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

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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.

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## COMMONLY USED ABBREVIATIONS

### A B C

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>arachidonic acid</td>
</tr>
<tr>
<td>ACC</td>
<td>acetyl-CoA Carboxylase</td>
</tr>
<tr>
<td>ACS</td>
<td>acyl-CoA synthetase</td>
</tr>
<tr>
<td>ACOD</td>
<td>acyl-CoA oxidase</td>
</tr>
<tr>
<td>ad libitum</td>
<td>to any desired extent</td>
</tr>
<tr>
<td>ADD-1</td>
<td>adipocyte determination and differentiation-1</td>
</tr>
<tr>
<td>ALA</td>
<td>alpha-linolenic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ASP</td>
<td>acylation-stimulating protein</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ATGL</td>
<td>adipose triglyceride lipase</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>BAT</td>
<td>brown adipose tissue</td>
</tr>
<tr>
<td>BHT</td>
<td>butylated hydroxyl toluene</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>C</td>
<td>control</td>
</tr>
<tr>
<td>CAF</td>
<td>cafetera</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CART</td>
<td>cocaine- and amphetamine-regulated transcript</td>
</tr>
<tr>
<td>C/EBPα</td>
<td>CCAAT/enhancer-binding protein-alpha</td>
</tr>
<tr>
<td>C/EBPβ</td>
<td>CCAAT/enhancer-binding protein-beta</td>
</tr>
<tr>
<td>C/EBPγ</td>
<td>CCAAT/enhancer-binding protein-gamma</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
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### D E F G

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>day(s)</td>
</tr>
<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOHaD</td>
<td>Developmental Origins of Health and Disease</td>
</tr>
<tr>
<td>dsDNA</td>
<td>double stranded deoxyribonucleic acid</td>
</tr>
<tr>
<td>DR</td>
<td>diet resistant</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine tetraacetic acid</td>
</tr>
<tr>
<td>EGF</td>
<td>epidermal growth factor</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>EIA</td>
<td>enzyme immunoassay</td>
</tr>
<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ERRα</td>
<td>estrogen related receptor alpha</td>
</tr>
<tr>
<td>FABP</td>
<td>fatty acid binding protein</td>
</tr>
<tr>
<td>FAME</td>
<td>fatty acid methyl esters</td>
</tr>
<tr>
<td>FATP</td>
<td>fatty acid transport protein</td>
</tr>
<tr>
<td>FAS</td>
<td>fatty acid synthase</td>
</tr>
<tr>
<td>FFA</td>
<td>free fatty acids</td>
</tr>
<tr>
<td>FIAF</td>
<td>fasting–induced adipose factor</td>
</tr>
<tr>
<td>FID</td>
<td>flame ionisation detector</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>GDM</td>
<td>gestational diabetes mellitus</td>
</tr>
<tr>
<td>GH</td>
<td>growth hormone</td>
</tr>
<tr>
<td>G3PDH</td>
<td>glycerol 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>G6PD</td>
<td>glucose-6-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GPAT</td>
<td>glycerol-3-phosphate acyltransferase</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>HSL</td>
<td>hormone sensitive lipase</td>
</tr>
<tr>
<td>IGFs</td>
<td>insulin-like growth factors</td>
</tr>
<tr>
<td>IGF-I</td>
<td>insulin-like growth factor I</td>
</tr>
<tr>
<td>IPGTT</td>
<td>Intraperitoneal glucose tolerance tests</td>
</tr>
<tr>
<td>IRS</td>
<td>insulin receptor substrate</td>
</tr>
<tr>
<td>LA</td>
<td>linoleic acid</td>
</tr>
<tr>
<td>LDL-R</td>
<td>lipoprotein receptor</td>
</tr>
<tr>
<td>LPL</td>
<td>lipoprotein lipase</td>
</tr>
<tr>
<td>LCPUFA</td>
<td>long chain polyunsaturated fatty acids</td>
</tr>
<tr>
<td>ME</td>
<td>malic enzyme</td>
</tr>
<tr>
<td>MEFA</td>
<td>methy-N-ethyl-N(β-hydroxyethyl)-aniline</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>MGL</td>
<td>monoglyceride lipase</td>
</tr>
<tr>
<td>NEFA</td>
<td>non-esterified free fatty acids</td>
</tr>
<tr>
<td>n-3</td>
<td>omega-3</td>
</tr>
<tr>
<td>n-6</td>
<td>omega-6</td>
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### P Q R S

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>PEPCK</td>
<td>phosphoenolpyruvate carboxykinase</td>
</tr>
<tr>
<td>POD</td>
<td>peroxidase</td>
</tr>
<tr>
<td>PGAR</td>
<td>PPAR-γ angiopoietin related peptide</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>6-PG</td>
<td>6-phosphogluconate</td>
</tr>
<tr>
<td>PND1</td>
<td>postnatal day 1</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acids</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>red blood cells</td>
</tr>
<tr>
<td>rRNA</td>
<td>ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>quantitative real time PCR</td>
</tr>
<tr>
<td>RXR</td>
<td>retinoid-acid receptor</td>
</tr>
<tr>
<td>SC</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>SCD-1</td>
<td>stearoyl-CoA desaturase-1</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SPSS</td>
<td>statistical package for social sciences</td>
</tr>
<tr>
<td>SREBP</td>
<td>sterol regulatory element binding proteins</td>
</tr>
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</table>

### T U V W X Y Z

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>T2DM</td>
<td>type 2 diabetes mellitus</td>
</tr>
<tr>
<td>TG</td>
<td>triglyceride</td>
</tr>
<tr>
<td>TGFα</td>
<td>transforming growth factor-alpha</td>
</tr>
<tr>
<td>TGFβ</td>
<td>transforming growth factor-beta</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetramethylbenzidine</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumour necrosis factor-α</td>
</tr>
<tr>
<td>UCP-1</td>
<td>uncoupling protein 1</td>
</tr>
<tr>
<td>WAT</td>
<td>white adipose tissue</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

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RELATED PUBLICATIONS


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.