

**THE EARLY ORIGINS OF OBESITY: THE IMPORTANCE
OF PRENATAL VS POSTNATAL ENVIRONMENT**

Mini A. Vithayathil B.Sc. (Hons)

FOODplus Research Centre

Faculty of Sciences

School of Agriculture, Food & Wine

The University of Adelaide

South Australia

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

September 2015



DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any other university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except myself and where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give my permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

.....

Mini Aprem Vithayathil
BSc. (Honours, First Class)

TABLE OF CONTENTS

DECLARATION	II
TABLE OF CONTENTS	III
LIST OF FIGURES	XI
LIST OF TABLES	XVII
COMMONLY USED ABBREVIATIONS	XX
ACKNOWLEDGEMENTS.....	XXIII
RELATED PUBLICATIONS	XXV
ABSTRACT	XXVI
CHAPTER 1: LITERATURE REVIEW.....	2
1.1 OBESITY: A PUBLIC HEALTH EPIDEMIC	2
1.1.1 Childhood obesity.....	2
1.1.2 Obesity in pregnancy.....	3
1.1.3 Maternal obesity, high birth weight and obesity in later life	4
1.2 DEVELOPMENTAL PROGRAMMING OF OBESITY.....	7
1.2.1 The developmental origins of health and disease hypothesis	7
1.2.2 The role of nutrition in developmental programming	7
1.2.2.1 Epidemiological studies	10
1.2.2.2 Sheep models.....	13
1.2.2.3 Rodent models.....	13
1.2.3 Programming of obesity by maternal overnutrition: the proposed mechanisms	14
1.2.4 Prenatal/ perinatal programming of postnatal obesity	17

1.2.5	The role of early postnatal environment in the programming of obesity	19
1.2.5.1	Protective effect of breastfeeding against obesity?	20
1.2.5.2	The effect of maternal high-fat feeding on breast-milk composition	22
1.3	CURRENT OBESITY EPIDEMIC: THE ROLE OF CHANGES IN THE COMPOSITION OF MATERNAL DIET	24
1.3.1	The n-3 and n-6 fats: physiological roles	25
1.3.2	The effect of maternal cafeteria/Western diet during lactation on proximate and fatty acid composition of breast-milk	25
1.3.3	The role of dietary fat types as early determinants of adiposity in the offspring	27
1.3.4	Cafeteria diet feeding- A robust model	28
1.4	THE ADIPOCYTE: AN IMPORTANT TARGET FOR DEVELOPMENTAL PROGRAMMING	29
1.4.1	Development and function of adipose tissue	29
1.4.1.1	White and brown adipose tissue	29
1.4.2	Adipose tissue development	31
1.4.3	Regulation of adipogenesis	32
1.4.3.1	Determinants of adipose cell maturation	32
1.4.3.2	Transcriptional regulation of adipocyte differentiation	33
1.4.4	Regulation of lipogenesis and lipolysis	36
1.4.4.1	Transcriptional regulation of lipogenesis	36
1.4.4.2	Enzymes involved in lipogenesis	37
1.4.4.3	Hormonal regulation of lipogenesis	38
1.4.4.4	Lipolysis	41
1.4.5	Adipose tissue as an endocrine organ	41
1.4.5.1	Leptin	42
1.4.5.2	Adiponectin	43
1.4.6	Adipose cell development in the offspring: The importance of the prenatal and early postnatal periods in humans, sheep and rodents	45
1.5	SEX DIFFERENCES	46

1.5.1	Sex differences in the programming of obesity in response to maternal under/overnutrition during the prenatal and postnatal period.....	47
1.6	SUMMARY	49
1.7	EXPERIMENTAL HYPOTHESES.....	51
CHAPTER 2: THE CONTRIBUTION OF MATERNAL CAFETERIA DIETS DURING PREGNANCY AND LACTATION TO BODY WEIGHT, FAT MASS AND GLUCOSE TOLERANCE IN THE OFFSPRING.....		56
2.1	INTRODUCTION	56
2.2	MATERIALS AND METHODS.....	57
2.2.1	Animals and feeding regime	57
2.2.2	Mating and pregnancy.....	60
2.2.3	Cross-fostering.....	60
2.2.4	Determination of glucose tolerance	61
2.2.5	Post-mortem and tissue collection.....	61
2.2.6	Determination of plasma glucose and NEFA concentrations	62
2.2.7	Determination of plasma insulin and leptin concentrations.....	62
2.2.7.1	Validation of insulin and leptin ELISAs	63
2.2.7.2	Variations from standard protocol.....	64
2.2.8	Statistical analyses.....	64
2.3	RESULTS.....	65
2.3.1	Maternal nutritional intake	65
2.3.1.1	Nutritional intake of dams before pregnancy, during pregnancy and lactation.....	65
2.3.2	Maternal body weight	67
2.3.3	Birth and neonatal outcomes.....	67
2.3.4	Postnatal growth.....	69
2.3.4.1	Suckling period (Growth to weaning).....	69
2.3.4.2	Post-weaning period	69
2.3.5	Effect of prenatal and postnatal nutritional exposure on offspring body composition at 3 weeks and at 6 weeks of age	73

2.3.5.1	3 Weeks.....	73
2.3.5.2	6 Weeks.....	73
2.3.6	Effect of prenatal and postnatal nutritional exposure on offspring plasma hormone and metabolite concentrations at 3 weeks and 6 weeks of age.....	78
2.3.7	Relationship between plasma hormone and metabolite concentrations and total relative fat mass and individual fat mass at 3 weeks and 6 weeks of age.....	80
2.3.8	Glucose tolerance	81
2.4	DISCUSSION	84
2.4.1	Birth and neonatal outcomes.....	84
2.4.2	Postnatal growth.....	85
2.4.3	Body fat mass.....	87
2.4.4	Plasma leptin and insulin concentrations	89
2.4.5	Plasma glucose and glucose tolerance	90
2.5	SUMMARY	91
CHAPTER 3: EFFECT OF A ‘CAFETERIA DIET’ ON MATERNAL MILK AND OFFSPRING RED BLOOD CELL FATTY ACID COMPOSITION AND ITS RELATIONSHIP TO OFFSPRING FAT MASS		93
3.1	INTRODUCTION	93
3.2	MATERIALS AND METHODS.....	94
3.2.1	Animals and feeding regime.....	94
3.2.2	Measurement of food intake	95
3.2.3	Mating and pregnancy.....	95
3.2.4	Cross-fostering.....	95
3.2.5	Blood sample collection.....	96
3.2.6	Milk collection	96
3.2.7	Determination of total fat content and fatty acid composition	96
3.2.8	Statistical analysis	97
3.3	RESULTS.....	98

3.3.1	Fatty acid composition of the cafeteria diet	98
3.3.2	Maternal nutritional intake	99
3.3.3	Milk composition.....	102
3.3.4	Effect of maternal diet on offspring fatty acid status	104
3.3.4.1	Postnatal day 1	104
3.3.4.2	3 weeks	104
3.3.4.3	6 weeks	105
3.3.5	The relationship between maternal diet, milk composition and fat mass in the male and female offspring at weaning	109
3.4	DISCUSSION	111
3.4.1	Maternal diet and milk composition	111
3.4.2	Maternal cafeteria diets and offspring fatty acid status.....	113
3.4.3	Maternal dietary fatty acid intake, milk composition and offspring adiposity at weaning	114
3.5	SUMMARY	116
CHAPTER 4: EFFECT OF EXPOSURE TO MATERNAL CAFETERIA DIET DURING THE FETAL AND/OR SUCKLING PERIOD ON ADIPOCYTE GENE EXPRESSION IN THE OFFSPRING		118
4.1	INTRODUCTION.....	118
4.2	MATERIALS AND METHODS	120
4.2.1	Animals and feeding regime	120
4.2.2	Mating and pregnancy.....	120
4.2.3	Cross-fostering.....	120
4.2.4	Determination of plasma glucose, NEFA, insulin and leptin concentrations	121
4.2.5	Post-mortem and tissue collection.....	121
4.2.5	RNA extraction and reverse transcription.....	123
4.2.6	Determination of gene expression in the subcutaneous and retroperitoneal adipose tissue.....	123
4.2.7	Statistical analyses.....	126

4.3	RESULTS.....	127
4.3.1	Expression of adipogenic and lipogenic genes in subcutaneous and retroperitoneal adipose tissue at 3 weeks of age.....	127
4.3.1.1	SREBP-1c mRNA expression.....	127
4.3.1.2	PPAR- γ mRNA expression	131
4.3.1.3	G3PDH and FAS mRNA expression.....	133
4.3.1.4	Adiponectin mRNA expression	136
4.3.1.5	Leptin mRNA expression	139
4.3.2	Expression of adipogenic and lipogenic genes in subcutaneous and retroperitoneal adipose tissue at 6 weeks of age.....	142
4.3.2.1	SREBP-1c mRNA expression.....	142
4.3.2.2	PPAR- γ mRNA expression	145
4.3.2.3	G3PDH and FAS mRNA expression.....	147
4.3.2.4	Adiponectin mRNA expression	150
4.3.2.5	Leptin mRNA expression	153
4.3.3	Differences in the expression of adipogenic and lipogenic genes between subcutaneous and retroperitoneal adipose tissue in the male and female offspring at 3 weeks and 6 weeks of age	156
4.3.3.1	3 weeks	156
4.3.3.2	6 weeks	156
4.4	DISCUSSION	159
4.4.1	Impact of increased maternal nutrition during the suckling period on the expression of adipogenic and lipogenic genes in subcutaneous and retroperitoneal adipose tissue at 3 weeks and 6 weeks of age.....	159
4.4.1.1	SREBP-1c mRNA expression.....	159
4.4.1.2.	PPAR- γ mRNA expression	161
4.4.1.3	G3PDH and FAS mRNA expression.....	164
4.4.1.4	Adiponectin.....	165
4.4.1.5	Leptin.....	167
4.4.2	Differential expression of adipogenic and lipogenic genes in subcutaneous and retroperitoneal adipose tissue in the male and female offspring at 3 weeks and 6 weeks of age.....	169

4.5. SUMMARY	171
CHAPTER 5: EXPOSURE TO MATERNAL HIGH-FAT AND HIGH-SUGAR CAFETERIA DIET DURING LACTATION INCREASES OFFSPRING SUSCEPTIBILITY TO DIET-INDUCED OBESITY.....	174
5.1 INTRODUCTION	174
5.2 MATERIALS AND METHODS.....	175
5.2.1 Animals and feeding regime	175
5.2.2 Mating and pregnancy.....	175
5.2.3 Cross-fostering.....	175
5.2.4 Offspring feeding regime	176
5.2.5 Post-mortem and tissue collection.....	176
5.2.6 Determination of plasma glucose, NEFA, insulin and leptin concentrations	178
5.2.7 RNA extraction and reverse transcription.....	178
5.2.8 Determination of gene expression in the subcutaneous and retroperitoneal adipose tissue.....	178
5.2.9 Statistical analyses.....	178
5.3 RESULTS.....	180
5.3.1 Offspring growth during the control diet period and food preference diet period.....	180
5.3.2 The effect of prenatal and postnatal diet on offspring body fat mass at 3 months of age.....	182
5.3.3 Effect of prenatal and postnatal maternal diet on plasma hormones and metabolite concentrations at 3 months of age.	186
5.3.4 The relationship between percentage fat mass and plasma glucose, NEFA, insulin and leptin concentrations at 3 months of age.	186
5.3.5 Effect of prenatal and postnatal nutrition on the expression of adipogenic and lipogenic genes in subcutaneous and retroperitoneal tissue of male and female offspring at 3 months of age.....	190
5.3.5.1 SREBP-1c mRNA expression.....	190
5.3.5.2 PPAR- γ mRNA expression.....	193

5.3.5.3	G3PDH and FAS mRNA expression.....	196
5.3.5.4	Adiponectin mRNA expression	201
5.3.5.5	Leptin mRNA expression	203
5.3.6	Differences in the expression of adipogenic and lipogenic genes between subcutaneous and retroperitoneal adipose tissue in the male and female offspring at 3 months of age	206
5.4	DISCUSSION	208
5.4.1	Effect of prenatal and postnatal exposure to the cafeteria diet on offspring growth.	208
5.4.2	The role of prenatal and postnatal nutritional environment as a determinant of the susceptibility to diet-induced obesity in the offspring	209
5.4.3	The effect of prenatal and postnatal maternal diet on plasma hormones and metabolite concentrations at 3 months of age.	211
5.4.4	Effect of prenatal and postnatal nutrition on the expression of adipogenic and lipogenic genes in subcutaneous and retroperitoneal tissue at 3 months of age.....	212
5.4.4.1	SREBP-1c mRNA expression.....	212
5.4.4.2	PPAR- γ mRNA expression	213
5.4.4.3	G3PDH and FAS mRNA expression.....	214
5.4.4.4	Adiponectin mRNA expression	216
5.4.4.5	Leptin mRNA expression	217
5.4.5	Differential expression of adipogenic and lipogenic genes in subcutaneous and retroperitoneal adipose tissue in male and female offspring at 3 months of age.....	218
5.5	SUMMARY	220
CHAPTER 6: GENERAL DISCUSSION.....		223
BIBLIOGRAPHY.....		235

LIST OF FIGURES

Figure 1.1 Schematic representation of the intergenerational cycle of obesity.

Figure 1.2 Schematic representation of the effects of maternal nutrition on the health of the offspring.

Figure 1.3 The proposed pathway for the intergenerational transmission of T2DM.

Figure 1.4 A summary of the potential mechanisms which have been proposed to underlie the development of obesity after exposure to maternal nutrition or maternal obesity before birth.

Figure 1.5 Overview of stages in adipocyte differentiation.

Figure 1.6 Regulation of lipogenesis in adipocytes.

Figure 2.1 Mean daily intake of fat, protein, carbohydrate and total energy of Control dams and CAF dams before pregnancy (A), during pregnancy (B) and during the lactation period (C).

Figure 2.2 Body weight at weaning (3 weeks of age) for male (A) and female (B) offspring in the C-C, CAF-C, C-CAF and CAF-CAF groups.

Figure 2.3 Body weight at 6 weeks of age (3 weeks after weaning) for male (A) and female (B) offspring in the C-C, CAF-C, C-CAF and CAF-CAF groups.

Figure 2.4 Body fat mass (expressed as a percentage of total body weight) in male (A) and female (B) offspring in the C-C, CAF-C, C-CAF and CAF-CAF groups at 3 weeks of age.

Figure 2.5 Body fat mass (expressed as a percentage of total body weight) in male (A) and female (B) offspring in the C-C, C-CAF, CAF-C and CAF-CAF groups 6 weeks of age.

Figure 2.6 Blood glucose concentrations during the 2hr glucose tolerance test (2.0g/kg, intraperitoneal injection) results in male (A) and female (B) offspring at 6 weeks of age.

Figure 3.1 The effect of cafeteria feeding on total protein and total fat percentage (A) and fatty acid composition as a percentage of total lipids (B) in the milk of Control dams and Cafeteria dams.

Figure 3.2 The effect of cafeteria feeding on offspring fatty acid status as a percentage of total lipids in the postnatal day 1 red blood cell phospholipids of pups exposed to control diet and cafeteria diet during pregnancy.

Figure 4.1 The relative expression of SREBP-1c mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 weeks of age.

Figure 4.2 The relationship between SREBP-1c mRNA expression in subcutaneous adipose tissue and fat mass in subcutaneous fat depot in the male (A) and female (B) offspring and the relationship between SREBP-1c expression in retroperitoneal adipose tissue and fat mass in retroperitoneal fat depot in the male (C) and female (D) offspring at 3 weeks of age.

Figure 4.3 The relative expression of PPAR- γ mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 weeks of age.

Figure 4.4 The relative expression of G3PDH mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 weeks of age.

Figure 4.5 The relative expression of FAS mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 weeks of age.

Figure 4.6 The relative expression of adiponectin mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 weeks of age.

Figure 4.7 The relationship between adiponectin mRNA expression in subcutaneous adipose tissue and the relative mass of this fat depot in male (A) and female (B) offspring and the relationship between adiponectin mRNA expression in retroperitoneal adipose tissue and the relative mass of this fat depot in male (C) and female (D) offspring at 3 weeks of age.

Figure 4.8 The relative expression of leptin mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 weeks of age.

Figure 4.9 The relationship between leptin mRNA expression in subcutaneous adipose tissue and the relative mass of this fat depot in male (A) and female (B) offspring and the relationship between leptin mRNA expression in retroperitoneal adipose tissue and relative mass of this fat depot in male (C) and female (D) offspring at 3 weeks of age.

Figure 4.10 The relative expression of SREBP-1c mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 6 weeks of age.

Figure 4.11 The relative expression of PPAR- γ mRNA in subcutaneous (A, B) and retroperitoneal (C, D) adipose tissue in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 6 weeks of age.

Figure 4.12 The relative expression of G3PDH mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 6 weeks of age.

Figure 4.13 The relative expression of FAS mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 6 weeks of age.

Figure 4.14 The relative expression of adiponectin mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 6 weeks of age.

Figure 4.15 The relationship between adiponectin mRNA expression in subcutaneous adipose tissue and the relative mass of this fat depot in male (A) and female (B) offspring and the relationship between adiponectin mRNA expression in retroperitoneal adipose tissue and relative mass of this fat depot in male (C) and female (D) offspring at 6 weeks of age.

Figure 4.16 The relative expression of leptin mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 6 weeks of age.

Figure 4.17 The relationship between leptin mRNA expression in subcutaneous adipose tissue and the relative mass of this fat depot in male (A) and female (B) offspring and the relationship between leptin mRNA expression in retroperitoneal adipose tissue and the relative mass of this fat depot in male (C) and female (D) offspring at 6 weeks of age.

Figure 5.1 Experimental design

Figure 5.2 Body weight of male (A) and female (B) offspring during the post weaning control diet period and food preference diet period in C-C, CAF-C, C-CAF and CAF-CAF groups at 3 months of age.

Figure 5.3 Total body fat mass (expressed as a percentage of body weight) in male (A) and female offspring (B) in the C-C, CAF-C, C-CAF and CAF-CAF groups at 3 months of age.

Figure 5.4 Subcutaneous (A, B) and retroperitoneal fat mass (C, D) (expressed as a percentage of body weight) in male (A, C) and female (B, D) offspring in the C-C, CAF-C, C-CAF and CAF-CAF groups at 3 months of age.

Figure 5.5 The relative expression of SREBP-1c mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 months of age.

Figure 5.6 The relative expression of PPAR- γ mRNA in subcutaneous (A, B) and retroperitoneal (C, D) adipose tissue in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 months of age.

Figure 5.7 The relationship between PPAR- γ mRNA expression in subcutaneous adipose tissue and the relative mass of this fat depot in male offspring at 3 months of age.

Figure 5.8 The relative expression of G3PDH mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 months of age.

Figure 5.9 The relationship between G3PDH mRNA expression in subcutaneous adipose tissue and the relative mass of this fat depot in male offspring at 3 months of age.

Figure 5.10 The relative expression of FAS mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 months of age.

Figure 5.11 The relative expression of adiponectin mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female offspring (B, D) at 3 months of age.

Figure 5.12 The relative expression of leptin mRNA in subcutaneous (A, B) and retroperitoneal adipose tissue (C, D) in the C-C, CAF-C, C-CAF and CAF-CAF groups of male (A, C) and female (B, D) offspring at 3 months of age.

Figure 5.13 The relationship between leptin mRNA expression in subcutaneous adipose tissue and the relative mass of this fat depot in male offspring at 3 months of age.

LIST OF TABLES

Table 1.1 Metabolic disorders and diseases of adulthood that have been associated with nutritional imbalances during fetal life.

Table 2.1 Nutritional details of cafeteria diet and standard rodent feed.

Table 2.2 Birth outcomes for Control and CAF pregnancies.

Table 2.3 Mass of individual fat depots and major organs expressed as a percentage of bodyweight in male and female offspring in the C-C, CAF-C, C-CAF and CAF-CAF groups at 3 weeks of age.

Table 2.4 Mass of individual fat depots and major organs expressed as a percentage of body weight in male and female offspring in the C-C, CAF-C, C-CAF and CAF-CAF groups at 6 weeks of age.

Table 2.5 Plasma concentrations of glucose, NEFA and leptin in male and female offspring in the C-C, C-CAF, CAF-C and CAF-CAF groups at 3 weeks and plasma concentrations of glucose, NEFA, insulin and leptin in male and female offspring in the C-C, C-CAF, CAF-C and CAF-CAF groups at 6 weeks of age.

Table 2.6 The relationship between percentage total body fat mass and percentage individual fat masses with plasma glucose, NEFA, insulin and leptin concentrations, independent of treatment groups, in the male and female offspring at 3 weeks and 6 weeks of age.

Table 3.1 Fatty acid composition (percent) of the total fat in each diet item

Table 3.2 Maternal intake of fat (g/day), protein (g/day), total energy (KJ/day) and key fatty acids as a proportion of daily energy intake (%en) during pregnancy and lactation in control and cafeteria fed groups.

Table 3.3 Red blood cell phospholipid fatty acid composition expressed as a percentage of total fatty acids in male and female offspring of C-C, CAF-C, C-CAF and CAF-CAF groups at 3 weeks and 6 weeks of age.

Table 3.4 The relationship between maternal total fat (%en) and fatty acid intake (%en), total fat (%) and fatty acid composition (%) in the milk and total fat mass relative to body weight in the male and female offspring at weaning.

Table 4.1 Total number of animals included in each group at 3 weeks and 6 weeks in males and females.

Table 4.2 Primers sequences used for the determination of gene expression in adipose tissue by qRT-PCR.

Table 4.3 The relationship between the normalised expression of adipogenic and lipogenic genes in the subcutaneous and retroperitoneal adipose tissues and plasma glucose, NEFA, insulin and leptin concentrations, independent of treatment groups, in male and female offspring at 3 weeks of age.

Table 4.4 The relationship between the normalised expression of adipogenic and lipogenic genes in the subcutaneous and retroperitoneal adipose tissues and plasma glucose, NEFA, insulin and leptin concentrations, independent of treatment group, in male and female offspring at 6 weeks of age.

Table 4.5 The normalised expression of adipogenic and lipogenic genes between subcutaneous and retroperitoneal adipose tissue in the male and female offspring, independent of treatment groups, at 3 weeks and 6 week of age.

Table 5.1 Mass of individual fat depots expressed as a percentage of body weight in male and female offspring in the C-C, CAF-C, C-CAF and CAF-CAF groups at 3 months of age.

Table 5.2 Plasma concentrations of glucose, NEFA, insulin and leptin in male and female offspring in the C-C, C-CAF, CAF-C and CAF-CAF groups at 3 months of age.

Table 5.3 The relationship between percentage total body fat mass and percentage individual fat mass with plasma glucose, NEFA, insulin and leptin concentrations, independent of treatment groups, in the male and female offspring at 3 months of age.

Table 5.4 The relationship between the normalised expression of adipogenic and lipogenic genes in the subcutaneous and retroperitoneal adipose tissues and plasma glucose, NEFA, insulin and leptin concentrations, independent of treatment groups, in male and female offspring at 3 months of age.

Table 5.5 The normalised expression of adipogenic and lipogenic genes between subcutaneous and retroperitoneal adipose tissue in the male and female offspring, independent of treatment groups, at 3 months of age.

COMMONLY USED ABBREVIATIONS

A B C

AA	arachidonic acid
ACC	acetyl-CoA Carboxylase
ACS	acyl-CoA synthetase
ACOD	acyl-CoA oxidase
<i>ad libitum</i>	to any desired extent
ADD-1	adipocyte determination and differentiation-1
ALA	alpha-linolenic acid
ANOVA	analysis of variance
ASP	acylation-stimulating protein
ATP	adenosine triphosphate
ATGL	adipose triglyceride lipase
AUC	area under the curve
BAT	brown adipose tissue
BHT	butylated hydroxyl toluene
BMI	body mass index
C	control
CAF	cafeteria
cDNA	complementary deoxyribonucleic acid
cAMP	cyclic adenosine monophosphate
CART	cocaine- and amphetamine-regulated transcript
C/EBP α	CCAAT/enhancer-binding protein-alpha
C/EBP β	CCAAT/enhancer-binding protein-beta
C/EBP γ	CCAAT/enhancer-binding protein-gamma
CoA	Coenzyme A

D E F G

D	day(s)
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
DOHaD	Developmental Origins of Health and Disease
dsDNA	double stranded deoxyribonucleic acid
DR	diet resistant
EDTA	ethylenediamine tetraacetic acid
EGF	epidermal growth factor
ELISA	enzyme linked immunosorbent assay
EIA	enzyme immunoassay
EPA	eicosapentaenoic acid

ERR α	estrogen related receptor alpha
FABP	fatty acid binding protein
FAME	fatty acid methyl esters
FATP	fatty acid transport protein
FAS	fatty acid synthase
FFA	free fatty acids
FIAF	fasting-induced adipose factor
FID	flame ionisation detector
GDP	guanosine diphosphate
GDM	gestational diabetes mellitus
GH	growth hormone
G3PDH	glycerol 3-phosphate dehydrogenase
G6PD	glucose-6-phosphate dehydrogenase
GPAT	glycerol-3-phosphate acyltransferase

HIJKL

HRP	horseradish peroxidase
HSL	hormone sensitive lipase
IGFs	insulin-like growth factors
IGF-I	insulin-like growth factor I
IPGTT	Intraperitoneal glucose tolerance tests
IRS	insulin receptor substrate
LA	linoleic acid
LDL-R	lipoprotein receptor
LPL	lipoprotein lipase
LCPUFA	long chain polyunsaturated fatty acids

MNO

ME	malic enzyme
MEFA	methy-N-ethyl-N(β -hydroxyethyl)-aniline
mRNA	messenger ribonucleic acid
min	minute(s)
MGL	monoglyceride lipase
NEFA	non-esterified free fatty acids
n-3	omega-3
n-6	omega-6

P Q R S

PEPCK	phosphoenolpyruvate carboxykinase
POD	peroxidase
PGAR	PPAR- γ angiopoietin related peptide
PI3K	phosphoinositide 3-kinase
6-PG	6-phosphogluconate
PND1	postnatal day 1
PPAR- γ	peroxisome proliferator- activated receptor gamma
PUFA	polyunsaturated fatty acids

RBC	red blood cells
rRNA	ribosomal ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
qRT-PCR	quantitative real time PCR
RXR	retinoid-acid receptor

SC	subcutaneous
SCD-1	stearoyl-CoA desaturase-1
SEM	standard error of the mean
SPSS	statistical package for social sciences
SREBP	sterol regulatory element binding proteins

T U V W X Y Z

T2DM	type 2 diabetes mellitus
TG	triglyceride
TGF α	transforming growth factor-alpha
TGF β	transforming growth factor-beta
TLC	thin layer chromatography
TMB	Tetramethylbenzidine
TNF- α	tumour necrosis factor- α
UCP-1	uncoupling protein 1
WAT	white adipose tissue
WHO	World Health Organization

ACKNOWLEDGEMENTS

My PhD journey wouldn't have been an interesting one without the help of many people around me. First and foremost, I would like to acknowledge my sincere gratitude to my primary supervisor Dr. Beverly Muhlhausler for her invaluable help, support and guidance throughout my PhD candidature. Thank you Bev, for giving me all the opportunities to improve my knowledge and skills and also for making my research life smooth and rewarding. Your dedication to research has always inspired me and your encouragement and constructive comments throughout the course of my study have helped me to develop the skills that are required to become a good researcher.

I would like to express my sincere thanks to my co-supervisor Professor Robert Gibson for all his guidance and support. His constructive criticism and efforts have helped to improve my presentation skills as well as my research career. Thank you for all your kind help, Bob.

A special thanks to Dr. John Carragher, for his invaluable support throughout my PhD candidature. Your critical reviews have helped me a lot to improve my presentation skills. Thank you for all your advice and encouragement John.

I would also like to thank Dr. Zhi Yi Ong, Pamela Sim, Lauren Astill, and Romain Lacaze for all their support and assistance with animal experiments. Special thanks to Dr. Zhi Yi Ong and Pamela Sim for teaching me valuable animal handling techniques. My sincere thanks and appreciation also goes to Dr. Wei-Chun Tu, Dr. Zhi Yi Ong, Pamela Sim, David Apps and Ela Zielinski for helping me to learn the lab techniques that were required to complete my project. Thank you everyone for all the support.

Also, I would like to thank all my lab buddies at the Food and Nutrition laboratory and the entire FOODplus research group who all made it a convivial

place to work. I would particularly like to acknowledge my wonderful colleagues Jess Gugusheff and Dao Hunh for all the support.

In addition, I would like to acknowledge the financial support I have received from University of Adelaide Australian Postgraduate Award and Healthy Development Adelaide and Channel 7 Children's Research Foundation.

My deepest gratitude and appreciation are devoted to my mother and father (in memorial), my parents in law and all other family members and friends for their constant love, unwavering support and prayers during all these years. I am indebted to my husband, Jacob Pakrath, for his unflagging love, care, concern and support throughout my life and to my sweet little angels; Alina, Catherine and Donna for their understanding and boundless love. This dissertation is simply impossible without them. Thank you dear ones, this thesis is dedicated to you all....

Last but not least, thanks be to God for my life through all tests in the past years. You have made my life more bountiful. May your name be exalted, honoured, and glorified.

RELATED PUBLICATIONS

1. Gugusheff, JR., **Vithayathil, M.**, Ong, ZY., & Muhlhausler, B. S. (2013). The effects of prenatal exposure to a 'junk food'diet on offspring food preferences and fat deposition can be mitigated by improved nutrition during lactation. *Journal of Developmental Origins of Health and Disease*, 4(05), 348-357.
2. Muhlhausler, BS., Gugusheff, JR., Ong, ZY., & **Vithayathil, M. A.** (2013). Pregnancy, obesity and insulin resistance: maternal overnutrition and the target windows of fetal development. *Hormone Molecular Biology and Clinical Investigation*, 15(1), 25-36.
3. Muhlhausler BS, Gugusheff JR, Ong ZY, **Vithayathil MA**. Nutritional approaches to breaking the intergenerational cycle of obesity. *Canadian Journal of Physiology and Pharmacology*. 2013; 91:421-8.
4. Muhlhausler, B. S, **Vithayathil, M. A.** Impact of maternal obesity on offspring adipose tissue: lessons for the clinic. *Expert Review of Endocrinology & Metabolism* 10/2014; 9(6).

ABSTRACT

There is growing evidence that maternal obesity, maternal hyperglycemia or maternal intake of diets high in fat, sugar or total calories during pregnancy and lactation is associated with an increased risk of obesity and metabolic diseases in the offspring. The majority of studies to date, however, have examined the impact of maternal overnutrition during the entire perinatal period. While a small number of studies have provided clues that the impact of exposure to nutritional excess before birth in comparison to exposure during the early postnatal period may not be equivalent, the results of these studies have been inconsistent. Therefore, the relative contribution of prenatal and postnatal nutritional environment to obesity risk in the offspring remains unclear. The central aim of this thesis was to investigate the separate contributions of exposure to a maternal cafeteria diet during the prenatal and suckling periods on the metabolic outcomes of the offspring, specifically body weight, fat mass and the expression of key adipogenic and lipogenic genes at weaning, in early adolescence and in young adulthood using a cross-fostering approach in a rat model.

The results of this thesis demonstrated that exposure to a maternal cafeteria diet during the suckling period is more important for determining fat mass at weaning than exposure before birth. Importantly, this thesis provided considerable evidence to suggest that exposure to a nutritionally-balanced diet during the suckling period has the capacity to prevent the negative effects of exposure to a high-fat/high-sugar diet before birth. In addition, this thesis has demonstrated that the effects of being exposed to a high-fat/high-sugar diet during the perinatal period on offspring adiposity could be reversed/controlled by consuming a nutritionally-balanced diet post-weaning.

The results of this thesis also demonstrated that the levels of total fat, saturated and trans fats and omega-6 polyunsaturated fatty acids (n-6 PUFA) in the dams

milk were directly related to their levels in the maternal diet, and were higher in dams consuming a cafeteria diet. This supported the hypothesis that altered fat content and fatty acid composition of the milk is likely to play an important role in mediating the effects of maternal cafeteria diets on offspring fat mass, and may well account for the higher adiposity at weaning in offspring suckled by cafeteria-diet fed dams. Exposure to a cafeteria diet during the suckling period also resulted in altered expression of key adipogenic and lipogenic genes in visceral and subcutaneous fat depots and an increased susceptibility to diet-induced obesity in females. Importantly, this thesis provided evidence of clear sex-differences in the relative impact of prenatal and postnatal nutritional exposures on adipocyte gene expression and the susceptibility to diet-induced obesity in the offspring, suggesting that the timing of nutritional interventions aimed to re-program the offspring may be different in males and females.

Overall, this thesis identifies the early postnatal period in rodents as a 'critical window' for the programming of fat mass and susceptibility to diet-induced obesity in the offspring, and has provided important insights into the mechanisms underlying the early origins of obesity.