

Effects of Maternal Cigarette Smoke Exposure on Renal and Other Health Outcomes in Mice Offspring

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

By

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Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

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Date:

Dedication

To the memory of my *late*
mother

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List of Abbreviations

ACE	angiotensin-I-converting enzyme
AT	angiotensin type
ATGL	adipose triglyceride lipase
AUC	area under the curve
BMI	body mass index
BMP	bone morphogenetic protein
BW	body weight
CKD	Chronic kidney disease
CPT	carnitine palmitoyl transferase
DDti3	DNA damage-inducible transcript 3
E	embryonic day
ECM	extracellular matrix protein
eNOS	Endothelial nitric oxide synthase
ET	endothelin
Eya1	eyes absent 1
FASN	Fatty acid synthase
FGF	Fibroblast growth factor
FOXO	forkhead box protein O
GDNF	glial-cell line-derived neurotrophic factor
GFR	Glomerular Filtration Rate
GFR α 1	GDNF-family receptor α 1
Glut	Glucose transporter
h	hour
H&E	hematoxylin and eosin
HRP	horse radish peroxidase
HSL	hormone-sensitive lipase
IHC	immunohistochemistry
i.p	intraperitoneally
IPGTT	Intraperitoneal glucose tolerance test
IGF	Insulin-like growth factor
IL	interleukin
IM	intermediate mesoderm

IUGR	intrauterine growth retardation
LBW	low birth weight
LC	L-Carnitine
LIF	leukaemia inhibiting factor
mA	milliAmpere
MCP	monocyte chemoattractant protein
MM	metanephric mesenchyma
MSA	murine serum albumin
NC	nephrogenic cord
NEFA	non-esterified fatty acids
NHEBSA	normal horse serum with bovine serum albumin
NFκB	nuclear factor kappa B
NO	nitric oxide
NOS	NO synthase
Pax	paired box transcription factor
PGC	Peroxisome proliferator-activated receptor gamma coactivator
P	postnatal day
PAS	periodic acid Schiff
PPAR	peroxisome proliferator-activated receptor
RAS	renin-angiotensin system
RCF	relative centrifuge force
RET	receptor tyrosine kinase
ROS	reactive oxygen species
RV	renal vesicle
SE	smoke exposure
SEM	standard error of the mean
TG	triglyceride
TGF-β	transforming growth factor-β
TLR	Toll-like receptor
TMB	Tetramethylbenzidine
TNFα	tumour necrosis factor α
T-TBS	tween twenty trise buffer saline
UB	ureteric bud

UV	ultra violet
W13	week 13
WNT	wingless–type MMTV integration site family member
Wt1	Wilms tumor 1
Xbp	X-box binding protein

List of peer reviewed Publications

- **Al-Odat I**, Chen H, Chan Y-L, Sawiris A. Wong MG, Gill A, Pollock C, Saad S, (2014). The impact of maternal cigarette smoke exposure in a rodent model on renal development in the offspring. *PLoS ONE* 9(7): e103443.
- Stangenberg, S., Nguyen, L.T., Chen, H., **Al-Odat, I.**, Killingsworth, M.C., Gosnell, M.E., Anwer, A.G., Goldys, E.M., Pollock, C.A. & Saad, S. 2015, 'Oxidative stress, mitochondrial perturbations and fetal programming of renal disease induced by maternal smoking', *The International Journal of Biochemistry & Cell Biology*, vol. 64, pp. 81-90.
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List of conference abstracts

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Poster presentations

- **Ibrahim Al-Odat**, Hui Chen, Amgad Zaky, Carol Pollock and Sonia Saad, Maternal L-Carnitine Supplementation Reversed Kidney Underdevelopment and Adulthood Renal Dysfunction in Male Mice Offspring of Cigarette Smoke Exposed Dams. *New Horizons Conference 2015*, University of Technology Sydney, Sydney.
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- **Ibrahim Al-Odat**, Hui Chen, Amgad Sawiris, Carol Pollock and Sonia Saad. l-carnitine supplementation during gestation and lactation improve glucose intolerance induced by maternal smoking in the offspring. *The 50th Annual Scientific Meeting of the Australian and New Zealand Society of Nephrology*, 2014. Melbourne, Victoria, Australia
- **Al-Odat, Ibrahim**, Chen, Hui, Chan, Yik Lung, Sawaris, Amgad, Pollock, Carol and Saad, Sonia. Gender related differences in susceptibility to kidney disease in the mice offspring due to maternal cigarette smoke exposure. *New Horizons Conference 2014*, Kolling Institute of Medical Research, Sydney.
- **Ibrahim Al-Odat**, Sonia Saad, Carol Pollock and Hui Chen. Maternal smoking as an intrauterine factor to disturb fetal renal development and cause renal disorders in postnatal life. *RNSH/UTS/KIMR/USYD Scientific Research Meeting*, 2012.

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Abstract

Background

The burden of kidney disease is significantly increased worldwide. Developmental programming of non-communicable diseases is an established paradigm. Therefore, there is increasing attention to the contribution of intrauterine and early post-natal environmental insults to the risk of adulthood kidney diseases. One of such insults is maternal cigarette smoke exposure (SE), which is associated with intrauterine growth retardation (IUGR) and some adulthood diseases in offspring. However, it is unknown whether maternal SE can increase the risk of developing chronic kidney disease (CKD) in offspring. Maternal SE was also shown to increase renal oxidative stress in offspring; while the anti-oxidants L-carnitine (LC) supplementation has been shown to be beneficial in many human diseases, which may benefit such offspring.

Objectives

This thesis aimed to study the effect of maternal SE on, 1) kidney development and renal function; 2) glucose and lipid metabolic markers in the liver, and; 3) the effect of maternal LC supplementation during gestation and lactation on health outcome of the SE offspring.

Methods

Female Balb/c breeder mice were exposed to cigarette smoke for 6 weeks prior to mating, during gestation and lactation; with sham exposure as control. A subgroup of the SE dams was treated with LC (SE+L-C) during gestation and lactation via drinking water. The offspring were sacrificed at postnatal day (P)1, P20 (weaning age) and 13 weeks (mature age). Blood, urine, kidneys and livers were collected. Renal Morphology and function, renal development factors, and metabolic markers in the liver were examined in the offspring.

Results

Reduced nephrons number, enlarged glomerular size and altered renal expression of developmental factors such as glial cell line-derived neurotrophic factor (GDNF) and paired box binding protein (Pax)2 were observed in the offspring. This was linked to increased mRNA expression of pro-inflammatory marker, monocyte chemoattractant

protein (MCP)-1, and urine albumin/creatinine ratio at adulthood in the male offspring. However female offspring were protected from such maternal effect. Both genders developed glucose intolerance. mRNA expression of IL-1 β and TNF- α was upregulated in the liver in the female offspring, with hyperlipidemia. Maternal LC supplementation during gestation and lactation ameliorated these changes in the offspring by maternal SE.

Conclusion

Maternal SE led to kidney underdevelopment, adulthood renal dysfunction, lipid and glucose metabolic disorders, and increased renal and liver inflammation in the offspring in a gender-specific manner. Maternal LC supplementation has a beneficial role in ameliorating the detrimental impact of maternal SE on the offspring.