



- 9.00am Welcome and Introduction
Dr Janna Morrison
- 9.05am Building a Durable Heart
Prof Kent Thornburg, Director, Heart Research Centre, Oregon Health Sciences University, Portland, Oregon, USA
- 10.00am Hypoxia in Embryonic Heart Development
Assoc Prof Sally Dunwoodie, Victor Chang Cardiac Research Institute

10.30am **Morning Tea / Postgraduate Posters**

Growth and Development of the Heart: Placental and Fetal Determinants

- 11.00am Heart Development in Small Babies
Dr Janna Morrison, Sansom Institute for Health Research, UniSA
- 11.30am Adult Cardiac Hypertrophy - an outcome of disturbed growth signalling and cardiomyocyte loss in the neonatal heart
Assoc Prof Lea Delbridge, University of Melbourne

- 12.00pm Role of IGFs in Heart Health
Dr Julie McMullen, Baker Heart Research Institute

12.30pm **Lunch / Postgraduate Posters**

Growth and Development of the Heart: Growth factors and Cardiomyocytes

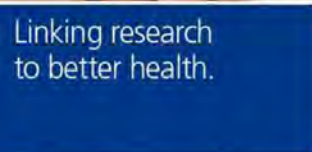
- 1.30pm Interactions between glucocorticoids and the renin-angiotensin system; effects on cardiac development and function
Prof Eugenie Lumbers, University of New South Wales

- 2.00pm Preterm Birth and Heart Development
Dr Jane Black, Monash University

- 2.30pm Linking Myocardial Morphology to Function in the Preterm Piglet
Dr Barbara Lingwood, University of Queensland

- 3.00pm What do we know and what questions still need answering?
Panel Discussion led by Prof Kent Thornburg and Prof Eugenie Lumbers

4.00pm **Network Session / Afternoon Tea / Postgraduate Posters**





Professor Kent Thornburg
Director, Heart Research Centre
Oregon Health Science University

Kent Thornburg is Professor and Associate Chief for Research in Cardiovascular Medicine at Oregon Health & Science University (OHSU). He holds the M. Lowell Edwards Chair and professorial appointments in 5 departments in the School of Medicine. He also serves as the director of the OHSU Heart Research Center. Outside of OHSU, Dr. Thornburg serves on scientific advisory panels at the National Institutes of Health and the National American Heart Association. He also serves on the Medical Advisory Board for the National Children's Heart Foundation in Chicago.

The Thornburg laboratory studies how poor nutrition before birth leads to adult-onset heart disease. He directs an NIH funded program that studies how pregnant mothers provide chemical signals to their babies before birth and how some signals can lead to disease in their offspring. He runs an NIH program that trains student-cardiologists in research. He also directs research projects on maternal nutrition and fetal growth in women who live in rural Oregon. He collaborates with scientists in Southampton, UK; Auckland, New Zealand; Marseille, France; Helsinki, Finland; Adelaide, Australia and Mumbai, India.



Associate Professor Sally Dunwoodie
Victor Chang Cardiac Research Institute, Sydney

Sally Dunwoodie is the head of the Embryology Laboratory at the Victor Chang Cardiac Research Institute, and is an Associate Professor in the Faculty of Medicine at the University of New South Wales. In 1993 Sally gained a PhD researching the genetics of muscle development, from the Children's Medical Research Institute and University of Sydney. In 1993 she undertook postdoctoral training in the Mammalian Development Unit at the National Institute for Medical Research, in London, UK.

There she identified novel genes active during mouse embryo development. In 2000, Sally returned to Australia to take up a faculty position at the Victor Chang Cardiac Research Institute, and to establish the Embryology Laboratory within the Developmental Biology Division. In 2003 Sally was awarded the inaugural Pfizer Foundation Australia Senior Research Fellowship, and currently holds a NHMRC Senior Research Fellowship. Sally's research goals are to define molecular and cellular interactions that orchestrate mammalian development through a mechanistic understanding of genetic and environmental interactions, and how they impact upon the developing form and function of the mammalian embryo. In particular her research has identified that Notch signalling is central to somite and vertebral column formation in mouse and humans, and that hypoxia is a feature of normal embryo and placenta development.



University of
South Australia

Sansom Institute
for Health Research

Dr Janna Morrison
CoHead, Early Origins of Adult Health
Research Group, Sansom Institute for
Health Research, UniSA

Dr. Morrison currently holds a Heart Foundation and NHMRC Career Development Award (2008-2012) in the Early Origins of Adult Health Research Group in the Sansom Institute for Health Research at the University of South Australia. Dr. Morrison was trained in fetal physiology at the University of Western Ontario (Masters, 1997) and the University of British Columbia (PhD, 2001). Dr. Morrison's early work focussed on the neural regulation of fetal sleep states and the impact of maternal antidepressant use on fetal development during late gestation to provide pregnant women with evidence to decide whether or not to take antidepressant during pregnancy. These interests led to a post doctoral position at the University of Toronto investigating the neurotransmitters controlling the tongue muscle during sleep in the search for therapeutic interventions in the treatment of obstructive sleep apnea. In October 2002, Dr. Morrison moved to the University of Adelaide to join the Centre for the Early Origins of Adult Health, returning to the world of fetal physiology. Studies in Adelaide focus on the impact of low birth weight on cardiovascular development, including both regulation of blood pressure and development of the heart. With the award of a second National Heart Foundation Research Fellowship, Dr. Morrison joined the Sansom Institute in early 2006 where investigations of cardiovascular development continue. These studies are funded by 4 National Health and Medical Research Council Project grants.

Dr. Morrison is an enthusiastic science communicator who has participated in community programs such as Let's Talk Science, Tall Poppies Campaign and Science meets Parliament over the past decade. These have provided her with opportunities to speak about her passion for science with students of all ages, their teachers and members of parliament. Our community's children are our community's future nurses, engineers, teachers, doctors and scientists.



Associate Professor Lea Delbridge
Head, Cardiac Phenomics Laboratory,
Dept Physiology
University of Melbourne

Lea is an Associate Professor in the Department of Physiology at the University of Melbourne, and heads the Cardiac Phenomics Laboratory. The Cardiac Phenomics Lab focus is to understand the alterations in heart structure and function which occur in different forms of hypertrophic cardiomyopathy associated with hormonal disturbances. This includes research into the early developmental factors which shape the heart and study of the adult phenotypes associated with primary cardiac hypertrophy. Integrated molecular, cellular, in vitro and in vivo mechanistic investigations are pursued.

Lea completed her undergraduate training at Monash University, obtaining a BSc (Hons) in Physiology. After graduating with her PhD in 1993 at the University of Melbourne, she was appointed for a 3 year term as International Fellow of the American Heart Association in Chicago. On returning to Australia she took up a Post-doctoral Fellowship awarded by the National Heart Foundation of Australia for two years, and then an academic appointment in the Department of Physiology at the University of Melbourne followed.

Currently Lea is World Council Member and President of the Australasian Section Council of the International Society of Heart Research (ISHR) and a Council member for the Australian Physiological Society (AuPS). She is an elected Fellow, Cardiac Society of Aust & New Zealand and appointed to the CSANZ Scientific Committee and has recently served as Grant Review Panel member, for NHMRC, Nat Heart Foundation, and the NZ Health (Biomed & Clinical).



Dr Julie McMullen
Cardiac Hypertrophy Laboratory,
Baker IDI Heart & Diabetes Institute

Julie McMullen (PhD) heads the Cardiac Hypertrophy Laboratory at the Baker IDI Heart & Diabetes Institute, Melbourne, Australia. Dr McMullen graduated from the School of Physiology & Pharmacology at the University of New South Wales. She then trained as a Cardiology Research Fellow at Beth Israel Deaconess Medical Centre and Harvard Medical School in Boston. During this time she gained experience generating and characterising cardiac specific transgenic mice in the laboratory of Dr Seigo Izumo. In early 2005, Dr McMullen established her own laboratory at Baker IDI. Her research interests include cardiac hypertrophy and failure, specifically focusing on molecular mechanisms responsible for the induction of physiological and pathological cardiac hypertrophy. Dr McMullen is currently supported by an Australian Research Council Future Fellowship and holds an Honorary NHMRC Research Fellowship. She is a member of two Editorial Boards and is the current Treasurer of the International Society for Heart Research, Australasian section.



Professor Eugenie Lumbers
University of New South Wales

Eugenie graduated MBBS from University of Adelaide and was subsequently awarded a Doctorate in Medicine from that university. She was the first woman to be awarded a CJ Martin Fellowship by the NMHRC and studied fetal physiology at the Nuffield Institute for Medical Research in Oxford. Eugenie established her own laboratory at UNSW in 1974 and has been funded over the years by ARC, NHMRC, AKF and NHF. Eugenie was supported by NHMRC from 1975 onwards. She retired in 2003 and returned to research in 2006 and holds a fractional professorial position at University of Newcastle and conjoint positions at UNSW and University of Queensland. Currently Eugenie holds 2 NHMRC grants at University of Newcastle (one as CIA on the role (s) intrauterine renin-angiotensin system in human pregnancy and the other (CIB) on the impact of maternal stress on renal development) and she is also a CIC on an NHMRC grant at University of Queensland on low systemic blood flow in the newborn and CIB on an NHMRC grant at University of Adelaide on novel regulators of pregnancy success.

Eugenie's research has been broad based and has both studied cardiovascular and fluid and electrolyte physiology in adult non pregnant and pregnant animals, in the fetus and in the newborn. She has been particularly interested in the role and actions of the renin-angiotensin system (RAS), and has carried out human and animal experiments. She discovered inactive (prorenin) and with Brian Morris showed that it was activated by proteases. She has used the chronically catheterized pregnant ewe and her fetus as a model for studying fetal physiology. Most of her work relates to the development of the cardiovascular system and kidney including development of neural control of the circulation. Eugenie has worked in fetal gene therapy. Her current interests are related to the role of (pro)renin receptor/prorenin in prostaglandin synthesis in fetal membranes, decidua and placenta and in the programming of renal disease in animal and human populations and in the role of cardiac function in preterm neonatal hypotension.



Associate Professor Jane Black
Monash University

Associate Professor Black is the Deputy Head of Department (Teaching and Research Training) in the Department of Anatomy & Developmental Biology at Monash University. Associate Professor Black also heads a productive cardiovascular and renal cell biology research group in that department.

Associate Professor Black completed her PhD at the Baker Heart Research Institute in Melbourne and subsequently was the recipient of highly competitive post-doctoral fellowships from the Alfred Hospital (Prahran, Victoria) and the National Heart Foundation of Australia. She commenced her academic appointment in the Department of Anatomy and Developmental Biology at Monash University in 1999.

She has a strong background in cardiovascular cell biology and in the fetal programming of cardiovascular and renal diseases; her current research focuses on the effects of perturbations in early life on the development of the heart and kidney and the long-term consequences. Her research group has made major contributions to understanding the mechanisms of the developmental programming of cardiovascular disease. Her experimental studies have provided important insight into how intrauterine growth restriction and preterm birth affect the developing heart and kidney. Her research group is leading research worldwide into the effects of preterm birth on heart structure. In particular, her studies have provided seminal insight into the maladaptive remodelling of the heart in response to preterm birth.



Dr Barbara Lingwood
University of Queensland

Dr Barbara Lingwood completed her PhD in 1984 at the Howard Florey Institute of Experimental Physiology and Medicine in Melbourne. She commenced working at the Perinatal Research Centre, Brisbane, in 1998 after being awarded a University of Queensland Post-Doctoral Re-entry Fellowship. She, along with the rest of the Perinatal Research Centre, has recently joined the University of Queensland Centre for Clinical Research, a new \$70M facility located on the Royal Brisbane and Women's Hospital campus and designed to bridge the gap between biomedical science and modern patient care. Her work has focused on improving outcomes for babies born prematurely or who have suffered from lack of oxygen during birth.

Her current research comprises two inter-related areas: Studies of body composition in the neonate are focusing on infants born to obese women and women with gestational diabetes. Research projects are addressing optimal management of these women and its implications for long-term health of the offspring. Studies of cardiovascular function in the preterm neonate include parallel animal and human studies aimed at understanding the aetiology of poor cardiovascular function in the preterm neonate, and the development of targeted novel treatment strategies.

Abstracts

IMPACT OF INTRAUTERINE GROWTH RESTRICTION ON THE INSULIN-LIKE GROWTH FACTOR SYSTEM IN THE HEART

KCW Wang¹, L Zhang¹, D Brooks², KJ Botting^{1,3}, JA Duffield^{1,3}, IC McMillen^{1,3}, JL Morrison^{1,3}, Early Origins of Adult Health Research Group¹, Cell Biology of Diseases Research Group², Sansom Institute, School of Pharmacy and Medical Sciences, University of South Australia and Discipline of Physiology³, University of Adelaide, Australia 5000.

Background: Babies with a birth weight less than the 10th centile are defined as having intrauterine growth restricted (IUGR). We have previously shown that the heart of the IUGR sheep fetus has a greater proportion of mononucleated cardiomyocytes and larger binucleated cardiomyocytes relative to heart weight, however the reason for this is not known. The insulin-like growth factor (IGF) system is important in cardiomyocyte development. Many studies have shown that IGF-1 stimulates cardiac hypertrophy via IGF-1 receptor (IGF-1R) signalling cascade in adult life; however the relationship between cardiomyocyte development and IGFs in Control and IUGR heart development is not yet well-established.

Hypothesis: In the IUGR fetus, up-regulation of the cardiac IGF-2 stimulates IGF-1R signalling pathway to stimulate hypertrophy and protect cardiomyocytes from apoptosis. In the low birth weight lamb, there is a sustained up-regulation of the IGF-2 which may results in hypertrophy of cardiomyocytes.

Methods: IUGR was induced by the removal of most of the endometrial caruncles prior to mating. Ewes were humanely killed at >137d (term,150d) and fetuses were delivered by hysterotomy; post-natal lambs were humanely killed at 21d after birth. A section of left ventricle (LV) was frozen for later mRNA and protein extraction from both fetuses and 21d lambs. IGF-1, IGF-2, IGF-1R and IGF-2R mRNA expression were analysed by real time RT-PCR and normalised to the housekeeper, RpPO.

Results: Relative IGF-2 mRNA expression was significantly higher in both IUGR fetal heart and LBW postnatal heart compared to Controls. The fetal LV binucleated cardiomyocyte length relative to heart weight was significantly related to relative IGF-2 mRNA expression ($y=0.838x-0.403$; $r^2=0.622$; $P=0.001$), relative IGF-1R mRNA ($y=4.245x-0.096$; $r^2=0.53$; $P=0.005$) and protein expression ($y=0.032x+1.665$; $r^2=0.432$; $P=0.015$). In the heart of 21d lambs, LV weight relative to heart weight was significantly related to relative IGF-2 mRNA expression ($y=0.578x+2.467$; $r^2=0.37$; $P<0.05$).

Conclusion: This data suggests that small size at birth results in higher cardiac IGF-2 gene expression. We proposed that in fetal cardiomyocytes, IGF-2 acts on the IGF-1R to induce protective effect to induce hypertrophy; while IGF-2 has a potential role to induce hypertrophy in postnatal life.

ANALYSIS OF BIOCHEMICAL CHANGES IN THE HEARTS OF ADULT INTRAUTERINE GROWTH RESTRICTED RATS USING FTIR MICRO – SPECTROSCOPY IMAGING

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Aim: Intrauterine growth restriction is linked to the development of heart disease in adulthood. The aim of this study was to examine the effect of intrauterine growth restriction in rats, due to maternal protein restriction, on the biochemical composition of the heart in female adult offspring.

Methods: Wistar Kyoto (WKY) dams were administered either a low protein diet (LPD; 8.7% casein) or a normal protein diet (NPD; 20% casein) during pregnancy and lactation. At 14 weeks of age, hypertension was induced through a 4 week continuous infusion of angiotensin II (ANGII 200ng/kg/min) in female NPD and LPD offspring, while the control group received saline (N = 7-8 animals/group). Tail cuff systolic blood pressure was measured weekly. At 18 weeks of age the offspring were perfusion fixed. The hearts were weighed, sliced and the heart wall volumes stereologically determined. In left ventricle (LV) sections the biochemical composition of the myocardium was assessed using Fourier transform infrared (FTIR) micro-spectroscopy. FTIR images were analyzed using an Unsupervised Hierarchical Cluster Analysis (UHCA) approach.

Results: Body weights of LPD offspring were significantly lower compared to controls at postnatal day 4; however in adulthood this difference was no longer significant. ANGII infusion resulted in lower body weights in NPD and LPD offspring ($P < 0.0002$). Relative LV wall volume was significantly smaller as a result of ANGII administration. Systolic blood pressure was increased in both, NPD and LPD groups due to ANGII. FTIR spectra demonstrated significant differences in collagen distribution and density between NPD and LPD hearts; collagen appeared denser and disordered in the LPD hearts. The LPD hearts had overall higher optical density in the mid infrared spectra.

Conclusion: Intrauterine growth restricted hearts have an altered cardiac biochemical composition in adulthood compared to hearts from offspring that were not growth restricted *in utero*. This may contribute to their vulnerability to heart disease.

PRENATAL DEXAMETHASONE EXPOSURE IMPAIRS CARDIAC GROWTH IN FEMALE BUT NOT MALE OFFSPRING

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Background: Prenatal glucocorticoid (GC) exposure has been shown to be detrimental to adult health, resulting in diseases such as hypertension, by a process commonly known as 'developmental programming'. This study investigated the effects of short-term exposure to the synthetic GC dexamethasone (DEX) on heart size and cardiac gene expression offspring.

Method: C57/BL/6 mice were infused with DEX or saline (SAL) for 72 hours from embryonic day 12.5. Animals were allowed to litter down and hearts were collected from offspring at postnatal day 30 (PN30). Gene expression of cardiac growth factors was analysed by real-time PCR.

Results: At PN30 heart weight in males were similar in DEX and SAL exposed offspring. However in females, heart weight ($100.0 \pm 10.0\text{mg}$ vs $120.0 \pm 20\text{mg}$) and the heart to body weight ratio (7.30 ± 0.69 vs 8.36 ± 1.42) in DEX treated offspring were significantly decreased ($p < 0.05$). Studies in the hearts of female offspring showed no differences in VEGFa, AT1a, IGF-2 or TGF- β gene expression. AT1b expression was significantly lower in DEX treated female offspring (0.30 ± 0.06 vs 1.65 ± 0.44).

Conclusions: Prenatal DEX exposure significantly impaired cardiac growth in female animals only. The decrease in AT1b expression suggests possible alterations in the renin-angiotensin system which may influence heart growth and development. Retarded cardiac growth may contribute to long term impairments in cardiac function resulting in an increased risk of developing later cardiovascular disease. Future studies examining cardiomyocyte number and size, along with analysis of cardiac function are warranted to further understand the disease processes in this model.

THE EFFECT OF HYPOXIA ON NGF, AT1R AND AT2R EXPRESSION IN THE AORTA AND FEMORAL ARTERY IN THE GROWTH RESTRICTED SHEEP FETUS.

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Objective: Intrauterine growth restriction (IUGR) is associated with an increased risk of developing cardiovascular disease during adult life. We have previously demonstrated that the restriction of placental and fetal growth results in fetal hypoxia and fetal brain sparing suggesting a redistribution of cardiac output. Furthermore, placentally restricted (PR) hypoxic fetuses are more dependent on their sympathetic nervous system and Angiotensin II for the maintenance of blood pressure during late gestation. Nerve growth factor (NGF) plays a significant role in sympathetic innervation of blood vessels. Also the Angiotensin Receptors (AT1R and AT2R) are responsible for the vasoconstrictive action of Angiotensin II.

Hypothesis: We hypothesize that the expression of *NGF*, *AT1R* and *AT2R* will be higher in the aorta and femoral artery of the PR hypoxic compared to the control fetus.

Method: Carunclectomy was performed in 8 non-pregnant ewes to induce PR. Vascular catheters were inserted in 9 PR and 7 control (C) fetuses at 103-117d and arterial blood samples were collected for blood gas analysis. All PR fetuses were chronically hypoxic (mean gestational arterial $PO_2 < 17$ mmHg). Post mortem was performed at 139-141d. *NGF*, *AT1R* and *AT2R* mRNA expression was determined by qRT-PCR.

RESULTS: Fetal weight (C, 4.39 ± 0.3 kg; PR, 2.56 ± 0.2 kg) and mean gestational arterial PO_2 (C, 21.19 ± 1.3 ; PR, 14.1 ± 0.6 mmHg) were significantly lower in PR fetuses ($P < 0.001$). There was no difference in the expression of NGF in the aorta (C, $0.0005 \pm 6 \times 10^{-5}$; PR, $0.0006 \pm 18 \times 10^{-5}$) or femoral artery (C, 0.0026 ± 0.0006 ; PR, 0.003 ± 0.00038) between the control and PR fetal sheep ($P < 0.1$). There was also no significant difference in *AT1R* (C, 0.033 ± 0.006 ; PR, 0.046 ± 0.004) and *AT2R* (C, 0.006 ± 0.002 ; PR, 0.014 ± 0.003) expression in the aorta between control and PR fetal sheep ($P = 0.07$ and $P = 0.06$ respectively).

CONCLUSIONS: These data suggest that *NGF*, *AT1R* and *AT2R* expression at 139-141d gestation is not higher in the vasculature of the hypoxic fetus at a time when the maintenance of arterial blood pressure is more dependent on the sympathetic nervous system and Angiotensin II in the IUGR fetus

THE HEART'S RESPONSE TO CHRONIC HYPOXEMIA IN LATE GESTATION

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Intrauterine growth restriction (IUGR) is associated with an increased risk of cardiovascular disease in adulthood. Previous research in sheep demonstrates that chronic hypoxemia in late gestation results in delayed cardiomyocyte (CM) binucleation, decreased size and no change to the percentage of proliferating CMs. We describe the effect of hypoxemia in late gestation on the number of CMs, the percentage of apoptotic CMs, and the mRNA expression of genes involved in the response to cellular hypoxia.

IUGR was achieved by carunclectomy induced placental restriction (PR) in sheep. PR fetuses with a mean gestational $PO_2 < 17$ mmHg were defined as chronically hypoxemic. At *post mortem* (139±2d GA), fetal hearts (13 control (C); 12 PR) were collected and weighed. A portion of the left ventricle was snap frozen for RNA extraction, a portion of the right ventricle was weighed and fixed in 4% formaldehyde for CM number estimation and the remaining ventricles were enzymatically digested to isolate CMs. HIF-1a and -2a, VEGF-A, VEGFR-1 and VEGFR-2 mRNA expression was determined using qRT-PCR and normalised to Ribosomal Protein P0 (8C; 8PR). Fixed sections were systematically cut with a tissue slicer (blades 2mm apart), sampled using systematic, uniformly random sampling, and embedded in glycomethacrylate. CM nuclei were visualised in a 30mm slice by staining with 0.15% basic fuchsin and haematoxylin and the numerical density of nuclei determined with NewCAST software. The total number of CMs was calculated by dividing the total number of nuclei by the average number of nuclei per CM as determined from the isolated CM's (5C; 4PR). TUNEL was used to determine the percentage of isolated CMs undergoing apoptosis (6C; 7PR).

Fetuses exposed to chronic hypoxemia had decreased body weight and heart weight, increased numerical density of CM nuclei (C: $53.0 \times 10^4 \pm 1.6 \times 10^4$; PR: $62.5 \times 10^4 \pm 3.4 \times 10^4$ mm³; $P < 0.05$), but no difference in total CM number. Total CM number was, however, positively correlated to fetal weight (R^2 0.74; $P < 0.001$). There was no difference in the percentage of apoptotic CMs or of HIF-1a, HIF-2a, VEGF-A or VEGFR2 mRNA expression. There was an increase in VEGFR1 mRNA expression (C: 0.081 ± 0.015 ; PR: 0.131 ± 0.014 ; $P < 0.05$). This data suggests that smaller fetuses have a reduced total number of CMs. This data also suggest that in late gestation the heart of the PR fetus may not have been hypoxic when compared with controls.

PERICONCEPTIONAL UNDERNUTRITION IN NORMAL AND OVERWEIGHT EWES LEADS TO INCREASED ADRENAL GROWTH AND EPIGENETIC CHANGES IN ADRENAL IGF2/H19 GENE IN OFFSPRING

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Adverse conditions in early life result in an increased activation of the hypothalamo-pituitary-adrenal (HPA) axis and in stress responsiveness in the offspring. We have developed a model in which 'donor' ewes are either normally nourished or overnourished prior to a period of dietary restriction, before transfer of the embryo at 6-7 days after conception to a ewe of normal weight and nutritional history. A moderate restriction of energy intake during the periconceptual period in both normal weight and overweight ewes resulted in increased adrenal mass in male and female lambs and an increased cortisol response to stress in female lambs. The increase in adrenal weight in lambs exposed to periconceptual undernutrition was associated with a decrease in the adrenal mRNA expression of the insulin-like growth factor 2 (IGF2) and decreased methylation in the proximal CTCF binding site in the differentially methylated region (DMR) of the Igf2/H19 gene. Thus weight loss in both normal and overweight mothers during the periconceptual period results in epigenetic modification of Igf2 in the adrenal, adrenal overgrowth and an increased vulnerability to stress in the offspring. Determining the appropriate approach to weight loss in the periconceptual period may therefore be important in overweight or obese women seeking to become pregnant.

INTRAUTERINE GROWTH RESTRICTION COUPLED WITH HYPERGLYCEMIA LEADS TO ALTERED CARDIAC MORPHOLOGY IN ADULT RATS; AN ECHOCARDIOGRAPHY STUDY

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Introduction: Diabetes leads to pathological remodeling of the myocardium resulting in loss of cardiomyocytes and subsequent deposition of fibrosis. We propose that the hearts of subjects who were growth restricted *in utero* are more vulnerable to diabetic heart disease. Hence, the aim of the present study was to examine the effect of hyperglycemia in IUGR adult rat offspring when blood glucose levels were maintained at mild or moderate levels.

Methods: Female WKY rats were fed either a normal protein diet (NPD, 20% casein) or low protein diet (LPD, 8.7% casein) 2 weeks prior to mating, during pregnancy and lactation. At 23 weeks of age, streptozotocin (STZ; 50mg/kg) was administered to the offspring to induce hyperglycemia. From 2 days after STZ injection, blood glucose levels were measured daily; long acting insulin (prothophane) was injected (1-2U) daily to stratify blood glucose levels at mild, (7-10 mmol/L) or moderate, (10-15 mmol/L) levels (n=8males per group). Morphological and functional analyses of the heart were performed using non-invasive transthoracic echocardiography and 2D B-mode imaging. At 32 weeks of age, the rats were perfusion-fixed for the analyses of cardiac fibrosis. Data was analysed by a two-way ANOVA with maternal diet (P_D) and induction of hyperglycemia (P_T) as factors.

Results: LPD offspring were born with reduced body weight and they remained significantly smaller than NPD offspring throughout the experimental period ($P_D < 0.001$). Induction of hyperglycemia (mild or moderate) led to a further decrease in body weight ($P_T < 0.001$). Both maternal diet and induction of hyperglycemia directly influenced heart size, however, the response to hyperglycemia was not different between the NPD and LPD offspring. IUGR led to a significant increase in relative heart weight, posterior wall thickness and septum thickness at 32 weeks of age ($P_D < 0.0001$), however, there was no difference in fractional shortening indicating cardiac function was normal. Induction of hyperglycemia led to a significant increase in relative heart size, end diastolic dimensions and end systolic dimensions. Importantly, when hyperglycemia was tightly controlled, the effect of hyperglycemia was markedly attenuated. There was a significant increase in interstitial fibrosis in the left ventricle plus septum (LV+S) of LPD offspring ($P_D < 0.0001$) compared to NPD offspring and this was further increased with induction of hyperglycemia. The interstitial fibrosis was not attenuated by improved glycemetic control.

Conclusion: Intrauterine growth restriction is associated with hypertrophy of the heart and increased levels of interstitial fibrosis in the LV+S of adult rat offspring. Increased levels of blood glucose further exacerbates the effects of IUGR in the heart, however, tight glycemetic control attenuates these effects. Hence, our study emphasizes the importance of maintaining a tight glycemetic control in diabetic subjects in order to attenuate the risk of cardiac abnormalities in diabetic subjects.

EFFECT OF VITAMIN D DEFICIENCY ON CARDIAC FUNCTION AND SUSCEPTIBILITY TO ISCHEMIA/REPERFUSION INJURY IN THE ADULT RAT HEART

Okzan.Gezmish¹, Marianne Tare ², Helena C. Parkington² & M. Jane Black¹ De-
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sity, Clayton, VIC. 3800.

Exposure to vitamin D deficiency *in utero* and early life leads to delayed maturation and subsequent enhanced growth (proliferation and hypertrophy) of cardiomyocytes in the left ventricle. The implications of these changes on cardiac function later in life are unknown. The aim of the present study was to investigate the effect of vitamin D deficiency in adult rats on cardiac function and the susceptibility to ischemia/reperfusion injury. Four week old Sprague-Dawley female rats were fed either a vitamin D deplete or vitamin D replete (control) diet for 6 weeks prior to pregnancy, during pregnancy and throughout lactation. Offspring remained on their respective diets until adulthood. Hearts of 16 week old male and female offspring ($n=8/\text{group}$) were mounted on a Langendorff apparatus. Basal heart rate (HR), coronary flow, rate of contraction (+dp/dt) and relaxation (-dp/dt) and response to isoprenaline were recorded. The hearts were then subjected to 20 minutes ischemia and 1½ hours reperfusion. At the end of the reperfusion period the left ventricle was sliced and incubated in 1% 2, 3, 5 triphenyl tetrazolium solution (TTZ), to determine infarct area using computerized planimetry. Basal cardiac function (HR, +dp/dt, -dp/dt) was not different between groups. Basal coronary flow was lower in hearts of vitamin D deficient rats. The isoprenaline-induced increase in HR tended to be greater in vitamin D deficient males ($p = 0.06$), but there was no differences in contractile function between groups. After 55 minutes reperfusion, HR had declined by 30% of that before ischemia in both males and females, with HR being higher in vitamin D deficient males compared with control males. Infarct area was 2-fold greater in vitamin D deficient hearts of both males and females ($p = 0.006$ & $p = 0.03$, respectively). Basal and stimulated heart function was not altered, although coronary flow was significantly reduced in vitamin D deficient rats. In conclusion, the hearts of vitamin D deficient rats are particularly susceptible to ischemia/reperfusion injury. Dysregulation of coronary flow and the extent of vascularisation may be factors which contribute to the increased susceptibility to ischemia/reperfusion injury.

NGF GENE EXPRESSION IS ELEVATED IN THE HEART OF GROWTH RESTRICTED FETUSES AND IS NOT RELATED TO ENDOTHELIN RECEPTOR EXPRESSION

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Small size at birth is associated with an increased risk of perinatal and postnatal cardiovascular morbidity and mortality. Growth restricted (IUGR) fetuses have an increased dependence on the sympathetic nervous system in the maintenance of mean arterial pressure (MAP). Endothelin-1 (ET-1) mediates cardiac sympathetic innervation by regulating gene expression of nerve growth factor (NGF). The impact of IUGR on development of the ET system and the contribution of ET-1 to MAP regulation are not known. The aim of this study was to compare the effect of ET-1 blockade on MAP and HR and the mRNA expression of NGF, ETA receptor and ETB receptor in the coronary artery in control and growth restricted fetuses. Placental restriction leading to IUGR was induced by removing the majority of the endometrial caruncles from Merino ewes prior to mating. Vascular catheters were implanted at 120-130d gestation in Control (n=6) and IUGR (n=5) fetuses. Three different doses of endothelin-1 (ET-1) (0.4ug/kg; 0.8ug/kg; 1.2ug/kg) and on a different day a single dose of FR139317 (1.5mg/kg), an ETAR antagonist, were administered intravenously to each fetus and the MAP and HR responses were recorded. In a separate cohort of 9 Control and 9 IUGR fetuses, coronary arteries were collected at 137-142d gestation, snap frozen and then the RNA was extracted and real-time PCR was used to measure NGF, ETAR and ETBR gene expression. ET-1 increased MAP at a higher dose in IUGR (1.2ug/kg) compared to Control (0.8ug/kg) fetuses but there was no difference in the magnitude of the rise. Fetal MAP and HR responses during FR139317 infusion were not different between control and IUGR fetuses. NGF, but not ETA or ETB receptor, gene expression in the coronary artery was higher in IUGR compared to control fetuses. There was no relationship between NGF and ETAR or ETBR gene expression. These data suggest that ET does not play a different role in maintaining blood pressure in the IUGR fetus and suggest a potential role for NGF, through the recruitment of sympathetic neurons in the regulation of coronary blood flow in the IUGR fetus.

FETAL CARDIOVASCULAR RESPONSE TO CHRONIC HYPOXEMIA: REDISTRIBUTION OF CARDIAC OUTPUT

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Background: It is well established that in response to acute hypoxemia, fetuses redistribute their cardiac output to maintain adequate perfusion of key organs including the brain, heart and adrenal glands at the expense of peripheral tissues. It is not known, however, whether the redistribution of cardiac output persists in chronically hypoxemic fetuses. The surgical removal of uterine caruncles in the non pregnant ewe results in the restriction of placental growth (PR) and chronic fetal hypoxemia. We hypothesize that exposure of the fetus to PR and chronic hypoxemia results in increased blood flow to the brain, heart & adrenals.

Methods: At 124 d gestation, vascular catheters were implanted in control (C, n = 7) and PR (n = 7) fetuses to measure fetal blood gases. At 131 d gestation, fluorescent microspheres were injected into the right atrium to determine blood flow. At 133 d gestation (term ~150 d), ewes and fetuses were humanely killed and fetal weights recorded. The fetal organs including brain, heart and adrenals were dissected and weighed.

Results: Fetal weight (C, 4.0 ± 0.02 kg; PR, 2.5 ± 0.1 kg) and mean gestational PaO₂ (C, 22.3 ± 1.3 ; PR, 15.3 ± 0.1 mmHg) were significantly reduced in PR fetuses. The relative brain and adrenal weight in PR fetuses was higher compared to control fetuses, however, there was no difference in relative heart weight. Blood flow was increased to the brain and adrenals, but not the heart, in PR fetuses compared to control fetuses.

Conclusion: This blood flow data supports the hypothesis that as in acute hypoxemia, chronic hypoxemia leads to redistribution of cardiac output to the brain and adrenals, consistent with the observation of the sparing of brain and adrenal growth in chronically hypoxemic fetuses.