THE IMPACT OF MATERNAL OVERNUTRITION DURING THE PERICONCEPTIONAL PERIOD ON THE DEVELOPMENT OF POSTNATAL OBESITY IN THE SHEEP

Leewen Rattanatray B. Biomedical Sc. (Hons)

Discipline of Physiology

School of Molecular and Biomedical Science

The University of Adelaide

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy to the University of Adelaide

May 2010

TABLE OF CONTENTS

TABLE OF CONTENTS II

ABSTRACT VII

DECLARATION IX

ACKNOWLEDGEMENTS X

RELATED PUBLICATIONS XII

LIST OF FIGURES AND TABLES XIII

COMMONLY USED ABBREVIATIONS XVII

CHAPTER 1: LITERATURE REVIEW 2

1.1 INTRODUCTION 2

1.2 FETAL ORIGINS OF ADULT DISEASE 3

1.3 DEVELOPMENTAL PROGRAMMING OF THE METABOLIC SYNDROME: EPIDEMIOLOGICAL EVIDENCE 5

1.3.1 Maternal overnutrition and the developmental programming of obesity 7

1.3.2 The obesity epidemic 7

1.3.3 Maternal obesity 8

1.4 BENEFITS AND RISKS ASSOCIATED WITH DIETARY RESTRICTION OF WOMEN WITH A HIGH BMI 9

1.4.1 Benefits of dietary weight loss before entering pregnancy 9

1.5 DEVELOPMENTAL PROGRAMMING OF OBESITY: EPIDEMIOLOGICAL EVIDENCE 11

1.5.1 Relationship between maternal obesity and high birth weight 11

1.5.2 High maternal and fetal planes of nutrition result in a high birth weight infant 12

1.5.3 Maternal diabetes 12

1.5.4 High birth weight a predictor of adulthood adiposity, insulin resistance and diabetes 14

1.6 MATERNAL OVERNUTRITION AND OFFSPRING ADIPOSITY: EXPERIMENTAL ANIMAL STUDIES 16
1.7 CRITICAL WINDOWS OF EARLY DEVELOPMENT: INTRA-UTERINE GROWTH AND DEVELOPMENT 18
1.7.1 Follicular development and oogenesis 18
1.7.2 Fertilization and Embryogenesis 20
1.7.3 Gastrulation and the formation of the germ layers 21
1.8 IMPACT OF NUTRITION ON THE DEVELOPMENT OF THE OOCYTE AND EMBRYO 22
1.8.1 Nutrition and gonadotropin secretion 23
1.8.2 Nutrition and progesterone concentrations 25
1.8.3 Nutrition and ovarian function 25
1.8.4 Nutrition and embryo quality 25
1.9 FUNCTION AND REGULATION OF ADIPOSE TISSUE 27
1.9.1 Function of adipose tissue during fetal development and later in life 27
1.9.2 Adipose tissue development 29
1.9.3 Hormonal regulation of adipose tissue maturation 30
1.9.4 Regulation of lipogenesis and lipolysis 32
1.9.5 Adipose derived molecules 34
1.10 EXPERIMENTAL HYPOTHESES 37

CHAPTER 2: THE EFFECTS OF MATERNAL OVERNUTRITION AND/OR DIETARY RESTRICTION DURING THE PERICONCEPTIONAL PERIOD ON LAMB GROWTH AT BIRTH 42

2.1 INTRODUCTION 42
2.2 MATERIAL AND METHODS 45
2.2.1 Animals and nutritional feeding regime 45
2.2.2 Donor and recipient ewe synchronisation and pregnancy 46
2.2.2.1 Super ovulation protocol 46
2.2.2.2 Artificial insemination and embryo collection 47
2.2.3 Determination of major transitions in structure of the embryo during early embryogenesis 48
2.2.4 Embryo transfer protocol 48
2.2.5 Blood Sampling Regime 49
2.2.5.1 Donor and recipient ewe blood sampling 49
2.2.5.2 Lamb blood sampling 52

2.2.6 Lamb birth and growth measurements 52
2.2.7 Plasma non-esterified free fatty acid determination 52
2.2.8 Plasma insulin determination 52
2.2.9 Statistical Analyses 53
2.2.9.1 Ewe weights 53
2.2.9.2 Conception and pregnancy outcomes 54
2.2.9.3 Plasma glucose, non-esterified fatty acid and insulin concentrations ..54
2.2.9.4 Lamb birth measures 54
2.3 RESULTS 55
2.3.1 Donor ewe weights 55
2.3.2 Donor ewe plasma glucose and insulin concentrations 56
2.3.3 Periconceptional nutrition and embryo collection 56
2.3.4 Body condition and weight of recipient ewes 56
2.3.5 Plasma glucose and insulin concentrations in recipient ewes 64
2.3.6 Lamb birth and growth measurements 64
2.3.7 Lamb plasma glucose, insulin and free fatty acid concentrations 64
2.4 DISCUSSION 70
2.4.1 Weights of non-pregnant donor ewes and pregnant recipient ewes 70
2.4.2 Maternal plasma glucose and insulin concentrations 71
2.4.3 Pregnancy outcomes 72
2.4.4 Lamb birth and growth measurements 75
2.5 SUMMARY 76

CHAPTER 3: THE EFFECTS OF MATERNAL OVERNUTRITION AND/OR DIETARY RESTRICTION DURING THE PERICONCEPTIONAL PERIOD ON LAMB GROWTH FROM BIRTH TO 4 MONTHS OF AGE 78

3.1 INTRODUCTION 78
3.2 MATERIAL AND METHODS 79
3.2.1 Blood sampling regime 80
3.2.2 Lamb birth and growth measurements 81
3.2.3 Glucose tolerance test 81
3.2.4 Plasma glucose, NEFA and insulin concentrations 82
3.2.5 Post mortem and tissue collection 82
3.2.6 Statistical Analyses 82
3.2.6.1 Plasma glucose, non-esterified fatty acid and insulin concentrations ..83
3.2.6.2 Lamb growth parameters and organ and tissue weights 83

3.3 RESULTS 84
3.3.1 Lamb birth and growth measurements 84
3.3.2 Lamb plasma glucose, insulin and free fatty acid concentrations and glucose tolerance test 84
3.3.3 Liver, kidney, pancreas, lung and brain weights 85
3.3.4 Heart weight 94
3.3.5 Adrenal weights 94
3.3.6 Total adipose tissue weight 97
3.3.7 Proportions of fat mass in the adipose tissue depots 97
3.4 DISCUSSION 111
3.4.1 Lamb growth measurements 111
3.4.2 Lamb plasma glucose, non-esterified fatty acid and insulin concentrations 112
3.4.3 Lamb weights 112
3.4.4 Lamb organ weights 113
3.4.4.1 Lamb heart weights 113
3.4.4.2 Lamb brain weights 117
3.4.4.3 Lamb adrenal weights 118
3.4.5 Adipose tissue weight 120
3.5 SUMMARY 122

CHAPTER 4: IMPACT OF PERICONCEPTIONAL OVERNUTRITION AND/OR DIETARY RESTRICTION ON THE EXPRESSION OF ADIPOGENIC AND LIPOGENIC GENES IN THE POSTNATAL LAMB 124

4.1 INTRODUCTION 124
4.2 MATERIAL AND METHODS 126
4.2.1 Animals and nutritional feeding regime 126
4.2.2 Post mortem and adipose tissue collection 127
4.2.3 RNA extraction 128
4.2.4 Real Time Quantitative Reverse Transcription-PCR (qRT-PCR) 128
4.2.5 Statistical Analysis 131
4.2.5.1 Adipogenic and lipogenic gene expression 131
4.3 RESULTS 132
4.3.1 Subcutaneous adipose tissue 132
4.3.2 Perirenal adipose tissue 132
4.3.3 Omental adipose tissue 133

4.3.4 Comparison of adipogenic and lipogenic gene expression in all fat depots 133
4.4 DISCUSSION..... 148
4.5 SUMMARY..... 153
CHAPTER 5: SUMMARY AND CONCLUSIONS 155
BIBLIOGRAPHY..... 164

ABSTRACT

Women who enter pregnancy with an increased body weight have an increased risk of developing gestational diabetes later in pregnancy and of having a baby with a high birth weight and fat mass who is also at an increased risk of becoming overweight or obese in childhood or later adult life. It is not known, however, whether exposure of the oocyte and embryo during the periconceptional period alone to maternal obesity is associated with an increased risk of obesity in the offspring, and if so, whether the impact of maternal obesity can be ameliorated by maternal weight loss immediately before conception.

The present study has investigated in sheep, whether, a high plane of maternal nutrition before and immediately after conception leads to the programming of an increased expression of adipogenic and lipogenic genes and fat mass in the offspring and whether a period of dietary restriction in overnourished mothers reverses these changes. Non pregnant ewes (*n*=23) were randomly assigned to one of four treatment groups, either control-control (CC) maintained at 100% maintenance energy requirements (MER) for at least 5 months prior to conception, control-restricted (CR) maintained at 100% MER for the first 4 months, then 1 month before conception were placed on a dietary restriction to 70% MER, high-high (HH) maintained *ad libitum* (170-190% MER) for 5 months prior to conception or high-restricted (HR) maintained at *ad libitum* for 4 months, and then 1 month before conception were placed on an energy-restricted diet (70% MER). To determine the effect of overnutrition in the periconceptional

period only, single embryos were then transferred to recipient ewes which were maintained on a control diet (100% MER) for the remainder of pregnancy. All ewes were allowed to give birth naturally. At 4 months of age, lamb fat depots were weighed and samples collected for the measurement of mRNA expression for genes regulating adipogenesis and lipogenesis by quantitative real-time PCR.

The studies in this thesis have shown that periconceptional overnutrition increased total fat mass in female lambs at 4 months of age. This change was not associated with an increase in peroxisome proliferator-activated receptor γ, leptin or adiponectin expression in the perirenal, omental and subcutaneous fat depots. The period of dietary restriction in overnourished ewes ablated this effect. These findings suggest that the effects of periconceptional overnutrition on the oocyte or early embryo alters the subsequent development of adipose tissue, and that the impact of periconceptional overnutrition may be reduced by a period of dietary restriction prior to entering pregnancy.

DECLARATION

This body of scientific work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary Institution to Leewen Rattanatray 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 text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University‟s digital research repository, the Library Catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Leewen Rattanatray
November 2009

ACKNOWLEDGEMENTS

I would like to thank all colleagues, students and supervisors who contributed to this study and have shown great support throughout my Ph.D.

I am grateful to the members of the Early Origins of Adult Health Research Group for their tremendous team effort and support and friendship during the project. I would especially like to acknowledge the assistance of Dr. Severence Mac Laughlin and Dr. Beverly Muhlhausler. Thank you both dearly for giving me a “crash course” in running a large animal study, for your time, patience, advice and guidance throughout my PhD Your unwavering support meant a lot to me during the difficult moments during my PhD (and there were many).

Thank you also to those who assisted in blood sampling, glucose tolerance tests, post-mortems and assays, especially Laura O‟Carroll, Melissa Walker, Bernard Chuang, Pamela Sim, Petra Bos and the staff from Laboratory Animal Services. This project would not have been possible without this great team behind me.

We are grateful to Dr. Dave Kleemann and Dr. Simon Walker for their collaboration with the extensive animal protocol, and to the research staff at the South Australian Research and Development Institute at Turretfield, including Dr. Jen Kelly, and Skye Rudiger for their support during the preliminary stages of the project. I would especially like to acknowledge the assistance of Dr. Dave Kleemann and his expertise on nutrition and reproduction in the sheep and Dr. Simon Walker for his assistance with the embryo transfer protocols.

I wish to express my sincere gratitude to my wonderful family for their constant love, encouragement and support throughout my life; I could not have achieved this without the sacrifices that they have made in their lives for me. And to Thuong, thank you for your constant love, understanding, encouragement, unwavering support and your patience, thank you for being you.

Lastly, I would like to thank my supervisors Prof. Caroline McMillen and Assoc. Prof. Jeff Schwartz. Thank you both for your guidance and support during the start of my scientific career. I would especially wish to thank Caroline for her vision, invaluable guidance and assistance in making this research possible. I am greatly indebted to her for helping me to overcome obstacles in the process of conducting the research and for the opportunities she has given me during my PhD.

RELATED PUBLICATIONS

Rattanatray L, MacLaughlin SM, Kleemann DO, Walker SK, Muhlhausler BS and McMillen IC (2010) Impact of Maternal Periconceptional Overnutrition on Fat Mass and Expression of Adipogenic and Lipogenic Genes in Visceral and Subcutaneous Fat Depots in the Postnatal Lamb. Submitted.

Zhang S, Rattanatray L, MacLaughlin SM, Cropley J, Suter C Molloy L, Kleemann D, Walker S, Muhlhausler BS, Morrison JL and McMillen IC (2010) Periconceptional Undernutrition in Normal and Overweight Ewes Leads to Increased Adrenal Growth and Epigenetic Changes in Adrenal *Igf2*/*H19* Gene in Offspring. *The FASEB Journal*. In press.

McMillen IC, Rattanatray L, Duffield JA, Morrison JL, MacLaughlin SM, Gentili S, Muhlhausler BS. (2009) The Early Origins of Later Obesity: Pathways and Mechanisms. Advances in Experimental Medicine and Biology Volume 646: 71-81

McMillen IC, Rattanatray L, Duffield JA, Morrison JL, MacLaughlin SM, Gentili S, Muhlhausler BS. (2009) The Early Origins of Later Obesity: Pathways and Mechanisms. In B Koletzko,D Molnár, T Decsi and A de la Hunty (Eds) Early Nutrition Programming and Health Outcomes in Later Life. Obesity and Beyond, 71-82

LIST OF FIGURES AND TABLES

Figure 1.1 The intergenerational cycle of obesity 15

Figure 1.2 A schematic diagram showing the sequence of events in origin, growth and rupture of the tertiary follicle and degradation of the corpus luteum 24

Figure 2.2.1 Nutritional protocol 50

Figure 2.2.2 The major transitions in structure of the bovine embryo during early embryogenesis 51

Figure 2.2.1 Blood sampling regime in the donor and recipient ewes 57

Figure 2.3.1 Effect of periconceptional overnutrition and/or dietary restriction on the weight of donor ewes during the nutritional feeding protocol from 35 weeks before conception to 1 week after conception 58

Figure 2.3.2 Effect of periconceptional overnutrition and/or dietary restriction on the change in the weight of the non-pregnant donor ewes between 25 weeks before conception to the day of embryo transfer (day 6-7 pregnancy) 59

Table 2.3.1 Donor ewe body condition scores between 5 weeks prior to conception and conception 60

Table 2.3.2 Plasma glucose concentrations in donor ewes at 5, 4 and 1 week prior to conception and at conception 61

Figure 2.3.3 Plasma insulin concentration in donor ewes between 5 weeks before conception and conception 62

Table 2.3.3 Periconceptional nutrition and/or dietary restriction and the stage of embryo development 63

Table 2.3.4 Plasma glucose and insulin concentrations in recipient ewes at 5 weeks before and 7 weeks after conception 65

Table 2.3.5 Periconceptional nutrition and pregnancy outcomes and lamb survival 66

Table 2.3.6 Effect of periconceptional overnutrition and/or dietary restriction on growth parameters at birth 67

Figure 2.3.4 Effect of periconceptional overnutrition and/or dietary restriction on birth weight in male and female lambs 68

Table 2.3.7 Effect of periconceptional overnutrition and/or dietary restriction on plasma glucose, non-esterified free fatty acid and insulin concentrations at birth in the offspring 69

Figure 3.3.1 The growth rate of lambs from the 4 nutritional treatment groups between 1 and 16 weeks of age 86

Table 3.3.1 The effect of periconceptional overnutrition and/or dietary restriction on growth parameters at 4 months of age 87

Figure 3.3.2 Effect of periconceptional overnutrition and/or dietary restriction on male and female lamb weight at 4 months of age 88

Figure 3.3.3 Effect of periconceptional overnutrition and/or dietary restriction on plasma glucose concentration between birth and 4 months of age in lambs 89

Figure 3.3.4 Effect of periconceptional overnutrition and dietary restriction on the plasma non-esterified free fatty acid concentration from birth to 4 months of age in lambs 90

Figure 3.3.5 Effect of periconceptional overnutrition and/or dietary restriction on the plasma insulin concentration from birth to 4 months of age in lambs 91

Figure 3.3.6 Plasma glucose and insulin concentrations responses to intravenous glucose challenge at 3 months of age in lambs 92

Figure 3.3.7 Area under the glucose and insulin response curves after the intravenous glucose challenge 93

Figure 3.3.8 Effect of periconceptional nutrition on absolute organ weights and relative to body weight of 4 month old lambs 95

Figure 3.3.9a Effect of periconceptional overnutrition and dietary restriction on the absolute brain weight in female and male lambs at 4 months of age 98

Figure 3.3.9b Effect of periconceptional overnutrition and dietary restriction on the brain weight relative to body weight in female and male lambs at 4 months of age 99

Figure 3.3.10a Effect of periconceptional overnutrition and dietary restriction on absolute heart of 4 month old male and female lambs 100

Figure 3.3.10b Effect of periconceptional overnutrition and dietary restriction on heart weight relative to body weight of 4 month old male and female lambs 101

Figure 3.3.11 Effect of periconceptional overnutrition and/or dietary restriction on absolute adrenal weight and relative to body weight of 4 month old lambs 102

Figure 3.3.12 Effect of periconceptional overnutrition and/or dietary restriction on total fat mass of male and female lambs at 4 months of age 103

Figure 3.3.13 Relationship between total fat mass in 4 month old female offspring and the weight of the donor ewe at conception 104

Figure 3.3.14 Effect of periconceptional overnutrition and/or dietary restriction on omental adipose tissue depot weight of 4 months old male and female lambs 105

Figure 3.3.15 Effect of periconceptional overnutrition and/or dietary restriction on perirenal adipose tissue depot weight of 4 months old male and female lambs 106

Figure 3.3.16 Effect of periconceptional overnutrition and/or dietary restriction on subcutaneous adipose tissue depot weight of 4 months old male and female lambs 107

Figure 3.3.17 The proportion of the total fat mass in the different adipose depots in female lambs at 4months of age 108

Figure 3.3.18 The proportion of the total fat mass in the different adipose depots in male lambs at 4 months of age 109

Table 3.3.2 Differences between the proportion of the total adipose tissue in each fat depot between male and female lambs at 4 months of age 110

Table 4.2.1 Sequences of real time PCR primers for adipogenic, lipogenic and reference genes 130

Figure 4.3.1 Normalised expression of adipogenic and lipogenic genes in the subcutaneous adipose tissue depot of male and female lambs at 4 months of age 135

Figure 4.3.2 Normalised expression of adipogenic and lipogenic genes in the perirenal adipose tissue depot of male and female lambs at 4 months of age 137

Figure 4.3.3 Normalised expression of adipogenic and lipogenic genes in the omental adipose tissue depot of male and female lambs at 4 months of age 139

Figure 4.3.4 Correlation between donor ewe weight at conception and perirenal G3PDH mRNA expression in female lambs at 4 months of age 141

Table 4.3.1 Correlations between the relative mRNA expression of PPARγ and other adipogenic and lipogenic genes in the subcutaneous, perirenal and omental adipose tissue depots of lambs at 4 months of age 142

Figure 4.3.5 Relative adipose tissue depot specific expression of PPARγ mRNA of male and female lambs at 4 months of age 143

Figure 4.3.6 Relative adipose tissue depot specific expression of G3PDH mRNA of male and female lambs at 4 months of age 144

Figure 4.3.7 Relative adipose tissue depot specific expression of LPL mRNA of male and female lambs at 4 months of age 145

Figure 4.3.8 Relative adipose tissue depot specific expression of leptin mRNA of male and female lambs at 4 months of age 146

Figure 4.3.9 Relative adipose tissue depot specific expression of adiponectin mRNA of male and female lambs at 4 months of age 147

Figure 5.1 Proposed mechanism for the impact of periconceptional overnutrition on the intergenerational cycle of obesity 159

Figure 5.2 Proposed mechanism for the impact of late gestational overnutrition on the intergenerational cycle of obesity 161

Figure 5.3 Proposed mechanism for the impact of periconceptional overnutrition and late gestational overnutrition on the intergenerational cycle of obesity 162

COMMONLY USED ABBREVIATIONS

A B C

ACE	angiotensin-converting enzyme
ACTH	adrenocorticotrophic hormone
Ad libitum	to any desired extent
ANOVA	analysis of variance
BAT	brown adipose tissue
BCS	body condition score
BMI	body mass index
bp	base pairs
cAMP	cyclic adenosine monophosphate
CC	control-control
C/EBP	CCAAT enhancer binding protein
CR	control-restricted

D E F G

d	days
DNA	deoxyribonucleic acid
FFA	free fatty acids
FSH	follicle stimulating hormone
GDM	gestational diabetes mellitus
GLUT	glucose transporter
G3PDH	glycerol-3-phoshate dehydrogenase

H I J K L

h	hours
HFD	high fat diet
hGh	human growth hormone
HH	high-high
HPA	hypothalamic-pituitary-adrenal axis
HR	high-restricted
ICM	inner cell mass
IGF	insulin-like growth factor
IGFR	insulin-like growth factor receptor
I.V	intravenous
LGA	large for gestational age
LH	luteinizing hormone
LPL	lipoprotein lipase

M N O

MER	maintenance energy requirements
min	minute(s)
mRNA	messenger ribonucleic acid
n	number
NEFA	non-esterified free fatty acids

P Q R S

PCON	periconceptional overnutrition
PCR	polymerase chain reaction
PCUN	periconceptional undernutrition
PPARγ	peroxisome proliferator-activated receptor γ
PPRE	peroxisome proliferator response elements
qRT-PCR	real-time quantitative reverse transcription polymerase chain reaction
RAS	renin-angiotensin system
RNA	ribonucleic acid
RPLP0	ribosomal protein large subunit P0
RT-PCR	reverse transcription polymerase chain reaction
RxR	retinoid x receptor
SEM	standard error of the mean
SPSS	statistical package for the social sciences

T U V W X Y Z

TZD	thiazolidinediones
WAT	white adipose tissue