F., Krssak, M., Shen, J., Hundal, R. S., Trajanoski, Z., et al. (1999). Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N Engl J Med, 341*(4), 240-246.

Cnattingius, S., Bergstrom, R., Lipworth, L., & Kramer, M. S. (1998). Prepregnancy weight and the risk of adverse pregnancy outcomes. *N Engl J Med, 338*(3), 147-152.

Cohn, H. E., Sacks, E. J., Heymann, M. A., & Rudolph, A. M. (1974). Cardiovascular responses to hypoxemia and acidemia in fetal lambs. *Am J Obstet Gynecol, 120*(6), 817-824.

Colao, A., Pivonello, R., Spiezia, S., Faggiano, A., Ferone, D., Filippella, M., et al. (1999). Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *J Clin Endocrinol Metab, 84*(8), 2664-2672.

Cole, L., Anderson, M., Antin, P. B., & Limesand, S. W. (2009). One process for pancreatic beta-cell coalescence into islets involves an epithelial-mesenchymal transition. *J Endocrinol, 203*(1), 19-31.

Cole, T. J., Paul, A. A., & Whitehead, R. G. (2002). Weight reference charts for British long-term breastfed infants. *Acta Paediatr, 91*(12), 1296-1300.

Coletta, D. K., Balas, B., Chavez, A. O., Baig, M., Abdul-Ghani, M., Kashyap, S. R., et al. (2008). Effect of acute physiological hyperinsulinemia on gene expression in human skeletal muscle in vivo. *Am J Physiol Endocrinol Metab, 294*(5), E910-917.

Communal, C., Singh, K., Sawyer, D. B., & Colucci, W. S. (1999). Opposing effects of beta(1)- and beta(2)-adrenergic receptors on cardiac myocyte apoptosis : role of a pertussis toxin-sensitive G protein. *Circulation, 100*(22), 2210-2212.

Condon, J. C., Jeyasuria, P., Faust, J. M., & Mendelson, C. R. (2004). Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. *Proc Natl Acad Sci U S A, 101*(14), 4978-4983.

Cooke, R. J., McCormick, K., Griffin, I. J., Embleton, N., Faulkner, K., Wells, J. C., et al. (1999). Feeding preterm infants after hospital discharge: effect of diet on body composition. *Pediatr Res, 46*(4), 461-464.

Cooke, R. J., Rawlings, D. J., McCormick, K., Griffin, I. J., Faulkner, K., Wells, J. C., et al. (1999). Body composition of preterm infants during infancy. *Arch Dis Child Fetal Neonatal Ed, 80*(3), F188-191.

Cooke, R. W. (2007). Conventional birth weight standards obscure fetal growth restriction in preterm infants. *Arch Dis Child Fetal Neonatal Ed, 92*(3), F189-192.

Cooper, R., Atherton, K., & Power, C. (2009). Gestational age and risk factors for cardiovascular disease: evidence from the 1958 British birth cohort followed to mid-life. *Int J Epidemiol, 38*(1), 235-244.

Costeloe, K., Hennessy, E., Gibson, A. T., Marlow, N., & Wilkinson, A. R. (2000). The EPICure study: outcomes to discharge from hospital for infants born at the threshold of viability. *Pediatrics, 106*(4), 659-671.

Cowett, R. M., Susa, J. B., Warburton, D., Stonestreet, B., Schwartz, R., & Oh, W. (1980). Endogenous posthepatic insulin secretion and metabolic clearance rates in the neonatal lamb. *Pediatr Res, 14*(12), 1391-1394.

Cowie, C. C., Rust, F. K., Ford, E. S., Eberhardt, M. S., Byrd-Holt, D. D., Li, C., et al. (2009). Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. *Diabetes Care, 32*(2), 287-294.

Cox, P., & Marton, T. (2009). Pathological assessment of intrauterine growth restriction. *Best Pract Res Clin Obstet Gynaecol, 23*(6), 751-764.

Craig, L. C., McNeill, G., Macdiarmid, J. I., Masson, L. F., & Holmes, B. A. (2010). Dietary patterns of school-age children in Scotland: association with socio-economic indicators, physical activity and obesity. *Br J Nutr, 103*(3), 319-334.

Crowther, C., McKinlay, C., Middleton, P., & Harding, J. (2011). Repeat dose prenatal corticosteroids for women at risk of preterm birth: the Cochrane Review. *Journal of Paediatrics and Child Health, 47*(S1), 29-30.

Crowther, C. A., & Harding, J. E. (2007). Repeat doses of prenatal corticosteroids for women at risk of preterm birth for preventing neonatal respiratory disease. *Cochrane Database Syst Rev*(3), CD003935.

Crowther, N. J., Cameron, N., Trusler, J., Toman, M., Norris, S. A., & Gray, I. P. (2008). Influence of catch-up growth on glucose tolerance and beta-cell function in 7-year-old children: results from the birth to twenty study. *Pediatrics, 121*(6), e1715-1722.

Crump, C., Winkleby, M. A., Sundquist, K., & Sundquist, J. (2011). Risk of hypertension among young adults who were born preterm: A Swedish national study of 636,000 births. *Am J Epidemiol, 173*(7), 797-803.

Custodis, F., Baumhakel, M., Schlimmer, N., & al, E. (2008). Heart rate reduction by ivabradine reduces oxadative stress, improves endothelial function and prevents atherosclerosis in apolipoprotein E-deficient mice. *Circulation, 117*, 2377-2387.

Cutfield, W. S., Bergman, R. N., Menon, R. K., & Sperling, M. A. (1990). The modified minimal model: application to measurement of insulin sensitivity in children. *J Clin Endocrinol Metab, 70*(6), 1644-1650.

Dalziel, S. R., Liang, A., Parag, V., Rodgers, A., & Harding, J. E. (2004). Blood pressure at 6 years of age after prenatal exposure to betamethasone: follow-up results of a randomized, controlled trial. *Pediatrics, 114*(3), e373-377.

Dalziel, S. R., Parag, V., Rodgers, A., & Harding, J. E. (2007). Cardiovascular risk factors at age 30 following pre-term birth. *Int J Epidemiol, 36*(4), 907-915.

Dalziel, S. R., Walker, N. K., Parag, V., Mantell, C., Rea, H. H., Rodgers, A., et al. (2005). Cardiovascular risk factors after antenatal exposure to betamethasone: 30-year follow-up of a randomised controlled trial. *Lancet, 365*(9474), 1856-1862.

Danadian, K., Balasekaran, G., Lewy, V., Meza, M. P., Robertson, R., & Arslanian, S. A. (1999). Insulin sensitivity in African-American children with and without family history of type 2 diabetes. *Diabetes Care, 22*(8), 1325-1329.

Dandona, P., Weinstock, R., Thusu, K., Abdel-Rahman, E., Aljada, A., & Wadden, T. (1998). Tumour necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab, 83*(8), 2907-2910.

Darby, C. J., Clarke, L., Lomax, M. A., & Symonds, M. E. (1996). Brown adipose tissue and liver development during early postnatal life in hand-reared and ewe-reared lambs. *Reprod Fertil Dev, 8*(1), 137-145.

Darendeliler, F., Bas, F., Bundak, R., Coban, A., Sancakli, O., Eryilmaz, S. K., et al. (2008). Insulin resistance and body composition in preterm born children during prepubertal ages. *Clin Endocrinol (Oxf), 68*(5), 773-779.

Das, U. N. (2006). Essential Fatty acids - a review. *Curr Pharm Biotechnol, 7*(6), 467-482.

Daughaday, W. H., Parker, K. A., Borowsky, S., Trivedi, B., & Kapadia, M. (1982). Measurement of somatomedin-related peptides in fetal, neonatal, and maternal rat serum by insulin-like growth factor (IGF) I radioimmunoassay, IGF-II radioreceptor assay (RRA), and multiplication-stimulating activity RRA after acid-ethanol extraction. *Endocrinology, 110*(2), 575-581.

Davey Smith, G., Leary, S., Ness, A., & Lawlor, D. A. (2009). Challenges and novel approaches in the epidemiological study of early life influences on later disease. *Adv Exp Med Biol, 646*, 1-14.

David, M., Hirsch, M., Karin, J., Toledo, E., & Akselrod, S. (2007). An estimate of fetal autonomic state by time-frequency analysis of fetal heart rate variability. *J Appl Physiol, 102*(3), 1057-1064.

Davidoff, M. J., Dias, T., Damus, K., Russell, R., Bettegowda, V. R., Dolan, S., et al. (2006). Changes in the gestational age distribution among U.S. singleton births: impact on rates of late preterm birth, 1992 to 2002. *Semin Perinatol, 30*(1), 8-15.

Davis, E. P., Waffarn, F., Uy, C., Hobel, C. J., Glynn, L. M., & Sandman, C. A. (2009). Effect of prenatal glucocorticoid treatment on size at birth among infants born at term gestation. *J Perinatol, 29*(11), 731-737.

Davrath, L. R., & Goren, Y. (2003). Early autonomic malfunction in normotensive individuals with a genetic predisposition to essential hypertension. *Am J Physiol Heart Circ Physiol, 285*, H1697 - H1704.

de Beer, M., van Eijsden, M., Vrijkotte, T., & Gemke, R. (2009). Early growth patterns and cardiometabolic function at the age of 5 in a multiethnic birth cohort: the ABCD study. *BMC Pediatrics, 9*(23).

De Blasio, M. J., Blache, D., Gatford, K. L., Robinson, J. S., & Owens, J. A. (2010). Placental restriction increases adipose leptin gene expression and plasma leptin and alters their relationship to feeding activity in the young lamb. *Pediatr Res, 67*(6), 603-608.

De Blasio, M. J., Gatford, K. L., McMillen, I. C., Robinson, J. S., & Owens, J. A. (2007). Placental restriction of fetal growth increases insulin action, growth, and adiposity in the young lamb. *Endocrinology, 148*(3), 1350-1358.

De Blasio, M. J., Gatford, K. L., Robinson, J. S., & Owens, J. A. (2007). Placental restriction of fetal growth reduces size at birth and alters postnatal growth, feeding activity, and adiposity in the young lamb. *Am J Physiol Regul Integr Comp Physiol, 292*(2), R875-886.

de Boer, M. P., Ijzerman, R. G., de Jongh, R. T., Eringa, E. C., Stenhouwer, C. D. A., Smulders, Y. M., et al. (2008). Birth weight relates to salt sensitivity of blood pressure in healthy adults. *Hypertension, 51*(4), 928-932.

de Boo, H. A., Cranendonk, A., Kulik, W., Harding, J. E., & Lafeber, H. N. (2005). Whole body protein turnover and urea production of preterm small for gestational age infants fed fortified human milk or preterm formula. *J Pediatr Gastroenterol Nutr, 41*(1), 81-87.

de Ferranti, S. D., Gauvreau, K., Ludwig, D. S., Neufeld, E. J., Newburger, J. W., & Rifai, N. (2004). Prevalence of the metabolic syndrome in American adolescents: findings from the Third National Health and Nutrition Examination Survey. *Circulation, 110*(16), 2494-2497.

De Ferrari, G. M., Salvati, P., Grossoni, M., Ukmar, G., Vaga, L., Patrono, C., et al. (1993). Pharmacologic modulation of the autonomic nervous system in the prevention of sudden cardiac death. A study with propranolol, methacholine and oxotremorine in conscious dogs with a healed myocardial infarction. *J Am Coll Cardiol, 22*(1), 283-290.

de Jong, C. L., Gardosi, J., Dekker, G. A., Colenbrander, G. J., & van Geijn, H. P. (1998). Application of a customised birthweight standard in the assessment of perinatal outcome in a high risk population. *BJOG, 105*(5), 531-535.

De Matteo, R., Blasch, N., Stokes, V., Davis, P., & Harding, R. (2010). Induced preterm birth in sheep: a suitable model for studying the developmental effects of moderately preterm birth. *Reprod Sci, 17*(8), 724-733.

De Matteo, R., Stacy, V., Probyn, M., Desai, M., Ross, M., & Harding, R. (2008). The perinatal development of arterial pressure in sheep: effects of low birth weight due to twinning. *Reprod Sci, 15*(1), 66-74.

De Matteo, R., Stacy, V., Probyn, M., E. , Brew, N., Blasch, N., & Harding, R. (2008). Does moderate preterm birth lead to altered arterial pressure? Studies in sheep. *Clin Exp Pharmacol Physiol, 35*(12), 1426-1432.

de Moura, E. G., Lisboa, P. C., Custodio, C. M., Nunes, M. T., de Picoli Souza, K., & Passos, M. C. (2007). Malnutrition during lactation changes growth hormone mRNA expression in offspring at weaning and in adulthood. *J Nutr Biochem, 18*(2), 134-139.

de Onis, M., Garza, C., Onyango, A. W., & Borghi, E. (2007). Comparison of the WHO child growth standards and the CDC 2000 growth charts. *J Nutr, 137*(1), 144-148.

De Rogalski Landrot, I., Roche, F., Pichot, V., Teyssier, G., Gaspoz, J. M., Barthelemy, J. C., et al. (2007). Autonomic nervous system activity in premature and full-term infants from theoretical term to 7 years. *Auton Neurosci, 136*(1-2), 105-109.

de Rooij, S. R., Painter, R. C., Roseboom, T. J., Phillips, D. I., Osmond, C., Barker, D. J., et al. (2006). Glucose tolerance at age 58 and the decline of glucose tolerance in comparison with age 50 in people prenatally exposed to the Dutch famine. *Diabetologia, 49*(4), 637-643.

de Vries, A., Holmes, M. C., Heijnis, A., Seier, J. V., Heerden, J., Louw, J., et al. (2007). Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic-pituitary-adrenal axis function. *J Clin Invest, 117*(4), 1058-1067.

de Vries, W. B., Karemaker, R., Mooy, N. F., Strengers, J. L., Kemperman, H., Baerts, W., et al. (2008). Cardiovascular follow-up at school age after perinatal glucocorticoid exposure in prematurely born children: perinatal glucocorticoid therapy and cardiovascular follow-up. *Arch Pediatr Adolesc Med, 162*(8), 738-744.

Dechert, R., Wesley, J., Schafer, L., LaMond, S., Beck, T., Coran, A., et al. (1985). Comparison of oxygen consumption, carbon dioxide production, and resting energy expenditure in premature and full-term infants. *J Pediatr Surg, 20*(6), 792-798.

DeChiara, T. M., Efstratiadis, A., & Robertson, E. J. (1990). A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature, 345*(6270), 78-80.

Deeney, J. T., Prentki, M., & Corkey, B. E. (2000). Metabolic control of beta-cell function. *Semin Cell Dev Biol, 11*(4), 267-275.

DeFronzo, R. A., Gunnarsson, R., Bjorkman, O., Olsson, M., & Wahren, J. (1985). Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest, 76*(1), 149-155.

DeFronzo, R. A., Jacot, E., Jequier, E., Maeder, E., Wahren, J., & Felber, J. P. (1981). The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes, 30*(12), 1000-1007.

DeFronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol Endocrinol Metab, 237*(3), E214-223.

Dekker, J. M., Crow, R. S., Folsom, A. R., Hannan, P. J., Liao, D., Swenne, C. A., et al. (2000). Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: the ARIC Study. Atherosclerosis Risk In Communities. *Circulation, 102*(11), 1239-1244.

Despres, J. P., Lamarche, B., Mauriege, P., Cantin, B., Dagenais, G. R., Moorjani, S., et al. (1996). Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med, 334*(15), 952-957.

Dewey, K. G. (1998). Growth characteristics of breast-fed compared to formula-fed infants. *Biol Neonate, 74*(2), 94-105.

Dewey, K. G., Heinig, M. J., Nommsen, L. A., & Lonnerdal, B. (1991). Adequacy of energy intake among breast-fed infants in the DARLING study: relationships to growth velocity, morbidity, and activity levels. Davis Area Research on Lactation, Infant Nutrition and Growth. *J Pediatr, 119*(4), 538-547.

Dewey, K. G., Heinig, M. J., Nommsen, L. A., Peerson, J. M., & Lonnerdal, B. (1992). Growth of breast-fed and formula-fed infants from 0 to 18 months: the DARLING Study. *Pediatrics, 89*(6 Pt 1), 1035-1041.

Dewey, K. G., Heinig, M. J., Nommsen, L. A., Peerson, J. M., & Lonnerdal, B. (1993). Breast-fed infants are leaner than formula-fed infants at 1 y of age: the DARLING study. *Am J Clin Nutr, 57*(2), 140-145.

Dewey, K. G., & Lonnerdal, B. (1986). Infant self-regulation of breast milk intake. *Acta Paediatr Scand, 75*(6), 893-898.

Di Renzo, G. C., & Roura, L. C. (2006). Guidelines for the management of spontaneous preterm labor. *J Perinat Med, 34*(5), 359-366.

DiGiacomo, J. E., & Hay, W. W., Jr. (1990). Fetal glucose metabolism and oxygen consumption during sustained hypoglycemia. *Metabolism, 39*(2), 193-202.

Dodic, M., Abouantoun, T., O'Connor, A., Wintour, E. M., & Moritz, K. M. (2002). Programming effects of short prenatal exposure to dexamethasone in sheep. *Hypertension, 40*(5), 729-734.

Dodic, M., May, C. N., Wintour, E. M., & Coghlan, J. P. (1998). An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. *Clin Sci (Lond), 94*(2), 149-155.

Donovan, E. F., Tyson, J. E., Ehrenkranz, R. A., Verter, J., Wright, L. L., Korones, S. B., et al. (1999). Inaccuracy of Ballard scores before 28 weeks' gestation. National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr, 135*(2 Pt 1), 147-152.

Dor, Y., Brown, J., Martinez, O. I., & Melton, D. A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature, 429*(6987), 41-46.

Doyle, L. W., Ford, G. W., Davis, N. M., & Callanan, C. (2000). Antenatal corticosteroid therapy and blood pressure at 14 years of age in preterm children. *Clin Sci (Lond), 98*(2), 137-142.

Dumortier, O., Blondeau, B., Duvillie, B., Reusens, B., Breant, B., & Remacle, C. (2007). Different mechanisms operating during different critical time-windows reduce rat fetal beta cell mass due to a maternal low-protein or low-energy diet. *Diabetologia, 50*(12), 2495-2503.

Dunn, G. A., & Bale, T. L. (2009). Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. *Endocrinology, 150*(11), 4999-5009.

Eckman, D. M., Kerr, B. A., Fuloria, M., Simandle, S. A., Watt, S. E., Rose, J. C., et al. (2010). Antenatal betamethasone alters vascular reactivity in adult female ovine cerebral arteries. *Pediatr Res, 68*(4), 344-348.

Economides, D. L., Nicolaides, K. H., Linton, E. A., Perry, L. A., & Chard, T. (1988). Plasma cortisol and adrenocorticotropin in appropriate and small for gestational age fetuses. *Fetal Ther, 3*(3), 158-164.

Economides, D. L., Proudler, A., & Nicolaides, K. H. (1989). Plasma insulin in appropriate- and small-for-gestational-age fetuses. *Am J Obstet Gynecol, 160*(5 Pt 1), 1091-1094.

Edwards, C. R., Benediktsson, R., Lindsay, R. S., & Seckl, J. R. (1993). Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? *Lancet, 341*(8841), 355-357.

Ehrenberg, H. M., Dierker, L., Milluzzi, C., & Mercer, B. M. (2003). Low maternal weight, failure to thrive in pregnancy, and adverse pregnancy outcomes. *Am J Obstet Gynecol, 189*(6), 1726-1730.

Ehrenkranz, R. A., Dusick, A. M., Vohr, B. R., Wright, L. L., Wrage, L. A., & Poole, W. K. (2006). Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics, 117*(4), 1253-1261.

Ehrenkranz, R. A., Younes, N., Lemons, J. A., Fanaroff, A. A., Donovan, E. F., Wright, L. L., et al. (1999). Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics, 104*(2 Pt 1), 280-289.

Ehrhardt, R. A., Greenwood, P. L., Bell, A. W., & Boisclair, Y. R. (2003). Plasma leptin is regulated predominantly by nutrition in preruminant lambs. *J Nutr, 133*(12), 4196-4201.

Einhorn, D., Reaven, G. M., Cobin, R. H., Ford, E., Ganda, O. P., Handelsman, Y., et al. (2003). American College of Endocrinology position statement on the insulin resistance syndrome. *Endocr Pract, 9*(3), 237-252.

Ekelund, U., Ong, K. K., Linne, Y., Neovius, M., Brage, S., Dunger, D. B., et al. (2007). Association of weight gain in infancy and early childhood with metabolic risk in young adults. *J Clin Endocrinol Metab, 92*(1), 98-103.

Ellis, K. J. (2007). Evaluation of body composition in neonates and infants. *Semin Fetal Neonatal Med, 12*(1), 87-91.

Ellmann, S., Sticht, H., Thiel, F., Beckmann, M. W., Strick, R., & Strissel, P. L. (2009). Estrogen and progesterone receptors: from molecular structures to clinical targets. *Cell Mol Life Sci, 66*(15), 2405-2426.

Embleton, N. E., Pang, N., & Cooke, R. J. (2001). Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? *Pediatrics, 107*(2), 270-273.

Engle, W. A. (2006). A recommendation for the definition of "late preterm" (near-term) and the birth weight-gestational age classification system. *Semin Perinatol, 30*(1), 2-7.

Engle, W. A., & Committee on Fetus and Newborn. (2004). Age terminology during the perinatal period. *Pediatrics, 114*(5), 1362-1364.

Engle, W. A., & Kominiarek, M. A. (2008). Late preterm infants, early term infants, and timing of elective deliveries. *Clin Perinatol, 35*(2), 325-341.

Engle, W. A., Tomashek, K. M., & Wallman, C. (2007). "Late-preterm" infants: a population at risk. *Pediatrics, 120*(6), 1390-1401.

Eremia, S. C., de Boo, H. A., Bloomfield, F. H., Oliver, M. H., & Harding, J. E. (2007). Fetal and amniotic insulin-like growth factor-I supplements improve growth rate in intrauterine growth restriction fetal sheep. *Endocrinology, 148*(6), 2963-2972.

Eriksson, J., Forsen, T., Tuomilehto, J., Osmond, C., & Barker, D. (2002). Size at birth, fat-free mass and resting metabolic rate in adult life. *Horm Metab Res, 34*(2), 72-76.

Eriksson, J., Lindstrom, J., Valle, T., Aunola, S., Hamalainen, H., Ilanne-Parikka, P., et al. (1999). Prevention of type II diabetes in subjects with impaired glucose tolerance: the Diabetes Prevention Study (DPS) in Finland. Study design and 1-year interim report on the feasibility of the lifestyle intervention programme. *Diabetologia, 42*(7), 793-801.

Eriksson, J. G. (2011). Early growth and coronary heart disease and type 2 diabetes: findings from the Helsinki Birth Cohort Study (HBCS). *Am J Clin Nutr, 94*(6), 1799S-1802S.

Eriksson, J. G., Forsen, T., Tuomilehto, J., Osmond, C., & Barker, D. J. (2001). Early growth and coronary heart disease in later life: longitudinal study. *BMJ, 322*(7292), 949-953.

Eriksson, J. G., Osmond, C., Kajantie, E., Forsen, T. J., & Barker, D. J. (2006). Patterns of growth among children who later develop type 2 diabetes or its risk factors. *Diabetologia, 49*(12), 2853-2858.

Erlinger, T. P., Vollmer, W. M., Svetkey, L. P., & Appel, L. J. (2003). The potential impact of nonpharmacologic population-wide blood pressure reduction on coronary heart disease events: pronounced benefits in African-Americans and hypertensives. *Prev Med, 37*(4), 327-333.

Escobar, G. J., Clark, R. H., & Greene, J. D. (2006). Short-term outcomes of infants born at 35 and 36 weeks gestation: we need to ask more questions. *Semin Perinatol, 30*(1), 28-33.

European Commission. (1991). Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae. Retrieved October 2010, from Off J Eur Union http://eur-lex.europa.eu/LexUniServ/LexUniServ.do?uri=OJ:L:2003:041:0037:0040:EN:PDF

Euser, A. M., de Wit, C. C., Finken, M. J., Rijken, M., & Wit, J. M. (2008). Growth of preterm born children. *Horm Res, 70*(6), 319-328.

Euser, A. M., Finken, M. J., Keijzer-Veen, M. G., Hille, E. T., Wit, J. M., & Dekker, F. W. (2005). Associations between prenatal and infancy weight gain and BMI, fat mass, and fat distribution in young adulthood: a prospective cohort study in males and females born very preterm. *Am J Clin Nutr, 81*(2), 480-487.

Evensen, K. A. I., Steinshamn, S., Tjønna, A. E., Stølen, T., Høydal, M. A., Wisløff, U., et al. (2009). Effects of preterm birth and fetal growth retardation on cardiovascular risk factors in young adulthood. *Early Hum Dev, 85*(4), 239-245.

Fall, C. H., Sachdev, H. S., Osmond, C., Lakshmy, R., Biswas, S. D., Prabhakaran, D., et al. (2008). Adult metabolic syndrome and impaired glucose tolerance are associated with different patterns of BMI gain during infancy: Data from the New Delhi birth cohort. *Diabetes Care, 31*(12), 2349-2356.

Fanaro, S., Ballardini, E., & Vigi, V. (2010). Different pre-term formulas for different pre-term infants. *Early Hum Dev, 86 Suppl 1*, 27-31.

Farrag, H. M., Nawrath, L. M., Healey, J. E., Dorcus, E. J., Rapoza, R. E., Oh, W., et al. (1997). Persistent glucose production and greater peripheral sensitivity to insulin in the neonate vs. the adult. *Am J Physiol Endocrinol Metab, 272*(1 Pt 1), E86-93.

Fehrman-Ekholm, I., Duner, F., Brink, B., Tyden, G., & Elinder, C. G. (2001). No evidence of accelerated loss of kidney function in living kidney donors: results from a cross-sectional follow-up. *Transplantation, 72*(3), 444-449.

Feig, D. I., & Johnson, R. J. (2003). Hyperuricemia in childhood primary hypertension. *Hypertension, 42*(3), 247-252.

Feig, D. I., Soletsky, B., & Johnson, R. J. (2008). Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. *JAMA, 300*(8), 924-932.

Feldt, K., Raikkonen, K., Eriksson, J. G., Andersson, S., Osmond, C., Barker, D. J., et al. (2007). Cardiovascular reactivity to psychological stressors in late adulthood is predicted by gestational age at birth. *J Hum Hypertens, 21*(5), 401-410.

Felton, A. M., Felton, A., Raubenheimer, D., Simpson, S. J., Foley, W. J., Wood, J. T., et al. (2009). Protein content of diets dictates the daily energy intake of a free-ranging primate. *Behavioral Ecology, 20*(4), 685-690.

Ferrannini, E., Buzzigoli, G., Bonadonna, R., Giorico, M. A., Oleggini, M., Graziadei, L., et al. (1987). Insulin resistance in essential hypertension. *N Engl J Med, 317*(6), 350-357.

Ferrannini, E., & Pilo, A. (1979). Pattern of insulin delivery after intravenous glucose injection in man and its relation to plasma glucose disappearance. *J Clin Invest, 64*(1), 243-254.

Fewtrell, M. S., Lucas, A., Cole, T. J., & Wells, J. C. (2004). Prematurity and reduced body fatness at 8-12 y of age. *Am J Clin Nutr, 80*(2), 436-440.

Filho, J. C., Hazel, S. J., Anderstam, B., Bergstrom, J., Lewitt, M., & Hall, K. (1999). Effect of protein intake on plasma and erythrocyte free amino acids and serum IGF-I and IGFBP-1 levels in rats. *Am J Physiol Endocrinol Metab, 277*(4 Pt 1), E693-701.

Finken, M. J., Keijzer-Veen, M. G., Dekker, F. W., Frolich, M., Walther, F. J., Romijn, J. A., et al. (2008). Antenatal glucocorticoid treatment is not associated with long-term metabolic risks in individuals born before 32 weeks of gestation. *Arch Dis Child Fetal Neonatal Ed, 93*(6), F442-447.

Flaa, A., Aksnes, T. A., Kjeldsen, S. E., Eide, I., & Rostrup, M. (2008). Increased sympathetic reactivity may predict insulin resistance: an 18-year follow-up study. *Metabolism, 57*(10), 1422-1427.

Fleischman, A. R., Oinuma, M., & Clark, S. L. (2010). Rethinking the definition of "term pregnancy". *Obstet Gynecol, 116*(1), 136-139.

Ford, S. P., Hess, B. W., Schwope, M. M., Nijland, M. J., Gilbert, J. S., Vonnahme, K. A., et al. (2007). Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. *J Anim Sci, 85*(5), 1285-1294.

Forhead, A. J., Broughton Pipkin, F., & Fowden, A. L. (2000). Effect of cortisol on blood pressure and the renin-angiotensin system in fetal sheep during late gestation. *J Physiol, 526 Pt 1*, 167-176.

Forsdahl, A. (1977). Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? *Br J Prev Soc Med, 31*(2), 91-95.

Fowden, A. L. (1992). The role of insulin in fetal growth. *Early Hum Dev, 29*(1-3), 177-181.

Fowden, A. L. (2003). The insulin-like growth factors and feto-placental growth. *Placenta, 24*(8-9), 803-812.

Fowden, A. L., & Forhead, A. J. (2004). Endocrine mechanisms of intrauterine programming. *Reproduction, 127*(5), 515-526.

Fowden, A. L., & Hill, D. J. (2001). Intra-uterine programming of the endocrine pancreas. *Br Med Bull, 60*, 123-142.

Fowden, A. L., Hughes, P., & Comline, R. S. (1989). The effects of insulin on the growth rate of the sheep fetus during late gestation. *Q J Exp Physiol, 74*(5), 703-714.

Fowden, A. L., Li, J., & Forhead, A. J. (1998). Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proc Nutr Soc, 57*(1), 113-122.

Fowden, A. L., Szemere, J., Hughes, P., Gilmour, R. S., & Forhead, A. J. (1996). The effects of cortisol on the growth rate of the sheep fetus during late gestation. *J Endocrinol, 151*(1), 97-105.

Fox, Massaro, J. M., Hoffmann, U., Pou, K. M., Maurovich-Horvat, P., Liu, C. Y., et al. (2007). Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation, 116*(1), 39-48.

Fox, K., Borer, J. S., Camm, A. J., Danchin, N., Ferrari, R., Lopez Sendon, J. L., et al. (2007). Resting heart rate in cardiovascular disease. *J Am Coll Cardiol, 50*(9), 823-830.

Franko, K. L., Forhead, A. J., & Fowden, A. L. (2010). Differential effects of prenatal stress and glucocorticoid administration on postnatal growth and glucose metabolism in rats. *J Endocrinol, 204*(3), 319-329.

Fried, S. K., Bunkin, D. A., & Greenberg, A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab, 83*(3), 847-850.

Friedman, T. C., Mastorakos, G., Newman, T. D., Mullen, N. M., Horton, E. G., Costello, R., et al. (1996). Carbohydrate and lipid metabolism in endogenous hypercortisolism: shared features with metabolic syndrome X and NIDDM. *Endocr J, 43*(6), 645-655.

Froguel, P., Zouali, H., Vionnet, N., Velho, G., Vaxillaire, M., Sun, F., et al. (1993). Familial hyperglycemia due to mutations in glucokinase. Definition of a subtype of diabetes mellitus. *N Engl J Med, 328*(10), 697-702.

Frydman, R., Lelaidier, C., Baton-Saint-Mleux, C., Fernandez, H., Vial, M., & Bourget, P. (1992). Labor induction in women at term with mifepristone (RU 486): a double-blind, randomized, placebo-controlled study. *Obstet Gynecol, 80*(6), 972-975.

Galland, B. C., Taylor, B. J., Bolton, D. P., & Sayers, R. M. (2006). Heart rate variability and cardiac reflexes in small for gestational age infants. *J Appl Physiol, 100*(3), 933-939.

Gambling, L., & McArdle, H. J. (2004). Iron, copper and fetal development. *Proc Nutr Soc, 63*(4), 553-562.

Gardner, D. S., Tingey, K., Van Bon, B. W., Ozanne, S. E., Wilson, V., Dandrea, J., et al. (2005). Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition. *Am J Physiol Regul Integr Comp Physiol, 289*(4), R947-954.

Gardosi, J. (2004). Customized fetal growth standards: rationale and clinical application. *Semin Perinatol, 28*(1), 33-40.

Gardosi, J., Mongelli, M., Wilcox, M., & Chang, A. (1995). An adjustable fetal weight standard. *Ultrasound Obstet Gynecol, 6*(3), 168-174.

Garg, A. X., Muirhead, N., Knoll, G., Yang, R. C., Prasad, G. V., Thiessen-Philbrook, H., et al. (2006). Proteinuria and reduced kidney function in living kidney donors: A

systematic review, meta-analysis, and meta-regression. *Kidney Int, 70*(10), 1801-1810.

Garg, P., Abdel-Latif, M. E., Bolisetty, S., Bajuk, B., & Lui, K. (2010). Perinatal characteristics and outcome of very preterm singleton, twin and triplet infants in NSW and the ACT, Australia (1994-2005). *Arch Dis Child Fetal Neonatal Ed, 95*, F20-F24.

Garofano, A., Czernichow, P., & Breant, B. (1997). In utero undernutrition impairs rat beta-cell development. *Diabetologia, 40*(10), 1231-1234.

Garofano, A., Czernichow, P., & Breant, B. (1998). Postnatal somatic growth and insulin contents in moderate or severe intrauterine growth retardation in the rat. *Biol Neonate, 73*(2), 89-98.

Garofano, A., Czernichow, P., & Breant, B. (1999). Effect of ageing on beta-cell mass and function in rats malnourished during the perinatal period. *Diabetologia, 42*(6), 711-718.

Gaster, M., Handberg, A., Beck-Nielsen, H., & Schroder, H. D. (2000). Glucose transporter expression in human skeletal muscle fibers. *Am J Physiol Endocrinol Metab, 279*(3), E529-538.

Gatford, K. L., De Blasio, M. J., Thavaneswaran, P., Robinson, J. S., McMillen, I. C., & Owens, J. A. (2004). Postnatal ontogeny of glucose homeostasis and insulin action in sheep. *Am J Physiol Endocrinol Metab, 286*, E1050 - E1059.

Gatford, K. L., Mohammad, S. N., Harland, M. L., De Blasio, M. J., Fowden, A. L., Robinson, J. S., et al. (2008). Impaired beta-cell function and inadequate compensatory increases in beta-cell mass after intrauterine growth restriction in sheep. *Endocrinology, 149*(10), 5118-5127.

Gatford, K. L., Simmons, R. A., De Blasio, M. J., Robinson, J. S., & Owens, J. A. (2010). Review: Placental programming of postnatal diabetes and impaired insulin action after IUGR. *Placenta, 31* (Suppl), S60-65.

Gentz, J., Persson, B., Kellum, M., Bengtsson, G., & Thorell, J. (1971). Effect of feeding on intravenous glucose tolerance and insulin response in piglets during the first day of life. *Life Sci II, 10*(3), 137-144.

Gerich, J. E. (2002). Is reduced first-phase insulin release the earliest detectable abnormality in individuals destined to develop type 2 diabetes? *Diabetes, 51 Suppl 1*, S117-121.

Giangrande, P. H., Kimbrel, E. A., Edwards, D. P., & McDonnell, D. P. (2000). The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. *Mol Cell Biol, 20*(9), 3102-3115.

Gianni, M. L., Mora, S., Roggero, P., Amato, O., Piemontese, P., Orsi, A., et al. (2008). Regional fat distribution in children born preterm evaluated at school age. *J Pediatr Gastroenterol Nutr, 46*(2), 232-235.

Gianni, M. L., Roggero, P., Taroni, F., Liotto, N., Piemontese, P., & Mosca, F. (2009). Adiposity in small for gestational age preterm infants assessed at term equivalent age. *Arch Dis Child Fetal Neonatal Ed, 94*(5), F368-372.

Gillman, M. W., & Rich-Edwards, J. W. (2000). The fetal origin of adult disease: from sceptic to convert. *Paediatr Perinat Epidemiol, 14*(3), 192-193.

Girard, J. (1986). Gluconeogenesis in late fetal and early neonatal life. *Biol Neonate, 50*(5), 237-258.

Giudice, L. C., de Zegher, F., Gargosky, S. E., Dsupin, B. A., de las Fuentes, L., Crystal, R. A., et al. (1995). Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. *J Clin Endocrinol Metab, 80*(5), 1548-1555.

Gluckman, P. D., & Butler, J. H. (1983). Parturition-related changes in insulin-like growth factors-I and -II in the perinatal lamb. *J Endocrinol, 99*(2), 223-232.

Gluckman, P. D., & Hanson, M. A. (2004). The developmental origins of the metabolic syndrome. *Trends Endocrinol Metab, 15*(4), 183-187.

Gluckman, P. D., & Harding, J. E. (1997). Fetal growth retardation: underlying endocrine mechanisms and postnatal consequences. *Acta Paediatr Suppl, 422*, 69-72.

Gluckman, P. D., Mallard, C., & Boshier, D. P. (1991). The effect of hypothalamic lesions on the length of gestation in fetal sheep. *Am J Obstet Gynecol, 165*(5 Pt 1), 1464-1468.

Gluckman, P. D., & Pinal, C. S. (2003). Regulation of fetal growth by the somatotrophic axis. *J Nutr, 133*(5 Suppl 2), 1741S-1746S.

Godfrey, K. M., Lillycrop, K. A., Burdge, G. C., Gluckman, P. D., & Hanson, M. A. (2007). Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. *Pediatr Res, 61*(5 Pt 2), 5R-10R.

Gohlke, B. C., Fahnenstich, H., Dame, C., & Albers, N. (2004). Longitudinal data for intrauterine levels of fetal IGF-I and IGF-II. *Horm Res, 61*(4), 200-204.

Goldenberg, R. L., Culhane, J. F., Iams, J. D., & Romero, R. (2008). Epidemiology and causes of preterm birth. *Lancet, 371*(9606), 75-84.

Goldfine, A. B., Fonseca, V., Jablonski, K. A., Pyle, L., Staten, M. A., & Shoelson, S. E. (2010). The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. *Ann Intern Med, 152*(6), 346-357.

Gray, I. P., Cooper, P. A., Cory, B. J., Toman, M., & Crowther, N. J. (2002). The intrauterine environment is a strong determinant of glucose tolerance during the neonatal period, even in prematurity. *J Clin Endocrinol Metab, 87*(9), 4252-4256.

Grayson, B. E., Allen, S. E., Billes, S. K., Williams, S. M., Smith, M. S., & Grove, K. L. (2006). Prenatal development of hypothalamic neuropeptide systems in the nonhuman primate. *Neuroscience, 143*(4), 975-986.

Green, A., Rozance, P., & Limesand, S. (2010). Consequences of a compromised intrauterine environment on islet function. *J Endocrinol, 205*(3), 211-224.

Greenwood, J. P., Stoker, J. B., & Mary, D. (1999). Single-unit sympathetic discharge-quantitative assessment in human hypertensive disease. *Circulation, 100*(12), 1305-1310.

Greenwood, P. L., Hunt, A. S., Hermanson, J. W., & Bell, A. W. (1998). Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition, and some aspects of energetic efficiency. *J Anim Sci, 76*(9), 2354-2367.

Greenwood, P. L., Hunt, A. S., Hermanson, J. W., & Bell, A. W. (2000). Effects of birth weight and postnatal nutrition on neonatal sheep: II. Skeletal muscle growth and development. *J Anim Sci, 78*(1), 50-61.

Greenwood, P. L., Hunt, A. S., Slepetis, R. M., Finnerty, K. D., Alston, C., Beermann, D. H., et al. (2002). Effects of birth weight and postnatal nutrition on neonatal sheep: III. Regulation of energy metabolism. *J Anim Sci, 80*(11), 2850-2861.

Groh-Wargo, S., & Sapsford, A. (2009). Enteral nutrition support of the preterm infant in the neonatal intensive care unit. *Nutr Clin Pract, 24*(3), 363-376.

Gross, S. J. (1997). Intrauterine growth restriction: a genetic perspective. *Clin Obstet Gynecol, 40*(4), 730-739.

Grote, V., von Kries, R., Closa-Monasterolo, R., Scaglioni, S., Gruszfeld, D., Sengier, A., et al. (2010). Protein intake and growth in the first 24 months of life. *J Pediatr Gastroenterol Nutr, 51 Suppl 3*, S117-118.

Grove, K. L., & Smith, M. S. (2003). Ontogeny of the hypothalamic neuropeptide Y system. *Physiol Behav, 79*(1), 47-63.

Grundy, S. M., Cleeman, J. I., Daniels, S. R., Donato, K. A., Eckel, R. H., Franklin, B. A., et al. (2005). Diagnosis and management of the metabolic syndrome: an American Heart

Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation, 112*(17), 2735-2752.

Grupe, A., Hultgren, B., Ryan, A., Ma, Y. H., Bauer, M., & Stewart, T. A. (1995). Transgenic knockouts reveal a critical requirement for pancreatic beta cell glucokinase in maintaining glucose homeostasis. *Cell, 83*(1), 69-78.

Guillam, M. T., Hummler, E., Schaerer, E., Yeh, J. I., Birnbaum, M. J., Beermann, F., et al. (1997). Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. *Nat Genet, 17*(3), 327-330.

Gunther, A. L., Buyken, A. E., & Kroke, A. (2007). Protein intake during the period of complementary feeding and early childhood and the association with body mass index and percentage body fat at 7 y of age. *Am J Clin Nutr, 85*(6), 1626-1633.

Gunther, A. L., Remer, T., Kroke, A., & Buyken, A. E. (2007). Early protein intake and later obesity risk: which protein sources at which time points throughout infancy and childhood are important for body mass index and body fat percentage at 7 y of age? *Am J Clin Nutr, 86*(6), 1765-1772.

Gyomorey, S., Gupta, S., Lye, S. J., Gibb, W., Labrie, F., & Challis, J. R. (2000). Temporal expression of prostaglandin H synthase type 2 (PGHS-2) and P450(C17)in ovine placentomes with the natural onset of labour. *Placenta, 21*(5-6), 478-486.

Haeri, S., Khoury, J., Kovilam, O., & Miodovnik, M. (2008). The association of intrauterine growth abnormalities in women with type 1 diabetes mellitus complicated by vasculopathy. *Am J Obstet Gynecol, 199*(3), 278 e271-275.

Haffner, S. M., Stern, M. P., Mitchell, B. D., Hazuda, H. P., & Patterson, J. K. (1990). Incidence of type II diabetes in Mexican Americans predicted by fasting insulin and glucose levels, obesity, and body-fat distribution. *Diabetes, 39*(3), 283-288.

Haggarty, P. (2002). Placental regulation of fatty acid delivery and its effect on fetal growth--a review. *Placenta, 23 Suppl A*, S28-38.

Haggarty, P., Ashton, J., Joynson, M., Abramovich, D. R., & Page, K. (1999). Effect of maternal polyunsaturated fatty acid concentration on transport by the human placenta. *Biol Neonate, 75*(6), 350-359.

Haggarty, P., Campbell, D. M., Bendomir, A., Gray, E. S., & Abramovich, D. R. (2004). Ponderal index is a poor predictor of in utero growth retardation. *BJOG, 111*(2), 113-119.

Haldre, K., Rahu, K., Karro, H., & Rahu, M. (2007). Is a poor pregnancy outcome related to young maternal age? A study of teenagers in Estonia during the period of major socio-economic changes (from 1992 to 2002). *Eur J Obstet Gynecol Reprod Biol, 131*(1), 45-51.

Hales, C. N., & Barker, D. J. (1992). Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia, 35*(7), 595-601.

Hales, C. N., Barker, D. J., Clark, P. M., Cox, L. J., Fall, C., Osmond, C., et al. (1991). Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ, 303*(6809), 1019-1022.

Haluska, G. J., Cook, M. J., & Novy, M. J. (1997). Inhibition and augmentation of progesterone production during pregnancy: effects on parturition in rhesus monkeys. *Am J Obstet Gynecol, 176*(3), 682-691.

Hamilton, B. E., Martin, J. A., & Ventura, S. J. (2005). Births: Preliminary Data for 2005. Retrieved August 2009: http://www.cdc.gov.ezproxy.auckland.ac.nz/nchs/products/pubs/pubd/hestats/prelimbirths05/prelimbirths05.htm

Hancock, S. N., Oliver, M. H., McLean, C., Jaquiery, A. L., & Bloomfield, F. H. (2011). Size at birth and adult fat mass in twin sheep are determined in early gestation. *J Physiol, published ahead of print December 19, 2011*.

Hanebutt, F. L., Demmelmair, H., Schiessl, B., Larque, E., & Koletzko, B. (2008). Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. *Clin Nutr, 27*(5), 685-693.

Hans, D. M., Pylipow, M., Long, J. D., Thureen, P. J., & Georgieff, M. K. (2009). Nutritional practices in the neonatal intensive care unit: analysis of a 2006 neonatal nutrition survey. *Pediatrics, 123*(1), 51-57.

Harder, T., Bergmann, R., Kallischnigg, G., & Plagemann, A. (2005). Duration of breastfeeding and risk of overweight: a meta-analysis. *Am J Epidemiol, 162*(5), 397-403.

Harding, J. E. (2001). The nutritional basis of the fetal origins of adult disease. *Int J Epidemiol, 30*(1), 15-23.

Haslam, D. W., & James, W. P. (2005). Obesity. *Lancet, 366*(9492), 1197-1209.

Hattersley, A. T., & Tooke, J. E. (1999). The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet, 353*(9166), 1789-1792.

Hauser, J., Dettling-Artho, A., Pilloud, S., Maier, C., Knapman, A., Feldon, J., et al. (2007). Effects of prenatal dexamethasone treatment on postnatal physical, endocrine, and social development in the common marmoset monkey. *Endocrinology, 148*(4), 1813-1822.

Hawdon, J. M., Ward Platt, M. P., & Aynsley-Green, A. (1992). Patterns of metabolic adaptation for preterm and term infants in the first neonatal week. *Arch Dis Child, 67*(4 Spec No), 357-365.

Hay, W. W., Jr., Sparks, J. W., Wilkening, R. B., Battaglia, F. C., & Meschia, G. (1984). Fetal glucose uptake and utilization as functions of maternal glucose concentration. *Am J Physiol Endocrinol Metab, 246*(3 Pt 1), E237-242.

Haymond, M. W., Karl, I. E., & Pagliara, A. S. (1974). Increased gluconeogenic substrates in the small-for-gestational-age infant. *N Engl J Med, 291*(7), 322-328.

Hediger, M. L., Overpeck, M. D., Ruan, W. J., & Troendle, J. F. (2000). Early infant feeding and growth status of US-born infants and children aged 4-71 mo: analyses from the third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr, 72*(1), 159-167.

Heine, R. J., & Dekker, J. M. (2002). Beyond postprandial hyperglycaemia: metabolic factors associated with cardiovascular disease. *Diabetologia, 45*(4), 461-475.

Heinig, M. J., Nommsen, L. A., Peerson, J. M., Lonnerdal, B., & Dewey, K. G. (1993). Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: the DARLING Study. *Am J Clin Nutr, 58*(2), 152-161.

Heird, W. C. (2007). Progress in promoting breast-feeding, combating malnutrition, and composition and use of infant formula, 1981-2006. *J Nutr, 137*(2), 499S-502S.

Helmerhorst, F. M., Perquin, D. A., Donker, D., & Keirse, M. J. (2004). Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ, 328*(7434), 261.

Helmrich, S. P., Ragland, D. R., Leung, R. W., & Paffenbarger, R. S., Jr. (1991). Physical activity and reduced occurrence of non-insulin-dependent diabetes mellitus. *N Engl J Med, 325*(3), 147-152.

Hemachandra, A. H., Howards, P. P., Furth, S. L., & Klebanoff, M. A. (2007). Birth weight, postnatal growth, and risk for high blood pressure at 7 years of age: results from the Collaborative Perinatal Project. *Pediatrics, 119*(6), e1264-1270.

Henderson, G., Fahey, T., & McGuire, W. (2005). Calorie and protein-enriched formula versus standard term formula for improving growth and development in preterm or low birth weight infants following hospital discharge. *Cochrane Database Syst Rev*(2), CD004696.

Henderson, J. J., Hartmann, P. E., Moss, T. J., Doherty, D. A., & Newnham, J. P. (2008). Disrupted secretory activation of the mammary gland after antenatal glucocorticoid treatment in sheep. *Reproduction, 136*(5), 649-655.

Hendler, I., Goldenberg, R. L., Mercer, B. M., Iams, J. D., Meis, P. J., Moawad, A. H., et al. (2005). The Preterm Prediction study: Association between maternal body mass index and spontaneous and indicated preterm birth. *Am J Obstet Gynecol, 192*(3), 882-886.

Hendrix, N., & Berghella, V. (2008). Non-placental causes of intrauterine growth restriction. *Semin Perinatol, 32*(3), 161-165.

Hennige, A. M., Burks, D. J., Ozcan, U., Kulkarni, R. N., Ye, J., Park, S., et al. (2003). Upregulation of insulin receptor substrate-2 in pancreatic beta cells prevents diabetes. *J Clin Invest, 112*(10), 1521-1532.

Henquin, J. C., Ishiyama, N., Nenquin, M., Ravier, M. A., & Jonas, J. C. (2002). Signals and pools underlying biphasic insulin secretion. *Diabetes, 51 Suppl 1*, S60-67.

Henriksen, C., Westerberg, A. C., Ronnestad, A., Nakstad, B., Veierod, M. B., Drevon, C. A., et al. (2009). Growth and nutrient intake among very-low-birth-weight infants fed fortified human milk during hospitalisation. *Br J Nutr, 102*(8), 1179-1186.

Henriksen, E. J., Bourey, R. E., Rodnick, K. J., Koranyi, L., Permutt, M. A., & Holloszy, J. O. (1990). Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *Am J Physiol, 259*(4 Pt 1), E593-598.

Henriksen, T. B., Wilcox, A. J., Hedegaard, M., & Secher, N. J. (1995). Bias in studies of preterm and postterm delivery due to ultrasound assessment of gestational age. *Epidemiology, 6*(5), 533-537.

Hernandez, C. E., Harding, J. E., Oliver, M. H., Bloomfield, F. H., Held, S. D., & Matthews, L. R. (2009). Effects of litter size, sex and periconceptional ewe nutrition on side preference and cognitive flexibility in the offspring. *Behav Brain Res, 204*(1), 82-87.

Hildreth, V., Anderson, R. H., & Henderson, D. J. (2009). Autonomic innervation of the developing heart: origins and function. *Clin Anat, 22*(1), 36-46.

Hill, D. J. (2011). Nutritional programming of pancreatic beta-cell plasticity. *World J Diabetes, 2*(8), 119-126.

Hillier, T. A., & Pedula, K. L. (2001). Characteristics of an adult population with newly diagnosed type 2 diabetes: the relation of obesity and age of onset. *Diabetes Care, 24*(9), 1522-1527.

Hirst, J. J., Parkington, H. C., Young, I. R., Palliser, H. K., Peri, K. G., & Olson, D. M. (2005). Delay of preterm birth in sheep by THG113.31, a prostaglandin F2alpha receptor antagonist. *Am J Obstet Gynecol, 193*(1), 256-266.

Hodges, E. A., Hughes, S. O., Hopkinson, J., & Fisher, J. O. (2008). Maternal decisions about the initiation and termination of infant feeding. *Appetite, 50*(2-3), 333-339.

Hoek, H. W., Susser, E., Buck, K. A., Lumey, L. H., Lin, S. P., & Gorman, J. M. (1996). Schizoid personality disorder after prenatal exposure to famine. *Am J Psychiatry, 153*(12), 1637-1639.

Hofman, P. L., Regan, F., Jackson, W. E., Jefferies, C., Knight, D. B., Robinson, E. M., et al. (2004). Premature birth and later insulin resistance. *N Engl J Med, 351*(21), 2179-2186.

Hokken-Koelega, A. C., De Ridder, M. A., Lemmen, R. J., Den Hartog, H., De Muinck Keizer-Schrama, S. M., & Drop, S. L. (1995). Children born small for gestational age: do they catch up? *Pediatr Res, 38*(2), 267-271.

Holland, M. G., Refuerzo, J. S., Ramin, S. M., Saade, G. R., & Blackwell, S. C. (2009). Late preterm birth: how often is it avoidable? *Am J Obstet Gynecol, 201*(4), 404 e401-404.

Holme, I., Aastveit, A. H., Hammar, N., Jungner, I., & Walldius, G. (2009). Uric acid and risk of myocardial infarction, stroke and congestive heart failure in 417 734 men and women in the Apolipoprotein MOrtality RISk study (AMORIS). *J Intern Med, 266*(6), 558-570.

Homma, M., Tanaka, A., Hino, K., Takamura, H., Hirano, T., Oka, K., et al. (2001). Assessing systemic 11 beta hydroxysteroid dehydrogenase with serum cortisone/cortisol ratios in healthy subjects and patients with diabetes mellitus. *Metabolism, 50*(7), 801-804.

Hon, E. H., & Lee, S. T. (1963). Electronic evaluation of the fetal heart rate, Viii. Patterns preceding fetal death, further observations. *Am J Obstet Gynecol, 87*, 814-826.

Hoppe, C., Molgaard, C., Dalum, C., Vaag, A., & Michaelsen, K. F. (2009). Differential effects of casein versus whey on fasting plasma levels of insulin, IGF-1 and IGF-1/IGFBP-3: results from a randomized 7-day supplementation study in prepubertal boys. *Eur J Clin Nutr, 63*(9), 1076-1083.

Hoppe, C., Molgaard, C., Juul, A., & Michaelsen, K. F. (2004). High intakes of skimmed milk, but not meat, increase serum IGF-I and IGFBP-3 in eight-year-old boys. *Eur J Clin Nutr, 58*(9), 1211-1216.

Hoppe, C., Molgaard, C., Vaag, A., Barkholt, V., & Michaelsen, K. F. (2005). High intakes of milk, but not meat, increase s-insulin and insulin resistance in 8-year-old boys. *Eur J Clin Nutr, 59*(3), 393-398.

Horton, J. D., Goldstein, J. L., & Brown, M. S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest, 109*(9), 1125-1131.

Hovi, P., Andersson, S., Eriksson, J. G., Jarvenpaa, A. L., Strang-Karlsson, S., Makitie, O., et al. (2007). Glucose regulation in young adults with very low birth weight. *N Engl J Med, 356*(20), 2053-2063.

Hovorka, R., Chassin, L., Luzio, S. D., Playle, R., & Owens, D. R. (1998). Pancreatic beta-cell responsiveness during meal tolerance test: model assessment in normal subjects and subjects with newly diagnosed noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab, 83*(3), 744-750.

Howarth, C., Gazis, A., & James, D. (2007). Associations of Type 1 diabetes mellitus, maternal vascular disease and complications of pregnancy. *Diabet Med, 24*(11), 1229-1234.

Hu, G., Qiao, Q., Tuomilehto, J., Balkau, B., Borch-Johnsen, K., & Pyorala, K. (2004). Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. *Arch Intern Med, 164*(10), 1066-1076.

Huang, S., & Czech, M. P. (2007). The GLUT4 glucose transporter. *Cell Metab, 5*(4), 237-252.

Huang, T. T., Nansel, T. R., Belsheim, A. R., & Morrison, J. A. (2008). Sensitivity, specificity, and predictive values of pediatric metabolic syndrome components in relation to adult metabolic syndrome: the Princeton LRC follow-up study. *J Pediatr, 152*(2), 185-190.

Hughson, M., Farris, A. B., 3rd, Douglas-Denton, R., Hoy, W. E., & Bertram, J. F. (2003). Glomerular number and size in autopsy kidneys: the relationship to birth weight. *Kidney Int, 63*(6), 2113-2122.

Huh, J., Riggs, N. R., Spruijt-Metz, D., Chou, C. P., Huang, Z., & Pentz, M. (2011). Identifying patterns of eating and physical activity in children: a latent class analysis of obesity risk. *Obesity (Silver Spring), 19*(3), 652-658.

Hume, R., & Burchell, A. (1993). Abnormal expression of glucose-6-phosphatase in preterm infants. *Arch Dis Child, 68*(2), 202-204.

Hume, R., McGeechan, A., & Burchell, A. (1999). Failure to detect preterm infants at risk of hypoglycemia before discharge. *J Pediatr, 134*(4), 499-502.

Huvers, F. C., Popa, C., Netea, M. G., van den Hoogen, F. H., & Tack, C. J. (2007). Improved insulin sensitivity by anti-TNFalpha antibody treatment in patients with rheumatic diseases. *Ann Rheum Dis, 66*(4), 558-559.

Huxley, R., Owen, C. G., Whincup, P. H., Cook, D. G., Rich-Edwards, J., Smith, G. D., et al. (2007). Is birth weight a risk factor for ischaemic heart disease in later life? *Am J Clin Nutr, 85*(5), 1244-1250.

Huxley, R. R., Shiell, A. W., & Law, C. M. (2000). The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. *J Hypertens, 18*(7), 815-831.

Ibanez, L., Ong, K., Dunger, D. B., & de Zegher, F. (2006). Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. *J Clin Endocrinol Metab, 91*(6), 2153-2158.

Ikegami, M., Jobe, A. H., Newnham, J., Polk, D. H., Willet, K. E., & Sly, P. (1997). Repetitive prenatal glucocorticoids improve lung function and decrease growth in preterm lambs. *Am J Respir Crit Care Med, 156*(1), 178-184.

Ilcol, Y. O., Hizli, Z. B., & Eroz, E. (2008). Resistin is present in human breast milk and it correlates with maternal hormonal status and serum level of C-reactive protein. *Clin Chem Lab Med, 46*(1), 118-124.

Ingemarsson, I. (2003). Gender aspects of preterm birth. *BJOG, 110* (Suppl 20), 34-38.

Innis, S. M. (2005). Essential fatty acid transfer and fetal development. *Placenta, 26 Suppl A*, S70-75.

Innis, S. M. (2007). Dietary (n-3) fatty acids and brain development. *J Nutr, 137*(4), 855-859.

Inoue, T., Matsuoka, H., Higashi, Y., Ueda, S., Sata, M., Shimada, K. E., et al. (2008). Flow-mediated vasodilation as a diagnostic modality for vascular failure. *Hypertens Res, 31*(12), 2105-2113.

Irving, R. J., Shore, A. C., Belton, N. R., Elton, R. A., Webb, D. J., & Walker, B. R. (2004). Low birth weight predicts higher blood pressure but not dermal capillary density in two populations. *Hypertension, 43*(3), 610-613.

Isacco, L., Lazaar, N., Ratel, S., Thivel, D., Aucouturier, J., Dore, E., et al. (2010). The impact of eating habits on anthropometric characteristics in French primary school children. *Child Care Health Dev, 36*(6), 835-842.

Itskovitz, J., LaGamma, E. F., & Rudolph, A. M. (1987). Effects of cord compression on fetal blood flow distribution and O2 delivery. *Am J Physiol Heart Circ Physiol, 252*(1), H100-109.

Izumi, H., Ishizuka, S., Inafune, A., Hira, T., Ozawa, K., Shimizu, T., et al. (2009). Alpha-lactalbumin hydrolysate stimulates glucagon-like peptide-2 secretion and small intestinal growth in suckling rats. *J Nutr, 139*(7), 1322-1327.

Jackson, R. A., Gibson, K. A., Wu, Y. W., & Croughan, M. S. (2004). Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol, 103*(3), 551-563.

Jansson, T., & Powell, T. L. (2006). IFPA 2005 Award in Placentology Lecture. Human placental transport in altered fetal growth: does the placenta function as a nutrient sensor? *Placenta, 27 Suppl A*, S91-97.

Jaquiery, A. L., Oliver, M. H., Bloomfield, F. H., Connor, K. L., Challis, J. R., & Harding, J. E. (2006). Fetal exposure to excess glucocorticoid is unlikely to explain the effects of periconceptional undernutrition in sheep. *J Physiol, 572*(Pt 1), 109-118.

Jaquiery, A. L., Oliver, M. H., Bloomfield, F. H., & Harding, J. E. (2011). Periconceptional events perturb postnatal growth regulation in sheep. *Pediatr Res, 70*(3), 261-266.

Jenkins, J., Gardner, E., McCall, E., Casson, K., & Dolk, H. (2009). Socioeconomic inequalities in neonatal intensive care admission rates. *Arch Dis Child Fetal Neonatal Ed*.

Jensen, C. B., Storgaard, H., Madsbad, S., Richter, E. A., & Vaag, A. A. (2007). Altered skeletal muscle fiber composition and size precede whole-body insulin resistance in young men with low birth weight. *J Clin Endocrinol Metab, 92*(4), 1530-1534.

Jensen, D. M., Damm, P., Sorensen, B., Molsted-Pedersen, L., Westergaard, J. G., Ovesen, P., et al. (2003). Pregnancy outcome and prepregnancy body mass index in 2459 glucose-tolerant Danish women. *Am J Obstet Gynecol, 189*(1), 239-244.

Jensen, G. M., & Moore, L. G. (1997). The effect of high altitude and other risk factors on birthweight: independent or interactive effects? *Am J Public Health, 87*(6), 1003-1007.

Jensen, M. D., Haymond, M. W., Rizza, R. A., Cryer, P. E., & Miles, J. M. (1989). Influence of body fat distribution on free fatty acid metabolism in obesity. *J Clin Invest, 83*(4), 1168-1173.

Jimenez-Chillaron, J. C., Hernandez-Valencia, M., Lightner, A., Faucette, R. R., Reamer, C., Przybyla, R., et al. (2006). Reductions in caloric intake and early postnatal growth prevent glucose intolerance and obesity associated with low birthweight. *Diabetologia, 49*(8), 1974-1984.

Jimenez-Chillaron, J. C., Hernandez-Valencia, M., Reamer, C., Fisher, S., Joszi, A., Hirshman, M., et al. (2005). Beta-cell secretory dysfunction in the pathogenesis of low birth weight-associated diabetes: a murine model. *Diabetes, 54*(3), 702-711.

Joeckel, R. J., & Phillips, S. K. (2009). Overview of infant and pediatric formulas. *Nutr Clin Pract, 24*(3), 356-362.

Johansson, S., Iliadou, A., Bergvall, N., Tuvemo, T., Norman, M., & Cnattingius, S. (2005). Risk of high blood pressure among young men increases with the degree of immaturity at birth. *Circulation, 112*(22), 3430-3436.

Johansson, S., Norman, M., Legnevall, L., Dalmaz, Y., Lagercrantz, H., & Vanpee, M. (2007). Increased catecholamines and heart rate in children with low birth weight: perinatal contributions to sympathoadrenal overactivity. *J Intern Med, 261*(5), 480-487.

Johnson, R. J., Feig, D. I., Herrera-Acosta, J., & Kang, D. H. (2005). Resurrection of uric acid as a causal risk factor in essential hypertension. *Hypertension, 45*(1), 18-20.

Jones, A., Beda, A., Osmond, C., Godfrey, K. M., Simpson, D. M., & Phillips, D. I. (2008). Sex-specific programming of cardiovascular physiology in children. *Eur Heart J, 29*(17), 2164-2170.

Jones, A., Beda, A., Ward, A. M., Osmond, C., Phillips, D. I., Moore, V. M., et al. (2007). Size at birth and autonomic function during psychological stress. *Hypertension, 49*(3), 548-555.

Jones, J. N., Gercel-Taylor, C., & Taylor, D. D. (1999). Altered cord serum lipid levels associated with small for gestational age infants. *Obstet Gynecol, 93*(4), 527-531.

Joseph, K. S., Fahey, J., Platt, R. W., Liston, R. M., Lee, S. K., Sauve, R., et al. (2009). An outcome-based approach for the creation of fetal growth standards: do singletons and twins need separate standards? *Am J Epidemiol, 169*(5), 616-624.

Joseph, K. S., & Kramer, M. S. (1996). Review of the evidence on fetal and early childhood antecedents of adult chronic disease. *Epidemiol Rev, 18*(2), 158-174.

Jouven, X., Empana, J. P., Schwartz, P. J., Desnos, M., Courbon, D., & Ducimetiere, P. (2005). Heart-rate profile during exercise as a predictor of sudden death. *N Engl J Med, 352*(19), 1951-1958.

Jovanovic-Peterson, L., Fuhrmann, K., Hedden, K., Walker, L., & Peterson, C. M. (1989). Maternal milk and plasma glucose and insulin levels: studies in normal and diabetic subjects. *J Am Coll Nutr, 8*(2), 125-131.

Kaijser, M., Bonamy, A. K., Akre, O., Cnattingius, S., Granath, F., Norman, M., et al. (2008). Perinatal risk factors for ischemic heart disease: disentangling the roles of birth weight and preterm birth. *Circulation, 117*(3), 405-410.

Kaijser, M., Bonamy, A. K., Akre, O., Cnattingius, S., Granath, F., Norman, M., et al. (2009). Perinatal risk factors for diabetes in later life. *Diabetes, 58*(3), 523-526.

Kalhan, S. C., D'Angelo, L. J., Savin, S. M., & Adam, P. A. (1979). Glucose production in pregnant women at term gestation. Sources of glucose for human fetus. *J Clin Invest, 63*(3), 388-394.

Kalhoff, H., & Manz, F. (2001). Nutrition, acid-base status and growth in early childhood. *Eur J Nutr, 40*(5), 221-230.

Kalish, R. B., Thaler, H. T., Chasen, S. T., Gupta, M., Berman, S. J., Rosenwaks, Z., et al. (2004). First- and second-trimester ultrasound assessment of gestational age. *Am J Obstet Gynecol, 191*(3), 975-978.

Kaplan, J. R., Manuck, S. B., & Clarkson, T. B. (1987). The influence of heart rate on coronary artery atherosclerosis. *J Cardiovasc Pharmacol, 10* (Suppl 2), S100-103.

Karlberg, J., & Albertsson-Wikland, K. (1995). Growth in full-term small-for-gestational-age infants: from birth to final height. *Pediatr Res, 38*(5), 733-739.

Kasuga, M., Karlsson, F. A., & Kahn, C. R. (1982). Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. *Science, 215*(4529), 185-187.

Kaufman, C. L., Kaiser, D. R., Steinberger, J., & Dengel, D. R. (2007). Relationships between heart rate variability, vascular function, and adiposity in children. *Clin Auton Res, 17*(3), 165-171.

Keller, G., Zimmer, G., Mall, G., Ritz, E., & Amann, K. (2003). Nephron number in patients with primary hypertension. *N Engl J Med, 348*(2), 101-108.

Kennedy, K., Ross, S., Isaacs, E. B., Weaver, L. T., Singhal, A., Lucas, A., et al. (2010). The 10-year follow-up of a randomised trial of long-chain polyunsaturated fatty acid supplementation in preterm infants: effects on growth and blood pressure. *Arch Dis Child, 95*(8), 588-595.

Ketelslegers, J. M., Maiter, D., Maes, M., Underwood, L. E., & Thissen, J. P. (1996). Nutritional regulation of the growth hormone and insulin-like growth factor-binding proteins. *Horm Res, 45*(3-5), 252-257.

Khovidhunkit, W., Memon, R. A., Feingold, K. R., & Grunfeld, C. (2000). Infection and inflammation-induced proatherogenic changes of lipoproteins. *J Infect Dis, 181* (Suppl 3), S462-472.

Kierson, J. A., Dimatteo, D. M., Locke, R. G., Mackley, A. B., & Spear, M. L. (2006). Ghrelin and cholecystokinin in term and preterm human breast milk. *Acta Paediatr, 95*(8), 991-995.

Kim, J., Peterson, K. E., Scanlon, K. S., Fitzmaurice, G. M., Must, A., Oken, E., et al. (2006). Trends in overweight from 1980 through 2001 among preschool-aged children

enrolled in a health maintenance organization. *Obesity (Silver Spring), 14*(7), 1107-1112.

Kimball, S. R. (2007). The role of nutrition in stimulating muscle protein accretion at the molecular level. *Biochem Soc Trans, 35*(Pt 5), 1298-1301.

Kind, K. L., Roberts, C. T., Sohlstrom, A. I., Katsman, A., Clifton, P. M., Robinson, J. S., et al. (2005). Chronic maternal feed restriction impairs growth but increases adiposity of the fetal guinea pig. *Am J Physiol Regul Integr Comp Physiol, 288*(1), R119-126.

Kiserud, T., Ebbing, C., Kessler, J., & Rasmussen, S. (2006). Fetal cardiac output, distribution to the placenta and impact of placental compromise. *Ultrasound Obstet Gynecol, 28*(2), 126-136.

Kitsommart, R., Janes, M., Mahajan, V., Rahman, A., Seidlitz, W., Wilson, J., et al. (2009). Outcomes of late-preterm infants: a retrospective, single-center, Canadian study. *Clin Pediatr (Phila), 48*(8), 844-850.

Kitts, D. D., Anderson, G. B., BonDurant, R. H., & Stabenfeldt, G. H. (1984). Temporal patterns of delta 4 C-21 steroids in coexisting, genetically dissimilar twin lamb fetuses throughout late gestation. *Endocrinology, 114*(3), 703-711.

Klammt, J., Pfaffle, R., Werner, H., & Kiess, W. (2008). IGF signaling defects as causes of growth failure and IUGR. *Trends Endocrinol Metab, 19*(6), 197-205.

Klein, C. J. (2002). Nutrient requirements for preterm infant formulas. *J Nutr, 132*(6 Suppl 1), 1395S-1577S.

Klover, P. J., Zimmers, T. A., Koniaris, L. G., & Mooney, R. A. (2003). Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes, 52*(11), 2784-2789.

Koletzko, B., Broekaert, I., Demmelmair, H., Franke, J., Hannibal, I., Oberle, D., et al. (2005). Protein intake in the first year of life: a risk factor for later obesity? The E.U. childhood obesity project. *Adv Exp Med Biol, 569*, 69-79.

Koletzko, B., von Kries, R., Closa, R., Escribano, J., Scaglioni, S., Giovannini, M., et al. (2009). Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. *Am J Clin Nutr, 89*(6), 1836-1845.

Koletzko, B., von Kries, R., Monasterolo, R. C., Subias, J. E., Scaglioni, S., Giovannini, M., et al. (2009). Infant feeding and later obesity risk. *Adv Exp Med Biol, 646*, 15-29.

Koo, W. W., & Hockman, E. M. (2006). Posthospital discharge feeding for preterm infants: effects of standard compared with enriched milk formula on growth, bone mass, and body composition. *Am J Clin Nutr, 84*(6), 1357-1364.

Kotelevtsev, Y., Holmes, M. C., Burchell, A., Houston, P. M., Schmoll, D., Jamieson, P., et al. (1997). 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci U S A, 94*(26), 14924-14929.

Koupil, I., Leon, D. A., & Lithell, H. O. (2005). Length of gestation is associated with mortality from cerebrovascular disease. *J Epidemiol Community Health, 59*(6), 473-474.

Koutcherov, Y., Mai, J. K., & Paxinos, G. (2003). Hypothalamus of the human fetus. *J Chem Neuroanat, 26*(4), 253-270.

Kramer, M. S., Chalmers, B., Hodnett, E. D., Sevkovskaya, Z., Dzikovich, I., Shapiro, S., et al. (2001). Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. *JAMA, 285*(4), 413-420.

Kramer, M. S., Guo, T., Platt, R. W., Vanilovich, I., Sevkovskaya, Z., Dzikovich, I., et al. (2004). Feeding effects on growth during infancy. *J Pediatr, 145*(5), 600-605.

Kramer, M. S., & Kakuma, R. (2002). Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev*(1), CD003517.

Kramer, M. S., Matush, L., Vanilovich, I., Platt, R. W., Bogdanovich, N., Sevkovskaya, Z., et al. (2009). A randomized breast-feeding promotion intervention did not reduce child obesity in Belarus. *J Nutr, 139*(2), 417S-421S.

Kramer, M. S., Séguin, L., Lydon, J., & Goulet, L. (2000). Socio-economic disparities in pregnancy outcome: why do the poor fare so poorly? *Paediatr Perinat Epidemiol, 14*(3), 194-210.

Kumari, M., Shipley, M., Stafford, M., & Kivimaki, M. (2011). Association of diurnal patterns in salivary cortisol with all cause and cardiovascular mortality: Findings from the Whitehall II Study. *J Clin Endocrinol Metab, 96*, 1478-1485.

Kuschel, C. A., & Harding, J. E. (2004). Multicomponent fortified human milk for promoting growth in preterm infants. *Cochrane Database Syst Rev*(1), CD000343.

Labayen, I., Ruiz, J. R., Ortega, F. B., Harro, J., Merenakk, L., Oja, L., et al. (2011). Insulin sensitivity at childhood predicts changes in total and central adiposity over a 6-year period. *Int J Obes (Lond)*.

Labbok, M. H., Clark, D., & Goldman, A. S. (2004). Breastfeeding: maintaining an irreplaceable immunological resource. *Nat Rev Immunol, 4*(7), 565-572.

Lackman, F., Capewell, V., Richardson, B., daSilva, O., & Gagnon, R. (2001). The risks of spontaneous preterm delivery and perinatal mortality in relation to size at birth according to fetal versus neonatal growth standards. *Am J Obstet Gynecol, 184*(5), 946-953.

Lacroix, M. C., Guibourdenche, J., Frendo, J. L., Muller, F., & Evain-Brion, D. (2002). Human placental growth hormone--a review. *Placenta, 23* (Suppl A), S87-94.

Lage, M., Baldelli, R., Camina, J. P., Rodriguez-Garci, J., Penalva, A., Dieguez, C., et al. (2002). Presence of bovine leptin in edible commercial milk and infant formula. *J Endocrinol Invest, 25*(8), 670-674.

Lahiri, M. K., Kannankeril, P. J., & Goldberger, J. J. (2008). Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. *J Am Coll Cardiol, 51*(18), 1725-1733.

Lambert, M., Paradis, G., O'Loughlin, J., Delvin, E. E., Hanley, J. A., & Levy, E. (2004). Insulin resistance syndrome in a representative sample of children and adolescents from Quebec, Canada. *Int J Obes Relat Metab Disord, 28*(7), 833-841.

Landmann, E., Reiss, I., Misselwitz, B., & Gortner, L. (2006). Ponderal index for discrimination between symmetric and asymmetric growth restriction: percentiles for neonates from 30 weeks to 43 weeks of gestation. *J Matern Fetal Neonatal Med, 19*(3), 157-160.

Lane, R. H., Flozak, A. S., & Simmons, R. A. (1996). Measurement of GLUT mRNA in liver of fetal and neonatal rats using a novel method of quantitative polymerase chain reaction. *Biochem Mol Med, 59*(2), 192-199.

Lange, S., Van Leeuwen, P., Schneider, U., Frank, B., Hoyer, D., Geue, D., et al. (2009). Heart rate features in fetal behavioural states. *Early Hum Dev, 85*(2), 131-135.

Langille, B. L., Brownlee, R. D., & Adamson, S. L. (1990). Perinatal aortic growth in lambs: relation to blood flow changes at birth. *Am J Physiol Heart Circ Physiol, 259*(4 Pt 2), H1247-1253.

Langley-Evans, S. C. (1997). Maternal carbenoxolone treatment lowers birthweight and induces hypertension in the offspring of rats fed a protein-replete diet. *Clin Sci (Lond), 93*(5), 423-429.

Langley-Evans, S. C., Phillips, G. J., Benediktsson, R., Gardner, D. S., Edwards, C. R., Jackson, A. A., et al. (1996). Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. *Placenta, 17*(2-3), 169-172.

Large, V., Peroni, O., Letexier, D., Ray, H., & Beylot, M. (2004). Metabolism of lipids in human white adipocyte. *Diabetes Metab, 30*(4), 294-309.

Larque, E., Demmelmair, H., Berger, B., Hasbargen, U., & Koletzko, B. (2003). In vivo investigation of the placental transfer of (13)C-labeled fatty acids in humans. *J Lipid Res, 44*(1), 49-55.

Larsson, L., & Maunsbach, A. B. (1980). The ultrastructural development of the glomerular filtration barrier in the rat kidney: a morphometric analysis. *J Ultrastruct Res, 72*(3), 392-406.

Law, C. M., Shiell, A. W., Newsome, C. A., Syddall, H. E., Shinebourne, E. A., Fayers, P. M., et al. (2002). Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. *Circulation, 105*(9), 1088-1092.

Lawlor, D. A., Hubinette, A., Tynelius, P., Leon, D. A., Smith, G. D., & Rasmussen, F. (2007). Associations of gestational age and intrauterine growth with systolic blood pressure in a family-based study of 386,485 men in 331,089 families. *Circulation, 115*(5), 562-568.

Lazdam, M., de la Horra, A., Pitcher, A., Mannie, Z., Diesch, J., Trevitt, C., et al. (2010). Elevated blood pressure in offspring born premature to hypertensive pregnancy. Is endothelial dysfunction the underlying vascular mechanism? *Hypertension, 56*(1), 159-165.

Lee, P. D., Conover, C. A., & Powell, D. R. (1993). Regulation and function of insulin-like growth factor-binding protein-1. *Proc Soc Exp Biol Med, 204*(1), 4-29.

Leen, W. G., Klepper, J., Verbeek, M. M., Leferink, M., Hofste, T., van Engelen, B. G., et al. (2010). Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. *Brain, 133*(Pt 3), 655-670.

Leger, J., Noel, M., Limal, J. M., & Czernichow, P. (1996). Growth factors and intrauterine growth retardation. II. Serum growth hormone, insulin-like growth factor (IGF) I, and IGF-binding protein 3 levels in children with intrauterine growth retardation compared with normal control subjects: prospective study from birth to two years of age. Study Group of IUGR. *Pediatr Res, 40*(1), 101-107.

Leguizamon, G., & von Stecher, F. (2003). Third trimester glycemic profiles and fetal growth. *Curr Diab Rep, 3*(4), 323-326.

Lembo, G., Napoli, R., Capaldo, B., Rendina, V., Iaccarino, G., Volpe, M., et al. (1992). Abnormal sympathetic overactivity evoked by insulin in the skeletal muscle of patients with essential hypertension. *J Clin Invest, 90*(1), 24-29.

Leon, D. A., Lithell, H. O., Vagero, D., Koupilova, I., Mohsen, R., Berglund, L., et al. (1998). Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. *BMJ, 317*(7153), 241-245.

Leroith, D., & Accili, D. (2008). Mechanisms of disease: using genetically altered mice to study concepts of type 2 diabetes. *Nat Clin Pract Endocrinol Metab, 4*(3), 164-172.

Leunissen, R. W., Oosterbeek, P., Hol, L. K., Hellingman, A. A., Stijnen, T., & Hokken-Koelega, A. C. (2008). Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab, 93*(2), 445-451.

Levy-Marchal, C., Arslanian, S., Cutfield, W., Sinaiko, A., Druet, C., Marcovecchio, M. L., et al. (2010). Insulin resistance in children: consensus, perspective, and future directions. *J Clin Endocrinol Metab, 95*(12), 5189-5198.

Lewis, D. S., Bertrand, H. A., McMahan, C. A., McGill, H. C., Jr., Carey, K. D., & Masoro, E. J. (1986). Preweaning food intake influences the adiposity of young adult baboons. *J Clin Invest, 78*(4), 899-905.

Lewis, D. S., Bertrand, H. A., McMahan, C. A., McGill, H. C., Jr., Carey, K. D., & Masoro, E. J. (1989). Influence of preweaning food intake on body composition of young adult baboons. *Am J Physiol Regul Integr Comp Physiol, 257*(5 Pt 2), R1128-1135.

Lewis, D. S., Jackson, E. M., & Mott, G. E. (1992). Effect of energy intake on postprandial plasma hormones and triglyceride concentrations in infant female baboons (Papio species). *J Clin Endocrinol Metab, 74*(4), 920-926.

Lewis, M. E., Al-Khalidi, A. H., Bonser, R. S., Clutton-Brock, T., Morton, D., Paterson, D., et al. (2001). Vagus nerve stimulation decreases left ventricular contractility in vivo in the human and pig heart. *J Physiol, 534*(Pt. 2), 547-552.

Li, C., Levitz, M., Hubbard, G. B., Jenkins, S. L., Han, V., Ferry, R. J., Jr., et al. (2007). The IGF axis in baboon pregnancy: placental and systemic responses to feeding 70% global ad libitum diet. *Placenta, 28*(11-12), 1200-1210.

Li, J., Forhead, A. J., Dauncey, M. J., Gilmour, R. S., & Fowden, A. L. (2002). Control of growth hormone receptor and insulin-like growth factor-I expression by cortisol in ovine fetal skeletal muscle. *J Physiol, 541*(Pt 2), 581-589.

Li, J., Owens, J. A., Owens, P. C., Saunders, J. C., Fowden, A. L., & Gilmour, R. S. (1996). The ontogeny of hepatic growth hormone receptor and insulin-like growth factor I gene expression in the sheep fetus during late gestation: developmental regulation by cortisol. *Endocrinology, 137*(5), 1650-1657.

Li, R., Fein, S. B., & Grummer-Strawn, L. M. (2008). Association of breastfeeding intensity and bottle-emptying behaviors at early infancy with infants' risk for excess weight at late infancy. *Pediatrics, 122* (Suppl 2), S77-84.

Li, R., Fein, S. B., & Grummer-Strawn, L. M. (2010). Do infants fed from bottles lack self-regulation of milk intake compared with directly breastfed infants? *Pediatrics, 125*(6), e1386-1393.

Liao, D., Arnett, D. K., Tyroler, H. A., Riley, W. A., Chambless, L. E., Szklo, M., et al. (1999). Arterial stiffness and the development of hypertension. The ARIC study. *Hypertension, 34*(2), 201-206.

Licht, C. M., Vreeburg, S. A., van Reedt Dortland, A. K., Giltay, E. J., Hoogendijk, W. J., DeRijk, R. H., et al. (2010). Increased sympathetic and decreased parasympathetic activity rather than changes in hypothalamic-pituitary-adrenal axis activity is associated with metabolic abnormalities. *J Clin Endocrinol Metab, 95*(5), 2458-2466.

Lien, E. L. (2003). Infant formulas with increased concentrations of alpha-lactalbumin. *Am J Clin Nutr, 77*(6), 1555S-1558S.

Lievre, M., & Sitruk-Ware, R. (2009). Meta-analysis of 200 or 600 mg mifepristone in association with two prostaglandins for termination of early pregnancy. *Contraception, 80*(1), 95-100.

Liggins, G. C. (1976). Adrenocortical-related maturational events in the fetus. *Am J Obstet Gynecol, 126*(7), 931-941.

Liggins, G. C., & Howie, R. N. (1972). A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics, 50*(4), 515-525.

Limesand, S. W., Jensen, J., Hutton, J. C., & Hay, W. W., Jr. (2005). Diminished beta-cell replication contributes to reduced beta-cell mass in fetal sheep with intrauterine growth restriction. *Am J Physiol Regul Integr Comp Physiol, 288*(5), R1297-1305.

Limesand, S. W., Rozance, P. J., Smith, D., & Hay, W. W., Jr. (2007). Increased insulin sensitivity and maintenance of glucose utilization rates in fetal sheep with placental insufficiency and intrauterine growth restriction. *Am J Physiol Endocrinol Metab, 293*(6), E1716-1725.

Limesand, S. W., Rozance, P. J., Zerbe, G. O., Hutton, J. C., & Hay, W. W., Jr. (2006). Attenuated insulin release and storage in fetal sheep pancreatic islets with intrauterine growth restriction. *Endocrinology, 147*(3), 1488-1497.

Lindsay, R. S., Lindsay, R. M., Waddell, B. J., & Seckl, J. R. (1996). Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: studies with the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone. *Diabetologia, 39*(11), 1299-1305.

Lingohr, M. K., Dickson, L. M., Wrede, C. E., McCuaig, J. F., Myers, M. G., Jr., & Rhodes, C. J. (2003). IRS-3 inhibits IRS-2-mediated signaling in pancreatic beta-cells. *Mol Cell Endocrinol, 204*(1-2), 85-99.

Lithell, H. O., McKeigue, P. M., Berglund, L., Mohsen, R., Lithell, U. B., & Leon, D. A. (1996). Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50-60 years. *BMJ, 312*(7028), 406-410.

Liu, J. K., Fleseriu, M., Delashaw, J. B., Jr., Ciric, I. S., & Couldwell, W. T. (2007). Treatment options for Cushing disease after unsuccessful transsphenoidal surgery. *Neurosurg Focus, 23*(3), E8.

Long, N. M., George, L. A., Uthlaut, A. B., Smith, D. T., Nijland, M. J., Nathanielsz, P. W., et al. (2010). Maternal obesity and increased nutrient intake before and during gestation in the ewe results in altered growth, adiposity, and glucose tolerance in adult offspring. *J Anim Sci, 88*(11), 3546-3553.

Lopuhaa, C. E., Roseboom, T. J., Osmond, C., Barker, D. J., Ravelli, A. C., Bleker, O. P., et al. (2000). Atopy, lung function, and obstructive airways disease after prenatal exposure to famine. *Thorax, 55*(7), 555-561.

Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M., & Sitzer, M. (2007). Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation, 115*(4), 459-467.

Lubchenco, L. O., Searls, D. T., & Brazie, J. V. (1972). Neonatal mortality rate: relationship to birth weight and gestational age. *J Pediatr, 81*(4), 814-822.

Lucas, A., Boyes, S., Bloom, S. R., & Aynsley-Green, A. (1981). Metabolic and endocrine responses to a milk feed in six-day-old term infants: differences between breast and cow's milk formula feeding. *Acta Paediatr Scand, 70*(2), 195-200.

Lucas, A., Fewtrell, M. S., & Cole, T. J. (1999). Fetal origins of adult disease-the hypothesis revisited. *BMJ, 319*(7204), 245-249.

Lucas, A., Morley, R., & Cole, T. J. (1998). Randomised trial of early diet in preterm babies and later intelligence quotient. *BMJ, 317*(7171), 1481-1487.

Lucas, A., Morley, R., Cole, T. J., Gore, S. M., Lucas, P. J., Crowle, P., et al. (1990). Early diet in preterm babies and developmental status at 18 months. *Lancet, 335*(8704), 1477-1481.

Lucini, D., Mela, G. S., Malliani, A., & Pagani, M. (2002). Impairment in cardiac autonomic regulation preceding arterial hypertension in humans: insights from spectral analysis of beat-by-beat cardiovascular variability. *Circulation, 106*(21), 2673-2679.

Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey proteins in the regulation of food intake and satiety. *J Am Coll Nutr, 26*(6), 704S-712S.

Lupu, F., Terwilliger, J. D., Lee, K., Segre, G. V., & Efstratiadis, A. (2001). Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol, 229*(1), 141-162.

Lyall, H., Scott, H. M., & Burchell, A. (1997). Hepatic glucose-6-phosphatase development in preterm and full-term guinea-pigs: comparison with rat and human development. *Comp Biochem Physiol A Physiol, 116*(3), 261-265.

Lyle, R. E., Kincaid, S. C., Bryant, J. C., Prince, A. M., & McGehee, R. E., Jr. (2001). Human milk contains detectable levels of immunoreactive leptin. *Adv Exp Med Biol, 501*, 87-92.

Macdonald, P. D., Ross, S. R., Grant, L., & Young, D. (2003). Neonatal weight loss in breast and formula fed infants. *Arch Dis Child Fetal Neonatal Ed, 88*(6), F472-476.

MacIsaac, R. J., Bell, R. J., McDougall, J. G., Tregear, G. W., Wang, X., & Wintour, E. M. (1985). Development of the hypothalamic-pituitary-axis in the ovine fetus: ontogeny of action of ovine corticotropin-releasing factor. *J Dev Physiol, 7*(5), 329-338.

MacKay, D. F., Smith, G. C., Dobbie, R., & Pell, J. P. (2010). Gestational age at delivery and special educational need: retrospective cohort study of 407,503 schoolchildren. *PLoS Med, 7*(6), e1000289.

Madar, J., Richmond, S., & Hey, E. (1999). Surfactant-deficient respiratory distress after elective delivery at 'term'. *Acta Paediatr, 88*(11), 1244-1248.

Madsen, G., Zakar, T., Ku, C. Y., Sanborn, B. M., Smith, R., & Mesiano, S. (2004). Prostaglandins differentially modulate progesterone receptor-A and -B expression in human myometrial cells: evidence for prostaglandin-induced functional progesterone withdrawal. *J Clin Endocrinol Metab, 89*(2), 1010-1013.

Maedler, K., Sergeev, P., Ris, F., Oberholzer, J., Joller-Jemelka, H. I., Spinas, G. A., et al. (2002). Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J Clin Invest, 110*(6), 851-860.

Magiakou, M. A., Smyrnaki, P., & Chrousos, G. P. (2006). Hypertension in Cushing's syndrome. *Best Pract Res Clin Endocrinol Metab, 20*(3), 467-482.

Malik, S., Wong, N. D., Franklin, S. S., Kamath, T. V., L'Italien, G. J., Pio, J. R., et al. (2004). Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation, 110*(10), 1245-1250.

Malliani, A. (2005). Heart rate variability: from bench to bedside. *Eur J Intern Med, 16*(1), 12-20.

Malloy, M. H., & Freeman, D. H., Jr. (2000). Birth weight- and gestational age-specific sudden infant death syndrome mortality: United States, 1991 versus 1995. *Pediatrics, 105*(6), 1227-1231.

Manalich, R., Reyes, L., Herrera, M., Melendi, C., & Fundora, I. (2000). Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. *Kidney Int, 58*(2), 770-773.

Manesso, E., Toffolo, G. M., Butler, A. E., Butler, P. C., & Cobelli, C. (2011). Shortened beta-cell life leads to a beta-cell deficit in a rodent model of type 2 diabetes. *Am J Physiol Endocrinol Metab, 300*, E933-E938.

Mangham, L. J., Petrou, S., Doyle, L. W., Draper, E. S., & Marlow, N. (2009). The cost of preterm birth throughout childhood in England and Wales. *Pediatrics, 123*(2), e312-327.

Mangoni, A. A., Mircoli, L., Giannattasio, C., Ferrari, A. U., & Mancia, G. (1996). Heart rate-dependence of arterial distensibility in vivo. *J Hypertens, 14*(7), 897-901.

Mann, D. L., Kent, R. L., Parsons, B., & Cooper, G. (1992). Adrenergic effects on the biology of the adult mammalian cardiocyte. *Circulation, 85*(2), 790-804.

Marconi, A. M., Cetin, I., Davoli, E., Baggiani, A. M., Fanelli, R., Fennessey, P. V., et al. (1993). An evaluation of fetal glucogenesis in intrauterine growth-retarded pregnancies. *Metabolism, 42*(7), 860-864.

Marconi, A. M., Paolini, C., Buscaglia, M., Zerbe, G., Battaglia, F. C., & Pardi, G. (1996). The impact of gestational age and fetal growth on the maternal-fetal glucose concentration difference. *Obstet Gynecol, 87*(6), 937-942.

Marconi, A. M., & Paolini, C. L. (2008). Nutrient transport across the intrauterine growth-restricted placenta. *Semin Perinatol, 32*(3), 178-181.

Mardones, F., Villarroel, L., Karzulovic, L., Barja, S., Arnaiz, P., Taibo, M., et al. (2008). Association of perinatal factors and obesity in 6- to 8-year-old Chilean children. *Int J Epidemiol, 37*(4), 902-910.

Marette, A. (2002). Mediators of cytokine-induced insulin resistance in obesity and other inflammatory settings. *Curr Opin Clin Nutr Metab Care, 5*(4), 377-383.

Mari, A., Schmitz, O., Gastaldelli, A., Oestergaard, T., Nyholm, B., & Ferrannini, E. (2002). Meal and oral glucose tests for assessment of beta -cell function: modeling analysis in normal subjects. *Am J Physiol Endocrinol Metab, 283*(6), E1159-1166.

Mark, P. J., Augustus, S., Lewis, J. L., Hewitt, D. P., & Waddell, B. J. (2009). Changes in the placental glucocorticoid barrier during rat pregnancy: impact on placental corticosterone levels and regulation by progesterone. *Biol Reprod, 80*(6), 1209-1215.

Markestad, T., Kaaresen, P. I., Ronnestad, A., Reigstad, H., Lossius, K., Medbo, S., et al. (2005). Early death, morbidity, and need of treatment among extremely premature infants. *Pediatrics, 115*(5), 1289-1298.

Marshall, B. A., Ren, J. M., Johnson, D. W., Gibbs, E. M., Lillquist, J. S., Soeller, W. C., et al. (1993). Germline manipulation of glucose homeostasis via alteration of glucose transporter levels in skeletal muscle. *J Biol Chem, 268*(25), 18442-18445.

Martin, B., Ji, S., Maudsley, S., & Mattson, M. P. (2010). "Control" laboratory rodents are metabolically morbid: why it matters. *Proc Natl Acad Sci U S A, 107*(14), 6127-6133.

Martin, J. A. (2007). United States vital statistics and the measurement of gestational age. *Paediatr Perinat Epidemiol, 21*(Suppl 2), 13-21.

Martin, L. J., Woo, J. G., Geraghty, S. R., Altaye, M., Davidson, B. S., Banach, W., et al. (2006). Adiponectin is present in human milk and is associated with maternal factors. *Am J Clin Nutr, 83*(5), 1106-1111.

Martin, P. A., & Faulkner, A. (1994). Gastric inhibitory polypeptide concentrations in lambs fed milk or milk constituents. *Comp Biochem Physiol Comp Physiol, 108*(2-3), 371-375.

Martin, R. M., Holly, J. M., Smith, G. D., Ness, A. R., Emmett, P., Rogers, I., et al. (2005). Could associations between breastfeeding and insulin-like growth factors underlie associations of breastfeeding with adult chronic disease? The Avon Longitudinal Study of Parents and Children. *Clin Endocrinol (Oxf), 62*(6), 728-737.

Martos-Moreno, G. A., Barrios, V., Saenz de Pipaon, M., Pozo, J., Dorronsoro, I., Martinez-Biarge, M., et al. (2009). Influence of prematurity and growth restriction on the adipokine profile, IGF1, and ghrelin levels in cord blood: relationship with glucose metabolism. *Eur J Endocrinol, 161*(3), 381-389.

Mason, J. I., France, J. T., Magness, R. R., Murry, B. A., & Rosenfeld, C. R. (1989). Ovine placental steroid 17 alpha-hydroxylase/C-17,20-lyase, aromatase and sulphatase in dexamethasone-induced and natural parturition. *J Endocrinol, 122*(1), 351-359.

Masuo, K., Kawaguchi, H., Mikami, H., Ogihara, T., & Tuck, M. L. (2003). Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. *Hypertension, 42*(4), 474-480.

Masuzaki, H., Paterson, J., Shinyama, H., Morton, N. M., Mullins, J. J., Seckl, J. R., et al. (2001). A transgenic model of visceral obesity and the metabolic syndrome. *Science, 294*(5549), 2166-2170.

Matsumoto, M., Han, S., Kitamura, T., & Accili, D. (2006). Dual role of transcription factor FoxO1 in controlling hepatic insulin sensitivity and lipid metabolism. *J Clin Invest, 116*(9), 2464-2472.

Mazor, M., Hershkovitz, R., Chaim, W., Levy, J., Sharony, Y., Leiberman, J. R., et al. (1994). Human preterm birth is associated with systemic and local changes in progesterone/17 beta-estradiol ratios. *Am J Obstet Gynecol, 171*(1), 231-236.

Mazzali, M., Hughes, J., Kim, Y. G., Jefferson, J. A., Kang, D. H., Gordon, K. L., et al. (2001). Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension, 38*(5), 1101-1106.

Mazzali, M., Kanellis, J., Han, L., Feng, L., Xia, Y. Y., Chen, Q., et al. (2002). Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. *Am J Physiol Renal Physiol, 282*(6), F991-997.

McCowan, L., Stewart, A. W., Francis, A., & Gardosi, J. (2004). A customised birthweight centile calculator developed for a New Zealand population. *Aust N Z J Obstet Gynaecol, 44*(5), 428-431.

McDonald, T. J., & Nathanielsz, P. W. (1991). Bilateral destruction of the fetal paraventricular nuclei prolongs gestation in sheep. *Am J Obstet Gynecol, 165*(3), 764-770.

McDonough, A. A. (2010). Mechanisms of proximal tubule sodium transport regulation that link extracellular fluid volume and blood pressure. *Am J Physiol Regul Integr Comp Physiol, 298*(4), R851-861.

McLean, M., Bisits, A., Davies, J., Woods, R., Lowry, P., & Smith, R. (1995). A placental clock controlling the length of human pregnancy. *Nat Med, 1*(5), 460-463.

McMillen, I. C., Adams, M. B., Ross, J. T., Coulter, C. L., Simonetta, G., Owens, J. A., et al. (2001). Fetal growth restriction: adaptations and consequences. *Reproduction, 122*(2), 195-204.

McMillen, I. C., & Robinson, J. S. (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev, 85*(2), 571-633.

Mecenas, C. A., Giussani, D. A., Owiny, J. R., Jenkins, S. L., Wu, W. X., Honnebier, B. O., et al. (1996). Production of premature delivery in pregnant rhesus monkeys by androstenedione infusion. *Nat Med, 2*(4), 443-448.

Meier, J. J., Hucking, K., Holst, J. J., Deacon, C. F., Schmiegel, W. H., & Nauck, M. A. (2001). Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes, 50*(11), 2497-2504.

Meier, P. P., Furman, L. M., & Degenhardt, M. (2007). Increased lactation risk for late preterm infants and mothers: evidence and management strategies to protect breastfeeding. *J Midwifery Womens Health, 52*(6), 579-587.

Melamed, N., Klinger, G., Tenenbaum-Gavish, K., Herscovici, T., Linder, N., Hod, M., et al. (2009). Short-term neonatal outcome in low-risk, spontaneous, singleton, late preterm deliveries. *Obstet Gynecol, 114*(2 Pt 1), 253-260.

Melloul, D. (2004). Transcription factors in islet development and physiology: role of PDX-1 in beta-cell function. *Ann N Y Acad Sci, 1014*, 28-37.

Mennella, J. A., Ventura, A. K., & Beauchamp, G. K. (2011). Differential growth patterns among healthy infants fed protein hydrolysate or cow-milk formulas. *Pediatrics, 127*(1), 110-118.

Merchant, J. R., Worwa, C., Porter, S., Coleman, J. M., & deRegnier, R. A. (2001). Respiratory instability of term and near-term healthy newborn infants in car safety seats. *Pediatrics, 108*(3), 647-652.

Merewood, A., Brooks, D., Bauchner, H., MacAuley, L., & Mehta, S. D. (2006). Maternal birthplace and breastfeeding initiation among term and preterm infants: a statewide assessment for Massachusetts. *Pediatrics, 118*(4), e1048-1054.

Mericq, V., Ong, K. K., Bazaes, R., Pena, V., Avila, A., Salazar, T., et al. (2005). Longitudinal changes in insulin sensitivity and secretion from birth to age three years

in small- and appropriate-for-gestational-age children. *Diabetologia, 48*(12), 2609-2614.

Mesiano, S., Chan, E. C., Fitter, J. T., Kwek, K., Yeo, G., & Smith, R. (2002). Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. *J Clin Endocrinol Metab, 87*(6), 2924-2930.

Mikkila, V., Rasanen, L., Raitakari, O. T., Marniemi, J., Pietinen, P., Ronnemaa, T., et al. (2007). Major dietary patterns and cardiovascular risk factors from childhood to adulthood. The Cardiovascular Risk in Young Finns Study. *Br J Nutr, 98*(1), 218-225.

Mikkila, V., Rasanen, L., Raitakari, O. T., Pietinen, P., & Viikari, J. (2005). Consistent dietary patterns identified from childhood to adulthood: the cardiovascular risk in Young Finns Study. *Br J Nutr, 93*(6), 923-931.

Mildenhall, L., Battin, M., Bevan, C., Kuschel, C., & Harding, J. E. (2009). Repeat prenatal corticosteroid doses do not alter neonatal blood pressure or myocardial thickness: randomized, controlled trial. *Pediatrics, 123*(4), e646-652.

Miller, D. R., Blache, D., Jackson, R. B., Downie, E. F., & Roche, J. R. (2010). Metabolic maturity at birth and neonate lamb survival: association among maternal factors, litter size, lamb birth weight, and plasma metabolic and endocrine factors on survival and behavior. *J Anim Sci, 88*(2), 581-593.

Mills, J. L., Graubard, B. I., Harley, E. E., Rhoads, G. G., & Berendes, H. W. (1984). Maternal alcohol consumption and birth weight. How much drinking during pregnancy is safe? *JAMA, 252*(14), 1875-1879.

Milsom, S. R., Blum, W. F., & Gunn, A. J. (2008). Temporal changes in insulin-like growth factors I and II and in insulin-like growth factor binding proteins 1, 2, and 3 in human milk. *Horm Res, 69*(5), 307-311.

Miralles, O., Sanchez, J., Palou, A., & Pico, C. (2006). A physiological role of breast milk leptin in body weight control in developing infants. *Obesity (Silver Spring), 14*(8), 1371-1377.

Misra, A., Pandey, R. M., Devi, J. R., Sharma, R., Vikram, N. K., & Khanna, N. (2001). High prevalence of diabetes, obesity and dyslipidaemia in urban slum population in northern India. *Int J Obes Relat Metab Disord, 25*(11), 1722-1729.

Mitanchez-Mokhtari, D., Lahlou, N., Kieffer, F., Magny, J. F., Roger, M., & Voyer, M. (2004). Both relative insulin resistance and defective islet beta-cell processing of proinsulin are responsible for transient hyperglycemia in extremely preterm infants. *Pediatrics, 113*(3 Pt 1), 537-541.

Mitanchez, D. (2007). Glucose regulation in preterm newborn infants. *Horm Res, 68*(6), 265-271.

Mitchell, B. F., Lye, S. J., Lukash, L., & Challis, J. R. (1986). Androstenedione metabolism in the late gestation sheep fetus. *Endocrinology, 118*(1), 63-68.

Mitoff, P. R., Gam, D., Ivanov, J., Al-hesayen, A., Azevedo, E. R., Newton, G. E., et al. (2011). Cardiac-specific sympathetic activation in men and women with and without heart failure. *Heart, 97*(5), 382-387.

Mitrakou, A., Vuorinen-Markkola, H., Raptis, G., Toft, I., Mokan, M., Strumph, P., et al. (1992). Simultaneous assessment of insulin secretion and insulin sensitivity using a hyperglycemia clamp. *J Clin Endocrinol Metab, 75*(2), 379-382.

Mittelman, S. D., Van Citters, G. W., Kim, S. P., Davis, D. A., Dea, M. K., Hamilton-Wessler, M., et al. (2000). Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced beta-cell response. *Diabetes, 49*(12), 2116-2125.

Miyawaki, K., Yamada, Y., Yano, H., Niwa, H., Ban, N., Ihara, Y., et al. (1999). Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc Natl Acad Sci U S A, 96*(26), 14843-14847.

Mongelli, M., & Gardosi, J. (1995). Longitudinal study of fetal growth in subgroups of a low-risk population. *Ultrasound Obstet Gynecol, 6*(5), 340-344.

Monteiro, P. O., & Victora, C. G. (2005). Rapid growth in infancy and childhood and obesity in later life--a systematic review. *Obes Rev, 6*(2), 143-154.

Montel-Hagen, A., Blanc, L., Boyer-Clavel, M., Jacquet, C., Vidal, M., Sitbon, M., et al. (2008). The Glut1 and Glut4 glucose transporters are differentially expressed during perinatal and postnatal erythropoiesis. *Blood, 112*(12), 4729-4738.

Mook-Kanamori, D. O., Steegers, E. A., Eilers, P. H., Raat, H., Hofman, A., & Jaddoe, V. W. (2010). Risk factors and outcomes associated with first-trimester fetal growth restriction. *JAMA, 303*(6), 527-534.

Moore, C. C., Mellon, S. H., Murai, J., Siiteri, P. K., & Miller, W. L. (1993). Structure and function of the hepatic form of 11 beta-hydroxysteroid dehydrogenase in the squirrel monkey, an animal model of glucocorticoid resistance. *Endocrinology, 133*(1), 368-375.

Moore, V. M., & Davies, M. J. (2005). Diet during pregnancy, neonatal outcomes and later health. *Reprod Fertil Dev, 17*(3), 341-348.

Morgan, J. A., Young, L., McCormick, F. M., & McGuire, W. (2011). Promoting growth for preterm infants following hospital discharge. *Arch Dis Child Fetal Neonatal Ed, epub ahead of print*.

Mori, A., Uchida, N., Inomo, A., & Izumi, S. (2006). Stiffness of systemic arteries in appropriate- and small-for-gestational-age newborn infants. *Pediatrics, 118*(3), 1035-1041.

Mori, H., Matsuda, K. I., Tsukahara, S., & Kawata, M. (2010). Intrauterine position affects estrogen receptor alpha expression in the ventromedial nucleus of the hypothalamus via promoter DNA methylation. *Endocrinology, 151*(12), 5775-5781.

Morise, A., Seve, B., Mace, K., Magliola, C., Le Huerou-luron, I., & Louveau, I. (2009). Impact of intrauterine growth retardation and early protein intake on growth, adipose tissue, and the insulin-like growth factor system in piglets. *Pediatr Res, 65*(1), 45-50.

Moritz, K. M., & Wintour, E. M. (1999). Functional development of the meso- and metanephros. *Pediatr Nephrol, 13*(2), 171-178.

Moritz, K. M., Wintour, E. M., & Dodic, M. (2002). Fetal uninephrectomy leads to postnatal hypertension and compromised renal function. *Hypertension, 39*(6), 1071-1076.

Morton, N. M., Emilsson, V., Liu, Y. L., & Cawthorne, M. A. (1998). Leptin action in intestinal cells. *J Biol Chem, 273*(40), 26194-26201.

Moss, T. J., Sloboda, D. M., Gurrin, L. C., Harding, R., Challis, J. R., & Newnham, J. P. (2001). Programming effects in sheep of prenatal growth restriction and glucocorticoid exposure. *Am J Physiol Regul Integr Comp Physiol, 281*(3), R960-970.

Mounien, L., Marty, N., Tarussio, D., Metref, S., Genoux, D., Preitner, F., et al. (2010). Glut2-dependent glucose-sensing controls thermoregulation by enhancing the leptin sensitivity of NPY and POMC neurons. *FASEB J, 24*(6), 1747-1758.

Moutquin, J.-M. (2003). Socio-economic and psychosocial factors in the management and prevention of preterm labour. *BJOG, 110*(Suppl 20), 56-60.

Muhlhausler, B. S., Adam, C. L., Marrocco, E. M., Findlay, P. A., Roberts, C. T., McFarlane, J. R., et al. (2005). Impact of glucose infusion on the structural and functional characteristics of adipose tissue and on hypothalamic gene expression for appetite regulatory neuropeptides in the sheep fetus during late gestation. *J Physiol, 565*(Pt 1), 185-195.

Muhlhausler, B. S., Duffield, J. A., Ozanne, S. E., Pilgrim, C., Turner, N., Morrison, J. L., et al. (2009). The transition from fetal growth restriction to accelerated postnatal growth: a potential role for insulin signalling in skeletal muscle. *J Physiol, 587*(Pt 17), 4199-4211.

Muniyappa, R., Lee, S., Chen, H., & Quon, M. J. (2008). Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab, 294*(1), E15-26.

Muniyappa, R., Montagnani, M., Koh, K. K., & Quon, M. J. (2007). Cardiovascular actions of insulin. *Endocr Rev, 28*(5), 463-491.

Murphy, M. J., Metcalf, B. S., Voss, L. D., Jeffery, A. N., Kirkby, J., Mallam, K. M., et al. (2004). Girls at five are intrinsically more insulin resistant than boys: The Programming Hypotheses Revisited--The EarlyBird Study (EarlyBird 6). *Pediatrics, 113*(1 Pt 1), 82-86.

Murphy, V. E., Smith, R., Giles, W. B., & Clifton, V. L. (2006). Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev, 27*(2), 141-169.

Murthy, K., Grobman, W. A., Lee, T. A., & Holl, J. L. (2011). Trends in induction of labor at early-term gestation. *Am J Obstet Gynecol, 204*(5), 435e431-436.

Mzayek, F., Sherwin, R., Hughes, J., Hassig, S., Srinivasan, S., Chen, W., et al. (2009). The association of birth weight with arterial stiffness at mid-adulthood: the Bogalusa Heart Study. *J Epidemiol Community Health, 63*(9), 729-733.

Naeye, R. L., Blanc, W., Leblanc, W., & Khatamee, M. A. (1973). Fetal complications of maternal heroin addiction: abnormal growth, infections, and episodes of stress. *J Pediatr, 83*(6), 1055-1061.

Nagai, M., Kamide, K., Rakugi, H., Takiuchi, S., Imai, M., Kida, I., et al. (2003). Role of endothelin-1 induced by insulin in the regulation of vascular cell growth. *Am J Hypertens, 16*(3), 223-228.

Nathanielsz, P. W., Jenkins, S. L., Tame, J. D., Winter, J. A., Guller, S., & Giussani, D. A. (1998). Local paracrine effects of estradiol are central to parturition in the rhesus monkey. *Nat Med, 4*(4), 456-459.

National Institutes for Health. (2002). *Third Report of the National Cholesterol Education Program Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults 2002:*.

National Institutes of Health. (1998). Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults--the evidence report. *Obes Res, 6* (Suppl 2), 51S-209S.

Nauck, M. A. (2011). Incretin-based therapies for type 2 diabetes mellitus: properties, functions, and clinical implications. *Am J Med, 124*(1 Suppl), S3-18.

Nelson, S. E., Rogers, R. R., Ziegler, E. E., & Fomon, S. J. (1989). Gain in weight and length during early infancy. *Early Hum Dev, 19*(4), 223-239.

New Zealand Health Information Service. (2007). *Report on Maternity: Maternal and Newborn Information 2004*. Retrieved from http://www.moh.govt.nz.

Newnham, J. P., Evans, S. F., Godfrey, M., Huang, W., Ikegami, M., & Jobe, A. (1999). Maternal, but not fetal, administration of corticosteroids restricts fetal growth. *J Matern Fetal Med, 8*(3), 81-87.

Newsholme, P., Bender, K., Kiely, A., & Brennan, L. (2007). Amino acid metabolism, insulin secretion and diabetes. *Biochem Soc Trans, 35*(Pt 5), 1180-1186.

Newsome, C. A., Shiell, A. W., Fall, C. H., Phillips, D. I., Shier, R., & Law, C. M. (2003). Is birth weight related to later glucose and insulin metabolism?--A systematic review. *Diabet Med, 20*(5), 339-348.

Nicolaides, K. H., Economides, D. L., & Soothill, P. W. (1989). Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses. *Am J Obstet Gynecol, 161*(4), 996-1001.

Nicolas, L., Martinez-Gomez, M., Hudson, R., & Bautista, A. (2011). Littermate presence enhances motor development, weight gain and competitive ability in newborn and juvenile domestic rabbits. *Dev Psychobiol, 53*(1), 37-46.

Nicolini, U., Hubinont, C., Santolaya, J., Fisk, N. M., & Rodeck, C. H. (1990). Effects of fetal intravenous glucose challenge in normal and growth retarded fetuses. *Horm Metab Res, 22*(8), 426-430.

Ninomiya, J. K., L'Italien, G., Criqui, M. H., Whyte, J. L., Gamst, A., & Chen, R. S. (2004). Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. *Circulation, 109*(1), 42-46.

Noble, S., & Emmett, P. (2006). Differences in weaning practice, food and nutrient intake between breast- and formula-fed 4-month-old infants in England. *J Hum Nutr Diet, 19*(4), 303-313.

Nolan, C. J., & Prentki, M. (2008). The islet beta-cell: fuel responsive and vulnerable. *Trends Endocrinol Metab, 19*(8), 285-291.

Northstone, K., & Emmett, P. (2005). Multivariate analysis of diet in children at four and seven years of age and associations with socio-demographic characteristics. *Eur J Clin Nutr, 59*(6), 751-760.

Northstone, K., & Emmett, P. M. (2008). Are dietary patterns stable throughout early and mid-childhood? A birth cohort study. *Br J Nutr, 100*(5), 1069-1076.

Nusken, K. D., Schneider, H., Plank, C., Trollmann, R., Nusken, E., Rascher, W., et al. (2011). Fetal programming of gene expression in growth-restricted rats depends on the cause of low birth weight. *Endocrinology, 152*(4), 1327-1335.

Nyirenda, M. J., Lindsay, R. S., Kenyon, C. J., Burchell, A., & Seckl, J. R. (1998). Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest, 101*(10), 2174-2181.

O'Connor, D. L., Khan, S., Weishuhn, K., Vaughan, J., Jefferies, A., Campbell, D. M., et al. (2008). Growth and nutrient intakes of human milk-fed preterm infants provided with extra energy and nutrients after hospital discharge. *Pediatrics, 121*(4), 766-776.

O'Dowd, R., Kent, J. C., Moseley, J. M., & Wlodek, M. E. (2008). Effects of uteroplacental insufficiency and reducing litter size on maternal mammary function and postnatal offspring growth. *Am J Physiol Regul Integr Comp Physiol, 294*(2), R539-548.

O'Leary, D. H., Polak, J. F., Kronmal, R. A., Manolio, T. A., Burke, G. L., & Wolfson, S. K., Jr. (1999). Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med, 340*(1), 14-22.

O'Sullivan, J., Iyer, S., Taylor, N., & Cheetham, T. (2007). Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is associated with a prolonged gestational age. *Arch Dis Child, 92*(8), 690-692.

Oberbach, A., Bossenz, Y., Lehmann, S., Niebauer, J., Adams, V., Paschke, R., et al. (2006). Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. *Diabetes Care, 29*(4), 895-900.

Ogata, E. S., Bussey, M. E., & Finley, S. (1986). Altered gas exchange, limited glucose and branched chain amino acids, and hypoinsulinism retard fetal growth in the rat. *Metabolism, 35*(10), 970-977.

Ogden, C. L., Carroll, M. D., Curtin, L. R., McDowell, M. A., Tabak, C. J., & Flegal, K. M. (2006). Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA, 295*(13), 1549-1555.

Oh, S. Y., Romero, R., Shim, S. S., Park, J. S., Jun, J. K., & Yoon, B. H. (2006). Fetal plasma cortisol and dehydroepiandrosterone sulfate concentrations in pregnancy and term parturition. *J Matern Fetal Neonatal Med, 19*(9), 529-536.

Okamura, K., Watanabe, T., Tanigawara, S., Endo, H., Iwamoto, M., Murotsuki, J., et al. (1990). Catecholamine levels and their correlation to blood gases in umbilical venous blood obtained by cordocentesis. *Fetal Diagn Ther, 5*(3-4), 147-152.

Oliver, M. H., Harding, J. E., Breier, B. H., & Gluckman, P. D. (1996). Fetal insulin-like growth factor (IGF)-I and IGF-II are regulated differently by glucose or insulin in the sheep fetus. *Reprod Fertil Dev, 8*(1), 167-172.

Oliver, P., Pico, C., De Matteis, R., Cinti, S., & Palou, A. (2002). Perinatal expression of leptin in rat stomach. *Dev Dyn, 223*(1), 148-154.

Olsen, I. E., Lawson, M. L., Meinzen-Derr, J., Sapsford, A. L., Schibler, K. R., Donovan, E. F., et al. (2009). Use of a body proportionality index for growth assessment of preterm infants. *J Pediatr, 154*(4), 486-491.

Olshansky, S. J., Passaro, D. J., Hershow, R. C., Layden, J., Carnes, B. A., Brody, J., et al. (2005). A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med, 352*(11), 1138-1145.

Olson, A. L., & Pessin, J. E. (1996). Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. *Annu Rev Nutr, 16*, 235-256.

Ong, K. K., Ahmed, M. L., Emmett, P. M., Preece, M. A., & Dunger, D. B. (2000). Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ, 320*(7240), 967-971.

Ong, K. K., & Loos, R. J. (2006). Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. *Acta Paediatr, 95*(8), 904-908.

Ong, K. K., Petry, C. J., Emmett, P. M., Sandhu, M. S., Kiess, W., Hales, C. N., et al. (2004). Insulin sensitivity and secretion in normal children related to size at birth, postnatal growth, and plasma insulin-like growth factor-I levels. *Diabetologia, 47*(6), 1064-1070.

Ortiz, L. A., Quan, A., Weinberg, A., & Baum, M. (2001). Effect of prenatal dexamethasone on rat renal development. *Kidney Int, 59*(5), 1663-1669.

Ortiz, L. A., Quan, A., Zarzar, F., Weinberg, A., & Baum, M. (2003). Prenatal dexamethasone programs hypertension and renal injury in the rat. *Hypertension, 41*(2), 328-334.

Oste, M., Van Haver, E., Thymann, T., Sangild, P., Weyns, A., & Van Ginneken, C. J. (2010). Formula induces intestinal apoptosis in preterm pigs within a few hours of feeding. *J Parenter Enteral Nutr, 34*(3), 271-279.

Owen, C. G., Martin, R. M., Whincup, P. H., Smith, G. D., & Cook, D. G. (2006). Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. *Am J Clin Nutr, 84*(5), 1043-1054.

Owens, J. A., Falconer, J., & Robinson, J. S. (1989). Glucose metabolism in pregnant sheep when placental growth is restricted. *Am J Physiol Regul Integr Comp Physiol, 257*(2 Pt 2), R350-357.

Owens, J. A., Gatford, K. L., De Blasio, M. J., Edwards, L. J., McMillen, I. C., & Fowden, A. L. (2007). Restriction of placental growth in sheep impairs insulin secretion but not sensitivity before birth. *J Physiol, 584*(Pt 3), 935-949.

Ozanne, S. E., Jensen, C. B., Tingey, K. J., Martin-Gronert, M. S., Grunnet, L., Brons, C., et al. (2006). Decreased protein levels of key insulin signalling molecules in adipose

tissue from young men with a low birthweight: potential link to increased risk of diabetes? *Diabetologia, 49*(12), 2993-2999.

Ozanne, S. E., Jensen, C. B., Tingey, K. J., Storgaard, H., Madsbad, S., & Vaag, A. A. (2005). Low birthweight is associated with specific changes in muscle insulin-signalling protein expression. *Diabetologia, 48*(3), 547-552.

Ozanne, S. E., Olsen, G. S., Hansen, L. L., Tingey, K. J., Nave, B. T., Wang, C. L., et al. (2003). Early growth restriction leads to down regulation of protein kinase C zeta and insulin resistance in skeletal muscle. *J Endocrinol, 177*(2), 235-241.

Ozanne, S. E., Wang, C. L., Coleman, N., & Smith, G. D. (1996). Altered muscle insulin sensitivity in the male offspring of protein-malnourished rats. *Am J Physiol Endocrinol Metab, 271*(6 Pt 1), E1128-1134.

Padbury, J. F., Martinez, A. M., Thio, S. L., Burnell, E. E., & Humme, J. A. (1989). Free and sulfoconjugated catecholamine responses to hypoxia in fetal sheep. *Am J Physiol Endocrinol Metab, 257*(2 Pt 1), E198-202.

Padidela, R., Patterson, M., Sharief, N., Ghatei, M., & Hussain, K. (2009). Elevated basal and post-feed glucagon-like peptide 1 (GLP-1) concentrations in the neonatal period. *Eur J Endocrinol, 160*(1), 53-58.

Pagani, M., & Lucini, D. (2001). Autonomic dysfunction in essential hypertension: insight from heart rate and arterial pressure variability. *Auton Neurosci, 90*(1-2), 76-82.

Painter, R. C., De Rooij, S. R., Bossuyt, P. M., Osmond, C., Barker, D. J., Bleker, O. P., et al. (2006). A possible link between prenatal exposure to famine and breast cancer: a preliminary study. *Am J Hum Biol, 18*(6), 853-856.

Palatini, P., Thijs, L., Staessen, J. A., Fagard, R. H., Bulpitt, C. J., Clement, D. L., et al. (2002). Predictive value of clinic and ambulatory heart rate for mortality in elderly subjects with systolic hypertension. *Arch Intern Med, 162*(20), 2313-2321.

Pankow, J. S., Jacobs, D. R., Jr., Steinberger, J., Moran, A., & Sinaiko, A. R. (2004). Insulin resistance and cardiovascular disease risk factors in children of parents with the insulin resistance (metabolic) syndrome. *Diabetes Care, 27*(3), 775-780.

Paolini, C. L., Marconi, A. M., Ronzoni, S., Di Noio, M., Fennessey, P. V., Pardi, G., et al. (2001). Placental transport of leucine, phenylalanine, glycine, and proline in intrauterine growth-restricted pregnancies. *J Clin Endocrinol Metab, 86*(11), 5427-5432.

Pardo, J. V., Sheikh, S. A., Kuskowski, M. A., Surerus-Johnson, C., Hagen, M. C., Lee, J. T., et al. (2007). Weight loss during chronic, cervical vagus nerve stimulation in depressed patients with obesity: an observation. *Int J Obes (Lond), 31*(11), 1756-1759.

Parizkova, J., & Rolland-Cachera, M. F. (1997). High proteins early in life as a predisposition for later obesity and further health risks. *Nutrition, 13*(9), 818-819.

Park, B., Park, E., Cho, S. J., Kim, Y., Lee, H., Min, J., et al. (2009). The association between fetal and postnatal growth status and serum levels of uric acid in children at 3 years of age. *Am J Hypertens, 22*(4), 403-408.

Park, J. H., Stoffers, D. A., Nicholls, R. D., & Simmons, R. A. (2008). Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. *J Clin Invest, 118*(6), 2316-2324.

Patel, A. L., Engstrom, J. L., Meier, P. P., & Kimura, R. E. (2005). Accuracy of methods for calculating postnatal growth velocity for extremely low birth weight infants. *Pediatrics, 116*(6), 1466-1473.

Patural, H., Pichot, V., Jaziri, F., Teyssier, G., Gaspoz, J. M., Roche, F., et al. (2008). Autonomic cardiac control of very preterm newborns: a prolonged dysfunction. *Early Hum Dev, 84*(10), 681-687.

Patural, H., St-Hilaire, M., Pichot, V., Beuchee, A., Samson, N., Duvareille, C., et al. (2010). Postnatal autonomic activity in the preterm lamb. *Res Vet Sci, 89*(2), 242-249.

Paul, I. M., Savage, J. S., Anzman, S. L., Beiler, J. S., Marini, M. E., Stokes, J. L., et al. (2010). Preventing obesity during infancy: a pilot study. *Obesity (Silver Spring), 19*(2), 353-361.

Peltoniemi, O. M., Kari, M. A., Lano, A., Yliherva, A., Puosi, R., Lehtonen, L., et al. (2009). Two-year follow-up of a randomised trial with repeated antenatal betamethasone. *Arch Dis Child Fetal Neonatal Ed, 94*(6), F402-406.

Pesonen, A. K., Raikkonen, K., Heinonen, K., Kajantie, E., Forsen, T., & Eriksson, J. G. (2007). Depressive symptoms in adults separated from their parents as children: a natural experiment during World War II. *Am J Epidemiol, 166*(10), 1126-1133.

Petersen, K. F., & Shulman, G. I. (2002). Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am J Cardiol, 90*(5A), 11G-18G.

Petrik, J., Reusens, B., Arany, E., Remacle, C., Coelho, C., Hoet, J. J., et al. (1999). A low protein diet alters the balance of islet cell replication and apoptosis in the fetal and neonatal rat and is associated with a reduced pancreatic expression of insulin-like growth factor-II. *Endocrinology, 140*(10), 4861-4873.

Pham, T. D., MacLennan, N. K., Chiu, C. T., Laksana, G. S., Hsu, J. L., & Lane, R. H. (2003). Uteroplacental insufficiency increases apoptosis and alters p53 gene methylation in the full-term IUGR rat kidney. *Am J Physiol Regul Integr Comp Physiol, 285*(5), R962-970.

Phillips, D. I., & Barker, D. J. (1997). Association between low birthweight and high resting pulse in adult life: is the sympathetic nervous system involved in programming the insulin resistance syndrome? *Diabet Med, 14*(8), 673-677.

Pick, A., Clark, J., Kubstrup, C., Levisetti, M., Pugh, W., Bonner-Weir, S., et al. (1998). Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes, 47*(3), 358-364.

Pico, C., Oliver, P., Sanchez, J., Miralles, O., Caimari, A., Priego, T., et al. (2007). The intake of physiological doses of leptin during lactation in rats prevents obesity in later life. *Int J Obes (Lond), 31*(8), 1199-1209.

Pieltain, C., De Curtis, M., Gerard, P., & Rigo, J. (2001). Weight gain composition in preterm infants with dual energy X-ray absorptiometry. *Pediatr Res, 49*(1), 120-124.

Pilgaard, K., Faerch, K., Carstensen, B., Poulsen, P., Pisinger, C., Pedersen, O., et al. (2010). Low birthweight and premature birth are both associated with type 2 diabetes in a random sample of middle-aged Danes. *Diabetologia, 53*(12), 2526-2530.

Pillot, M. H., Gautrais, J., Gouello, J., Michelena, P., Sibbald, A., & Bon, R. (2010). Moving together: Incidental leaders and naive followers. *Behav Processes, 83*(3), 235-241.

Plagemann, A., Harder, T., Franke, K., & Kohlhoff, R. (2002). Long-term impact of neonatal breast feeding on body weight and glucose tolerance in children of diabetic mothers. *Diabetes Care, 25*(1), 16-11.

Platz, E., & Newman, R. (2008). Diagnosis of IUGR: traditional biometry. *Semin Perinatol, 32*(3), 140-147.

Poore, K. R., Cleal, J. K., Newman, J. P., Boullin, J. P., Noakes, D. E., Hanson, M. A., et al. (2007). Nutritional challenges during development induce sex-specific changes in glucose homeostasis in the adult sheep. *Am J Physiol Endocrinol Metab, 292*(1), E32-39.

Popkin, B. M., & Gordon-Larsen, P. (2004). The nutrition transition: worldwide obesity dynamics and their determinants. *Int J Obes Relat Metab Disord, 28* (Suppl 3), S2-9.

Porte, D., Jr., Baskin, D. G., & Schwartz, M. W. (2005). Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. *Diabetes, 54*(5), 1264-1276.

Postic, C., Leturque, A., Printz, R. L., Maulard, P., Loizeau, M., Granner, D. K., et al. (1994). Development and regulation of glucose transporter and hexokinase expression in rat. *Am J Physiol Endocrinol Metab, 266*(4 Pt 1), E548-559.

Potenza, M. A., Marasciulo, F. L., Chieppa, D. M., Brigiani, G. S., Formoso, G., Quon, M. J., et al. (2005). Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between NO and ET-1 production. *Am J Physiol Heart Circ Physiol, 289*(2), H813-822.

Poulsen, P., & Vaag, A. (2006). The intrauterine environment as reflected by birth size and twin and zygosity status influences insulin action and intracellular glucose metabolism in an age- or time-dependent manner. *Diabetes, 55*(6), 1819-1825.

Power, M. L., & Schulkin, J. (2006). Functions of corticotropin-releasing hormone in anthropoid primates: from brain to placenta. *Am J Hum Biol, 18*(4), 431-447.

Power, M. L., & Schulkin, J. (2008). Anticipatory physiological regulation in feeding biology: cephalic phase responses. *Appetite, 50*(2-3), 194-206.

Prins, J. B., & O'Rahilly, S. (1997). Regulation of adipose cell number in man. *Clin Sci (Lond), 92*(1), 3-11.

Probyn, M. E., Stacy, V., Desai, M., Ross, M., & Harding, R. (2008). Spontaneously occurring differences in fetal weight do not affect blood pressure, the hypothalamic-pituitary-adrenal axis or the renin-angiotensin system in the late-gestation ovine fetus. *Reprod Fertil Dev, 20*(4), 451-459.

Puigserver, P., & Spiegelman, B. M. (2003). Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev, 24*(1), 78-90.

Pulver, L. S., Guest-Warnick, G., Stoddard, G. J., Byington, C. L., & Young, P. C. (2009). Weight for gestational age affects the mortality of late preterm infants. *Pediatrics, 123*(6), e1072-e1077.

Pumprla, J., Howorka, K., Groves, D., Chester, M., & Nolan, J. (2002). Functional assessment of heart rate variability: physiological basis and practical applications. *Int J Cardiol, 84*(1), 1-14.

Quigley, M. (2007). Non-human primates: the appropriate subjects of biomedical research? *J Med Ethics, 33*(11), 655-658.

Rabbia, F., Silke, B., Conterno, A., Grosso, T., De Vito, B., Rabbone, I., et al. (2003). Assessment of cardiac autonomic modulation during adolescent obesity. *Obes Res, 11*(4), 541-548.

Rahier, J., Wallon, J., & Henquin, J. C. (1981). Cell populations in the endocrine pancreas of human neonates and infants. *Diabetologia, 20*(5), 540-546.

Ravelli, A. C., van der Meulen, J. H., Michels, R. P., Osmond, C., Barker, D. J., Hales, C. N., et al. (1998). Glucose tolerance in adults after prenatal exposure to famine. *Lancet, 351*(9097), 173-177.

Ravelli, G. P., Stein, Z. A., & Susser, M. W. (1976). Obesity in young men after famine exposure in utero and early infancy. *N Engl J Med, 295*(7), 349-353.

Rea, S., & James, D. E. (1997). Moving GLUT4: the biogenesis and trafficking of GLUT4 storage vesicles. *Diabetes, 46*(11), 1667-1677.

Reaven, G. M. (1993). Role of insulin resistance in human disease (syndrome X): an expanded definition. *Annu Rev Med, 44*, 121-131.

Rebuffe-Scrive, M., Krotkiewski, M., Elfverson, J., & Bjorntorp, P. (1988). Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. *J Clin Endocrinol Metab, 67*(6), 1122-1128.

Redberg, R. F., Vogel, R. A., Criqui, M. H., Herrington, D. M., Lima, J. A., & Roman, M. J. (2003). 34th Bethesda Conference: Task force #3--What is the spectrum of current and emerging techniques for the noninvasive measurement of atherosclerosis? *J Am Coll Cardiol, 41*(11), 1886-1898.

Reddy, U. M., Bettegowda, V. R., Dias, T., Yamada-Kushnir, T., Ko, C. W., & Willinger, M. (2011). Term pregnancy: a period of heterogeneous risk for infant mortality. *Obstet Gynecol, 117*(6), 1279-1287.

Reinartz, G., Angermaier, A., Buchfelder, M., Fahlbusch, R., & Georgieff, M. (1995). Pre- and postoperative investigations of hepatic glucose production and leucine turnover in Cushing's disease utilizing stable isotope techniques. *Horm Metab Res, 27*(9), 425-431.

Relton, C. L., Pearce, M. S., & O'Sullivan, J. J. (2008). The relationship between gestational age, systolic blood pressure and pulse pressure in children. *J Hum Hypertens, 22*(5), 352-357.

Ren, J. M., Marshall, B. A., Gulve, E. A., Gao, J., Johnson, D. W., Holloszy, J. O., et al. (1993). Evidence from transgenic mice that glucose transport is rate-limiting for glycogen deposition and glycolysis in skeletal muscle. *J Biol Chem, 268*(22), 16113-16115.

Resto, M., O'Connor, D., Leef, K., Funanage, V., Spear, M., & Locke, R. (2001). Leptin levels in preterm human breast milk and infant formula. *Pediatrics, 108*(1), E15.

Reusens, B., & Remacle, C. (2006). Programming of the endocrine pancreas by the early nutritional environment. *Int J Biochem Cell Biol, 38*(5-6), 913-922.

Rhodes, C. J. (2005). Type 2 diabetes-a matter of beta-cell life and death? *Science, 307*(5708), 380-384.

Rich-Edwards, J. W., Colditz, G. A., Stampfer, M. J., Willett, W. C., Gillman, M. W., Hennekens, C. H., et al. (1999). Birthweight and the risk for type 2 diabetes mellitus in adult women. *Ann Intern Med, 130*(4 Pt 1), 278-284.

Rich-Edwards, J. W., Stampfer, M. J., Manson, J. E., Rosner, B., Hankinson, S. E., Colditz, G. A., et al. (1997). Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976. *BMJ, 315*(7105), 396-400.

Ricketts, M. L., Shoesmith, K. J., Hewison, M., Strain, A., Eggo, M. C., & Stewart, P. M. (1998). Regulation of 11 beta-hydroxysteroid dehydrogenase type 1 in primary cultures of rat and human hepatocytes. *J Endocrinol, 156*(1), 159-168.

Rigo, J., Nyamugabo, K., Picaud, J. C., Gerard, P., Pieltain, C., & De Curtis, M. (1998). Reference values of body composition obtained by dual energy X-ray absorptiometry in preterm and term neonates. *J Pediatr Gastroenterol Nutr, 27*(2), 184-190.

Risnes, K. R., Vatten, L. J., Baker, J. L., Jameson, K., Sovio, U., Kajantie, E., et al. (2011). Birthweight and mortality in adulthood: a systematic review and meta-analysis. *Int J Epidemiol, 40*(3), 647-661.

Roberts, D., & Dalziel, S. (2006). Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev, 3*, CD004454.

Robinson, S. M., Marriott, L. D., Crozier, S. R., Harvey, N. C., Gale, C. R., Inskip, H. M., et al. (2009). Variations in infant feeding practice are associated with body composition in childhood: a prospective cohort study. *J Clin Endocrinol Metab, 94*(8), 2799-2805.

Rochow, N., Jochum, F., Redlich, A., Korinekova, Z., Linnemann, K., Weitmann, K., et al. (2011). Fortification of breast milk in VLBW infants: Metabolic acidosis is linked to

the composition of fortifiers and alters weight gain and bone mineralisation. *Clin Nutr, 30*(1), 99-105.

Rodekamp, E., Harder, T., Kohlhoff, K. J., Dudenhausen, J. W., & Plagemann, A. (2005). Long-term impact of breast feeding on body weight and glucose tolerance in children of diabetic mothers: role of the late neonatal period and early infancy. *Diabetes Care, 28*(6), 1457-1462.

Rodel, H. G., Prager, G., Stefanski, V., von Holst, D., & Hudson, R. (2008). Separating maternal and litter-size effects on early postnatal growth in two species of altricial small mammals. *Physiol Behav, 93*(4-5), 826-834.

Rodriguez, M. M., Gomez, A. H., Abitbol, C. L., Chandar, J. J., Duara, S., & Zilleruelo, G. E. (2004). Histomorphometric analysis of postnatal glomerulogenesis in extremely preterm infants. *Pediatr Dev Pathol, 7*(1), 17-25.

Rogers, I. (2003). The influence of birthweight and intrauterine environment on adiposity and fat distribution in later life. *Int J Obes Relat Metab Disord, 27*(7), 755-777.

Roggero, P., Gianni, M. L., Amato, O., Orsi, A., Piemontese, P., Puricelli, V., et al. (2008). Influence of protein and energy intakes on body composition of formula-fed preterm infants after term. *J Pediatr Gastroenterol Nutr, 47*(3), 375-378.

Rolland-Cachera, M. F., Deheeger, M., Akrout, M., & Bellisle, F. (1995). Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 months to 8 years of age. *Int J Obes Relat Metab Disord, 19*(8), 573-578.

Romero, R., Scoccia, B., Mazor, M., Wu, Y. K., & Benveniste, R. (1988). Evidence for a local change in the progesterone/estrogen ratio in human parturition at term. *Am J Obstet Gynecol, 159*(3), 657-660.

Rose, G., & Marmot, M. G. (1981). Social class and coronary heart disease. *Br Heart J, 45*(1), 13-19.

Roseboom, T., de Rooij, S., & Painter, R. (2006). The Dutch famine and its long-term consequences for adult health. *Early Hum Dev, 82*(8), 485-491.

Roseboom, T. J. (2000). Coronary heart disease in adults after prenatal exposure to famine. In T. J. Roseboom (Ed.), *Prenatal Exposure to the Dutch Famine and Health in Later Life* (pp. 93-104). Enschede, The Netherlands: Ipskamp Printing Partners.

Rosenstock, J., Banarer, S., Fonseca, V. A., Inzucchi, S. E., Sun, W., Yao, W., et al. (2010). The 11-beta-hydroxysteroid dehydrogenase type 1 inhibitor INCB13739 improves hyperglycemia in patients with type 2 diabetes inadequately controlled by Metformin monotherapy. *Diabetes Care, 33*(7), 1516-1522.

Ross, J. T., Phillips, I. D., Simonetta, G., Owens, J. A., Robinson, J. S., & McMillen, I. C. (2000). Differential effects of placental restriction on IGF-II, ACTH receptor and steroidogenic enzyme mRNA levels in the foetal sheep adrenal. *J Neuroendocrinol, 12*(1), 79-85.

Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature, 362*(6423), 801-809.

Roth, C. L., Kratz, M., Ralston, M. M., & Reinehr, T. (2011). Changes in adipose-derived inflammatory cytokines and chemokines after successful lifestyle intervention in obese children. *Metabolism, 60*(4), 445-452.

Rother, E., Konner, A. C., & Bruning, J. C. (2008). Neurocircuits integrating hormone and nutrient signaling in control of glucose metabolism. *Am J Physiol Endocrinol Metab, 294*(5), E810-816.

Rotteveel, J., van Weissenbruch, M. M., Twisk, J. W., & Delemarre-Van de Waal, H. A. (2008a). Abnormal lipid profile and hyperinsulinaemia after a mixed meal: additional cardiovascular risk factors in young adults born preterm. *Diabetologia, 51*(7), 1269-1275.

Rotteveel, J., van Weissenbruch, M. M., Twisk, J. W., & Delemarre-Van de Waal, H. A. (2008b). Infant and childhood growth patterns, insulin sensitivity, and blood pressure in prematurely born young adults. *Pediatrics, 122*(2), 313-321.

Rowe, J. W., Young, J. B., Minaker, K. L., Stevens, A. L., Pallotta, J., & Landsberg, L. (1981). Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes, 30*(3), 219-225.

Rowland, A. S., Baird, D. D., Long, S., Wegienka, G., Harlow, S. D., Alavanja, M., et al. (2002). Influence of medical conditions and lifestyle factors on the menstrual cycle. *Epidemiology, 13*(6), 668-674.

Royal College of Obstetricians and Gynaecologists. (2010). Antenatal corticosteroids to reduce neonatal morbidity. Retrieved November 2011, from Royal College of Obstetricians and Gynaecologists: http://www.rcog.org.uk/files/rcog-corp/GTG%207.pdf

Rozance, P. J., Limesand, S. W., Barry, J. S., Brown, L. D., & Hay, W. W., Jr. (2009). Glucose replacement to euglycemia causes hypoxia, acidosis, and decreased insulin secretion in fetal sheep with intrauterine growth restriction. *Pediatr Res, 65*(1), 72-78.

Rubaltelli, F. F., Bonafe, L., Tangucci, M., Spagnolo, A., & Dani, C. (1998). Epidemiology of neonatal acute respiratory disorders. A multicenter study on incidence and fatality rates of neonatal acute respiratory disorders according to gestational age, maternal age, pregnancy complications and type of delivery. Italian Group of Neonatal Pneumology. *Biol Neonate, 74*(1), 7-15.

Ryder, J. W., Yang, J., Galuska, D., Rincon, J., Bjornholm, M., Krook, A., et al. (2000). Use of a novel impermeable biotinylated photolabeling reagent to assess insulin- and hypoxia-stimulated cell surface GLUT4 content in skeletal muscle from type 2 diabetic patients. *Diabetes, 49*(4), 647-654.

Sacca, L., Vigorito, C., Cicala, M., Corso, G., & Sherwin, R. S. (1983). Role of gluconeogenesis in epinephrine-stimulated hepatic glucose production in humans. *Am J Physiol Endocrinol Metab, 245*(3), E294-302.

Sachdev, H. S., Fall, C. H., Osmond, C., Lakshmy, R., Dey Biswas, S. K., Leary, S. D., et al. (2005). Anthropometric indicators of body composition in young adults: relation to size at birth and serial measurements of body mass index in childhood in the New Delhi birth cohort. *Am J Clin Nutr, 82*(2), 456-466.

Salmenpera, L., Perheentupa, J., Siimes, M. A., Adrian, T. E., Bloom, S. R., & Aynsley-Green, A. (1988). Effects of feeding regimen on blood glucose levels and plasma concentrations of pancreatic hormones and gut regulatory peptides at 9 months of age: comparison between infants fed with milk formula and infants exclusively breast-fed from birth. *J Pediatr Gastroenterol Nutr, 7*(5), 651-656.

Samara, M., Marlow, N., & Wolke, D. (2008). Pervasive behavior problems at 6 years of age in a total-population sample of children born at </= 25 weeks of gestation. *Pediatrics, 122*(3), 562-573.

Sanchez, J., Oliver, P., Miralles, O., Ceresi, E., Pico, C., & Palou, A. (2005). Leptin orally supplied to neonate rats is directly uptaken by the immature stomach and may regulate short-term feeding. *Endocrinology, 146*(6), 2575-2582.

Sanchez, J., Priego, T., Palou, M., Tobaruela, A., Palou, A., & Pico, C. (2008). Oral supplementation with physiological doses of leptin during lactation in rats improves insulin sensitivity and affects food preferences later in life. *Endocrinology, 149*(2), 733-740.

Sandman, C. A., Glynn, L., Schetter, C. D., Wadhwa, P., Garite, T., Chicz-DeMet, A., et al. (2006). Elevated maternal cortisol early in pregnancy predicts third trimester levels of

placental corticotropin releasing hormone (CRH): priming the placental clock. *Peptides, 27*(6), 1457-1463.

Sangild, P. T. (2006). Gut responses to enteral nutrition in preterm infants and animals. *Exp Biol Med (Maywood), 231*(11), 1695-1711.

Sarici, S. U., Serdar, M. A., Korkmaz, A., Erdem, G., Oran, O., Tekinalp, G., et al. (2004). Incidence, course, and prediction of hyperbilirubinemia in near-term and term newborns. *Pediatrics, 113*(4), 775-780.

Sarkar, S. A., Kobberup, S., Wong, R., Lopez, A. D., Quayum, N., Still, T., et al. (2008). Global gene expression profiling and histochemical analysis of the developing human fetal pancreas. *Diabetologia, 51*(2), 285-297.

Sarwar, N., Gao, P., Seshasai, S. R., Gobin, R., Kaptoge, S., Di Angelantonio, E., et al. (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet, 375*(9733), 2215-2222.

Sasidharan, K., Dutta, S., & Narang, A. (2009). Validity of New Ballard Score until 7th day of postnatal life in moderately preterm neonates. *Arch Dis Child Fetal Neonatal Ed, 94*(1), F39-44.

Sato, T., Nagafuku, M., Shimizu, K., Taira, T., Igarashi, Y., & Inokuchi, J. (2008). Physiological levels of insulin and IGF-1 synergistically enhance the differentiation of mesenteric adipocytes. *Cell Biol Int, 32*(11), 1397-1404.

Savino, F., Fissore, M. F., Grassino, E. C., Nanni, G. E., Oggero, R., & Silvestro, L. (2005). Ghrelin, leptin and IGF-I levels in breast-fed and formula-fed infants in the first years of life. *Acta Paediatr, 94*(5), 531-537.

Sawyer, E., & Jurkovic, D. (2007). Ultrasonography in the diagnosis and management of abnormal early pregnancy. *Clin Obstet Gynecol, 50*(1), 31-54.

Scaglia, L., Cahill, C. J., Finegood, D. T., & Bonner-Weir, S. (1997). Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. *Endocrinology, 138*(4), 1736-1741.

Schaffer, L., Burkhardt, T., Muller-Vizentini, D., Rauh, M., Tomaske, M., Mieth, R. A., et al. (2008). Cardiac autonomic balance in small-for-gestational-age neonates. *Am J Physiol Heart Circ Physiol, 294*(2), H884-890.

Schaffer, L., Burkhardt, T., Tomaske, M., Schmidt, S., Luzi, F., Rauh, M., et al. (2010). Effect of antenatal betamethasone administration on neonatal cardiac autonomic balance. *Pediatr Res, 68*(4), 286-291.

Schanler, R. J., Lau, C., Hurst, N. M., & Smith, E. O. (2005). Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. *Pediatrics, 116*(2), 400-406.

Schanler, R. J., Shulman, R. J., & Lau, C. (1999). Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics, 103*(6 Pt 1), 1150-1157.

Schiess, S., Grote, V., Scaglioni, S., Luque, V., Martin, F., Stolarczyk, A., et al. (2010). Introduction of complementary feeding in 5 European countries. *J Pediatr Gastroenterol Nutr, 50*(1), 92-98.

Schisterman, E., Whitcomb, B., & Bowers, K. (2011). Invited Commentary: Causation or "noitasuac"? *Am J Epidemiol, 173*(9), 984-987.

Schmelzle, H. R., Quang, D. N., Fusch, G., & Fusch, C. (2007). Birth weight categorization according to gestational age does not reflect percentage body fat in term and preterm newborns. *Eur J Pediatr, 166*(2), 161-167.

Schneider, U., Fiedler, A., Schroder, B., Jaekel, S., Stacke, A., Hoyer, D., et al. (2010). The effect of antenatal steroid treatment on fetal autonomic heart rate regulation revealed by fetal magnetocardiography (fMCG). *Early Hum Dev, 86*(5), 319-325.

Schwartz, M. W., Woods, S. C., Porte, D., Jr., Seeley, R. J., & Baskin, D. G. (2000). Central nervous system control of food intake. *Nature, 404*(6778), 661-671.

Scott, J. M., Esch, B. T., Haykowsky, M. J., Isserow, S., Koehle, M. S., Hughes, B. G., et al. (2007). Sex differences in left ventricular function and beta-receptor responsiveness following prolonged strenuous exercise. *J Appl Physiol, 102*(2), 681-687.

Scrocchi, L. A., Brown, T. J., MaClusky, N., Brubaker, P. L., Auerbach, A. B., Joyner, A. L., et al. (1996). Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat Med, 2*(11), 1254-1258.

Seccareccia, F., Pannozzo, F., Dima, F., Minoprio, A., Menditto, A., Lo Noce, C., et al. (2001). Heart rate as a predictor of mortality: the MATISS project. *Am J Public Health, 91*(8), 1258-1263.

Seckl, J. R. (2004). Prenatal glucocorticoids and long-term programming. *Eur J Endocrinol, 151* (Suppl 3), 49-62.

Segar, J. L., Lumbers, E. R., Nuyt, A. M., Smith, O. J., & Robillard, J. E. (1998). Effect of antenatal glucocorticoids on sympathetic nerve activity at birth in preterm sheep. *Am J Physiol Regul Integr Comp Physiol, 274*(1 Pt 2), R160-167.

Senat, M. V., Minoui, S., Multon, O., Fernandez, H., Frydman, R., & Ville, Y. (1998). Effect of dexamethasone and betamethasone on fetal heart rate variability in preterm labour: a randomised study. *BJOG, 105*(7), 749-755.

Setia, S., Sridhar, M. G., Bhat, V., Chaturvedula, L., Vinayagamoorti, R., & John, M. (2006). Insulin sensitivity and insulin secretion at birth in intrauterine growth retarded infants. *Pathology, 38*(3), 236-238.

Shah, T., Jonnalagadda, S. S., Kicklighter, J. R., Diwan, S., & Hopkins, B. L. (2005). Prevalence of metabolic syndrome risk factors among young adult Asian Indians. *J Immigr Health, 7*(2), 117-126.

Shaltout, H. A., Rose, J. C., Figueroa, J. P., Chappell, M. C., Diz, D. I., & Averill, D. B. (2010). Acute AT(1)-receptor blockade reverses the hemodynamic and baroreflex impairment in adult sheep exposed to antenatal betamethasone. *Am J Physiol Heart Circ Physiol, 299*(2), H541-547.

Shaw, G. M., Carmichael, S. L., Naelson, V., Selvin, S., & Schaffer, D. M. (2004). Occurrence of low birthweight and preterm delivery among Californian infants before and after compulsory food fortification with folic acid. *Public Health Rep, 119*(2), 170-173.

Shelly, H. J. (1961). Glycogen reserves and their changes at birth and in anoxia. *BMJ, 17*, 137-143.

Shepherd, P. R., & Kahn, B. B. (1999). Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. *N Engl J Med, 341*(4), 248-257.

Shimomura, I., Matsuda, M., Hammer, R. E., Bashmakov, Y., Brown, M. S., & Goldstein, J. L. (2000). Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. *Mol Cell, 6*(1), 77-86.

Shyu, K. G. (2009). Cellular and molecular effects of mechanical stretch on vascular cells and cardiac myocytes. *Clin Sci (Lond), 116*(5), 377-389.

Siebe, H., Baude, G., Lichtenstein, I., Wang, D., Buhler, H., Hoyer, G. A., et al. (1993). Metabolism of dexamethasone: sites and activity in mammalian tissues. *Ren Physiol Biochem, 16*(1-2), 79-88.

Sievers, E., Oldigs, H. D., Santer, R., & Schaub, J. (2002). Feeding patterns in breast-fed and formula-fed infants. *Ann Nutr Metab, 46*(6), 243-248.

Silver, M. (1988). Effects on maternal and fetal steroid concentrations of induction of parturition in the sheep by inhibition of 3{beta}-hydroxysteroid dehydrogenase. *J Reprod Fertil, 82*(2), 457-465.

Simeoni, U., & Barker, D. J. (2009). Offspring of diabetic pregnancy: Long term outcomes. *Semin Fetal Neonatal Med, 14*(2), 119-124.

Simpson, S. J., & Raubenheimer, D. (2005). Obesity: the protein leverage hypothesis. *Obes Rev, 6*(2), 133-142.

Sims, E. A. (2001). Are there persons who are obese, but metabolically healthy? *Metabolism, 50*(12), 1499-1504.

Singh, A. S., Mulder, C., Twisk, J. W., van Mechelen, W., & Chinapaw, M. J. (2008). Tracking of childhood overweight into adulthood: a systematic review of the literature. *Obes Rev, 9*(5), 474-488.

Singh, J. P., Larson, M. G., Tsuji, H., Evans, J. C., O'Donnell, C. J., & Levy, D. (1998). Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. *Hypertension, 32*(2), 293-297.

Singhal, A., Cole, T. J., Fewtrell, M., Deanfield, J., & Lucas, A. (2004). Is slower early growth beneficial for long-term cardiovascular health? *Circulation, 109*(9), 1108-1113.

Singhal, A., Cole, T. J., Fewtrell, M., Kennedy, K., Stephenson, T., Elias-Jones, A., et al. (2007). Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? *Circulation, 115*(2), 213-220.

Singhal, A., Cole, T. J., & Lucas, A. (2001). Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. *Lancet, 357*(9254), 413-419.

Singhal, A., Fewtrell, M., Cole, T. J., & Lucas, A. (2003). Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet, 361*(9363), 1089-1097.

Singhal, A., Kattenhorn, M., Cole, T. J., Deanfield, J., & Lucas, A. (2001). Preterm birth, vascular function, and risk factors for atherosclerosis. *Lancet, 358*(9288), 1159-1160.

Sinha, R., Fisch, G., Teague, B., Tamborlane, W. V., Banyas, B., Allen, K., et al. (2002). Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *N Engl J Med, 346*(11), 802-810.

Sipola-Leppanen, M., Hovi, P., Andersson, S., Wehkalampi, K., Vaarasmaki, M., Strang-Karlsson, S., et al. (2011). Resting energy expenditure in young adults born preterm--the Helsinki study of very low birth weight adults. *PLoS One, 6*(3), e17700.

Slack, J. M. (1995). Developmental biology of the pancreas. *Development, 121*(6), 1569-1580.

Sladkevicius, P., Saltvedt, S., Almstrom, H., Kublickas, M., Grunewald, C., & Valentin, L. (2005). Ultrasound dating at 12-14 weeks of gestation. A prospective cross-validation of established dating formulae in in-vitro fertilized pregnancies. *Ultrasound Obstet Gynecol, 26*(5), 504-511.

Slattery, M. M., & Morrison, J. J. (2002). Preterm delivery. *Lancet, 360*(9344), 1489-1497.

Sloboda, D. M., Moss, T. J., Li, S., Doherty, D. A., Nitsos, I., Challis, J. R., et al. (2005). Hepatic glucose regulation and metabolism in adult sheep: effects of prenatal betamethasone. *Am J Physiol Endocrinol Metab, 289*(4), E721-728.

Smith, C. A. (1947). The effect of wartime starvation in Holland upon pregnancy and its product. *Am J Obstet Gynecol, 53*(4), 599-608.

Smith, G. C., Smith, M. F., McNay, M. B., & Fleming, J. E. (1998). First-trimester growth and the risk of low birth weight. *N Engl J Med, 339*(25), 1817-1822.

Smith, G. C., Stenhouse, E. J., Crossley, J. A., Aitken, D. A., Cameron, A. D., & Connor, J. M. (2002). Early pregnancy levels of pregnancy-associated plasma protein a and the

risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J Clin Endocrinol Metab, 87*(4), 1762-1767.

Smith, L. K., Draper, E. S., Manktelow, B. N., Dorling, J. S., & Field, D. J. (2007). Socioeconomic inequalities in very preterm birth rates. *Arch Dis Child Fetal Neonatal Ed, 92*(1), F11-14.

Smith, M. A., Thomford, P. J., Mattison, D. R., & Slikker, W., Jr. (1988). Transport and metabolism of dexamethasone in the dually perfused human placenta. *Reprod Toxicol, 2*(1), 37-43.

Socha, P., Janas, R., Dobrzanska, A., Koletzko, B., Broekaert, I., Brasseur, D., et al. (2005). Insulin like growth factor regulation of body mass in breastfed and milk formula fed infants. Data from the E.U. Childhood Obesity Programme. *Adv Exp Med Biol, 569*, 159-163.

Soothill, P. W., Nicolaides, K. H., & Campbell, S. (1987). Prenatal asphyxia, hyperlacticaemia, hypoglycaemia, and erythroblastosis in growth retarded fetuses. *BMJ (Clin Res Ed), 294*(6579), 1051-1053.

Sorensen, A., Mayntz, D., Raubenheimer, D., & Simpson, S. J. (2008). Protein-leverage in mice: the geometry of macronutrient balancing and consequences for fat deposition. *Obesity (Silver Spring), 16*(3), 566-571.

Soto, N., Bazaes, R. A., Pena, V., Salazar, T., Avila, A., Iniguez, G., et al. (2003). Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. *J Clin Endocrinol Metab, 88*(8), 3645-3650.

Spalding, K. L., Arner, E., Westermark, P. O., Bernard, S., Buchholz, B. A., Bergmann, O., et al. (2008). Dynamics of fat cell turnover in humans. *Nature, 453*(7196), 783-787.

Spassov, L., Curzi-Dascalova, L., Clairambault, J., Kauffmann, F., Eiselt, M., Medigue, C., et al. (1994). Heart rate and heart rate variability during sleep in small-for-gestational age newborns. *Pediatr Res, 35*(4 Pt 1), 500-505.

Sperling, M. A., DeLamater, P. V., Phelps, D., Fiser, R. H., Oh, W., & Fisher, D. A. (1974). Spontaneous and amino acid-stimulated glucagon secretion in the immediate postnatal period. Relation to glucose and insulin. *J Clin Invest, 53*(4), 1159-1166.

Sperling, M. A., Ganguli, S., Leslie, N., & Landt, K. (1984). Fetal-perinatal catecholamine secretion: role in perinatal glucose homeostasis. *Am J Physiol Endocrinol Metab, 247*(1 Pt 1), E69-74.

Srinivasan, G., Pildes, R. S., Cattamanchi, G., Voora, S., & Lilien, L. D. (1986). Plasma glucose values in normal neonates: a new look. *J Pediatr, 109*(1), 114-117.

Stanner, S. A., Bulmer, K., Andres, C., Lantseva, O. E., Borodina, V., Poteen, V. V., et al. (1997). Does malnutrition in utero determine diabetes and coronary heart disease in adulthood? Results from the Leningrad siege study, a cross sectional study. *BMJ, 315*(7119), 1342-1348.

Stanner, S. A., & Yudkin, J. S. (2001). Fetal programming and the Leningrad Siege study. *Twin Res, 4*(5), 287-292.

Stein, A. D., Zybert, P. A., van de Bor, M., & Lumey, L. H. (2004). Intrauterine famine exposure and body proportions at birth: the Dutch Hunger Winter. *Int J Epidemiol, 33*(4), 831-836.

Stein, C. E., Fall, C. H., Kumaran, K., Osmond, C., Cox, V., & Barker, D. J. (1996). Fetal growth and coronary heart disease in south India. *Lancet, 348*(9037), 1269-1273.

Steinberger, J., Daniels, S. R., Eckel, R. H., Hayman, L., Lustig, R. H., McCrindle, B., et al. (2009). Progress and challenges in metabolic syndrome in children and adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular

Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. *Circulation, 119*(4), 628-647.

Steinberger, J., Moran, A., Hong, C. P., Jacobs, D. R., Jr., & Sinaiko, A. R. (2001). Adiposity in childhood predicts obesity and insulin resistance in young adulthood. *J Pediatr, 138*(4), 469-473.

Steiner, D. F., Clark, J. L., Nolan, C., Rubenstein, A. H., Margoliash, E., Aten, B., et al. (1969). Proinsulin and the biosynthesis of insulin. *Recent Prog Horm Res, 25*, 207-282.

Steinhoff-Wagner, J., Gors, S., Junghans, P., Bruckmaier, R. M., Kanitz, E., Metges, C. C., et al. (2011). Intestinal glucose absorption but not endogenous glucose production differs between colostrum- and formula-fed neonatal calves. *J Nutr, 141*(1), 48-55.

Stella, C. L., & Sibai, B. M. (2006). Thrombophilia and adverse maternal-perinatal outcome. *Clin Obstet Gynecol, 49*(4), 850-860.

Stellingwerff, T., Leblanc, P. J., Hollidge, M. G., Heigenhauser, G. J., & Spriet, L. L. (2006). Hyperoxia decreases muscle glycogenolysis, lactate production, and lactate efflux during steady-state exercise. *Am J Physiol Endocrinol Metab, 290*(6), E1180-1190.

Stettler, N., Stallings, V. A., Troxel, A. B., Zhao, J., Schinnar, R., Nelson, S. E., et al. (2005). Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. *Circulation, 111*(15), 1897-1903.

Stewart, P. M., Murry, B. A., & Mason, J. I. (1994). Human kidney 11 beta-hydroxysteroid dehydrogenase is a high affinity nicotinamide adenine dinucleotide-dependent enzyme and differs from the cloned type I isoform. *J Clin Endocrinol Metab, 79*(2), 480-484.

Stewart, P. M., Walker, B. R., Holder, G., O'Halloran, D., & Shackleton, C. H. L. (1995). 11b-hydroxysteroid dehydrogenase activity in Cushings Syndrome: explaining the mineralocorticoid excess state of the ectopic adrenocorticotropin syndrome. *J Clin Endocrinol Metab, 80*, 3617-3620.

Stockhorst, U., Huenig, A., Ziegler, D., & Scherbaum, W. A. (2011). Unconditioned and conditioned effects of intravenous insulin and glucose on heart rate variability in healthy men. *Physiol Behav, 103*(1), 31-38.

Stoffers, D. A. (2004). The development of beta-cell mass: recent progress and potential role of GLP-1. *Horm Metab Res, 36*(11-12), 811-821.

Stoffers, D. A., Desai, B. M., DeLeon, D. D., & Simmons, R. A. (2003). Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes, 52*(3), 734-740.

Stolarczyk, E., Guissard, C., Michau, A., Even, P. C., Grosfeld, A., Serradas, P., et al. (2010). Detection of extracellular glucose by GLUT2 contributes to hypothalamic control of food intake. *Am J Physiol Endocrinol Metab, 298*(5), E1078-1087.

Stout, R. W. (1992). Insulin and atherogenesis. *Eur J Epidemiol, 8* (Suppl 1), 134-135.

Strandberg, T. E., Jaervenpaa, A. L., Vanhanen, H., & McKeigue, P. M. (2001). Preterm birth and liquorice consumption during pregnancy. *Am J Epidemiol, 156*, 803-805.

Stratton, R. J., Stubbs, R. J., & Elia, M. (2008). Bolus tube feeding suppresses food intake and circulating ghrelin concentrations in healthy subjects in a short-term placebo-controlled trial. *Am J Clin Nutr, 88*(1), 77-83.

Stumvoll, M., Meyer, C., Mitrakou, A., Nadkarni, V., & Gerich, J. E. (1997). Renal glucose production and utilization: new aspects in humans. *Diabetologia, 40*(7), 749-757.

Stutchfield, P., Whitaker, R., & Russell, I. (2005). Antenatal betamethasone and incidence of neonatal respiratory distress after elective caesarean section: pragmatic randomised trial. *BMJ, 331*(7518), 662.

Sugino, T., Hasegawa, Y., Kikkawa, Y., Yamaura, J., Yamagishi, M., Kurose, Y., et al. (2002). A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. *Biochem Biophys Res Commun, 295*(2), 255-260.

Sun, K., Yang, K., & Challis, J. R. (1998). Glucocorticoid actions and metabolism in pregnancy: implications for placental function and fetal cardiovascular activity. *Placenta, 19*(5-6), 353-360.

Sun, X. J., Miralpeix, M., Myers, M. G., Jr., Glasheen, E. M., Backer, J. M., Kahn, C. R., et al. (1992). Expression and function of IRS-1 in insulin signal transmission. *J Biol Chem, 267*(31), 22662-22672.

Sun, X. J., Wang, L. M., Zhang, Y., Yenush, L., Myers, M. G., Jr., Glasheen, E., et al. (1995). Role of IRS-2 in insulin and cytokine signalling. *Nature, 377*(6545), 173-177.

Supramaniam, V. G., Jenkin, G., Loose, J., Wallace, E. M., & Miller, S. L. (2006). Chronic fetal hypoxia increases activin A concentrations in the late-pregnant sheep. *BJOG, 113*(1), 102-109.

Svec, F., Nastasi, K., Hilton, C., Bao, W., Srinivasan, S. R., & Berenson, G. S. (1992). Black-white contrasts in insulin levels during pubertal development. The Bogalusa Heart Study. *Diabetes, 41*(3), 313-317.

Swamy, G. K., Ostbye, T., & Skjaerven, R. (2008). Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. *JAMA, 299*(12), 1429-1436.

Swinburn, B. A., Nyomba, B. L., Saad, M. F., Zurlo, F., Raz, I., Knowler, W. C., et al. (1991). Insulin resistance associated with lower rates of weight gain in Pima Indians. *J Clin Invest, 88*(1), 168-173.

Takimoto, H., Yokoyama, T., Yoshiike, N., & Fukuoka, H. (2005). Increase in low-birth-weight infants in Japan and associated risk factors, 1980-2000. *J Obstet Gynaecol Res, 31*(4), 314-322.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation, 93*(5), 1043-1065.

Tatarczuch, L., Philip, C., & Lee, C. S. (1997). Involution of the sheep mammary gland. *J Anat, 190 (Pt 3)*, 405-416.

Tate, R. B., Manfreda, J., & Cuddy, T. E. (1998). The effect of age on risk factors for ischemic heart disease: the Manitoba Follow-Up Study, 1948-1993. *Ann Epidemiol, 8*(7), 415-421.

Taveras, E. M., Rifas-Shiman, S. L., Belfort, M. B., Kleinman, K. P., Oken, E., & Gillman, M. W. (2009). Weight status in the first 6 months of life and obesity at 3 years of age. *Pediatrics, 123*(4), 1177-1183.

Taveras, E. M., Scanlon, K. S., Birch, L., Rifas-Shiman, S. L., Rich-Edwards, J. W., & Gillman, M. W. (2004). Association of breastfeeding with maternal control of infant feeding at age 1 year. *Pediatrics, 114*(5), e577-583.

te Velde, S. J., Ferreira, I., Twisk, J. W., Stehouwer, C. D., van Mechelen, W., & Kemper, H. C. (2004). Birthweight and arterial stiffness and blood pressure in adulthood--results from the Amsterdam Growth and Health Longitudinal Study. *Int J Epidemiol, 33*(1), 154-161.

The Joint National Committee on prevention detection evaluation and treatment of high blood pressure. (1997). The sixth report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Arch Intern Med, 157*(21), 2413-2446.

Thiriez, G., Bouhaddi, M., Mourot, L., Nobili, F., Fortrat, J. O., Menget, A., et al. (2009). Heart rate variability in preterm infants and maternal smoking during pregnancy. *Clin Auton Res, 19*(3), 149-156.

Thomas, P., Peabody, J., Turnier, V., & Clark, R. H. (2000). A new look at intrauterine growth and the impact of race, altitude, and gender. *Pediatrics, 106*(2), E21.

Thong, F. S., Dugani, C. B., & Klip, A. (2005). Turning signals on and off: GLUT4 traffic in the insulin-signaling highway. *Physiology (Bethesda), 20*, 271-284.

Thorburn, G. D., Hollingworth, S. A., & Hooper, S. B. (1991). The trigger for parturition in sheep: fetal hypothalamus or placenta? *J Dev Physiol, 15*(2), 71-79.

Thorn, S. R., Regnault, T. R., Brown, L. D., Rozance, P. J., Keng, J., Roper, M., et al. (2009). Intrauterine growth restriction increases fetal hepatic gluconeogenic capacity and reduces messenger ribonucleic acid translation initiation and nutrient sensing in fetal liver and skeletal muscle. *Endocrinology, 150*(7), 3021-3030.

Thorsell, M., Kaijser, M., Almstrom, H., & Andolf, E. (2008). Expected day of delivery from ultrasound dating versus last menstrual period--obstetric outcome when dates mismatch. *BJOG, 115*(5), 585-589.

Thureen, P. J. (2007). The neonatologist's dilemma: catch-up growth or beneficial undernutrition in very low birth weight infants-what are optimal growth rates? *J Pediatr Gastroenterol Nutr, 45* (Suppl 3), S152-154.

Thymann, T., Moller, H. K., Stoll, B., Stoy, A. C., Buddington, R. K., Bering, S. B., et al. (2009). Carbohydrate maldigestion induces necrotizing enterocolitis in preterm pigs. *Am J Physiol Gastrointest Liver Physiol, 297*(6), G1115-1125.

Tilling, K., Davies, N., Windmeijer, F., Kramer, M. S., Bogdanovich, N., Matush, L., et al. (2011). Is infant weight associated with childhood blood pressure? Analysis of the Promotion of Breastfeeding Intervention Trial (PROBIT) cohort. *Int J Epidemiol, 40*(5), 1227-1237.

Tobi, E. W., Lumey, L. H., Talens, R. P., Kremer, D., Putter, H., Stein, A. D., et al. (2009). DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet, 18*(21), 4046-4053.

Todd, S. E., Oliver, M. H., Jaquiery, A. L., Bloomfield, F. H., & Harding, J. E. (2009). Periconceptional undernutrition of ewes impairs glucose tolerance in their adult offspring. *Pediatr Res, 65*(4), 409-413.

Todros, T., Plazzotta, C., & Pastorin, L. (1996). Body proportionality of the small-for-date fetus: is it related to aetiological factors? *Early Hum Dev, 45*(1-2), 1-9.

Toft-Nielsen, M. B., Damholt, M. B., Madsbad, S., Hilsted, L. M., Hughes, T. E., Michelsen, B. K., et al. (2001). Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab, 86*(8), 3717-3723.

Toft, I., Bonaa, K. H., Lindal, S., & Jenssen, T. (1998). Insulin kinetics, insulin action, and muscle morphology in lean or slightly overweight persons with impaired glucose tolerance. *Metabolism, 47*(7), 848-854.

Tomashek, K. M., Shapiro-Mendoza, C. K., Davidoff, M. J., & Petrini, J. R. (2007). Differences in mortality between late-preterm and term singleton infants in the United States, 1995-2002. *J Pediatr, 151*(5), 450-456, 456 e451.

Tookey, P. (2004). Rubella in England, Scotland and Wales. *Euro Surveill, 9*(4), 21-23.

Torrecilla, E., Fernandez-Vazquez, G., Vicent, D., Sanchez-Franco, F., Barabash, A., Cabrerizo, L., et al. (2011). Liver upregulation of genes involved in cortisol production and action is associated with metabolic syndrome in morbidly obese patients. *Obes Surg, epub ahead of print*.

Tran, T. T., Yamamoto, Y., Gesta, S., & Kahn, C. R. (2008). Beneficial effects of subcutaneous fat transplantation on metabolism. *Cell Metab, 7*(5), 410-420.

Traub, O., & Berk, B. C. (1998). Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol, 18*(5), 677-685.

Tsuji, H., Larson, M. G., Venditti, F. J., Jr., Manders, E. S., Evans, J. C., Feldman, C. L., et al. (1996). Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation, 94*(11), 2850-2855.

Tu, Y. K., West, R., Ellison, G. T., & Gilthorpe, M. S. (2005). Why evidence for the fetal origins of adult disease might be a statistical artifact: the "reversal paradox" for the relation between birth weight and blood pressure in later life. *Am J Epidemiol, 161*(1), 27-32.

Tucker, J., & McGuire, W. (2004). Epidemiology of preterm birth. *BMJ, 329*(7467), 675-678.

Tulchinsky, D., Hobel, C. J., Yeager, E., & Marshall, J. R. (1972). Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy. *Am J Obstet Gynecol, 112*(8), 1095-1100.

Tunon, K., Eik-Nes, S. H., & Grottum, P. (1996). A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. *Ultrasound Obstet Gynecol, 8*(3), 178-185.

Tverdal, A., Hjellvik, V., & Selmer, R. (2008). Heart rate and mortality from cardiovascular causes: a 12 year follow-up study of 379 843 men and women aged 40-45 years. *Eur Heart J, 29*(22), 2772-2781.

Unger, R. H. (1991). Diabetic hyperglycemia: link to impaired glucose transport in pancreatic beta cells. *Science, 251*(4998), 1200-1205.

Unger, R. H., & Eisentraut, A. M. (1969). Entero-insular axis. *Arch Intern Med, 123*, 262-266.

Unger, R. H., & Orci, L. (2001). Diseases of liporegulation: new perspective on obesity and related disorders. *FASEB J, 15*(2), 312-321.

Unger, R. H., & Zhou, Y. T. (2001). Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes, 50* (Suppl 1), S118-121.

Uthaya, S., Thomas, E. L., Hamilton, G., Dore, C. J., Bell, J., & Modi, N. (2005). Altered adiposity after extremely preterm birth. *Pediatr Res, 57*(2), 211-215.

Vagero, D., & Leon, D. (1994). Ischaemic heart disease and low birth weight: a test of the fetal-origins hypothesis from the Swedish Twin Registry. *Lancet, 343*(8892), 260-263.

Val-Laillet, D., Biraben, A., Randuineau, G., & Malbert, C. H. (2010). Chronic vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipigs. *Appetite, 55*(2), 245-252.

Van Assche, F. A., De Prins, F., Aerts, L., & Verjans, M. (1977). The endocrine pancreas in small-for-dates infants. *BJOG, 84*(10), 751-753.

Van De Borne, P., Hausberg, M., Hoffman, R. P., Mark, A. L., & Anderson, E. A. (1999). Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects. *Am J Physiol Regul Integr Comp Physiol, 276*(1 Pt 2), R178-183.

Van Leeuwen, P., Geue, D., Lange, S., Hatzmann, W., & Gronemeyer, D. (2003). Changes in the frequency power spectrum of fetal heart rate in the course of pregnancy. *Prenat Diagn, 23*(11), 909-916.

Van Leeuwen, P., Lange, S., Bettermann, H., Gronemeyer, D., & Hatzmann, W. (1999). Fetal heart rate variability and complexity in the course of pregnancy. *Early Hum Dev, 54*(3), 259-269.

Vanoli, E., De Ferrari, G. M., Stramba-Badiale, M., Hull, S. S., Jr., Foreman, R. D., & Schwartz, P. J. (1991). Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction. *Circ Res, 68*(5), 1471-1481.

Vegeto, E., Shahbaz, M. M., Wen, D. X., Goldman, M. E., O'Malley, B. W., & McDonnell, D. P. (1993). Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol Endocrinol, 7*(10), 1244-1255.

Verkauskiene, R., Beltrand, J., Claris, O., Chevenne, D., Deghmoun, S., Dorgeret, S., et al. (2007). Impact of fetal growth restriction on body composition and hormonal status at birth in infants of small and appropriate weight for gestational age. *Eur J Endocrinol, 157*(5), 605-612.

Verney, C., Rees, S., Biran, V., Thompson, M., Inder, T., & Gressens, P. (2010). Neuronal damage in the preterm baboon: impact of the mode of ventilatory support. *J Neuropathol Exp Neurol, 69*(5), 473-482.

Vernon, R. G. (1975). Effect of dietary safflower oil upon lipogenesis in neonatal lamb. *Lipids, 10*(5), 284-289.

Verwaerde, P., Senard, J. M., Galinier, M., Rouge, P., Massabuau, P., Galitzky, J., et al. (1999). Changes in short-term variability of blood pressure and heart rate during the development of obesity-associated hypertension in high-fat fed dogs. *J Hypertens, 17*(8), 1135-1143.

Vickers, M. H., Gluckman, P. D., Coveny, A. H., Hofman, P. L., Cutfield, W. S., Gertler, A., et al. (2005). Neonatal leptin treatment reverses developmental programming. *Endocrinology, 146*(10), 4211-4216.

Victora, C. G., Barros, F. C., Horta, B. L., & Martorell, R. (2001). Short-term benefits of catch-up growth for small-for-gestational-age infants. *Int J Epidemiol, 30*(6), 1325-1330.

Vielwerth, S. E., Jensen, R. B., Larsen, T., Holst, K. K., Molgaard, C., Greisen, G., et al. (2008). The effect of birthweight upon insulin resistance and associated cardiovascular risk factors in adolescence is not explained by fetal growth velocity in the third trimester as measured by repeated ultrasound fetometry. *Diabetologia, 51*(8), 1483-1492.

Villar, J., Knight, H. E., de Onis, M., Bertino, E., Gilli, G., Papageorghiou, A. T., et al. (2010). Conceptual issues related to the construction of prescriptive standards for the evaluation of postnatal growth of preterm infants. *Arch Dis Child, 95*(12), 1034-1038.

Vital, P., Larrieta, E., & Hiriart, M. (2006). Sexual dimorphism in insulin sensitivity and susceptibility to develop diabetes in rats. *J Endocrinol, 190*(2), 425-432.

Volkl, T. M., Schwobel, K., Simm, D., Beier, C., Rohrer, T. R., & Dorr, H. G. (2004). Spontaneous growth hormone secretion and IGF1:IGFBP3 molar ratios in children born small for gestational age (SGA). *Growth Horm IGF Res, 14*(6), 455-461.

von Kries, R., Koletzko, B., Sauerwald, T., von Mutius, E., Barnert, D., Grunert, V., et al. (1999). Breast feeding and obesity: cross sectional study. *BMJ, 319*(7203), 147-150.

Vuguin, P., Raab, E., Liu, B., Barzilai, N., & Simmons, R. (2004). Hepatic insulin resistance precedes the development of diabetes in a model of intrauterine growth retardation. *Diabetes, 53*(10), 2617-2622.

Wajchenberg, B. L. (2000). Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev, 21*(6), 697-738.

Wakatsuki, A., Murata, Y., Ninomiya, Y., Masaoka, N., Tyner, J. G., & Kutty, K. K. (1992). Autonomic nervous system regulation of baseline heart rate in the fetal lamb. *Am J Obstet Gynecol, 167*(2), 519-523.

Walker, B. R. (2006). Cortisol--cause and cure for metabolic syndrome? *Diabet Med, 23*(12), 1281-1288.

Walker, B. R., Irving, R. J., Andrew, R., & Belton, N. R. (2002). Contrasting effects of intrauterine growth retardation and premature delivery on adult cortisol secretion and metabolism in man. *Clin Endocrinol (Oxf), 57*(3), 351-355.

Walker, M. (2008). Breastfeeding the late preterm infant. *J Obstet Gynecol Neonatal Nurs, 37*(6), 692-701.

Wallace, J. M., Regnault, T. R., Limesand, S. W., Hay, W. W., Jr., & Anthony, R. V. (2005). Investigating the causes of low birth weight in contrasting ovine paradigms. *J Physiol, 565*(Pt 1), 19-26.

Waller, D. K., Spears, W. D., Gu, Y., & Cunningham, G. C. (2000). Assessing number-specific error in the recall of onset of last menstrual period. *Paediatr Perinat Epidemiol, 14*(3), 263-267.

Walsh, S. W., Stanczyk, F. Z., & Novy, M. J. (1984). Daily hormonal changes in the maternal, fetal, and amniotic fluid compartments before parturition in a primate species. *J Clin Endocrinol Metab, 58*(4), 629-639.

Wang, D., Pascual, J. M., Yang, H., Engelstad, K., Mao, X., Cheng, J., et al. (2006). A mouse model for Glut-1 haploinsufficiency. *Hum Mol Genet, 15*(7), 1169-1179.

Wang, M. L., Dorer, D. J., Fleming, M. P., & Catlin, E. A. (2004). Clinical outcomes of near-term infants. *Pediatrics, 114*(2), 372-376.

Wang, Z., & Thurmond, D. C. (2009). Mechanisms of biphasic insulin-granule exocytosis - roles of the cytoskeleton, small GTPases and SNARE proteins. *J Cell Sci, 122*(Pt 7), 893-903.

Wapner, R. J., Sorokin, Y., Mele, L., Johnson, F., Dudley, D. J., Spong, C. Y., et al. (2007). Long-term outcomes after repeat doses of antenatal corticosteroids. *N Engl J Med, 357*(12), 1190-1198.

Ward Platt, M., & Deshpande, S. (2005). Metabolic adaptation at birth. *Semin Fetal Neonatal Med, 10*(4), 341-350.

Warnes, D. M., Seamark, R. F., & Ballard, F. J. (1977). The appearance of gluconeogenesis at birth in sheep. Activation of the pathway associated with blood oxygenation. *Biochem J, 162*(3), 627-634.

Waterland, R. A., & Jirtle, R. L. (2004). Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition, 20*(1), 63-68.

Weiss, R., Dufour, S., Taksali, S. E., Tamborlane, W. V., Petersen, K. F., Bonadonna, R. C., et al. (2003). Prediabetes in obese youth: a syndrome of impaired glucose tolerance, severe insulin resistance, and altered myocellular and abdominal fat partitioning. *Lancet, 362*(9388), 951-957.

Weiss, R., Dziura, J., Burgert, T. S., Tamborlane, W. V., Taksali, S. E., Yeckel, C. W., et al. (2004). Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med, 350*(23), 2362-2374.

Weitz, G., Wellhoener, P., Heindl, S., Fehm, H. L., & Dodt, C. (2005). Relationship between metabolic parameters, blood pressure, and sympathoendocrine function in healthy young adults with low birth weight. *Exp Clin Endocrinol Diabetes, 113*(8), 444-450.

Wells, J. C. (2000). A Hattori chart analysis of body mass index in infants and children. *Int J Obes Relat Metab Disord, 24*(3), 325-329.

Wells, J. C., & Fewtrell, M. S. (2006). Measuring body composition. *Arch Dis Child, 91*(7), 612-617.

Wells, J. C., Fewtrell, M. S., Williams, J. E., Haroun, D., Lawson, M. S., & Cole, T. J. (2006). Body composition in normal weight, overweight and obese children: matched

case-control analyses of total and regional tissue masses, and body composition trends in relation to relative weight. *Int J Obes (Lond), 30*(10), 1506-1513.

Werner, E. D., Lee, J., Hansen, L., Yuan, M., & Shoelson, S. E. (2004). Insulin resistance due to phosphorylation of insulin receptor substrate-1 at serine 302. *J Biol Chem, 279*(34), 35298-35305.

Westby Wold, S. H., Sommerfelt, K., Reigstad, H., Ronnestad, A., Medbo, S., Farstad, T., et al. (2009). Neonatal mortality and morbidity in extremely preterm small for gestational age infants: a population based study. *Arch Dis Child Fetal Neonatal Ed, 94*(5), F363-367.

Whittle, W. L., Holloway, A. C., Lye, S. J., Gibb, W., & Challis, J. R. (2000). Prostaglandin production at the onset of ovine parturition is regulated by both estrogen-independent and estrogen-dependent pathways. *Endocrinology, 141*(10), 3783-3791.

WHO Expert Consultation. (2004). Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet, 363*(9403), 157-163.

Willemsen, R. H., Leunissen, R. W., Stijnen, T., & Hokken-Koelega, A. C. (2009). Prematurity is not associated with reduced insulin sensitivity in adulthood. *J Clin Endocrinol Metab, 94*(5), 1695-1700.

Wing, R. R., Marcus, M. D., Salata, R., Epstein, L. H., Miaskiewicz, S., & Blair, E. H. (1991). Effects of a very-low-calorie diet on long-term glycemic control in obese type 2 diabetic subjects. *Arch Intern Med, 151*(7), 1334-1340.

Wintour, E. M., Moritz, K. M., Johnson, K., Ricardo, S., Samuel, C. S., & Dodic, M. (2003). Reduced nephron number in adult sheep, hypertensive as a result of prenatal glucocorticoid treatment. *J Physiol, 549*(Pt 3), 929-935.

Wise, T., Roberts, A. J., & Christenson, R. K. (1997). Relationships of light and heavy fetuses to uterine position, placental weight, gestational age, and fetal cholesterol concentrations. *J Anim Sci, 75*(8), 2197-2207.

Wolf, M. M., Varigos, G. A., Hunt, D., & Sloman, J. G. (1978). Sinus arrhythmia in acute myocardial infarction. *Med J Aust, 2*(2), 52-53.

Wood, I. S., & Trayhurn, P. (2003). Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br J Nutr, 89*(1), 3-9.

Woods, K. A., Camacho-Hubner, C., Barter, D., Clark, A. J., & Savage, M. O. (1997). Insulin-like growth factor I gene deletion causing intrauterine growth retardation and severe short stature. *Acta Paediatr Suppl, 423*, 39-45.

Woods, L. L., Weeks, D. A., & Rasch, R. (2001). Hypertension after neonatal uninephrectomy in rats precedes glomerular damage. *Hypertension, 38*(3), 337-342.

Woods, L. L., Weeks, D. A., & Rasch, R. (2004). Programming of adult blood pressure by maternal protein restriction: role of nephrogenesis. *Kidney Int, 65*(4), 1339-1348.

Wooldridge, J., & Hall, W. A. (2003). Posthospitalization breastfeeding patterns of moderately preterm infants. *J Perinat Neonatal Nurs, 17*(1), 50-64.

Woolf, B. (1996). Studies on infant mortality--Part II. Social aetiology of stillbirths and infant deaths in county boroughs of England and Wales. *J Epidemiol Community Health, 50*(6), 613-619.

World Health Organisation. (1999). *Report of a WHO consultation: Definition of metabolic syndrome in definition, diagnosis and classification of diabetes mellitus and its complications*.

World Health Organisation. (2006). WHO Child Growth Standards based on length/height, weight and age. *Acta Paediatr Suppl, 450*, 76-85.

Wrede, C. E., Dickson, L. M., Lingohr, M. K., Briaud, I., & Rhodes, C. J. (2002). Protein kinase B/Akt prevents fatty acid-induced apoptosis in pancreatic beta-cells (INS-1). *J Biol Chem, 277*(51), 49676-49684.

Yancy, W. S., Jr., Olsen, M. K., Guyton, J. R., Bakst, R. P., & Westman, E. C. (2004). A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Ann Intern Med, 140*(10), 769-777.

Yang, K., Langlois, D. A., Campbell, L. E., Challis, J. R., Krkosek, M., & Yu, M. (1997). Cellular localization and developmental regulation of 11 beta-hydroxysteroid dehydrogenase type 1 (11 beta-HSD1) gene expression in the ovine placenta. *Placenta, 18*(7), 503-509.

Yang, S., Bergvall, N., Cnattingius, S., & Kramer, M. S. (2010). Gestational age differences in health and development among young Swedish men born at term. *Int J Epidemiol, 39*(5), 1240-1249.

Yeshaya, A., Orvieto, R., Ben-Shem, E., Dekel, A., Peleg, D., Dicker, D., et al. (1996). Uterine activity after betamethasone administration for the enhancement of fetal lung maturation. *Eur J Obstet Gynecol Reprod Biol, 67*(2), 139-141.

You, T., Yang, R., Lyles, M. F., Gong, D., & Nicklas, B. J. (2005). Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. *Am J Physiol Endocrinol Metab, 288*(4), E741-747.

Young, I. R. (2001). The comparative physiology of parturition in mammals. *Front Horm Res, 27*, 10-30.

Young, I. R., Deayton, J. M., Hollingworth, S. A., & Thorburn, G. D. (1996). Continuous intrafetal infusion of prostaglandin E2 prematurely activates the hypothalamo-pituitary-adrenal axis and induces parturition in sheep. *Endocrinology, 137*(6), 2424-2431.

Yu, C., Chen, Y., Cline, G. W., Zhang, D., Zong, H., Wang, Y., et al. (2002). Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem, 277*(52), 50230-50236.

Yu, Y., South, T., & Huang, X. F. (2009). Inter-meal interval is increased in mice fed a high whey, as opposed to soy and gluten, protein diets. *Appetite, 52*(2), 372-379.

Yuan, C. S., Attele, A. S., Wu, J. A., Zhang, L., & Shi, Z. Q. (1999). Peripheral gastric leptin modulates brain stem neuronal activity in neonates. *Am J Physiol Gastrointest Liver Physiol, 277*(3 Pt 1), G626-630.

Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., et al. (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet, 364*(9438), 937-952.

Zamudio, S., Palmer, S. K., Droma, T., Stamm, E., Coffin, C., & Moore, L. G. (1995). Effect of altitude on uterine artery blood flow during normal pregnancy. *J Appl Physiol, 79*(1), 7-14.

Zhang, Y., Popovic, Z. B., Bibevski, S., Fakhry, I., Sica, D. A., Van Wagoner, D. R., et al. (2009). Chronic vagus nerve stimulation improves autonomic control and attenuates systemic inflammation and heart failure progression in a canine high-rate pacing model. *Circ Heart Fail, 2*(6), 692-699.

Zhu, J. L., Obel, C., Hammer Bech, B., Olsen, J., & Basso, O. (2007). Infertility, infertility treatment, and fetal growth restriction. *Obstet Gynecol, 110*(6), 1326-1334.

Ziegler, E. E., O'Donnell, A. M., Nelson, S. E., & Fomon, S. J. (1976). Body composition of the reference fetus. *Growth, 40*(4), 329-341.

Zipes, D. P., & Wellens, H. J. J. (1998). Sudden Cardiac Death. *Circulation, 98*(21), 2334-2351.

Zuckerman, B., Frank, D. A., Hingson, R., Amaro, H., Levenson, S. M., Kayne, H., et al. (1989). Effects of maternal marijuana and cocaine use on fetal growth. *N Engl J Med, 320*(12), 762-768.

References

Zwicker, J. G., & Harris, S. R. (2008). Quality of life of formerly preterm and very low birth weight infants from preschool age to adulthood: a systematic review. *Pediatrics, 121*(2), e366-376.