

THE UNIVERSITY OF ADELAIDE

The Effect of Prenatal Hypoxia on Cardiomyocyte Development and Postnatal Heart Health

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ABSTRACT

Environmental factors can act in early life to increase the risk of disease in adulthood. Animal models demonstrate that intrauterine growth restriction (IUGR) results in a greater susceptibility to cardiac ischaemia/reperfusion injury and reduced cardiac power during reperfusion than Control offspring in postnatal life. Despite having an equivalent utilisation of fatty acids and glucose for cardiac ATP production prior to ischaemia/reperfusion, IUGR offspring have decreased utilisation of fatty acids and increased reliance on glycolysis for ATP production compared to Control offspring during reperfusion. We therefore aimed to determine if IUGR reduces cardiomyocyte endowment and alters the expression of cardiometabolic genes in postnatal life. We determined that IUGR due to placental restriction from conception, which causes chronic fetal hypoxaemia and hypoglycaemia, reduced the number of cardiomyocytes in the heart of sheep in late gestation. In addition, IUGR fetuses had the same percentage of apoptotic cardiomyocytes, length of coronary capillaries and expression of the majority of genes whose upregulation occurs during hypoxia, compared to Controls. Furthermore, we found that IUGR reduced cardiomyocyte endowment in adolescent guinea pigs if they were exposed to Maternal Hypoxia (MH) and were female, but not if they were male or if IUGR was induced by Maternal Nutrient Restriction (MNR). IUGR offspring exposed to MH had increased expression of the transcriptional regulator of fatty acid metabolism, *PPAR α* , and increased expression of fatty acid transporters, *FATP1*, *FAPT6* and *FABPpm*, but offspring exposed to MNR only had an increased expression of *FATP6*, compared to Control. Interestingly, IUGR male offspring, but not female offspring, had decreased expression of factors in the sarcoplasm that regulate fatty acid activation (*FACS*) and transport of active fatty acids into the mitochondria for fatty acid β -oxidation (*AMPK α 2* and *ACC*) if exposed to MNR, but a decrease in only *FACS* and *AMPK α 2* if exposed to MH. Interestingly, only IUGR females exposed to MH had increased activity of the metabolic fuel gauge, AMPK, suggesting that a decrease in ATP may be related to the deficit in

cardiomyocyte endowment. In conclusion, we have shown that in response to placental restriction, reducing cardiomyocyte endowment whilst maintaining the total length of coronary capillaries, results in the heart being normoxic, despite chronic hypoxaemia, in late gestation. Furthermore, this data suggests that females are more likely to have reduced cardiomyocyte endowment, following IUGR, in adolescence than males and that cardiomyocytes may be influenced by hypoxia more than nutrient restriction. Furthermore, we have demonstrated that IUGR programs changes in cardiometabolic gene expression in the absence of other IUGR pathologies such as cardiac hypertrophy, hypertension and increased plasma fatty acid and cortisol concentrations.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in my name, 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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RELATED PUBLICATIONS

List of Publications from Other Work Performed during Candidature

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COMMONLY USED ABBREVIATIONS

A-C

ACADL	Long chain acyl-CoA dehydrogenase
ACADM	Medium chain acyl-CoA dehydrogenases
ACADVL	Very Long chain acyl-CoA dehydrogenase
ACC	Acetyl-CoA Carboxylase
ACE	Angiotensin converting enzyme
ACTH	Adrenocorticotropic hormone
Adm	Adrenomedullin
ADP	Adenosine diphosphate
Akt	Protein kinase B
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate-activated protein kinase
Ang-II	Angiotensin-II
ANGPT	Angiopoietin
ANP	Atrial natriuretic peptide
ATP	Adenosine triphosphate
AT-R	Angiotensin receptor
β -AR	Adrenergic receptor - beta
CAs	Catecholamines
CD36	Fatty acid translocase
CDK	Cyclin dependent kinase
CPT-I β	Carnitine palmatonyl transport protein –I beta
CRH	Corticotrophin-releasing hormone
CVD	Cardiovascular disease

D-H

d	Day
DRs	Death receptors
ERK	Extracellular signal-related kinase
ETC	Electron transport chain
FABPpm	Plasma membrane specific fatty acid binding
FACS	Fatty-acyl CoA synthetase
FADD	Fas-Associated protein with Death Domain
FADH ₂	Flavin adenine dinucleotide
FATP	Fatty acid transport protein
FGF2	Fibroblast growth factor 2
FGFR	Fibroblast growth factor receptor
Flk-1	Vascular endothelial growth factor receptor 1
G ₀	Cell cycle - gap zero phase (resting/quiescent)
G ₁	Cell cycle - first gap phase
G ₂	Cell cycle - second gap phase
GLUT	Glucose transporter
GR	Glucocorticoid receptor
GS	Glycogen synthase
GSK-3 β	Glycogen synthase kinase-3 beta
H-FABP	Heart-type fatty acid binding protein
HIF	Hypoxia inducible factor
HK	Hexokinase
HPA	Hypothalamic-pituitary adrenal
HRE	Hypoxia response element

I-P

IGF-1	Insulin-like growth factor-1
IGF-1R	Insulin-like growth factor-1 receptor
IGF-2	Insulin-like growth factor-2
IGF-2R	Insulin-like growth factor-2 receptor
iNOS	Inducible nitric oxide synthase
IUGR	Intrauterine growth restriction
LBW	Low birth weight
LDH	Lactate dehydrogenase
LV	Left ventricle
LVH	Left ventricular hypertrophy
M	Cell cycle - mitosis
MCD	Manalyl CoA dehydrogenase
MH	Maternal hypoxia
miR	MicroRNA
NADH	Nicotinamide adenine dinucleotide
NEFA	Non-esterified fatty acid
NRG1	Neuregulin 1
PDH	Pyruvate dehydrogenase (PDH)
PFK	Phospho-6-fructose kinase I
PHD	Prolyl hydroxylase
PI3K	Phosphoinositide-3 kinase
PKC	Protein kinase C
PPAR	Peroxisome proliferator-activated receptor
PR	Placental restriction

R-Z

Rb	Retinoblastoma protein
RV	Right ventricle
RXR	Retinoid X receptor
S	Cell cycle - DNA synthesis phase
T ₃	Thyroid hormone
TCA	Tricarboxylic acid
Tie-2	Tyrosine-protein kinase receptor
UPE	Umbilicoplacental embolization
VEGF	Vascular endothelial growth factor