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*$\alpha$, 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 $\beta$-oxidation (*AMPK*$\alpha_2$ and *ACC*) if exposed to MNR, but a decrease in only *FACS* and *AMPK*$\alpha_2$ 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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### 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-Iβ | Carnitine palmatonyl transport protein –I beta |
| CRH | Corticotrophin-releasing hormone |
| CVD | Cardiovascular disease |

| | |
|---|---|
| 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-3β | 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 |

| | |
|---|---|
| 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 |

| | |
|---|---|
| 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 |