Placental Restriction and Endocrine Control of Postnatal Growth

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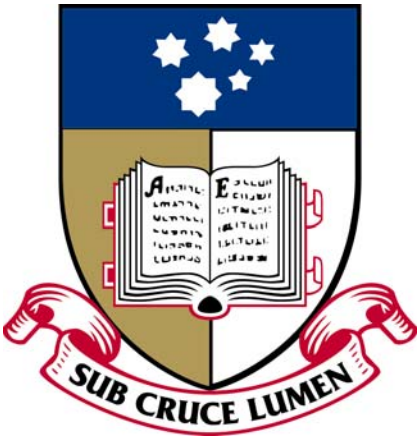

For my wife Zoe and my family and friends

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My specific contribution to the execution of the studies described here were as follows: carunclectomy surgery was performed on three cohorts, in which I was an assistant for the first, but organised and was one of two or more surgeons for the remaining two cohorts. Others who assisted with the work were: Melissa Walker, Damian Adams, Arkadi Katsman, Simon Fielke, Frank Carbone, Prema Thavaneswaran, Sanita Grover, Dr Mike Adams, Dr Tim Butler, Anne Jurisevic, Kirsty Warnes, Dr Lisa Edwards, and Severence MacLaughlin. Surgery to implant vascular catheters in lambs was organised and carried out by myself for about 90% of the lambs, together with Simon Fielke and others (10%). I also carried out routine maintenance of vascular catheters of the lambs (75%) together with Dr Kathy Gatford and Melissa Walker (25%). I was also responsible for carrying out the lamb growth measurements (95%) for the second and third cohorts together with Dr Kathy Gatford and Melissa Walker undertaking the remainder (5%), while Simon Fielke carried out the majority of these in the first cohort (75%) and I carried out the remainder (25%). I was responsible for the preparation and execution of insulin clamps, IGF clamps, and IVGTTs with assistance from Melissa Walker (40%), and Kathy Gatford (10%). I was responsible for most of the assays of plasma glucose, α-amino N, and FFA (95%), together with Damian Adams (plasma glucose assays, 5%). Plasma insulin assays were carried out equally by Dr Kathy Gatford and myself. I performed all of the plasma thyroid hormone assays. Plasma IGF-I and IGF-II

assays were performed by Pat Grant in the Department of Obstetrics and Gynaecology. I was present at each post-mortem and worked with a team of others that varied with each post-mortem including: Dr Kathy Gatford, Melissa Walker, Sanita Grover, Damian Adams, Arkadi Katsman, Dane Horton, Vicky Staikopoulos, Anne Jurisevic, Marta Gonzalez Warleta, Natasha Lloyd, Luke Parkinson, Katy Ramsden, and Brooke Summers. The workload for these post-mortems was shared equally.

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Thank you.

STATEMENT OF ORIGINALITY AND AUTHENTICITY

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university and or tertiary institution and, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying if accepted for the award of the degree.

Signed,

Miles J De Blasio,

Date:

TABLE OF ABBREVIATIONS AND BIOCHEMICAL NAMES

α-aN	Plasma α-amino nitrogen concentration
$\alpha\text{-aN}_{60'\text{-}120'}$	Plasma α-amino nitrogen concentration during the second hour of the hyperinsulinaemic euglycaemic clamp
AGA	Appropriate for gestational age
AGR	Absolute growth rate
ANOVA	Analysis of Variance (statistical test)
BMI	Body mass index
CFGR	Current fractional growth rate
CO_2	Carbon dioxide
CRL	Crown-rump length
CVD	Cardiovascular disease
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immuno-sorbent Assay
FFA	Free fatty acid concentration
$FFA_{60'\text{-}120'}$	Free fatty acid concentration during the second hour of the hyperinsulinaemic euglycaemic clamp
GH	Growth hormone
GIR	Glucose infusion rate
$GIR_{60'\text{-}120'}$	Glucose infusion rate during the second hour of the hyperinsulinaemic euglycaemic clamp
$GIR_{70\text{-}130'}$	Glucose infusion rate during the second hour of the hyper-IGF-I euglycaemic clamp
GLUT4	Glucose transporter protein 4
HEAAC	Hyperinsulinaemic euglycaemic aminoacidaemic clamp
HEC	Hyperinsulinaemic euglycaemic clamp
HIEC	Hyper-IGF-I euglycaemic clamp
HPAA	Hypothalamo-pituitary adrenal axis
HPLC	High performance liquid chromatography
HPTA	Hypothalamo-pituitary thyroid axis

i.m.	Intramuscular
ID	Internal diameter
IGFBP	Insulin-like growth factor binding protein
IGF-I	Insulin-like growth factor-I
IGF-II	Insulin-like growth factor-II
IGF-IR	Type 1 Insulin-like growth factor receptor
IGF-IIR	Type 2 Insulin-like growth factor receptor
IgG	Immunoglobulin G
IMVS	Institute of Medical and Veterinary Science
IR	Insulin receptor
IRS	Insulin receptor substrate
IUGR	Intrauterine growth restriction (or retardation)
IVGTT	Intravenous glucose tolerance test
kda	Kilodalton
kg	Kilogram
KHz	Kilohertz
M	Molar
Man-6-P	Mannose-6-Phosphate
mCi	Milli Curie
meq	Milli Equivalent
mg	Milligram
ml	Milli Litre
mM	Millimolar
mRNA	Messenger ribonucleic acid
ms	Millisecond
mU	Milli Unit
NFGR	Neonatal fractional growth rate
NIDDM	Non-insulin dependent diabetes mellitus
nmol	Nanomole
NQS	β-Napthoquinone sulphonate
O_2	Oxygen
^oC	Degrees centigrade
OH	Hydroxyl

pg	Picogram
PI	Ponderal index
pO_2	Partial pressure of oxygen
PR	Placental restriction or placentally restricted
rhIGF-I	Human recombinant insulin-like growth factor-I
RIA	Radioimmunoassay
SD	Standard deviation
SEM	Standard error of the mean
SGA	Small for gestational age
SSGIR	Steady state glucose infusion rate
TBG	Thyroxine-binding globulin
TG	Triglyceride
TH	Thyroid hormone
TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing hormone
TSH	Thyroid (thyrotropin)-stimulating hormone
TTR	Transthyrectin
μCi	Micro Curie
μg	Microgram
μl	Microlitre
%CV	Coefficient of variance
L-thyroxine (T4)	L-3,5,3',5'-tetraiodothyronine
L-triiodothyronine (T_3)	L-3,5,3'-triiodothyronine
3-(^{3}H)-Glucose	Carbon 3 tritiated labelled glucose
^3H_2O	Tritiated labelled water

PAPERS ARISING FROM THIS THESIS

De Blasio MJ, Walker MR, Gatford KL, Robinson JS, Owens JA. (2004). Placental restriction of fetal growth reduces size at birth and increases postnatal growth and adiposity in the young lamb. *'Accepted American Journal of Physiology – Regulatory, Integrative and Comparative Physiology'.*

De Blasio MJ, Gatford KL, Fielke SL, Robinson JS, McMillen IC, Owens JA. (2004). Fetal growth restriction increases growth rate and insulin action in the neonatal lamb. *'Submitted to J Physiol'.*

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De Blasio MJ, Walker MR, Gatford KL, Robinson JS, Owens JA. (2004). Placental restriction alters circulating thyroid hormone in the young lamb postnatally. *'Accepted American Journal of Physiology – Regulatory, Integrative and Comparative Physiology'.*

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Gatford, K. L., E. M. Wintour, **M. J. De Blasio,** J. A. Owens, M. Dodic, (2000). Differential timing for programming of glucose homeostasis, sensitivity to insulin and blood pressure by in utero exposure to dexamethasone in sheep. Clin Sci **98(5):** 553-560.

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Gatford, K. L., J. A. Owens, R. G. Campbell, J. M. Boyce, P. A. Grant, **M. J. De Blasio,** P. C. Owens. (2000). Treatment of underfed pigs with GH throughout the second quarter of pregnancy increases fetal growth. *J Endocrinol* **166(1):** 227-234.

Gatford, K. L., J. E. Ekert, K. Blackmore, **M. J. De Blasio,** J. M. Boyce, J. A. Owens, R. G. Campbell, P. C. Owens. (2003). Variable maternal nutrition and growth hormone treatment in the second quarter of pregnancy in pigs alter semitendinosus muscle in adolescent progeny. *Br J Nutr* **90:** 1-12.

ABSTRACT

Intrauterine Growth Restriction (IUGR) is evident in infants born with a reduced weight or length, and/or increased thinness for gestational age. IUGR is associated with altered postnatal growth and regulation, due to unknown mechanisms. Much clinical IUGR results from the reduced delivery of essential substrates (oxygen and nutrients) to the fetus, due to either maternal or placental limitations. Catch-up growth (accelerated rate of growth in absolute or fractional terms) occurs in the majority of IUGR infants, and returns an infant to their predetermined growth curve. IUGR is associated with increased risks of morbidity and mortality in the perinatal period, and with a reduced final adult stature and increased risk of adult onset diseases, particularly diabetes and cardiovascular disease. Catch-up growth after IUGR predicts improved health in terms of reduced hospital visits in infants and children, and an increased final adult stature but also predicts an increased risk of developing obesity, as well as diabetes and cardiovascular disease. The underlying mechanisms for catch-up growth may contribute to this range of outcomes in later life, but are poorly understood. Studies in IUGR infants have demonstrated increased absolute and/or fractional growth rates following birth, termed catch-up growth, in the presence of reduced or normal plasma concentrations of the thyroid hormones and major anabolic hormones (insulin and/or IGF-I). This suggests that increased sensitivity to, rather than increased production of insulin, IGF-I and thyroid hormone, causes catch-up growth following IUGR. We therefore hypothesised that placental restriction of fetal growth would reduce size at birth and increase postnatal growth and adiposity in association with increased metabolic sensitivity to insulin, IGFs and thyroid hormones. This study has

shown that the placentally restricted (PR) lamb has a reduced size at birth in terms of soft and skeletal tissues, has increased rates of growth postnatally, and has increased adiposity by six weeks of age. We have also shown that PR of fetal growth in the sheep did not alter gestational age at delivery, but reduced survival rate. PR lambs demonstrated catch-up growth in most parameters by 30 days of age and increased adiposity at six weeks of age compared to the control lambs. Placental restriction increased insulin and IGF sensitivity of circulating free fatty acids, which in turn, predicts increased adiposity. Neonatal catch-up growth after fetal growth restriction was substantially predicted by both abundance of, and metabolic sensitivity to insulin, suggesting increased insulin action as an underlying cause. Catch-up growth occurs in the neonate despite reduced concentrations of fasting plasma IGFs, along with increased IGF sensitivity of free fatty acid metabolism and adiposity. Plasma TH concentrations predicted growth of soft and skeletal tissue in lambs during early postnatal life, particularly in those undergoing catch-up growth following PR. Therefore neonatal catch-up growth after IUGR is associated with increased sensitivity to both insulin and IGFs, particularly of circulating free fatty acids, and appears to occur to the extent allowed by the prevailing abundance of these hormones and of thyroid hormones. If this altered endocrine state persists, increased adiposity and its subsequent amplification may contribute to the development of obesity, and related adverse metabolic and cardiovascular outcomes in adult life.