Diet-Induced Obesity Influences Oocyte Developmental Competence Via Peroxisome Proliferator-Activated Receptor Gamma (PPARG)-Mediated Mechanisms

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Declaration

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Cadence E Minge

November 2008
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Abstract

Across the world more women of childbearing age are becoming overweight and obese. Although overweight women have similar co-morbidity and stigmata as men they also experience problems specific to their gender. In particular, there is significant evidence that overweight and obese women require a longer time to successfully conceive, suggesting influence of bodyweight and adipose tissue mass upon the events surrounding conception.

This thesis investigated the interaction between diet-induced obesity and female reproductive function. To achieve this, the influence of maternal obesity-induced insulin resistance on ovulation and oocyte health, as indicated by subsequent embryonic developmental competence was determined.

Obesity adversely affects many aspects of health, and rodent models of diet-induced obesity are commonly used to investigate these consequences. However the impact of strain and genetic background on phenotypic response to diet, particularly in females, has not been systematically defined. We therefore characterised female metabolic responses of five different strains of laboratory mouse (Swiss, Balb/c, C57BL/6, CBA/CaH and 129T2Sv/Ems) to a “Western” high fat diet (22% fat, 0.15% cholesterol) and matched control diet (6% fat, 0% cholesterol). After 16 weeks of diet exposure the development and extent of hyperglycaemia, hyperinsulinaemia, insulin resistance, dyslipidaemia, and markers of chronically inflamed adipose tissue depots varied profoundly across the different strains.

To then determine if a perturbed metabolic profile triggers female infertility, these female mice were mated with strain matched, non-obese males, and zygotes extracted from the reproductive tract immediately following fertilization. Despite strain-dependent variation in susceptibility to the development of obesity, dyslipidaemia and insulin resistance, all mice investigated exhibit some degree of impaired reproductive potential following exposure to a high fat diet. We documented alteration to ovulation incidence and rate, fertilization, early embryo development to the blastocyst stage, and blastomere differentiation into the inner cell mass and trophectoderm cell lineages.

The nature of obesity-induced perturbation of female reproductive processes was more closely examined using statistical modelling which identified the specific metabolic parameters that were strongly associated with reproductive defects. These associations were consistent across the range of genetic backgrounds assessed and highlighted key mediators of this interaction, in particular, insulin resistance.
To determine if ovarian gene products already implicated in other reproductive outcomes are differentially regulated under conditions of obesity, ovarian mRNA collected at the pro-estrous (pre-ovulatory) stage of the reproductive cycle was applied to microarray slides developed through Suppressive Subtractive Hybridization. Two different gene chips that were enriched for ovarian genes were used. A number of genes were minimally regulated, and there was lack of significant validation in subsequent, and larger, sample cohorts. These findings have provided substantial technical information, and new experimental designs that overcome the current limitations have been established to obtain more informative data.

The role that insulin resistance plays in folliculogenesis and the development of oocyte developmental competence was more closely investigated. Hyperinsulinemia can interfere directly with ovarian cell function or be indirectly associated with other hormonal conditions detrimental to optimal fertility. To reverse the effects of obesity/hyperinsulinemia and identify the signalling pathways responsible for disruption of pre-implantation events, obese female mice were treated for 4 days prior to mating with three different insulin-sensitizing and plasma glucose-reducing pharmaceuticals: glucose and lipid-lowering AMP Kinase activator, AICAR, 30mg/kg/day; IκK inhibitor that reverses insulin resistance, sodium salicylate, 50mg/kg/day; or Peroxisome Proliferator-Activated Receptor Gamma (PPARG) agonist rosiglitazone, 10mg/kg/day. AICAR or sodium salicylate treatment did not have significant effects on the reproductive parameters examined. However, embryonic development to the blastocyst stage was significantly improved when diet-induced obese mice were treated with rosiglitazone, effectively repairing development rates. Rosiglitazone also normalized obesity-associated abnormal blastomere allocation to the inner cell mass. Such improvements to oocyte quality were coupled with weight loss, improved glucose metabolism and changes in ovarian mRNA expression of PPARG-regulated cholesterol transporters.

Overall, this thesis has demonstrated for the first time a link between maternal obesity and the ovarian follicle can impede oocyte health and developmental potential. As a result, the oocyte released at ovulation expresses impaired developmental competence following to conception. Key cellular pathways have been identified in this relationship, specifically PPARG-directed cell responses.
Publications arising from this thesis:

- Minge CE, Bennett BD, Norman RJ, Robker RL *Peroxisome Proliferator-Activated Receptor gamma Agonist Rosiglitazone Reverses the Adverse Effects of Diet-Induced Obesity on Oocyte Quality*. Endocrinology 2008, 149:2646-2656


- Minge CE, Bennett BD, Tsagareli V, Lane M, Owens JA, Norman RJ, Robker RL *Inflammatory and Metabolic Syndrome Phenotypes are Strain-Dependent in Female Mice with Diet-Induced Obesity* (in preparation)


Abstracts arising:

2007

- Minge CE *Peroxisome Proliferator-Activated Receptor gamma (PPARγ) Agonist Rosiglitazone Reverses the Adverse Effects of Diet-Induced Obesity on Ovarian Function and Female Fertility*. Young Investigator of the Year, October 2007, Adelaide, Australia. (The Young Investigator Award is a highly successful event rewarding excellence in South Australia's young researchers in both science and their ability to communicate and 'sell' that science.)

- Minge CE, Bennett BD, Lane M, Norman RJ, Robker RL *Impaired oocyte developmental competence arises from diet-induced obesity and can be reversed by peri-ovulatory rosiglitazone treatment*. Annual Meeting of the Society for Reproductive Biology, September 2007, Christchurch, New Zealand.

- Minge CE, Bennett BD, Lane M, Norman RJ, Robker RL *Obesity-Induced Female Infertility Arises From Impaired Oocyte Developmental Competence And Can Be Reversed By Peri-
Ovulatory Rosiglitazone Treatment. Ross Wishart Memorial Award, SA Australian Society for Medical Research (ASMR) Scientific Meeting, June 2007, Adelaide, Australia. (The Ross Wishart Memorial Award is presented to the most outstanding postgraduate presentation at the South Australian ASMR Annual Scientific Meeting.)

2006

- Minge CE, Bennett BD, Lane M, Norman RJ, Robker RL Obesity-Induced Female Infertility Arises From Impaired Oocyte Developmental Competence And Can Be Reversed By Peri-Ovulatory Rosiglitazone Treatment. 10th International Congress on Obesity, September 2006, Sydney, Australia.

- Minge CE PPARγ Agonist Rosiglitazone Reverses the Adverse Effects of Diet-Induced Obesity on Ovarian Function and Female Fertility. Young Investigator Award Semi-Finals, September 2006, Adelaide, Australia.

- Minge CE, Bennett BD, Lane M, Norman RJ, Robker RL Peri-Ovulatory Rosiglitazone Treatment Reverses Obesity-Induced Female Infertility Arising From Impaired Oocyte Developmental Competence. 39th Annual Meeting of the Society for the Study of Reproduction, August 2006, Omaha, USA.

- Minge CE, Bennett BD, Tsagareli V, Norman RJ, Lane M, Robker RL. Ovulation and Oocyte Quality are Reduced in Mice with Diet-Induced Obesity. 88th Annual Meeting of the Endocrine Society, June 2006, Boston, USA.

- Minge CE, Bennett BD, Tsagareli V, Norman RJ, Lane M, Robker RL. Ovulation and Oocyte Quality are Reduced in Mice with Diet-Induced Obesity. “Five minutes of excitement and fame”, “Womb to Tomb” Human Reproductive Health Throughout the Ages International Congress, March 2006, Adelaide Australia.

2005

## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>17β-HSD</td>
<td>17 beta-hydroxysteroid dehydrogenase</td>
</tr>
<tr>
<td>3β-HSD</td>
<td>3 beta-hydroxysteroid dehydrogenase</td>
</tr>
<tr>
<td>A</td>
<td>androgen</td>
</tr>
<tr>
<td>aaRNA</td>
<td>uridine 5’-triphosphate-amino allyl ribonucleic acid</td>
</tr>
<tr>
<td>AcLDL</td>
<td>acetylated low density lipoprotein</td>
</tr>
<tr>
<td>AGE</td>
<td>advanced glycated ends</td>
</tr>
<tr>
<td>AGER</td>
<td>advanced glycated ends receptor</td>
</tr>
<tr>
<td>AICAR</td>
<td>5-aminoimidazole 4-carboxamide-riboside</td>
</tr>
<tr>
<td>AMPK</td>
<td>5’AMP-activated protein kinase</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ART</td>
<td>assisted reproductive technologies</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BMPs</td>
<td>bone morphogenic proteins</td>
</tr>
<tr>
<td>CC</td>
<td>clomiphene citrate</td>
</tr>
<tr>
<td>CD</td>
<td>control diet</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
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<tr>
<td>CE</td>
<td>cholesterol ester</td>
</tr>
<tr>
<td>CETP</td>
<td>cholesteryl ester transfer protein</td>
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<tr>
<td>CL</td>
<td>corpus luteum</td>
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<tr>
<td>cm</td>
<td>centimetres</td>
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<tr>
<td>COC</td>
<td>cumulus oocyte complex</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CT</td>
<td>threshold cycle</td>
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<td>CYP17</td>
<td>cytochrome P450cyp17</td>
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<tr>
<td>DHEA</td>
<td>dehydroepiandrosterone</td>
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<tr>
<td>DHEA-S</td>
<td>dehydroepiandrosterone-sulfate</td>
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<tr>
<td>DIO</td>
<td>diet-induced obesity</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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E/E2 estrogen
EDTA ethylenediaminetetraacetic acid
FAI free androgen index
FF follicular fluid
FFA free fatty acids
FSH follicle stimulating hormone
g grams
GDF9 growth differentiation factor 9
GEE generalized estimating equation
GnRH gonadotrophin releasing hormone
GV germinal vesicle
GVBD germinal vesicle breakdown
h hours
HbA(1C) hemoglobin A1C (glycosylated hemoglobin)
HDL high density lipoprotein
HDL-C HDL-cholesterol
HFD high fat diet
HOMA homeostasis model of assessment
HOMA-IR HOMA-insulin resistance
ICM inner cell mass
ICSI intra-cytoplasmic sperm injection
IGF insulin-like growth factor
IGFBP IGF binding protein
IKK-b IκB kinase beta
IL interleukin
IPGTT intraperitoneal glucose tolerance test
IU international units
IVF in vitro fertilization
kg kilograms
L litres
LDL low density lipoprotein
LDL-C LDL-cholesterol
LH  luteinizing hormone
LMD laser microdissection
M  metres
mg milligrams
MJ mega joules
ml millilitres
mmol milli mole
mRNA messenger RNA
MZT maternal-zygotic transition
NF-κB nuclear factor-kappa B
ng nanograms
NSAID non-steroidal anti-inflammatory drugs
OGTT oral glucose tolerance test
oxLDL oxidized LDL
PBR peripheral benzodiazepine receptor
PCOS polycystic ovary syndrome
PCR polymerase chain reaction
pg picograms
pmol pico mole
PPARG peroxisome proliferator activated receptor-γ
PPRE PPAR response element
QUICKI quantitative insulin-sensitivity check index
R2 coefficient of determination
RIA radio-immuno assay
RNA ribonucleic acid
ROS reactive oxygen species
RT reverse transcription
RXR retinoid X receptor
SDS sodium dodecyl sulfate
SEM standard error
SHBG sex hormone binding globulin
SSH Suppression subtractive hybridization
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>STAR</td>
<td>steroidogenic acute regulatory protein</td>
</tr>
<tr>
<td>T</td>
<td>testosterone</td>
</tr>
<tr>
<td>TE</td>
<td>trophoderm</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>UCP</td>
<td>uncoupling protein</td>
</tr>
<tr>
<td>µg</td>
<td>micrograms</td>
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<tr>
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<td>micrometres</td>
</tr>
<tr>
<td>Vol</td>
<td>volume</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHR</td>
<td>waist-hip ratio</td>
</tr>
<tr>
<td>Wt</td>
<td>weight</td>
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