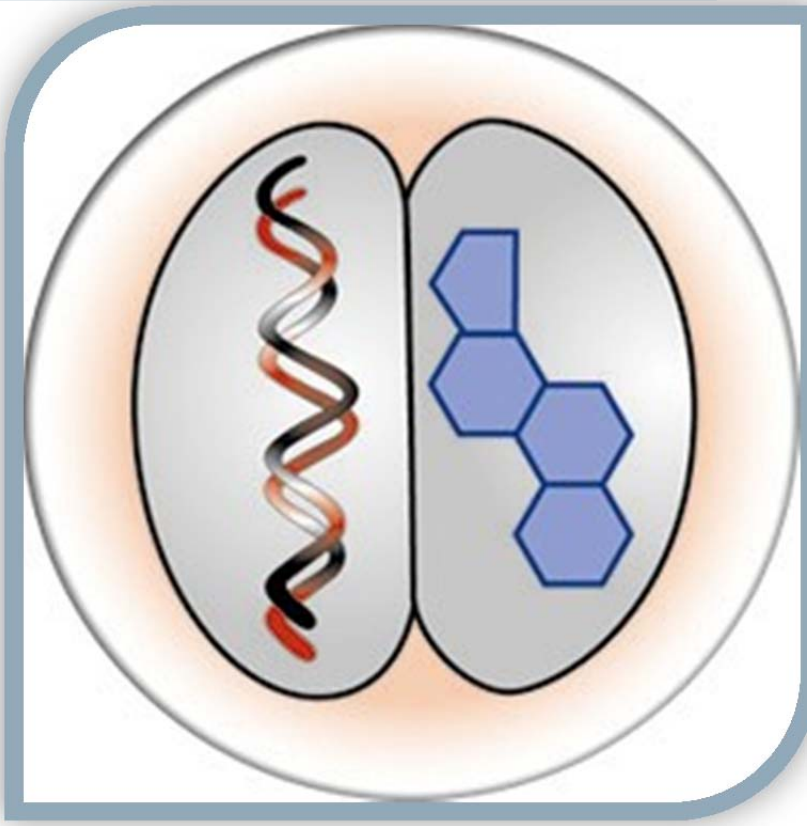
2012 Annual Research Symposium



Endocrinology & Reproductive Physiology Program
April 12, 2012
Fluno Center For Executive Education
601 University Ave.

Dedication to Dr. Paul Bertics, PhD

The Endocrinology & Reproductive Physiology Program dedicates this 2012 symposium to Dr. Paul Bertics, PhD who was a member of the ERP Program from 1988 until 2011. Dr. Bertics' contributions to the scientific and graduate education communities will be deeply missed.

Contents

Acknowledgements

2011 – 2021 Symposium Committee Members
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Schedule of Events

9:00 AM – 9:15 AM	**Welcome Remarks** by Dr. Richard Moss, Associate Dean, School of Medicine and Public Health
9:15 AM – 10:15 AM	**Invited Panel Presentation**: Dr. Christopher Bradfield & Dr. Patricia Keely "Collaborative Nature of Graduate Education: ERP, METC and MCP"
10:15 AM - 10:30 AM	**Break**
10:30 AM - 10:50 AM	**Student Session 1:** Meghan Maguire "Early Gestational Vitamin A Deficiency Causes Aberrant Signaling in Cyp1-b1-/-Mice"
10:50AM - 11:10 AM	**Student Session 1:** Brian Kenealy "Multiple Mechanisms of Rapid E2 Action in Primate GnRH Neurons"
11:10 AM - 11:30 AM	**Student Session 1:** Dr. Jennifer Krupp "Dysfunctional Vascular Adaptations in Human Umbilical Vein Endothelial Cells (HUVEC) and Preeclamptic Pregnancies"
11:30 AM - 12:15 PM	**Lunch** – Executive Dining Room
12:30PM - 1:30 PM	**Keynote Presentation:** Dr. Daniel Hardy, PhD University of Western Ontario "The role of LXRα in the Fetal Programming of the Liver"
1:30 PM - 2:30 PM	**Poster Session** – Foyer
2:30 PM – 3:00 PM	**Break**
3:00 PM - 3:20 PM	**Student Session 2:** Jason (Jun) Ren "The Effect of Protein Kinase C delta on the Transcriptome of Vascular Smooth Muscle Cells"
3:20 PM - 3:40 PM	**Student Session 2:** Bryan Ampey "Shear Stress, cAMP, and cGMP Modulate the Pregnancy-Related Phosphorylation State of Cx43-Gap Junction and eNOS Proteins in Uterine Artery Endothelial Cells"
3:40 PM – 4:00 PM	**Student Session 2:** Ashley Driver "RNA-Sequence Analysis Uncovers Transcriptomic Variations Between Morphologically Similar In-Vivo and In-Vitro- Derived Bovine Blastocytes"
4:10 PM - 4:30 PM	**Closing Remarks**
4:30 PM – 5:00 PM	Poster Take Down

Keynote Speaker: Daniel Hardy, PhD

Dr. Daniel B. Hardy, PhD completed his PhD in Physiology at the University of Western Ontario (UWO) under the supervision of Dr. Kaiping Yang. His thesis focused on placental glucocorticoid metabolism. His research interests in nuclear receptor biology then led him to pursue a postdoctoral fellowship in 2003 at the University of Texas Southwestern Medical Center (UTSW) at Dallas under the mentorship of Dr. Carole Mendelson, focusing on how the progesterone receptor (PR) plays a major anti-inflammatory role in both parturition and in breast cancer. In 2008, Dr. Hardy was recruited back to UWO by Dr. Bryan Richardson as an Assistant Professor in the Departments of Obstetrics and Gynaecology and Physiology and Pharmacology to examine the role of nuclear receptors in the fetal origins of adult diseases. He is also a Scientist with the Children's Health Research Institute (CHRI) and the Lawson Health Research Institute. Using animal models of under nutrition (*e.g.* maternal protein restriction) and hypoxia, Dr. Hardy has begun to uncover some of the transcriptional and epigenetic mechanisms involved in the long-term programming of the liver. He currently focuses on the role of yet another nuclear receptor, the Liver X Receptor (LXRα) in the *in utero* origins of hepatic cholesterol and glucose homeostasis. Since his transition to UWO, Dr. Hardy has been supported by the Canadian Institutes for Health Research (CHRI), the Molly Towell Perinatal Research Foundation, the National Science and Engineering Research Council of Canada (NSERC), and the Sick Kids Foundation. He has received several awards including the *Endocrine Scholar Award* from the Endocrine Society and most recently was honoured as the Perkin-Elmer Early Career Award Speaker from the *Perinatal Research Society*. He continues to participate in Canadian running clubs and is a director with the Irish Benevolent Society of London and Middlesex County (*est.* 1877).

Keynote Talk:

TITLE: **The role of LXRα in the Fetal Programming of the Liver**

Given that the Liver X Receptor (LXRα) plays an important role in both cholesterol, fatty acid, and glucose homeostasis, it is an attractive candidate to elucidate the molecular mechanisms underlying the etiology of the metabolic syndrome. Our laboratory has a great interest in understanding how perinatal insults such under nutrition, hypoxemia, and maternal nicotine exposure lead to long-term disease due to altered LXRα activity. I will discuss how the perinatal environment permanently alters the expression of LXR-target genes via transcriptional and epigenetic mechanisms. I also plan to highlight preliminary work investigating whether neonatal administration of LXRα agonists may prevent the impairment of glucose and cholesterol homeostasis by adulthood.

Dr. Hardy's talk is generously supported by NIH 5-T32 HD041921-8

Oral - Multiple Mechanisms of Rapid E_2 Action in Primate GnRH Neurons

Kenealy BP, Keen KL, and Terasawa E

Previously, we reported that 17β-estradiol (E_2) causes rapid stimulatory action in primate GnRH neurons. E_2 increases the frequency of intracellular calcium ($[Ca^{2+}]_i$) oscillations, their synchronization, and GnRH release within 10 minutes. This rapid E_2 action is, in part, mediated by a G-protein coupled receptor, GPR30, as the estrogen receptor (ER) inhibitor ICI182,780 failed to block E_2-induced $[Ca^{2+}]_i$, while the GPR30 agonist mimicked E_2-induced $[Ca^{2+}]_i$ changes and GPR30 specific siRNA significantly blocked these E_2 effects (Noel et al., 2009). However, because ERs, including ERα, ERβ and receptors sensitive to the diphenylacrylamide compound STX (STX-Rs) have been reported to initiate rapid effects on mouse GnRH neurons (Romano et al., 2008; Zhang et al., 2010; Sun et al., 2010), we 1) reinvestigated the role of ERα and ERβ using an siRNA transfection approach, 2) explored whether STX-Rs play a role in E_2-induced $[Ca^{2+}]_i$ changes and GnRH release, and 3) investigated the role of GPR30 in E_2-induced GnRH release using G1 and the specific antagonist G15. The results are 1) ERα or ERβ siRNA specific transfection did not block E_2-induced $[Ca^{2+}]_i$ changes, 2) treatment with STX was able to mimic E_2-induced $[Ca^{2+}]_i$ changes independently from GPR30 and STX significantly induced GnRH release although the duration was shorter than E_2 effects, and 3) G1 treatment induced GnRH release although the duration was also shorter than E_2 effects, while G15 was able to block E_2-induced GnRH secretion. The findings of the present studies indicate that rapid E_2 action on primate GnRH neurons are complex and are mediated by multiple ERs, specifically GPR30 and STX-Rs. The physiological role of rapid E_2 action in primate GnRH neurons remains to be determined.

Oral - Early Gestational Vitamin A Deficiency Causes Aberrant Signaling in Cyp1b1-/-Mice

Maguire, M; Jefocate CR

Cytochrome P450 1b1 (Cyp1b1) is a heme containing mono-oxygenase with a distinct spatio-temporal expression in embryonic development as well as expression in endothelial cells, macrophage, and numerous tissues including eye, liver, kidney, brain, and sex steroid responsive breast and prostate. Cyp1b1 has numerous substrates such as xenobiotics, estrogen, and retinol (Vitamin A). Cyp1b1 can participate in the conversion of retinol to retinaldehyde and the further metabolism to the bioactive retinoic acid (RA). However, it cannot inactivate retinoic acid, a process performed by the Cyp26 family. Cyp1b1 is retinoid responsive and is present in regions where traditional RA-generating enzymes are not observed. We hypothesize that the impact of an early gestational dietary vitamin A deficiency (VAD) maintained through pregnancy and lactation will alter local RA generation and signaling in the offspring. The combined insult of gestational VAD and Cyp1b1-/- causes aberrant signaling such that pups show a decreased weaning weight, while the body length remains the same. Furthermore, we show by in situ hybridization that VAD causes changes in gene expression at embryonic day (E) 9.5. Cyp1b1 is present in embryonic development and is involved in vitamin A signaling such that growth and development of the offspring are affected.

Oral - Dysfunctional Vascular Adaptations in Human Umbilical Vein Endothelial Cells (HUVEC) in Preeclamptic Pregancies

Jennifer Krupp, Derek Boeldt, Fu-Xian Yi, Ian Bird, Dinesh Shah

Objective: Preeclampsia (PE) occurs in 4-8% of all pregnancies. Endothelial cell dysfunction due to PE seen on the maternal side is also found in the umbilical vein. Our previous studies on umbilical vein endothelium (UV endo) of fresh cord vessels showed the sustained nitric oxide production and initial and sustained Ca2+ burst responses of normal pregnancy are impaired in PE. The basis of this dysfunction is unclear. Our hypothesis is these losses in Ca2+ response may either be due to failed programming of cell function or active suppression of such programming by local factors or inflammatory mediators. Removal of UV endo from the vessels and maintenance in vitro could indicate if recovery of function of HUVECs is possible, so suggesting that endothelial recovery on the maternal side at least may be possible given suitable therapy.

Study Design: HUVEC isolated from normal (N=7) and PE (N=6) subjects maintained in culture in vitro to passage 3 were imaged using Fura-2 to detect Ca2+ responses to stimulation with ATP (100um). Area under the curve (AUC), total number of bursts and mean Ca2+ response for the sustained phase were quantified.

Results: HUVEC from PE subjects do recover function (Figure) but with an increased frequency of Ca2+ bursts (* = $p < 0.05$) relative to control. There is no significant difference in the AUC between the normal and PE HUVEC. Despite the increased Ca2+ burst frequency in PE HUVEC, peak height is reduced and the overall AUC is not increased.

Conclusion: While intact PE UV endo lack sustained Ca2+ bursts in vivo, PE derived HUVEC maintained in vitro do show both restored Ca2+ bursting and increased frequency relative to control. Any such recovery of function in vitro suggests there was indeed a suppression of function by local factors in vivo. Further, since PE derived HUVEC manifest sustained Ca2+ burst responses which are both more rapid and of reduced size relative to control HUVEC, this suggests cell signaling programming is also altered relative to control. Funded by NIH Grant R21HD069181.

Oral - The Effect of Protein Kinase C delta (PKCδ) on the Transctiptome of Vascular Smooth Muscle Cells

Jun Ren, Yi Si, Bo Liu

Previous studies indicated that overexpression of PKCδ prevented neointima formation by causing apoptosis of smooth muscle cells (SMCs), while the reduction of intimal hyperplasia was attenuated by PKCδ overexpressing SMCs mediated adventitia cell migration. To elucidate the mechanism involved in the dual roles of PKCδ on smooth muscle cells (SMCs), we examined the effect of PKCδ on the transcriptome of vascular smooth muscle cells in vitro. Primary vascular smooth muscle cells were infected by AdPKCδ or AdNull and followed by incubating with PMA (1nM), and their gene expression was analysed by microarray. Pathway analysis and gene ontology analysis of differentially expressed genes in each comparison were performed. Identification of significantly overrepresented transcriptional regulatory elements (TREs) was performed using the Promoter Analysis and Interaction Network Toolset (PAINT) program. Differential gene expression of 15 genes was confirmed using quantitative real-time PCR. Overexpression of PKCδ caused a profound change in transcriptome of vascular smooth muscle cells in vitro. Pathway and gene ontology analysis identified multiple cellular pathways affected by PKCδ overexpression. Furthermore, both pathway and PAINT analyses indicated that the transcription factor NFkB played an important role in the transcriptome changes induced by PKCδ overexpression. Multiple genes involved in the cell migration were significantly increased by PKCδ overexpression in SMCs. These results indicate that apoptosis and chemokines expression induced by PKCδ overexpression may serve as the mechanism underline dual of PKCδ in neointima formation and specifically block chemoines induction without disturbing apoptosis effect of PKCδ may represent a novel therapeutic approach to prevent neointima formation.

Oral - Shear Stress, cAMP, and cGMP Modulate the Pregnancy-Related Phosphorylation State of Cx43-Gap Junction and eNOS Proteins in Uterine Artery Endothelial Cells

Bryan C Ampey, Ethan J. Schuler, Sheikh O Jobe, Daniel T.Thoresen, Gladys E. Lopez, Ian M. Bird, Ronald R Magness

Introduction: During normal pregnancy uterine artery endothelial cells (UAECs) are exposed to substantial elevations in shear stress, a very potent mechanical hemodynamic force which is a vital regulator of endothelial Nitric Oxide Synthase (eNOS) that mediates rapid and substantial rises in the vasodilator nitric oxide (NO). Enhanced vasodilatation during gestation is dependent upon UAEC cell-cell communication via Gap junctions to increase uterine blood flow (UBF). Although cyclic nucleotides (e.g. cAMP and/or cGMP) are known to increase Gap junction assembly, the effects of shear stress or these cyclic nucleotides on the associated phosphorylation state of the primary gap junction protein Cx43 and eNOS are unknown. **Hypothesis:** We hypothesized that shear stress and the cyclic nucleotides cAMP and/or cGMP will modify the "stimulatory" phosphorylation state of Cx43 and eNOS. **Methods:** Acute (0-60min) and prolonged (12-24hr) time course studies were performed exposing UAECs (n=4) from pregnant ewes (120-130 days; term=147) to either physiologic levels of pulsatile laminar shear stress (15 dynes/cm2) or exogenous treatment (1uM and 1mM) with either 8-Bromo-cAMP or 8-Bromo-cGMP. Western blotting was performed to determine phosphorylation states of Ser P365Cx43 compared to SerP368Cx43, and Ser P635 eNOS. The loading control □-Actin was unaltered by either shear stress or cyclic nucleotide treatment. **Results:** We observed that exposing P-UAECs to physiologic levels of shear stress stimulated acute (10-60min) and prolonged (24h) increases ($P<0.05$) in the phosphorylation patterns of 'cAMP dependent' Ser P365Cx43 (8-11 fold) and to a lesser extent the PKC dependent Ser P368Cx43 (2.3-2.5 fold), as well as NO stimulatory site Ser P635 eNOS (2.2-2.5 fold). Following treatment of P-UAECs with exogenous cAMP, we observed both time (30 min) and dose dependent maximum increases in phosphorylation patterns of the cAMP dependent Ser P365Cx43 (4.5-5.5 fold) and the Ser P635 eNOS (1.3-1.6 fold) stimulatory site, with no effect seen on the PKC dependent Ser P368Cx43 site. Compared to these observed cAMP responses, treatment with exogenous cGMP showed similar elevations of Ser P365Cx43 (4-6 fold) and Ser P635 eNOS (1.6-2.0 fold), but substantially greater rises in the PKC dependent Ser P368Cx43 (3.5-5.0 fold) phosphorylation sites. **Conclusion:** We demonstrate that shear stress and exogenous cAMP and cGMP all stimulate the cAMP-PKA dependent; but only shear stress and cGMP stimulate the PKC dependent modifications of Cx43 phosphorylation state in association with eNOS stimulatory activity state. Therefore, the rapid responses of Cx43 phosphorylation states to cAMP/PKA, cGMP/PKG, PKC pathways and also shear stress suggest a direct mechanistic signaling link between posttranslational modifications of Cx43 and Gap junction assembly and function in UAECs as part of the adaptation mechanism(s) needed to maintain NO production, UBF, and fetal growth during gestation. NIH GM083252, HL49210, HD38843, HL87144, HL079020.

Oral - RNA-Seq analysis uncovers transcriptomic variations between morphologically similar in vivo- and in vitro-derived bovine blastocysts

Driver AM, Peñagaricano F†, Huang W*, Ahmad KR‡, Hackbart KS*, Wiltbank MC*, and Khatib H†*

*Department of Dairy Science, University of Wisconsin, Madison, WI 53706 USA †Department of Animal Sciences, University of Wisconsin, Madison, WI 53706 USA ‡Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan The first two authors have equal contribution.

Background: A valuable tool for both research and industry, in vitro fertilization (IVF) has applications ranging from gamete selection and preservation of traits to cloning. Although IVF has achieved worldwide use, with approximately 339,685 bovine embryos transferred in 2010 alone, there are still continuing difficulties with efficiency. It is rare to have more than 40% of fertilized in vitro cattle oocytes reach blastocyst stage by day 8 of culture, and pregnancy rates are reported as less than 45% for in vitro produced embryos. Although numerous studies have been done to improve the IVF system itself, development rates remain seemingly low. As such, there has been a shift to better understand how the IVF process could influence the biological framework of the developing embryo. To investigate potential influences of IVF, this study compares in vivo- and in vitro-derived bovine blastocysts at a similar stage and quality grade (expanded, excellent quality) to determine the degree of transcriptomic variation beyond morphology using RNA-Sequencing. We will be able to go beyond total expression counts and characterize factors such as alternative splicing and novel transcribed units (NTU). Overall, we hypothesize that IVF will alter the transcriptomic landscape of the bovine pre-implantation embryo. **Results:** A total of 26,906,451 and 38,184,547 fragments were sequenced for in vitro and in vivo embryo pools, respectively. We detected expression for a total of 17,634 genes, with 793 genes showing differential expression between the two embryo populations with false discovery rate (FDR) < 0.05. There were also 395 novel transcribed units found, of which 45 were differentially expressed (FDR<0.05). In addition, 4,800 genes showed evidence of alternative splicing, with 873 genes displaying differential alternative splicing between the two pools (FDR<0.05). Interestingly we also detected 782 genes with differential alternative splicing without showing differential gene expression. Using GO enrichment analysis, multiple biological pathways were found to be significantly enriched for differentially expressed genes (FDR<0.01), including cholesterol and sterol synthesis, system development, and cell differentiation. **Conclusions:** Our results support that IVF influences at the transcriptomic level and that morphology is limited in full characterization of bovine pre-implantation embryos.

Poster - Increased Right Hand 2d:4d Ratio Accompanies Polycystic Ovary Syndrome-Like Traits in Female Monkeys Exposed to Testosterone During Early-to-Mid Gestation

AD Abbott, RJ Colman, R Tiefenthaler, DA Dumesic and DH Abbott

The length ratio for the second finger relative to the fourth finger (2D:4D ratio) in men is diminished compared to women. The 2D:4D ratio is repeatedly associated with male-typical fetal testosterone (T) exposure, and, therefore, is implicated as a biomarker for a variety of traits, including enhanced physical and cognitive abilities, diminished reproductive function, and susceptibility to several diseases. No human studies, however, have examined whether male-typical fetal T levels are responsible for altering the 2D:4D ratio. The present study utilizes the rhesus monkey, as a close relative of humans, to employ discrete gestational exposure of fetal female monkeys to male-typical T levels for 15-35 days in early (40-75 days) or late (94-139 days) gestation (term: 165 days) by daily subcutaneous injection of their dams with 5-10 mg T propionate. Such gestational exposures are known to enhance male-typical behavior and polycystic ovary syndrome-like traits. In this study, compared to control females, early gestation exposure alone virilized external genitalia, while concomitantly increasing the 2D:4D ratio of the right hand (RH), apparently from male-like elongation of RH2D, without elongation of RH4D. Increased RH2D length and 2D:4D ratio positively correlated with anogenital distance (AG); moreover RH2D and AG positively correlated with duration of early gestational T exposure. Male monkeys exhibited a diminished 2D:4D ratio in the right foot compared to control females, but this male trait was not emulated by either androgen-exposed female group. X-ray determined phalanx measurements indicated increased phalanx length in males and no differences between female groups. There were no differences in phalanx length ratios between any groups. Thus, discrete T exposure during early gestation in female rhesus monkeys appears to increase RH2D length through right-side biased, non-skeletal tissue growth. As variation in timing and duration of gestational T exposure alter male-like dimensions of RH2D independently of RH4D, postnatal RH2D:4D ratio provides a complex biomarker for fetal T exposure.

Poster- Electrical Properties of P-UAEC and the Role of Agonist Induced Hyperpolarization in Sustained Ca2+ Signaling

Roxanne Alvarez, Fu-Xian Yi, Bikash Pattnaik, Ian Bird

Uterine artery endothelial cells from pregnant ewes (P-UAEC) typically show sustained Ca2+ bursting in response to ATP stimulation due to periodic activation of TRPC channels. This in turn depends upon gap junction function, and the resulting bursts facilitate production of the vasodilator nitric oxide. Cells from nonpregnant ewes (NP-UAEC) fail to show prolonged burst responses and also show poor gap junction coupling. TRPC3 function is known to be sensitive to cell membrane potential, and may be enhanced as the result of cell-cell communication. We propose improved gap junction communication during pregnancy allows electrical coupling that shifts cells towards a membrane potential range supportive of TRPC3 activity necessary for sustained Ca2+ bursting. **Objective:** Our goal was to establish basic UAEC electrical properties and specifically the cell's resting membrane potential. Also, to then further determine possible changes in membrane potential in response to ATP stimulation, and to establish if a direct coupling relationship exists between Ca2+ bursting and the cell's membrane potential. **Methods:** Ovine P-UAEC (passage 4) were grown in 35 mm glass bottom dishes to <20% density for electrophysiology or 100% for use in dye imaging. Whole-cell patch clamping was performed on isolated, voltage clamped cells. Cells were loaded with Fura-2 (Ca2+ dye) followed by DIBAC4 (membrane potential dye). Simultaneous imaging of [Ca2+]i and membrane potential was acquired for 35 minutes under basal or ATP (100uM) stimulation. Data from stimulated cells were then compared to vehicle control cells. Results: Resting membrane potential of P-UAEC is negative 20mV ±2.0, capacitance is 14pF ±1.7, and resistance is 4.0GΩ ±0.54. ATP (100uM) stimulation of P-UAEC results in an agonist specific and progressive membrane hyperpolarization that continues for as long as sustained Ca2+ bursts occur. The approximate drop in membrane potential over 30 minutes is a further 20 mV. **Conclusion:** We have established the basic electrical properties in P-UAEC and that ATP stimulation does indeed result in further membrane potential hyperpolarization during Ca2+ bursting. We are currently extending these studies in NP-UAEC. Further studies are needed to determine the cause and effect relationship between changes in membrane potential and Ca2+ bursting in P- and NP-UAEC and the dependence of these responses on gap junctions. Funded by NIH R01 HL079020.

Poster – Glucose Metabolism in Immune Cells

Soma Banerjee, Arvinder Kapur, Kai Ludwig, Mildred Felder and Manish S. Patankar

Cancer cells have high metabolism and have specific molecular alterations that allow them to metabolize glucose and other nutrients at high levels. Tumor cells, by expressing high levels of glucose transporters and by developing specific mechanisms to sustain their proliferation by fermenting glucose to lactic acid, consume the available glucose and other carbohydrate nutrients at a very high rate. Immune cells likely do not have this ability. We are interested in the consequences of the interaction between immune cells and tumor cells in the tumor micro-environment. Here, we investigate the dynamics of glucose transport in naïve and stimulated immune cells isolated from the peripheral blood of healthy individuals. Uptake of glucose in T cell, B cell, NK cell, and monocytes was measured by multi-color flow cytometry where the individual immune cell subsets were identified by cell surface markers (CD3, CD19, NKp46, and CD14, respectively) and the fluorescent analogue of D-glucose [2-{N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose}, 2-NBDG] was used as a surrogate to determine sugar uptake. Naïve and IL-2 stimulated immune cells from three healthy donors were used in our assays. The naïve as well as IL-2 stimulated monocytes exhibited a 3-4-fold higher uptake of NBDG as compared the remaining immune cell subsets. The high uptake of glucose by the monocytes could be explained by the signifciantly higher level of expression of the glucose transporters, GLUT-1 and GLUT-3 in the monocytes as compared to the T, B, and NK cells. Our data also shows a higher level of the glucose-sodium cotransporters SGLT-1 and SGLT-2, on the human monocytes. We have also tested the effects of glucose deprivation on the immune cells especially as it relates to the stimulation of the autophagy pathway. Our on-going studies are focused on determining the effects of glucose deprivation or exposure to high levels of glucose that are observed in diabetic patients on immune cell function.

Poster - Pregnancy Enhancement of Endothelial Cell Function is Blocked by VEGF-165 Pretreatment in a Manner Reversible by the MEK/ERK Inhibitor U0126 in Uterine Artery (UA) Endothelial Cells.

Derek S. Boeldt, Mary A. Grummer, Ronald R. Magness, Fu-Xian Yi, Ian M. Bird

Introduction: Pregnancy-related increases in endothelial nitric oxide (NO) production are necessary for enhanced vasodilation and so increased blood perfusion of the uterus. Failure of this adaptation can result in preeclampsia and associated fetal growth restriction. Increases in sustained phase Ca2+ signaling are critically linked to increased endothelial nitric oxide synthase (eNOS) activity in response to agonists such as ATP, which stimulates repeated Ca2+ bursts in UA endothelial cells (UAEC) in a manner dependent on Cx43 gap junction function. Vascular endothelial growth factor (VEGF), which is elevated in preeclamptic pregnancies, is a known inhibitor of Cx43 function and we have recently reported VEGF inhibits Ca2+ burst and NO production on subsequent ATP challenge in intact UA endothelium. Herein we examine if VEGF inhibition of subsequent ATP stimulated sustained phase Ca2+ signaling in UAEC is associated with Cx43 phosphorylation and whether it is through VEGFR1 or VEGFR2. **Methods:** Primary UAEC (passage 4) from nonpregnant (NP) and pregnant (P) ewes were grown to 100% confluence on 35 mm glass bottom dishes. Cells were then loaded with Fura-2 and imaged under stimulation with ATP for 30 minutes (control). After washing, the same cells were pretreated as below, followed by a second ATP stimulation. Cx43 data phosphorylation data was acquired by western blot. **Results:** Pretreatment of P-UAEC with VEGF-165, VEGF-E (VEGFR2-specific), or a combination of VEGF-E and PlGF significantly inhibited ATP stimulated sustained phase Ca2+ bursts (79.6±3.5, 80.7±3.3, 80.3±2.9 respectively, expressed as % control), while PlGF (VEGFR1-specific) alone did not differ from vehicle (91.7±3.8, 90.3±3.2). Incubation with the MEK/ERK inhibitor U0126 completely reversed VEGF-165 inhibition (89.8±3.3). Western blot analysis for Cx43 phosphorylation revealed phosphorylation at s279/282 in response to VEGF-165 and full reversal by U0126. **Conclusion:** We have previously shown that ATP stimulated sustained phase Ca2+ bursts are dependent upon Cx43 function. Herein we propose that VEGF-165 mediates inhibition of sustained phase Ca2+ bursts through VEGFR2, mediated via MEK/ERK signaling, likely via direct phosphorylation of Cx43, with s279/282 being the key phosphorylation site. Funded by NIH HL079020, T32HD41921, HD38843.

Poster - Diet coordinately affects expression of multiple hepatocyte P450 Cytochromes by processes that are highly sensitive to external Cyp1b1

Justin Bushkofsky, Michelle Larsen, Colin Jefcoate

A change from a high carbohydrate diet to a high fat diet stimulates obesity and glucose intolerance in C57Bl/6j mice at maturity. Cyp1b1 exhibits many developmental functions in mice and humans. Deletion of Cyp1b1 in mice prevents this obesity response. Mouse liver expresses 48 Cyps at significant levels (total 82). Cyp1b1 is not expressed in mouse hepatocytes, but deletion dramatically redirects liver gene expression (>700 genes FC>2.0), even more than the dietary change. The same Cyp genes are affected in a coordinate manner, often as clusters within sub-families (2a, 2b, 2c, 3a and 4a). Cyp2c genes are stimulated by dietary fat and Cyp1b1 deletion, whereas fat-responsive genes in each of the 2a, 2b, 3a and 4a sub-families are highly suppressed. Cyp1b1 deletion appears to effect a general suppression of endogenous PPAR, CAR and PXR activities that extends to non-Cyp genes. This does not extend to external ligands, as PPAR induction is effectively stimulated by WY14623 in Cyp1b1-/- mice. One possibility is that endogenous ligands are either not formed or are sequestered by binding proteins. An extensive set of such proteins are expressed by chromosomally adjacent lipocalin and Mup families. Six such genes are highly induced by Cyp1b1 deletion. The diet change and Cyp1b1 deletion also suppress genes in the liver that are associated with oxidative stress and inflammation. Paradoxically, Cyp1b1 deletion in endothelia causes NF-kB activation. The external source of the Cyp1b1 regulation remains elusive.

These studies show that diet change and Cyp1b1 deletion exhibit similar selectivity for effects on several families of genes. Dietary flavonoids can inhibit Cyp1b1 and may affect metabolism of endogenous or dietary substrates thus altering hepatic glucose and fatty acid metabolism. This impact on hepatic Cyp expression could affect xenobiotic metabolism.

Poster - Stillbirth and Pregnancy Resorption in Female Mice Transgenic for Angiotensinogen or Renin

J. Morgan Denney, MD, Cynthia Bird, Annette Gendron, DVM, PhD, and Dinesh M. Shah, MD

Objective: Crossing a female mouse transgenic for human angiotensinogen (hAng) with a male mouse transgenic for human renin (hRen) has been reported as a model for preeclampsia. Previous in vitro studies have indicated no interaction between human RAS transgenes and mouse RAS genes due to species-specific biological actions. Because uterus has an independent RAS, especially in the gravid state, we compared wild type (WT) with transgenic (TG) gravid mice to determine the affect of transgene presence on fetal outcome. **Methods:** Mouse genotyping was performed for verification of lines. Colony lines of hAng, hRen, and WT were maintained separately during breeding protocol to avoid hAng x hRen interaction. Progeny from breeding were counted. Necropsy was performed on gestation day 19 of subjects designated for histologic analysis. Uteri and products of conception were resected en bloc, evaluated grossly, and weighed. Univariate analysis was used where appropriate. **Results:** 20 pregnancies were analyzed. TG females (hRen or hAng) delivered significantly less live born pups per gestation when compared to WT counterparts. TG females had a significantly higher stillbirth (SB) rate. Mean uterine-conceptus (U-C) weights of transgenic females were smaller compared to those achieved by their wild type counterparts (Table 1). Histologic examination of the uteroplacental interface revealed increased placental necrosis, fibrin deposits, inadequate vascular modeling, and inadequate development of the villous labyrinth in TG females. WT uterine-conceptus blocks were unremarkable. Table 1: Mean WT TG p-value LB/gestation 6.62 1.5 0.038 SB/gestation 0 1.5 0.026 U-C (gm) 2.3 0.8 NS **Conclusion:** Mice transgenic for either renin or angiotensinogen have lower live birth rates and higher still birth rates even when interactions of the hRen with hAng are avoided in breeding. Given significant effects on live birth rate and a trend toward low uterine-conceptus weight, these data suggest abnormality that may be a consequence of human transgene RAS interactions with native mouse RAS genes. Our observations indicate increased pregnancy resorption and failure to establish viable pregnancy in the transgenic mouse. Our observations also suggest placental hypoxia due to inadequate vasculature modeling. These data suggest interspecies interactions of RAS genes in vivo.

Poster - Tcf19 and regulation of beta cell proliferation

Danielle Fontaine, BS; Dawn Davis, MD, PhD; Kimberly Krautkramer, BS;Louise Meske, MS

Tcf19 is a largely uncharacterized transcription factor that is expressed during cell division, beginning at G1/S phase. In a mouse model of obesity-associated diabetes, we identified tcf19 within a group of cell cycle genes whose expression correlates with adaptive islet proliferation. In confirmation of these microarray data, we observed 3.3-fold upregulation of tcf19 mRNA in islets from obese non-diabetic C57Bl/6J (B6) mice vs. lean (n=5, p=0.01). This obesity-driven upregulation was not observed in islets from the diabetic BTBR strain. Tcf19 is most highly expressed in islet, which is an unusual pattern of expression for a cell cycle regulator since numerous tissues have higher basal proliferation rates, and suggests that tcf19 may play a more specific role in cell cycle regulation in the islet. The role of tcf19 in beta cell growth was then examined. After siRNA-mediated knockdown of tcf19 in INS-1 cells, a significant reduction in the number of viable cells was found. Proliferation was directly measured by 3H-thymidine incorporation and a 40% reduction was found with tcf19 knockdown (n=3, p=0.012). In summary, tcf19 is necessary for beta cell proliferation and may play a role in obesity-driven proliferation in mouse islets.

Poster - Systemic application of the kisspeptin antagonist, peptide 271, suppresses in vivo GnRH release in ovarian intact pubertal Rhesus monkeys.

Guerriero KA, Keen KL, Millar RP, Terasawa E

Kisspeptin (KP) and kisspeptin-1 receptor (KISS1R) signaling has been implicated as an important regulator in GnRH release, especially at the time of puberty. Our previous findings that direct infusion of the KP antagonist, peptide 234, into the stalk-median eminence (S-ME) suppresses in vivo GnRH release in ovarian intact monkeys (1), supports peptide 234's capacity as a potential tool for clinical treatment. Recent evidence in rodents, however, suggests that peptide 234 is unable to cross the blood-brain barrier (BBB), and therefore, systemic application is not practical (2). Accordingly, Millar and his colleagues developed a KP antagonist, peptide 271 (peptide 234 with penetratin attached to the N-terminus), which is capable of crossing the BBB, as intraperitoneal injection of peptide 271 in adult female rats inhibits the kisspeptin-induced stimulation of LH release (3). Currently, whether systemic administration of peptide 271 suppresses GnRH/LH release in primates is still unknown. Therefore, in this study we examined the effects of intravenously (iv) administered peptide 271 on in vivo GnRH release in ovarian intact pubertal monkeys using the microdialysis method. Results from a single bolus of 1 nmol/kg peptide 271 indicated that peptide 271 clearly suppressed GnRH release with a ~2-hour latency and this suppression continued for up to 6 hours post-injection. No significant adverse effects were observed from peptide 271 administration. These results further suggest that peptide 271 has a great potential for therapeutic application in the treatment of reproductive abnormality.

Poster - Effect of Insulin Resistance Induced by Excessive Growth Hormone on Ovarian Function

Katherine S. Hackbart, Milo C. Wiltbank

Polycystic ovarian syndrome (PCOS) is characterized by polycystic ovaries, anovulation, and hyperandrogenemia. Insulin resistance has also been shown to be associated with PCOS. Our hypothesis was that induction of insulin resistance by administration of excessive levels of growth hormone in cows would result in an anovulatory phenotype similar to that observed in women with PCOS. Nine non-lactating, non-pregnant cows were randomized to three different treatments: control (C; n=3) cows received s.q. injections of saline twice weekly (M and Th), 1X cows (n=3) received s.q. injections of 500 mg of a long-acting recombinant bovine somatotropin (rbST) on M and saline on Th, and 2X cows (n=3) received s.q. injections of 500 mg rbST on M and Th. Cows remained on their respective treatments for the course of the entire experiment. Blood was collected every M and Th for the duration of the trial to determine circulating insulin and glucose concentrations. Three intensive periods, lasting two weeks each and begun at weeks 5, 11, and 17 after treatment initiation, were separated by four weeks of continued treatments. The intensive periods consisted of follicular ablation to initiate a new follicular wave, followed by daily ovarian ultrasound until ovulation was observed. It was found that rbST treatment slightly but significantly increased circulating glucose in both 1X (61.3 ± 0.6 mg/dL; P=0.0024) and 2X (61.5 ± 0.6 mg/dL; P=0.0018) cows as compared to C (56.9 ± 0.6 mg/dL) cows. However, circulating insulin concentrations were increased (P=0.007) more than 2-fold in 2X cows (48.5 ± 4.6 μU/mL) compared to C cows (22.0 ± 4.6 μU/mL), with intermediate values in 1X cows (33.1 ± 4.6 μU/mL). A measure of insulin resistance, the HOMA-IR value, was elevated (P=0.006) in 2X cows (7.47 ± 0.7) compared to C cows (3.10 ± 0.7) with intermediate values in 1X cows (5.04 ± 0.7). There were no differences (P≥0.1) between treatments for number of follicles in different size categories (≤5 mm; 5.5-7.5 mm; 8-9.5 mm; ≥10mm) during the four days immediately following ablation, days from ablation to ovulation, or number of ovulations. There were, however, significant differences in the size of the ovulatory follicle. 2X cows (16.0 ± 0.5 mm) ovulated significantly larger follicles compared to C (13.7 ± 0.4 mm; P=0.0052) and 1X (12.1 ± 0.4 mm; P<0.0001) cows; C cows also ovulated significantly (P=0.0157) larger follicles than 1X cows. In each of the three intensive periods, all cows were capable of either ovulating or luteinizing a follicle, with no differences (P≥0.1) between treatment groups for number of animals that ovulated versus luteinized a follicle. Thus, induction of more than a 2-fold difference in circulating insulin for 4.5 months by treatment with excessive growth hormone had only minor effects on ovarian function of cows, inconsistent with our original idea that this model might be used to mimic the physiology associated with PCOS.

Poster - Oxytocin Regulation of Retinal Pigment Epithelium Cell Function

P. Halbach, MP Asuma, W. Luo, D. M. Pillers, B. R. Pattnaik

Purpose: Oxytocin (OT) is a neuropeptide that activates oxytocin receptor (OTR), a rhodopsin family G-protein coupled receptor. OTR signals the cellular phosphatidylinositol-calcium second messenger system. Within the retina, the cross talk between retinal pigment epithelium (RPE) and photoreceptor neurons is hypothesized to be mediated by one such phosphatidylinositol-calcium signaling mechanism. Several molecules, particularly ATP, are proposed to mediate RPE-retina communication. Since we have localized OTR transcripts to RPE, we were interested in knowing whether RPE cells also utilize oxitocinergic signaling for communication. **Methods:** Human fetal RPE (hRPE) cells were cultured as a tight monolayer using serum-free medium containing MEM alpha base medium, N1 supplement, non-essential aminoacids, taurine, hydrocortisone and triiodo-thryonin. Cultures were maintained at 37°C and 5% CO2 with a media change every 2-3 days. Cytoplasmic content of isolated cells were harvested and analyzed by single-cell RT-PCR. We used a two-step nested PCR method to amplify the transcripts. Immomix PCR mix and appropriate primer pairs were utilized to amplify OTR and other RPE-specific transcripts in individual cells. Localization of OTR to the RPE layer of monkey frozen sections was determined by standard immunofluorescence methods. Intracellular Ca2+ ([Ca2+]i) mobilization in response to OT and ATP was conducted via live-cell imaging and FURA-2 ratiometric measurements. **Results:** In four individual hRPE cells, a 256 bp transcript corresponding to the OTR messenger was amplified. These cells also tested positive for RPE-specific markers RPE65, Kir7.1, hBest1, NaKATPase, and ezrin transcripts. OTR was localized to the posterior aspects of monkey RPE cells. hRPE cells in culture, when stimulated by OT or ATP, exhibited a 70-120 nM increase of [Ca2+]i that was completely reversible. However, while ATP induced an instantaneous increase in Ca2+, OT induced a gradual rise, suggesting ATP and OT may be acting through independent signaling mechanisms. **Conclusions:** Localization of OTR in RPE is a novel finding that implicates an important regulatory role of OT in the RPE-retina communication. The increase of [Ca2+]i in response to OT stimulation, as well as previously established circadian regulation of the RPE, suggests that the RPE may utilize oxytocinergic signaling as one aspect of endocrine regulation of RPE function.

Poster - Steroidogeic Capacity as a Predictive Marker for Alzheimer's Disease

Kentaro Hayashi and Craig S. Atwood

Post-reproductive concentrations of circulating sex steroids are negatively correlated with the incidence of age-related diseases, including coronary heart disease, stroke, Alzheimer's disease and osteoporosis. Since circulating concentrations of hormones post-reproduction are derived from peripheral tissues, the synthetic capacity of peripheral tissues may be a useful marker for determining the likelihood of developing age-related diseases. Fibroblasts are peripheral tissue cell type that could be easily acquired to test this hypothesis. However, the capacity for steroidogenesis by fibroblasts has not previously been determined, although it is known that fibroblasts express p450scc protein (Slominski et al. 2004). Our initial experiments therefore measured whether fibroblasts secret progesterone (P4) following stimulation with luteinizing hormone (LH; 40 mIU/mL) or human chorionic gonadotropin (hCG; 50 mIU/mL). Both LH and hCG induced P4 secretion from fibroblasts; 3.26 ng/ul and 0.49 ng/ul, respectively. On the other hand, pregnenolone (P5) treatment did not increase P4 secretion compared to control. We next identified the expression of steroidogenic proteins in fibroblasts - StAR, p450scc and GnRHR proteins. LH increased the expression of the active form of StAR (1.2-fold) and GnRHR (1.4-fold) compared to control, indicating that LH promotes cholesterol transport into the mitochondrion for sex steroid synthesis. Interestingly, P4 (10 nM) and P5 (10 nM) decreased the expression of the active form of StAR (0.65-fold), and only P4 increased StAR inactive form (1.36-fold), compared with control. Both P4 and P5 decreased p450scc (0.5-fold) and GnRHR (0.2 to 0.4-fold) expression. These results suggest that P4 and P5 negatively feedback to decrease GnRH signaling, cholesterol transport into the mitochondria, and cholesterol utilization for P4 production. These results indicate both positive and negative feedback of gonadotropins and sex steroids in fibroblasts as has also been demonstrated in the brain, and suggest the potential for using skin fibroblasts as a marker of steroidogenic capacity.

Poster - Quantitative Study of Plasma Profiles of Estrogens and Estrogen Metabolites in Normotensive Pregnancy, Mild Preeclampsia and Severe Preeclampsia by Liquid Chromatography-Tandem Mass Spectrometry

Sheikh O Jobe, Chanel T Tyler, Ronald R Magness

Introduction: 2-methoxyestradiol (2-ME2), a metabolite of estradiol-17β (E2) synthesized from catecholestrogens by catechol-O-methyltransferase may play a critical role in the pathophysiology of preeclampsia. However, plasma levels of specific estrogens and/or estrogen metabolites remain undetermined and patterns of estrogen metabolism during normal (NP) and preeclamptic (PE) pregnancies are unclear. **Objective:** To compare plasma concentrations of total estrone (E1), E2, estriol (E3), 2-hydroxyestrone (2-OHE1) and 4-hydroxyestrone (4-OHE1), 16-hydroxyestrone (16-OHE1), 2-hydroxyestradiol (2-OHE2), 2-methoxyestrone (2-ME1), 3-methoxyestrone (3-ME1), 4-methoxyestrone (4-ME1), 2-ME2, 4-methoxyestradiol (4-ME2), 16-Keto-estradiol (16-Keto-E2), 16-epi-estriol (16-E3) and 17-epi-estriol (17-E3) in NP women to those of women with mild (mPE) and severe (sPE). **Methods:** Plasma samples were obtained from NP (n = 8), mPE (n = 8) and sPE (n = 8). Quantitative levels of estrogens and estrogen metabolites were measured by liquid chromatography mass spectrometry. **Results:** Compared to plasma levels of E1 and E2 in NP (50558±102, 8891±89 pg/ml respectively), levels in sPE (2631±102, 2039±89 pg/ml respectively) but not in mPE were lower (P< 0.001). Compared to levels of 2-OHE1 and 2-OHE2 in NP (490±53, 487±39 pg/ml respectively), levels were lower (P<0.001) in mPE (201±53, 221±39 pg/ml respectively) and sPE (176±53, 206±39 pg/ml respectively). 4-OHE1 and 16-OHE1 were unchanged. Compared to levels of 2-ME1, 4-ME1, 2-ME2 and 4-ME2 in NP (823±52, 698±78, 1967±47, 1151±90 pg/ml respectively), levels were lower (P<0.001) in sPE (508±52, 526±79, 957±47, 434±90 pg/ml respectively) but not in mPE (889±51.9, 734±78.2, 1988±46.9, 1434±89.4 pg/ml respectively). Interestingly, compared to plasma levels of 16-Keto-E2 in NP (883± 53pg/ml) and mPE (788± 53pg/ml), levels were higher in sPE (1939 ± 53 pg/ml). E3, 16-E3 and 17-E3 were unchanged. **Conclusions:** Since estrogens and estrogen metabolites play key roles in cardiovascular adaptations during normal pregnancy, these data suggest that aberrant synthesis and metabolism of estrogens may be a mechanism responsible for the dysfunctional cardiovascular function in pregnancies complicated with preeclampsia. NIH HL49210, HD38843, HL87144, T32-HD041921

Poster - Transcriptional Repression of TXNIP by FOXO1 in Pancreatic Beta Cells

Kibbe C, Shalev A.

Chronic hyperglycemia leads to a number of pathological problems including beta cell apoptosis. We have previously shown human pancreatic islet cells exposed to high levels of glucose have increased expression of thioredoxin-interacting protein (TXNIP). TXNIP binds and inhibits thioredoxin, a redox regulator, resulting in increased cell apoptosis. Using mouse models, we found that TXNIP deficiency inhibits beta cell apoptosis and protects against type 1 and type 2 diabetes. These studies suggest that TXNIP may be a possible target for therapies aimed to stop beta cell apoptosis in diabetic patients, however first more must be known about the mechanisms regulating TXNIP. The forkhead box O1 transcription factor (FOXO1) has been reported to regulate the expression of TXNIP, but the effects seem to be tissue-dependent and the effect of FOXO1 on TXNIP expression in the beta cell has yet to be described. Using transient transfections of a FOXO1 expression plasmid we first demonstrated that overexpression of FOXO1 in human islets and INS-1 cells significantly decreases endogenous TXNIP mRNA levels. We further showed that FOXO1 occupies the TXNIP promoter in human islets and INS-1 cells, as assessed by ChIP assays. Using luciferase reporter assays we also demonstrated that FOXO1 overexpression reduces TXNIP promoter activity in INS-1 cells. However, a promoter deletion analysis revealed that rather than a known consensus FOXO1 binding site, a downstream E-box-like motif was responsible for the regulation of TXNIP by FOXO1. In fact, we were able to show that this E-box-like motif is not only necessary, but also sufficient for FOXO1 mediated repression. We have previously shown that glucose-induced TXNIP expression is mediated through carbohydrate response element-binding protein (ChREBP) binding to this E-box-like motif in the TXNIP promoter. Using another set of transient transfections at low and high glucose we were able to show that FOXO1 overexpression also blunts glucose-induced TXNIP mRNA, protein and promoter activity. Furthermore, using ChIP assays we showed that FOXO1 overexpression is able to significantly reduce the glucose-induced binding of ChREBP at the TXNIP promoter. Using transient transfections employing a FOXO1 DNA-binding mutant (FOXO1-H215R) we have shown that DNA-binding is required for FOXO1 mediated repression of TXNIP. Together, these results demonstrate for the first time that FOXO1 inhibits TXNIP expression in pancreatic beta cells, and suggest that FOXO1 confers this inhibition by competing with ChREBP for binding to the TXNIP promoter.

Poster - Pleiotropy Involving Skeletal, Vascular, and Reproductive Phenotypes: Convergence via Endothelin Signaling and Nos3 Activity

Jasmin Kristianto, Jacqueline S Fisher, Michael G Johnson, Zhijie Wang, Chen Yen Ooi, Suzanne J Litscher , Naomi Chesler PhD, Robert D Blank MD PhD

There is a well-established epidemiological association between skeletal fragility and atherosclerosis. There is an equally recognized association between low birth weight and atherosclerosis. Through study of the recombinant congenic mouse strains HcB-8 and HcB-23, our laboratory has identified a pleiotropic quantitative trait locus (QTL) on mouse chromosome 4 that modulates skeletal modeling in response to mechanical loading. Recombination during the strains' construction limits the QTL region to ~6.5 Mb and includes Ece1, encoding endothelin converting enzyme 1. We hypothesize that the same QTL mediates modeling differences in response to mechanical loading in both bone and blood vessels and that Ece1 is the gene underlying the QTL. Further, we hypothesize that deficient vascular modeling in HcB-8 mice leads to impaired reproductive performance. HcB-8 carotid arteries have a smaller diameter at 90 mm Hg and are less compliant to increases in luminal pressure than HcB-23 carotids ($p=0.026$, $p=0.036$ respectively). HcB-8 mice also have larger fractional heart sizes ($0.71 \pm 0.02\%$ v $0.52 \pm 0.03\%$, $p<10\text{-}3$). HcB-23 heart expresses nearly 3-fold more Ece1 mRNA, $p <10\text{-}3$), with the protein data showing a similar trend. NOS3 protein is also more abundantly expressed in HcB-23 hearts. HcB-8 litters are smaller than HcB-23 litters ($4.4 + 1.8$ v $5.8 + 2.0$, $p< 10\text{-}3$) and HcB-8 pups are lighter at birth ($1.2 + 0.2$ g v $1.5 + 0.2$ g, $p <10\text{-}20$). Placental insufficiency may account for these differences. Immunohistochemistry of day 17 placentas shows higher ECE1 and NOS3 in HcB-23. Endothelin signaling through the B type receptor is known to induce Nos3, and endothelin signaling has also been shown to promote bone growth. This constellation of findings suggests that Ece1 expression differences underlie the multi-system pleiotropy of the chromosome 4 QTL. We speculate that an impaired response to mechanical loading may be an underlying cause of preeclampsia and intrauterine growth retardation.

Poster - Serotonin (5-HT) regulates calcium mobilization at the onset of lactation in rats

Laporta, J., T. L. Peters, K. E. Merriman, and L. L. Hernandez

Hernandez Serotonin (5-HT) is a known homeostastic regulator of lactation and was recently demonstrated to be a regulator of bone turnover. Circulating calcium (Ca2+) is known to decrease at the onset of lactation, and often results in milk fever in dairy cattle. 5-HT is synthesized in a two-step reaction from the amino acid L-tryptophan (L-TRP). The rate-limiting step is catalyzed by tryptophan hydroxylase (TPH1) isoform to form 5-hydroxytryptophan (5-HTP). To explore 5-HT's role on Ca2+ homeostasis in the transition period (10 d pre and post-partum) we fed 45 rats (n=15 per-treatment) 3 diets: control (CON), 5-HTP (0.2% total diet) and L-TRP (1.35% total diet) to increase endogenous 5-HT production. We collected milk samples on day 1, 5 and 9 of lactation to measure Ca2+ concentrations. We collected serum and plasma on d 20 of gestation and d 9 of lactation to measure circulating 5-HT, Ca2+ and parathyroid hormone-related protein (PTHrP) levels. Total mRNA was isolated from mammary gland tissue from d 9 lactating animals and analyzed for PTHrP, TPH1, plasma membrane Ca2+ ATPases 1 and 2 (PMCA1, 2), sodium- Ca2+ exchanger 1 (NCX1), secretory Ca2+ ATPase 1 and 2 (SPCA1, 2), and sarco(endo)plasmic reticulum Ca2+ ATPase 2 (SERCA2). The 5-HTP and L-TRP treatments effectively increased serum 5-HT over time ($P<0.001$), with a greater increase seen in the 5-HTP cohort. Plasma PTHrP was significantly increased ($P<0.05$) on d 9 of lactation in the 5-HTP cohort. There was a significant elevation of milk Ca2+ in the 5-HTP and L-TRP cohorts ($P<0.05$), and decreased serum Ca2+ on d 9 lactation in the L-TRP group ($P<0.05$). PTHrP, TPH1, NCX1, PMCA2, SPCA2, and SERCA2 mRNA were increased in the mammary glands of the 5-HTP cohort ($P<0.050$) and in the L-TRP cohort, except for PMCA2 and PTHrP ($P<0.05$). SPCA1 mRNA was decreased in both the 5-HTP and L-TRP cohorts ($P<0.05$). These results suggest that feeding 5-HTP increase PTHrP induction in the plasma and mammary glands of transition rats, as well as increase Ca2+ transport within the mammary gland. It is possible that the L-TRP cohort could be working through a different mechanism to increase Ca2+ transport.

Poster - Salt inducible kinase (SIK1) represses StAR expression in Y-1 adrenal and MA-10 testis cells through inhibition of processing mediated by CRTC2.

Lee J, Takemori H, Jefcoate CR

The steroidogenic acute regulatory protein (StAR) plays a central role in steroidogenesis by stimulating the transfer of cholesterol to pregnenolone by Cytochrome P450 11A1 (P450scc) in the inner mitochondrial membrane. Activation of this step depends on new synthesis of StAR and its phosphorylation by protein kinase A (PKA). Type 1 PKA orchestrates histone modifications and chromatin remodeling to activate StAR transcription. The activity of StAR depends on concommittant translation and phosphorylation of the new protein by PKA Type II. The control of StAR transcription by PKA additionally involves Salt Inducible Kinase 1 (SIK1). This kinase phosphorylates and inactivates CRTC2, which facilitates the recruitment of CREB and the histone acetyl transferase, CBP, to StAR. The inhibition of SIK1 by PKA is essential for sustained cAMP induced transcription of StAR. CREB binds CBP following phosphorylation at S133 by several kinases, including PKA. This binding occurs in the central KID region of CREB. In the absence of this phosphorylation, the C-terminal region of CREB interacts with the N-terminal region of CRTC2, which in turn further enhances CBP binding. When SIK is inhibited by PKA, dephosphorylation of CRTC2 ensues, which results in release from 14-3-3 and migration to the nucleus. In the nucleus a further dephosphorylation of CRTC2 is necessary prior to activation of CBP and CREB. SIK activity is rapidly restored by associated PP2A phosphatase, which returns CRTC2 to the cytoplasm. We hypothesize that this second step may be rate limiting in the activation of CRTC2 and that an additional combination with the processing co-factor p54/Nono may be important. In this work we examine the role of CRTC2 in the transcription of StAR in Y-1 (adrenal) and MA10 (testis) cells. We measure the kinetics of transfer of GFP-CRTC2 chimera to the nucleus and the impact of okadaic acid (OA) on this process. A further intra-nucl ear step is identified, which is sensitive to OA. These CRTC2 transfer processes are compared to the kinetics of transcription to pre-mRNA and of processing to mRNA.

Poster - Steroidogenic Factor 1 Expression Induces Steroidogenic Enzyme Expression and Steroid Synthesis To Fuel Growth of Aggressive Prostate Epithelial Cancer Cells.

S.R. Lewis, J.S. Jorgensen

The prostate depends on androgens synthesized by the testis both in disease and development. Although androgen deprivation therapy by medical or surgical castration is a cornerstone of treatment for metastatic prostate cancer, remission is only temporary and tumors inevitably progress to castration refractory prostate cancer. There are several theories to explain this aggressive resurgence, one of which suggests that prostate adenocarcinoma cells acquire machinery for de novo steroidogenesis. During development, and throughout life, Steroidogenic Factor 1 (NR5A1, ADBP4, SF1) is a key regulator of steroidogenesis in normal endocrine tissues. Normal and benign prostate tissues lacks SF1 expression and are non-steroidogenic. Therefore, we hypothesize that SF1 is expressed in castration refractive prostate cancer to stimulate steroidogenesis and fuel malignant growth. We examined SF1 expression in a panel of prostate epithelial cell lines and found SF1 present in each aggressive cancer cell line examined but absent in benign prostate cell lines. These results were supported by detection of SF1 in human prostate cancer tissue specimens from patients with castration refractory prostate cancer, whereas it is conspicuously absent from all specimens of localized prostate cancer and benign prostate thus far examined. To functionally link SF1 to steroidogenic production in prostate epithelial cells, we tested whether ectopic expression of SF1 was sufficient to induce steroid production in a benign prostate epithelial cell line (BPH-1). BPH-1 cells expressing ectopic SF1 exhibit increased mRNA levels of steroidogenic enzymes and secrete estradiol, which was inhibited by treatment with the aromatase inhibitor, anastrozole. These studies were followed by examining effects of shSF1 in an SF1-expressing cell line of malignant transformed prostate epithelial cells that synthesize estradiol in levels similar to SF1-transfected BPH-1 cells. shSF1-transfecting cells resulted in decreased estradiol production and a slower proliferation rate. Based on these data, we conclude that SF1 is induced in aggressive prostate cancers and is sufficient to stimulate steroidogenesis to promote aggressive growth. Our findings present a new potential mechanism and therapeutic target for deadly prostate cancer.

Poster - Estradiol-17β and its Metabolites Attenuate L-Ascorbic Acid-Suppressed

Huihui Li, Yingjie Zhao, Yan Li, Caifeng Dai, Sheikh O Jobe, Ronald R Magness, Jing Zheng

Estradiol-17β (E2β) plays a critical role in the growth, invasion and metastasis of human ovarian cancer cells. However, it is unknown whether its biologically active metabolites 2-hydroxyestradiol (2-OHE2), 4-hydroxyestradiol (4-OHE2), 2-methoxyestradiol (2-ME2) and 4-methoxyestradiol (4-ME2) have similar actions on human ovarian cancer cells. It has also been shown that L-ascorbic acid (AA) significantly inhibits ovarian cancer cell growth in vitro, but the reports on AA's in vivo effects on ovarian cancer are unclear. Herein, we examined expression of cytochrome P450s and catechol-O-methyltransferase (COMT) as well as if E2β and its metabolites and AA interactively modulate cell proliferation in OVCAR-3, SKOV-3, OVCAR429, OVCAR432 and IOSE385 cells. **Methods:** Western blotting was used to confirm expression of CYP1A1, CYP1B1, COMT, ERα and ERβ in these cells. Cells were treated with E2β and its metabolites (0.01-100 nM) for 6 days or AA (65-2000 μM) for 4 days. Cell proliferation was evaluated using crystal violet. Interactive effects of E2β and its metabolites with AA were also evaluated. **Results:** 1) CYP1B1, COMT, ERα and ERβ were expressed in all these five cell lines whereas CYP1A1 was not found in OVCAR-432 cell line; 2) E2β and its metabolites stimulated ($P \leq 0.05$) cell proliferation (~25%) in OVCAR-3 and IOSE383, but not SKOV-3, OVCAR429 and OVCAR432 cells with the maximum stimulatory effect at 0.1 nM; 3) AA dose-dependently suppressed ($P \leq 0.05$) cell proliferation in OVCAR-3, SKOV-3, OVCAR432, and IOSE385. In OVCAR429 cells, AA enhanced cell proliferation at doses < 125 μM, whereas attenuated cell proliferation at doses ≥ 125 μM; 4) E2β and its metabolites partly attenuated ($P \leq 0.05$) the AA's suppressive effect on growth of OVCAR-3, SKOV-3, OVCAR432 and IOSE385, but not OVCAR429 cells. **Conclusions**: E2β and its metabolites promote cell proliferation only in one ovarian cancer cell line studied, whereas AA inhibits cell proliferation in all five ovarian cell lines studied. Moreover, E2β and its metabolites attenuate AA-suppressed cell proliferation in three cancer cell lines studied. These data indicate that E2β and its metabolites stimulate growth of some ovarian cancer cells; however, E2β and its metabolites may play a more important role in rescuing ovarian cancer cells when challenged with AA or other anti-cancer agents, which will necessitate more research.

Poster - Roles of Aryl Hydrocarbon Receptor in Human Fetal Endothelial Cell Functions

Yan Li, Kai Wang, Yingjie Zhao, Huihui Li, Dongbao Chen, Jing Zheng

Aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, is a classic receptor mediating dioxin (TCDD)-induced adverse effect on pregnancy, causing increases in fetal and neonatal mortality and decreases in little sizes. However, AhR knockout in mice also leads to similar adverse phenotypes in the fetus and newborn, possibly partially due to abnormal vascular development. Herein, to examine physiological roles of AhR in fetal vasculature, we test the hypothesis that endogenous AhR ligands 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) and TCDD suppress endothelial cell growth, migration, and tube-formation and affect expression of eNOS in human umbilical cord vein (HUVE) and artery (HUAE) cells. **Methods:** The expressions of AhR in HUVE & HUAE cells as well as normal human term placentas were evaluated by immunohistochemistry and Western blotting. Cell proliferation and migration were determined using the crystal violet cell proliferation assay and the BD FluoroBlok Trans-wells system respectively. eNOS protein expression was evaluated by Western blotting. **Results**: We found that the AhR was immunolocalized in trophoblast cells and vascular endothelial cells of human placentas and umbilical cord Vessels. Both ITE and TCDD dose- and/or time-dependently inhibited ($p \leq 0.05$) HUAEC and HUVEC cell proliferation, while both ITE and TCDD inhibited ($p \leq 0.05$) HUAEC, but not HUVEC cell migration. A single dose of ITE and TCDD decreased ($p \leq 0.05$) AhR protein levels in HUVEC and HUAEC cells, indicating activation of the AhR. ITE increased eNOS protein levels ($p \leq 0.05$) in HUVEC while decreased eNOS protein levels in HUAEC ($p \leq 0.05$). **Conclusions:** These data indicate that ITE and TCDD suppress HUVE and HUAE cell proliferation, whereas differentially regulate HUVE and HUAE migration. ITE also differentially regulate eNOS protein expression in HUAE and HUVE cells. Thus, AhR may play important and differential roles in regulating fetal artery and vein endothelial cell functions. Supported by NIH HD38843 (JZ).

Poster - Visceral endoderm associated with the allantois exhibits unique properties in building the fetal-umbilical relationship.

Ka Yi, Ling and Karen Downs

By contrast with the anterior region of the mouse conceptus, where the brain and heart form, little is known about the posterior region, where the embryo is connected to its mother via the umbilical cord. A large number of umbilical-associated defects are associated with abnormalities in the fetal gastrointestinal system (Stevenson and Hall, 2006), but the developmental mechanisms that unify the umbilical cord and gut are not known. We have recently discovered several new morphological features within the allantois, or pre-umbilical cord, of the mouse conceptus. These include the Allantoic Core Domain (ACD), which contains progenitors of the allantois, posterior blood vessels, primordial germ cells and gut endoderm (Downs et al., 2009, M. Mikedis and K. Downs, 2011). In addition, we have discovered that the ventral proximal wall of the allantois, which we named Ventral Cuboidal Mesothelium (VCM), is associated with the formation of the posterior site of vascular arterial confluence, called the Vessel of Confluence (Daane et al., 2011). Overlying the ACD and the VCM are segments of visceral endoderm (VE) that may have important posterior functions; not only does VE, a temporary and nutritive tissue, contribute to the foregut (Franklin et al., 2008), but it also has powerful inductive properties (Thomas and Beddington, 1996; Belaoussoff et al., 1998). To investigate the role of allantois-associated visceral endoderm in posterior development, we have labeled it with Di-I, a lipophilic dye compatible with cell survival. After 20-24 hours in culture, the conceptuses were photobleached to convert the Di-I signal into a solid precipitate (Singleton et al., 1996). Our preliminary results indicate that the segment of visceral endoderm that overlies the allantois, previously called the allantois-associated extraembryonic visceral endoderm (AX), not only remains in place at the hindgut lip, but it also contributes a significant number of cells to both the ventral hindgut and the omphalomesenteric artery. By contrast, the VE immediately above the AX and which is associated with the VCM, remains fixed. Intriguingly, this is the point where the posterior arterial system converges. These results imply at least two critical functions for posterior VE. First, posterior VE provides a pool of stem cells that contribute to the hindgut and associated omphalomesenteric artery, and second, it may provide information that fixes the axial midline relative to the posterior vasculature and hindgut, ensuring their correct relationship to each other and to the umbilical vasculature. Grant acknowledgement: 1-FY09-511; RO1 HD042706

Poster - Neurosteroids - Plasticity and the Cytoskeleton

Millette MM, Dent EW

It is widely accepted that the cellular basis of learning and memory lies in the strengthening, weakening, formation, and elimination of chemical synapses between neurons. The vast majority of excitatory synapses occur at dendritic spines, tiny protrusions from neuronal dendrites which serve primarily to compartmentalize signaling. Dendritic spines are the most actin-rich structures in the brain, and in a given spine these filaments serve to organize the post-synaptic density, provide a framework on which receptors can be organized, and facilitate transport. Spines remain impressively plastic throughout one's lifespan. In-vivo two imaging has shown that learning and novel experience may both modify and even generate new spines and synaptic connections in adult animals. Numerous signaling pathways involved in learning and memory converge on the actin cytoskeleton, and a growing body of evidence shows tight correlation between spine morphology and function. Many serious human disorders, including schizophrenia, fragile X, and Down syndromes have been linked to pathological misregulation of these systems. Recent evidence from our lab and others illustrates dynamic microtubules (MTs) play related, equally important, and potentially cooperative roles. MTs are often thought to be fairly passive in mature neurons, serving as stable tracks on which transport may occur. We have shown, however, that dynamic MTs exhibit rapid bouts of polymerization from the dendritic shaft into spines, and that these transient invasions are linked to synaptic plasticity. These instances are correlated with NMDA receptor activation, and are required for sustained increases in spine volume following LTP induction as well as BDNF-induced accumulation of PSD-95. Additionally, work done by others suggests end-binding proteins, which localize at the plus ends of actively polymerizing MTs may mediate signaling directly to actin. In the two decades since the discovery that natural fluctuations in circulating estradiol mirror hippocampal spine density we have learned a great deal about estrogen signaling in the CNS. Of particular interest, estradiol is synthesized by synaptically located aromatase in an activity dependent manner. Estradiol then binds to extranuclear receptors, which mediate rapid signaling cascades. Instead of targeting the nucleus, these cascades appear to preferentially target cytoskeletal proteins and, as such, are of fundamental importance to our understanding of synaptic biology. Currently, I am investigating: (1) the effects of estradiol on microtubule dynamics, (2) how estrogen signaling may prompt functional interaction between cytoskeletal elements and (3) if MT invasion of spines is required for the effects of estradiol on the development and maintenance of new dendritic spines.

Poster - Identifying novel targets of pancreatic transcription factor 1a(PTF1A) in early stages of pancreas development using murine embryonic stem cells.

Gopika G Nair, Jon S Odorico

Pancreatic development in mammals is controlled, in part, by the temporal expression of numerous genes that encode transcription factors. Yet, how these factors regulate each other and their target genes is not completely understood. Pancreas-specific transcription factor 1a (PTF1a) is known be to be involved in pancreas specification in the foregut endoderm and transactivation of the PDX1 promoter. We have established a model of PTF1a driven pancreas development in mouse embryonic stem cells (mESC) using a tetracycline-inducible system. Morphological and molecular events characteristic of pancreas-organogenesis are observed in our model, including formation of PDX1+ bud-like structures, NKX6.1+HNF6+ PDX1+ pancreatic epithelium and more lineage restricted progenitors like NGN3+ endocrine cells and CPA+ exocrine cells. On further differentiation, terminal pancreatic islet cell types expressing hormones such as Insulin, glucagon, somatostatin and pancreatic polypeptide YY, and acinar cells expressing amylase are seen. To further investigate the molecular mechanism by which PTF1a activates the pancreatic developmental program, we have generated a new mESC with inducible PTF1a having an N-terminal flag. The flag tagged cell line showed robust differentiation as our previously established model and hence, could be used to interrogate novel direct targets of PTF1a early in development. This is almost impossible in an in vivo context due to limitation in embryonic tissue material and difficulty in identifying the putative pancreatic regions where PTF1a is first expressed. Through chromatin immunoprecipitation (ChIP), PTF1a bound DNA will be pulled down using an antibody against the flag epitope. Enrichment of known targets such as PDX1, amylase and elastase will be examined. The hypothesis whether PTF1a auto-regulates its own expression through a feedback loop will also be tested with this new cell line. Furthermore, we will perform a genome-wide promoter binding analysis to discover unidentified novel targets.

Poster - Analysis of Estrogen Receptors Alpha vs. Beta in the Uterine Endothelial Cell Plasma Membrane and their Interaction with Cav-1 for eNOS Activation

Mayra B. Pastore, Benjamin C. Hofeld, Jayanth Ramadoss, Ronald R. Magness

BACKGROUND: Uterine endothelial nitric oxide (NO) production is partly responsible for the maintenance of vasodilatation during physiologic states of high circulating estrogen levels such as pregnancy. Estrogen receptors (ER-α and/or ER-β) are classically thought of as nuclear transcription factor receptors; however, a small pool (3-5%) of ERs is localized to the plasma membrane of endothelial cells that are responsible for nongenomic vasodilatory responses. We and others have previously shown that ERs induce very rapid activation of ERK signaling and eNOS to increase NO production. In addition, these rapid changes were observed with estradiol-17β bound to plasma membrane impermeable BSA and the NO responses were ER-mediated since they were blocked by ICI, 182,780. Various estrogenic effects have been shown to be mediated by one or both ERs and may be dependent on interactions with Cav-1, the main caveolae scaffolding protein. It is unclear whether rapid NO productions are mediated by plasma membrane Cav-1 associated ER-α and/or ER-β. **HYPOTHESIS:** We hypothesize that ER-α and ER-β maintain similar spatial partitioning between the plasma membrane and nucleus of Uterine Artery Endothelial Cell (UAECs), similar interactions with Cav-1 protein at the plasma membrane, and are capable of changing the eNOS phosphorylation patterns indicative of temporal and special NO activation state. **METHOD:** UAECs lysates were subjected to Immunoisolation column chromatography using Cav-1 antibody to identify protein-protein interactions between Cav-1 and ER-α or ER-β. Transmission Electron Microscopy (TEM) was employ to visualized caveolae structures at the plasma membrane of UAECs under control and estrogen treatment. TEM ImmunoGold label visualization was employed to localize ER-α and ER-β at the UAEC plasma membrane and within the caveolae structures. Lastly, UAECs were treated with control or increasing concentrations of estradiol-17β (0.1-100nM) and changes in eNOS stimulatory phosphorylation sites, Ser 1177, Ser 635 vs. inhibitory site Thr 495 were evaluated via Western blot analyzes. **RESULTS:** UAEC protein Cav-1 immunoisolation columns showed that ER-a was tightly bound to Cav-1. In contrast, Cav-1 did not appear to interact with ER-β with high affinity. TEM revealed that UAECs have substantial numbers of caveolae structures on the plasma membrane. Immunogold labeling revealed that ER-α has relatively low expression bet very discrete focal caveolar allocations within the UAEC plasma membrane. However, ER-β showed a much higher expression patterns throughout the cell but similar focal patterns as ER-α at the plasma membrane. Increasing estradiol-17β concentrations directly increased stimulatory Ser 1177, Ser 635 phosphorylation with a concurrent decrease in inhibitory Thr 495 phosphorylation in eNOS, indicating elevations in eNOS activation. **CONCLUSION**: These data support the hypothesis that plasma membrane ERs mediate NO production through the activation of signaling cascades that alter eNOS multi-site phosphorylation state, protein-protein interactions with Cav-1, and its temporal spatial distribution. NIH GM083252, HL49210, HD38843, HL87144, AA19446.

Poster - Regulation of Ovine Fetal Renal Iron, Nephrogenesis and eNOS in Uterine Space Restriction (USR)

Sun MY, Meyer KM, Koch JM, Magness RR, Kling PJ

Aims: Intrauterine growth restriction (IUGR) deriving from many etiologies disrupt iron (Fe) transport and impair renal function in rodent models; Fe status has not been studied in IUGR sheep. Transferrin receptor (TfR) is the major placental cell-surface protein transporting Fe. TfR expression is controlled by iron regulatory proteins (IRP) modulated by nitric oxide (NO) via endothelial nitric oxide synthase (eNOS). We hypothesized that kidneys harvested from a USR model of IUGR will exhibit impaired development, in association with negative Fe status and reduced eNOS expression. Methods: We used an ovine IUGR model of USR (Meyer et. al, BOR, 2010) via single unilateral uterine horn ligation before breeding for 1-3 fetuses. These were compared to sheep fetuses of nonspace restricted (NSR) controls at gestation day (GD) 120 and GD130 (term=147). Blood total iron binding capacity (TIBC), plasma transferrin (Tf), Tf saturation, and fetal kidney and liver non-heme Fe were quantified. Immunoblotting was performed for TfR and eNOS expression. Cessation of nephrogenesis was determined by identifying an active or inactive nephrogenic zone. **Results:** Compared to NSR, USR fetal TIBC, Tf concentration was increased, and Tf saturation decreased at GD130, but not at GD120. Total fetal kidney Fe content (μg) was similar in NSR and USR at GD120 and 130. However when expressed proportionately, μg Fe /kg fetal wt fell from GD120 to 130. Because fetuses were smaller in USR, proportional Fe levels were greater in USR vs NSR at GD130. Liver Fe concentration and total Fe appeared to be increased between NSR 120 and NSR 130, but were significantly lower in USR 130, however no differences were observed across all groups when expressed proportional to fetal weight (μg Fe /kg).There was no difference in renal TfR expression between groups, although renal eNOS expression trended higher in GD130 USR vs both GD120 USR and GD130 NSR. In contrast to placenta, there was no correlation between eNOS and TfR in fetal kidneys. The nephrogenic zone had matured at GD120 in NSR, but matured later in USR. Conclusions: Thus, contrary to our hypothesis, the IUGR fetuses adapted to USR by delivering more Fe via the blood and accretion by the kidney. Unlike the placenta, renal TfR and eNOS were not associated. Of note, the non-heme Fe assay does not measure erythrocyte Fe, so relatively greater renal Fe is not derived from eNOS-mediated renal vascular dilation. It is important to note that fetal liver and kidney iron appear to be regulated differently as liver . The relatively greater renal Fe may be due in part to the tight regulation of fetal Fe homeostasis during the delay in active nephrogenesis and Fe needs for ongoing cell proliferation during nephrogenesis. NIH HL49210, HD38843, HL87144

Poster - Expression of G-protein subunit alpha-11 and 14 in Human Placentas

Yingjie Zhao, Yan Li, Huihui Li, Kai Wang, Jing Zheng

Background: During pregnancy, dramatic vascular growth in the fetus and placenta is critical for the remarkably increased fetal and placental blood flows required for supporting the developing fetus. G-protein coupled receptors represent by far the largest family of cell-surface molecules, which mediate numerous cell functions upon interacting with G-proteins. Their dysfunction contributes to some of the most prevalent human diseases such as hypertension and cancers. G-protein subunit alpha-11 (GNA11) and 14 (GNA14) are two members in the Gα family, which as transducers are involved in various transmembrane signaling including PLC and Ca++, key signaling molecules for endothelial functions. GNA11 has been implicated in modulating VEGF's signaling in endothelial cells; however, little is known about roles of GNA14 in any placental cell functions. We have found that physiological chronic hypoxia significantly enhanced VEGF- and FGF2-stimulated cell proliferation and migration, which are associated with robustly increases in mRNA of GNA14, but not GNA11 in human umbilical cord vein (HUVE) and artery (HUAE) endothelial cells. **Methods:** To explore potential roles of GNA11 and GNA14 in human placentas, in this study, we examined GNA14 and GNA11 protein expression in human placentas obtained from first and third trimester pregnancies as well as from normal (N) and severe preeclamptic (sPE) pregnancies. GNA14 protein expression was also determined in (HUVE and HUAE cells cultured under chronic normoxia (~ 20% O2) and hypoxia (3% O2) using Western blotting. **Results:** 1) No significant difference in GNA11 and GNA14 was found in in placentas from the first trimester vs. the third trimester pregnancies; 2) The levels of GNA14, but not GNA11 protein in sPE placentas were increased ~ 2.94 fold ($p \leq 0.01$) in sPE vs. N placentas; and 3). Physiological chronic hypoxia (3% O2) significantly promoted GNA14 (~ 1.63 fold, $p \leq 0.05$), but not GNA11 protein expression in HUVE, but not HUAE cells. **Conclusions:** Our data suggest that GNA14 may play an important role in placental and fetal vascular endothelial functions, especially under chronic hypoxia and in sPE pregnancies.

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Roxanne Alvarez	Ph.D.	Bird
Bryan Ampey	Ph.D.	Magness
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Justin Bushkofsky	Ph.D.	Jefcoate
Luca Clemente	Ph.D.	Bird (Bertics)
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Katherine Hackbart	Ph.D.	Wiltbank
Patrick Halbach	M.S.	Pattnaik / Bird
Amanda Hankes	Ph.D.	Bird
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Omar Jobe	Ph.D.	Magness
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Soraya Silva Arriga	M.S.	Wiltbank
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2011/ 2012 Faculty Directory

Name	Research Interests
Abbott, David	Neuroendocrine function, Polycystic Ovary Syndrome
Audhya, Jon	Membrane development and organization
Alarid, Elaine	Estrogen response
Atwood, Craig	Hormone regulation of aging and Alzheimer's Disease
Barry, Terence	Aquaculture, fish reproduction
Bertics, Paul	Cell regulation and proliferation by growth factors (Deceased -2011)
Bird, Ian	Uterine blood flow, eNOS, Adrenal Steroidogenesis.
Blank, Robert	Identifying genes that contribute to differences in bone's biomechanical performance
Bosu, William	Folliculogenesis, Corpus luteum function
Cezar, Gabriela	Stem cell safety
Davis, Dawn	Basic and translational research on diabetes and obesity
Downs, Karen	Developmental and genetic control of fetal and extraembryonic lineage formation during mouse gastrulation, use of mammalian stem cells in gene therapy
Drezner, Marc	Phosphatonins, Hormones
Duello, Theresa	Health Disparities in Underrepresented Populations
Ginther, Oliver	Equine reproduction
Golos, Thaddeus	Placenta biology, stem cells
Hernandez, Laura	Lactation biology
Jefcoate, Colin	StAR protein
Jorgensen, Joan	Gonad formation
Kessel, Julie	Neonatology
Khatib, Hasan	Genomic imprinting, genetic development of embryos in cattle, genetic traits that impact health and milk quality in cattle.
Kimple, Michelle	Role(s) that inhibitory guanine nucleotide binding proteins (Gproteins) play in pancreatic beta-cell biology
Kling, Pamela	Neonatal development, Growth factors

Kreeger, Pamela	The use of mathematical, and computational techniques to address cellular signaling questions relevant to women's health
Levine, Jon	Polycystic Ovary Syndrome
Liu, Bo	Molecular mechanism underlying vascular inflammation, molecular mechanism underlying occlusive vascular diseases, and development of new materials for biomedical applications (gene delivery and vascular grafts)
Magness, Ronald	Shear stress
Martin, Thomas	Cell Signaling, neuropeptides
Ntambi, James	Genetic regulation of metabolism
Odorico, Jon	Stem cells, Pancreatic islet development
Parrish, John	Sperm regulation and function, Equine Reproduction
Patankar, Manish	Epithelial Ovarian Cancer (EOS)
Payseur, Bret	Genetics of hybrid sterility
Pelegri, Francisco	Cellular and molecular level processes involved in early vertebrate development
Peterson, Richard	Prostate disease
Salih, Sana	Molecular Determinants of Oocyte Development, Fertilization, and Early Embryogenesis in Humans
Schuler, Linda	Prolactin, Growth hormones
Shah, Dinesh	Maternal-Fetal Medicine, mechanisms of preeclamptic hypertension
Terasawa, Ei	Neuroendocrinology
Thomson, James	Stem Cells
Vezina, Chad	Prostate Disease
Watters, Jyoti	Molecular mechanisms employed by microglia, Central Nervous System
Wiltbank, Milo	Hormonal interaction; intracellular regulation of cell death and steroidogenesis in the corpus luteum; regulation of ovarian function in dairy cattle.
Xu, Wei	Dissecting the epigenetic mechanisms controlling estrogen responsiveness
Zheng, Jing	Endothelial cell function