THE EFFECT OF FETAL GROWTH RESTRICTION AND SEX ON THE DEVELOPMENT AND FUNCTION OF ADIPOSE TISSUE

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Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the test.

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

Signed:……..…….

Date:…….

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Publications Arising From This Thesis

Duffield JA, Vuocolo T, Tellam RL, Yuen BS, Muhlhausler BS, McMillen IC. Placental restriction of fetal growth decreases IGF1 and leptin mRNA expression in the perirenal adipose tissue of late gestation fetal sheep. *Am J Physiol Regul Integr Comp Physiol.* 2008 Feb 13; [Epub ahead of print]

Related Publications

1. McMillen IC, MacLaughlin SM, Muhlhausler BS, Gentili S, Duffield JA and Morrison JL. Developmental Origins of Adult Health and Disease: The Role of Periconceptional and Fetal Nutrition. *Basic & Clinical Pharmacology & Toxicology.* 2008 102:82-89.

2. Muhlhausler BS, Duffield JA, McMillen IC. Increased Maternal Nutrition Increases Leptin Expression in Perirenal and Subcutaneous Adipose Tissue in the Postnatal Lamb. *Endocrinology.* 2007 Dec;148(12):6157-63.

3. Muhlhausler BS, Duffield JA, McMillen IC. Increased maternal nutrition stimulates Peroxisome Proliferator Activated Receptor-γ (PPAR-γ), adiponectin and leptin mRNA expression in adipose tissue before birth. *Endocrinology*. 2007 Feb;148(2):878-85.

4. Muhlhausler BS, Adam CL, Findlay PA, Duffield JA, McMillen IC. Increased maternal nutrition alters development of the appetite-regulating network in the brain. *FASEB J.* 2006 Jun;20(8):1257-9.

5. McMillen IC, Edwards LJ, Duffield J, Muhlhausler BS. Regulation of leptin synthesis and secretion before birth: implications for the early programming of adult obesity. *Reproduction.* 2006 Mar;131(3):415-27.

6. IC McMillen, JA Duffield and BS Muhlhausler. Chapter title: Prenatal Programming of Postnatal Obesity. In Hodgson, DM and Coe, CL (2006) *Perinatal Programming: Early Life Determinants of Adult Health and Disease.*

7. McMillen IC, Muhlhausler BS, Duffield JA, Yuen BS. Prenatal programming of postnatal obesity: fetal nutrition and the regulation of leptin synthesis and secretion before birth. *Proc Nutr Soc.* 2004 Aug;63(3):405-412.

Abstract

A world-wide series of epidemiological studies has demonstrated that there is an association between being born small and the risk of visceral obesity, a more central deposition of subcutaneous fat and insulin resistance in adult life. In the lamb, intrauterine growth restriction (IUGR) results in a low birth weight and an increased visceral fat mass by 45d of postnatal life. In this thesis I have investigated the effect of IUGR on adipose tissue development and function during fetal and early postnatal life in the sheep. IUGR was induced by removal of the majority of endometrial caruncles in non pregnant ewes prior to mating which resulted in the subsequent placental restriction of fetal growth (PR). Fetal blood samples were collected from 116d gestation and visceral perirenal adipose tissue (PAT) collected from PR and control fetuses at 145d. In lambs IUGR was defined as a birth weight less than 2 standard deviations below the mean of a cohort of singleton Merino lambs. Blood samples were collected throughout the first 3 weeks of life and PAT and subcutaneous adipose tissue (SAT) was collected at 21 d. It was determined whether IUGR alters the expression of genes which regulate adipogenesis (IGF1, IGFR1, IGF2, IGFR2, PPARγ, and RXRα), adipocyte metabolism (LPL, G3PDH, GAPDH) and adipokine signalling (leptin, adiponectin) in adipose tissue depots before and after birth using qRT-PCR.

PR fetuses were hypoglycaemic, hypoinsulinaemic, hypoxic, and had a lower body weight than Control fetuses. The expression of both IGF1 and leptin mRNA in PAT, the major fetal adipose depot, was lower in the PR fetuses, although

there was no difference in the expression of other adipokine or adipogenic genes in PAT between PR and control fetuses. Thus restriction of placental and hence fetal substrate supply results in decreased IGF1 and leptin expression in fetal visceral adipose tissue which may alter the functional development of the perirenal fat depot and contribute to altered leptin signalling in the growth restricted newborn and the subsequent emergence of an increased visceral adiposity.

At 21d of postnatal life there was no increase in the relative mass of perirenal or subcutaneous fat in IUGR lambs compared with controls. Thus, this study has investigated the effect of IUGR on the development of adipose tissue prior to the development of an obese phenotype.

At 21d of life there was a sex specific effect of IUGR on the expression of PPARγ and leptin mRNA in perirenal visceral fat such that PPARγ and leptin mRNA expression was decreased in male IUGR lambs, but not females. Interestingly PAT mass was greater in females than males, independent of birth weight. Plasma insulin concentrations during the first 24h after birth predicted the size of the adipocytes and expression of adiponectin in visceral adipose tissue in both males and females at 21d. Thus, the nutritional environment before, and immediately after birth, may program adipocyte growth and gene expression in visceral adipose tissue. The differential effect of sex and birth weight on PPARγ and leptin expression in visceral fat may be important in the subsequent development of visceral obesity and the insulin resistant phenotype in later life.

At 21d of life there was no difference between Control and IUGR lambs in the relative mass of subcutaneous fat, or the expression of PPARγ, RXRα, leptin, adiponectin, LPL, G3PDH, and GAPDH in subcutaneous fat at 21d of life. We have shown that the growth of the subcutaneous fat depot is related to plasma glucose, insulin and leptin concentrations, and to the development of perirenal fat. Thus, in contrast to perirenal adipose tissue, the postnatal, but not the fetal nutritional environment, programs subcutaneous adipocyte growth and gene expression. This thesis speculates that there may be a factor secreted from visceral fat that influences the development of the subcutaneous fat depot.

At 21d of life there was also an effect of sex, but not IUGR, on the expression of IGF mRNA in adipose tissue. Male lambs had a higher expression of IGF1 mRNA in both PAT and SAT, and a higher expression of IGF1R and IGF2R in SAT compared with female lambs. It is likely that these differences in IGF mRNA levels reflect sexual dimorphism of the GH-IGF axis. When male and female lambs were combined there was a higher expression of IGF1 mRNA in SAT compared with PAT, and a higher expression of IGF2, IGF1R and IGF2R mRNA in PAT compared with SAT. These differences in IGF mRNA expression provide a potential mechanism to explain the sex and depot specific variations in mitogenic potency of IGF1 and proliferative capacities of preadipocytes, the regional variation in adipocyte metabolism, and the difference in incidence of visceral obesity between men and women in adult life.

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Commonly Used Abbreviations

A	Acrp30	adipocyte complement-related protein of 30kDa
	AGA	average for gestational age
	AgRP	agouti-related protein
	ANOVA	analysis of variance
	ATP	adenosine triphosphate
B	BMI	body mass index
	bp	base pairs
C	cAMP	cyclic adenosine monophosphate
	CART	cocaine-amphetamine related transcript
	cDNA	complementary deoxyribonucleic acid
	C/EBP	CCAAT/enhancer binding protein
	CRL	crown rump length
	cT	comparative threshold
	CV	coefficient of variation
D	d	days
	DEXA	dual emission x-ray absorptiometry
	DM	dry matter
	DNA	deoxyribonucleic acid
E	ELISA	enzyme linked immunosorbent assay
F	FFA	free fatty acids
G	G3PDH	glycerol-3-phosphate dehydrogenase
	GAPDH	glyceraldehyde-3-phosphate dehydrogenase
	GA	gestational age
	GH	growth hormone

	GHRH	growth hormone releasing hormone
	GLUT	glucose transporter
H	h	hours
	HBW	high birth weight
	HDL	high density lipoprotein
	HOMA-IR	homeostasis model assessment to predict insulin resistance
I	IGF	insulin-like growth factor
	IGFBP	insulin-like growth factor binding protein
	IGFR	insulin-like growth factor receptor
	IR	insulin receptor
	i.m.	intramuscular
	i.v.	intravenous
	IUGR	intra-uterine growth retardation
J	JAK	janus kinase
K	kDA	kilodalton
	KO	knock out
L	LBW	low birth weight
	LDL	low density lipoprotein
	LGA	large for gestational age
	LPL	lipoprotein lipase
M	MAPK	mitogen-activated protein kinase
	MDI	methylisobutylxanthine, dexamethasone and IGF1 / insulin
	ME	metabolisable energy
	MRI	magnetic resonance imaging
	mRNA	messenger ribonucleic acid

N	NADH	nicotinamide adenine dinucleotide
	NEFA	non-esterified fatty acid
	NPY	neuropeptide Y
O	*Ob*	*obese* gene
	OB-Rb	leptin receptor type b
P	PAT	perirenal adipose tissue
	PI	ponderal index
	PO_2	partial pressure of oxygen
	POMC	pro-opiomelanocortin
	PPARγ	proliferator-activated receptor-γ
	PPRE	peroxisome proliferator response elements
	PR	placentally restricted
Q	qRTPCR	quantitative real-time polymerase chain reaction
R	RPLP0	acidic ribosomal protein large subunit P0
	RNA	ribonucleic acid
	RXR	retinoid-X receptor
S	SAT	subcutaneous adipose tissue
	SD	standard deviation
	SEM	standard error of the mean
	SES	socioeconomic status
	SGA	small for gestational age
	STAT	signal transducers and activators of transcription
T	TSW	time suckling withheld
	TZD	thiazolidinedione
U	UCP	uncoupling protein
	US	United States (of America)

	UTR	untranslated region
V	VAT	visceral adipose tissue
WXYZ		