OPS Abstracts

Oral Presentation

1. **Ablation of Na+/H+ Exchanger-3 Prevents Tissue Iron Loading in the Hfe Mouse Model of Hereditary Hemochromatosis**
Corbin Azucenas

Several common hereditary disorders are associated with toxic iron overloadwhichleads to liver cirrhosis, cardiomyopathy, and endocrine disorders. Hereditary hemochromatosis arises from mutations in the genes coding for any of several proteins involved in iron sensing (e.g. Hfe), hepcidin production (e.g. hemojuvelin),or hepcidin action, resulting inelevated iron absorption relative to the iron need. Divalent metal-ion transporter-1 (DMT1)is a H^+-coupled Fe^{2+}transporter and the principal or only mechanism by which nonheme iron is taken up at the intestinal brush border [Shawki A et al (2015) Am J Physiol Gastrointest Liver Physiol309, G635–G647]. DMT1 is a validated therapeutic target in treating iron overload. We have shown that Na^+/H^+exchanger-3 (NHE3) is required for adequate iron absorption, via its physiological role in generating at the intestinal brush border an acidic microclimate that energizes DMT1-mediated H^+-coupled Fe^{2+} transport [Shawki A et al (2016) Am J Physiol Gastrointest Liver Physiol311, G423–G430], so we have explored here the contribution of NHE3 to pathological iron loading. We tested the hypothesis that NHE3 activity is necessary for pathological iron loading. We examined the effect of ablating the SLC9A3 gene coding for NHE3 in the Hfe mouse model of hereditary hemochromatosis. We measured tissue iron levels, hematological and blood-iron variables, and the hepatic expression of Hamp1 (coding for hepcidin) by using qPCR in male and female FVB/N mice, age ≈ 120 d,fed a normal diet (NIH-07). We examined four genotypes in both sexes: wildtype mice, NHE3 knockout($NHE3^{-/-}$), Hfe knockout (hemochromatosis disease model, $Hfe^{-/-}$), and double knockout ($NHE3^{-/-}$| $Hfe^{-/-}$) (n= 12–26 mice per group). We chose $\alpha = 0.05$, and analyzed our data (mean, SD) by using multifactorial ANOVA. We found that ablation of NHE3 prevented the liver iron loading characteristic of the $Hfe^{-/-}$ mouse model, an effect that was independent of sex (Fig 1). We observed a similar effect on iron loading in the spleen. Ablation of NHE3 produced no sign of overt anemia. Hepatic expression of hepcidin was depressed in $NHE3^{-/-}$ and in $Hfe^{-/-}$mice relative to wild type mice, and remained low in the $NHE3^{-/-}$| $Hfe^{-/-}$ mice, providing a plausible explanation for the observation that, despitecorrection of the tissue iron levels in $NHE3^{-/-}$| $Hfe^{-/-}$ mice, iron levels remained high in the readily accessible serum iron pool (typically 0.1% of total body iron). We conclude that ablation of NHE3 prevents pathological tissue iron loading in the Hfe mouse model of hemochromatosis. Pharmacological blockade of NHE3 may offer a means of inhibiting iron absorption in hereditary hemochromatosis.

2. **Evidence for a Highly Cytokine-sensitive Network of Iron-associated Genes in Pancreatic Islets**
Grace P. Counts, Kira G. Slepchenko, Poonam R. Sharma, Kathryn L. Corbin, Farhan M. Qureshi, Robert A. Colvin, and Craig S. Nunemaker
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Among the complex factors contributing to β-cell failure is inflammation. Elevated levels of proinflammatory cytokines in obese individuals, such as interleukin (IL)-1β and IL-6, increases the risk of developing type-2 diabetes (T2D), and there is evidence that these low levels of circulating cytokines lead to islet dysfunction. Iron is closely connected to both the inflammatory response and diabetes. High levels of dietary iron increase risk of developing T2D, and excessive iron uptake by β-cells can cause oxidative stress and inhibit function. In this study, we provide evidence for a highly cytokine-sensitive network of iron-associated genes in pancreatic islets and

examine how these genes may play a role in β-cell responses to low-grade inflammation. Islets were treated for 48h with 10 pg/mL IL-1β + 20 pg/mL IL-6 as a model of low-grade inflammation. Analysis of gene microarray data identified three iron-associated genes among the most cytokine-sensitive: HAMP, STEAP4, and LCN2. These proteins are all involved directly or indirectly with increasing and/or sequestering cellular iron. RT-PCR following islet exposure to various stressors hypothesized to induce β-cell failure revealed upregulation of HAMP, STEAP4, and LCN2 to be cytokine-specific. Lentiviral transduction of STEAP4 upregulated HAMP and LCN2, implying interactions among these genes. β-cells scanned by powerful synchrotron X-Ray fluorescence at the Argonne National Lab provided unique data showing cytokine exposure alters iron distribution. Iron was found in discrete structures (area ~0.15-0.45 μm2) that were significantly smaller and more iron-dense in cytokine-treated β-cells, consistent with the function of these iron-associated genes. These data suggest a network of iron-regulating genes in β-cells plays a role in sequestering iron in response to low-grade inflammation.

3. **Fine-Tuning of Gi/o Signaling by RGS2 and 5 is Critical to Ensuring Normal Ventricular Rhythm**

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Cardiac arrhythmia is attributed partly to abnormal G protein signaling resulting from the loss of signaling regulation by regulators of G protein signaling (RGS) proteins. RGS proteins act as GTPase activating proteins (GAPs) to fine-tune G protein signaling. Previous studies have shown that G protein dysregulation due to the absence of Rgs2 or 5, separately, results in the impairment of cardiac structure and function, increasing susceptibility to hypertrophy and arrhythmia. We generated mice dually lacking Rgs2 and 5 (Rgs2/5dbKO) to test the hypothesis that dual absence of Rgs2 and 5 predisposes to stress induced cardiac arrhythmia. Echocardiographic assessment showed no difference in baseline cardiac structure between wild type (WT), Rgs2/5dbKO, Rgs2, andRgs5 single KO mice. However, mRNA expression of cardiomyopathy bio markers, Tnni3 and Mybpc3, were both increased in Rgs2/5dbKO relative to WT hearts. When subjected to surgery-induced stress, Rgs2/5dbKO mice had 75% mortality within 72 – 96 hours after surgery, accompanied by extreme hypertension and decreased cardiac contractile function. At a low frequency of electrical field stimulation, ventricular myocytes from Rgs2/5dbKO, and Rgs2 or 5 KO developed more arrhythmias than WT cells, and the difference was resolved by Gi/o blockade with pertussis. Cardio myocytes fro m Rgs2/5dbKO and Rgs2 or 5 KO also developed irregular excitation-contraction coupling and death in response to increasing concentrations ofβ-adrenergic receptors with isoproterenol. Forskolin-stimulated cAMP production was also attenuated inRgs2/5dbKO cardiomyocytes related to WT cells. Moreover, calcium extrusion via sodium/calcium exchanger in the presence of thapsigargin was impaired in the Rgs2/5dbKO cardiomyocytes. We conclude that RGS2 and 5 act synergistically to facilitate proper control of cardiomyocyte excitat ion-contraction coupling cardiac rhythm by regulating cytosolic calcium handling. Funding Sources: The study is funded in part by grants from the American Heart Association (16SDG27260276), and the National Inst itutes of Health – NHLBI (R01 HL139754) to Patrick Osei-Owusu. The funding agencies had no role in the study design or execution.

4. **miR-277 Targets hid to Ameliorate Aβ42-Mediated Neurodegeneration in Drosophila Eye Model of Alzheimer's Disease**

Prajakta Deshpande

Alzheimer's disease (AD), an age-related progressive neurodegenerative disorder, exhibits reduced cognitive functions with no cure to date. One of the reasons for AD is the extracellular accumulation of Amyloid-beta 42 (Aβ42) plaques. We misexpressed human Aβ42 in the developing retina of Drosophila, which exhibits AD-like neuropathology. Accumulation of Aβ42 plaque(s) triggers aberrant signaling resulting in neuronal cell death by unknown mechanism(s).

We screened for microRNAs which post-transcriptionally regulate expression of genes by degrading mRNA of the target genes. In a forward genetic screen with candidate miRNAs, we identified miR-277as a genetic modifier of Aβ42-mediated neurodegeneration. Gain-of-function of miR-277 rescues Aβ42 mediated neurodegeneration whereas loss-of-function of miR-277enhances Aβ42 mediated neurodegeneration. Moreover, misexpression of higher levels of miR-277 in the GMR>Aβ42 background restores the retinal axonal targeting indicating functional rescue. Furthermore, we have identified head involution defective (hid) as one of the targets ofmiR-277 by Fly TargetScan and validated by luciferase assay and qPCR. The hid transcript levels are decreased by one third when miR-277 is misexpressed in theGMR>Aβ42 background in comparison to the GMR>Aβ42 fly model. Hence, here we provide a mechanism of how miR-277 modulates Aβ42 mediated neurodegeneration by regulating hid transcript levels and demonstrate its neuroprotective role in Aβ42-mediated neuropathology.

5. **Rat models of low vs high exercise capacity demonstrate differences in risk for oral disease**
   Ana Maria Hardy

6. **Gas Permeation through human AQP5**
   Kowatz, T., Qin, X., Shinn, E.,Wang, H. H., Schwab,H., Lodowski,D. T., Tajkorshid, E. Vahedi-Faridi, A. and Boron, W. F.
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   Movement of water across membranes in cells is facilitated by membrane channels named aquaporins (AQPs). They are ubiquitous and are involved in many crucial physiological roles. AQPs arehomo-tetramers consisting of four monomeric or water pores (one pore per monomer for water conductance). Furthermore, tetrameric association generates an additional hydrophobic pore in the center (central pore). Besides water movement, gas transport through membranes plays an essential role in human physiology as well. Boron and coworkers published for the first time that the water channel aquaporin AQP1 plays an essentialrole in CO_2 conductance through membranes. There has still been an ongoing debate, whether CO_2 conductance occurs through the monomeric (water pores) or the central pore of AQPs. To get more insight into possible gas conductance through the central pore of human AQP5, residues T41 or/and L43 at the extracellular mouth of the central pore were mutated to H. Physiological data of human AQP5 T41H, L43H and T41H/L43H mutants in the presence of Ni^{2+} and Zn^{2+}, as well as crystallographic and Molecular Dynamic (MD) Simulations data of the T41H mutant with Ni2+prove that the four/eight histidine residues of the tetrameric AQP5 T41H, L43H and T41H/L43H mutants bind those divalent cations, leading to an occlusion of the central pore and, consequently, reducing/preventing gas (CO_2) permeation through it. Our structure of AQP5 T41H in complex with Ni^{2+} is the first example of an inhibitor blocking the central pore.

7. **Transmembrane CO_2 flux via AQP5 and NBCe1 quantified in a neutral-buoyancy assay**
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   Three discoveries demonstrate that Overton's rule—namely, membrane permeability of solute X depends uniquely on the lipid solubility of X—is not universal: (1) The existence of CO_2-impermeable membranes in gastric glands, (2) the CO_2-permeability of aquaporin 1 (AQP1), the first "gas channel"; and (3) the incorporation of "CO_2-blocking" proteins into artificial membranes. Certain AQPs conduct CO_2 in differing proportions; partially via their hydrophilic monomeric pores, and the remainder though the hydrophobic central pore (CP) of the tetramer. Rhesus (Rh) proteins can also conduct CO_2. A 3rd class of CO_2-conducting proteins lack a classical pore. NBCe1-A (SLC4A4) cotransports Na+ and CO_3=. Using out-of-equilibrium CO_2/HCO_3^- solutions and electrophysiological approaches with Xenopus oocytes, we showed that NBCe1-A also conducts CO_2, but only in the presence of HCO_3^-. We speculate that

transient pathways within the NBCe1 molecule arise during $Na^+/CO_3=$ cotransport, allowing CO_2 to pass via the protein. Here, we modify our Neutral Buoyancy Assay (NBA), originally developed to assessN_2 fluxes to measure transmembrane CO_2 flux via AQPs and NBCe1-A. We inject a precise volume of N_2 gas (number of gas molecules = nGas) into a Xenopus oocyte, which we transfer to a saline-containing tube. We then increase the pressure (PNB) in the air phase above the air-water interface so that it is sufficient to collapse the injected bubble enough to make the oocyte neutrally buoyant, 5 cm depth below the meniscus. As N_2 exits the bubble and ultimately diffuses into the extracellular fluid (ECF), the bubble shrinks, cell density increases, and the oocyte sinks. A camera/computer system detects the sinking and decreases PNB to re-establish neutral buoyancy at 5 cm depth. PNB decays exponentially over 1000 s. Calibrations allow us to compute the gas efflux from the ΔnGas time course. The NBA can also quantify gas influx. When we raise $[N_2]$ in the ECF from $[N_2]o = 0.56$ mM (room air at 1×ATA) to $[N_2]o =$ 2.06 mM (pre-equilibrating saline with 93% N_2/7% O_2 at 3×ATA) at constant $[O_2]o = 0.26$ mM, N_2 enters the cell and nGas rises. If we increase $[N_2]o$ further to 2.81 mM at fixed $[O_2]o$ (pre-equilibrating saline with 95% N_2/5% O_2 at 4×ATA), ΔnGas over 1000 s increases more. To perform the inflation assay with CO_2 (the high solubility of which translates to a low tendency to inflate the bubble), we expose the oocyte to 100% CO_2/95 mM HCO_3^-/pHo 6.66. nGas increases rapidly as CO_2 rushes into the cell, and peaks at <200 s, after which N_2 exit dominates and nGas decreases. Oocyte expressing AQP5 or NBCe1-A have a significantly greater maximal rate of nGas increase (i.e., CO_2 influx) c ompared to control oocytes. The peak ΔnGas from AQP5 or NBCe1-A oocytes is also larger and more delayed. Furthermore, when we express the AQP5-T41F mutant, which is hypothesized completely block of the extracellular entrance to the AQP5 CP, the maximal rate of nGas increase and peak ΔnGas is not significantly different from control oocytes. While we specifically devised the NBA to measure N_2 fluxes, here we demonstrate the adaptability of this method for measuring transmembrane CO_2 fluxes, and our novel approach confirms that both AQP5 and NBCe1-A conduct CO_2, and that the CP serves as the major conduit for CO_2 flux via AQP5.

8. **NFkB Mediates Renal Sodium Retention via the Sodium Chloride Cotransporter in Zinc Deficiency-Induced Hypertension**

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Background: Zinc deficiency (ZnD) is comorbid with diseases such as kidney disease and diabetes. Individuals in these populations have a higher prevalence of hypertension. Recently, we reported that ZnD promotes hypertension in mice. The blood pressure elevations were accompanied with increased Na+retention via the renal Sodium Chloride Cotransporter (NCC). Although our published results indicate that zinc plays a critical role in blood pressure and NCC regulation, the mechanisms involved were not investigated.
Hypothesis: Since nuclear factor-kB (NFκB) mediates ZnD-induced detrimental effects, we tested the hypothesis that NFκB drives ZnD-induced NCC upregulation and subsequently renal Na^+ retention and hypertension.
Experimental Design: To determine the role of NFκB in ZnD-induced renal Na+retention and hypertension, adult male C57Bl/6 mice were fed a ZnD diet (5 weeks) followed by administration of the NFκB inhibitor -caffeic acid phenethyl ester (CAPE; 1 week). Systolic blood pressure and urinary Na+concentrations were examined. To examine if NFκB mediates ZnD-inducedNCC upregulation, mouse distal convoluted tubule (mDCT) cells were treated with the zinc chelator N, N, N′, N′-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN) ± CAPE, a NFκB inhibitor. After 24 hours, NCC mRNA and protein expressions, as well as activation, were assessed by qRT-PCR, Western blot and immunofluorescence, respectively.

Results: In mice,ZnD promoted hypertension and renal Na+retention. Further, increased NCC expression was accompanied with enhanced NFκB expression in ZnD mice. However, CAPE administration lowered blood pressure and elevated urinary Na+concentrations. In mDCT cells, TPEN-induced NCC upregulation was preventedby CAPE treatment.
Conclusions: Together, these results demonstrate that 1) NFκB is a novel NCC regulator and 2) NFκB mediates ZnD-induced renal Na+retention and hypertension. These novel findings indicate that ZnD drives renal NFκB activation thereby stimulating NCC-mediated Na+retention and promoting hypertension.
Significance: This study identifies NFκB as a possible pharmacological target to mitigate hypertension.
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9. **Whole-Brain T1 and T2 Mapping in Mouse by 3D Magnetic Resonance Fingerprinting**
Yuran Zhu

Introduction: MRF allows simultaneous T1 and T2 mapping with unprecedented efficiency.1,2 Previously, we developed a single-slice MRF sequence that enabled simultaneous tracking of two MRI contrast agents in a single dynamic contrast-enhanced (DCE)-MRI scan.3,4 In the current study, we present further development of a 3D MRF sequence for brain-wide T1 and T2 mapping in mice. The potential of accelerated data acquisition for DCE-MRI studies was examined by comparing T1 and T2 mapping using retrospectively undersampled data.
Methods: Sequence Design: The FISP-based MRF sequence has 768 frames partitioned into 16 segments. Data acquisition in each segment used flip angles ramped up sinusoidally from 4° to a maximum value between 6° and 15°. Constant echo time (2.0 ms) and repetition time (10.0 ms) were used. The acquisition time of a single fingerprint was ~21 s.3D MRF data were acquired using a stack-of-spiral trajectory.5,6 For fully sampled acquisition, the in-plane k-space was sampled by 48 interleaves with an FOV of 30 × 30 mm2 for each kzvalue. It yields an in-plane resolution of 0.23 x 0.23 mm2, and a through-plane resolution of 1 mm. Data Acquisition: All experiments were performed on a Bruker 9.4T scanner with a 35-mm 1H volume coil. Fully-sampled 3D MRF data were acquired in vitro from a phantom with varied manganese concentrations, and in vivo from 3-month-old male C57BL/6 mice. Data Analysis: Prospective undersampling was performed on fully-sampled datasets. Different combinations of in-plane (Rxy) and through-plane (Rz) undersampling factors were evaluated by calculating the normalized root mean squared error (NRMSE).
Results: The accuracy of the MRF sequence was validated in vitro by comparing to the phantom T1 and T2 values from conventional method as shown in Figure 1. A total undersampling factor of 48 (Rxy = 16, Rz = 3) showed minimal impact on the accuracy of estimated T1 and T2 values (Figure 2C), with a NRMSE below 10%. Figure 3 demonstrates that a 16-fold in-plane acceleration combined with a 3-fold through-plane acceleration still preserved the majority of the mouse brain details with no obvious artifacts.
Conclusion: Our results demonstrate that up to 16-fold in-plane and 3-fold through-plane undersampling can be achieved with adequate accuracy. This undersampling capacity will enable whole brain T1 and T2 mapping in 3 to 5 minutes.

Posters

1. **Age-dependent deterioration of metabolic health in mice lacking Nkcc1in insulin-secreting β-cells**

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Insulin plays a key role in maintaining fuel homeostasis by regulating blood glucose and lipid levels. During obesity and other insulin resistant states, plasma insulin is insufficient to control metabolic demands leading to a vicious circle characterized by hyperglycemia,hyperlipidemia and hyperinsulinemia. These conditions increase the risk of enduring metabolic disorders including type-2 diabetes. Insulin secretion is predominantly regulated by ATP-dependent K^+(KATP) channels. Indeed, glucose increases ATP, closing these channels to depolarize the plasma membrane of insulin-secreting β-cells allowing Ca2+entry, the ultimate trigger of insulin secretion. However, it has long been suspected that bumetanide-sensitive $Na^+K^+2Cl^-$ cotransporters (Nkccs) also play arole in insulin secretion by maintaining $[Cl^-]i$ above Nernstian equilibrium thus making possible electrogenic Cl–effluxes via volume-regulated anion channels. In this study, we tested the hypothesis that elimination of the most abundant Nkcc expressed in β-cells i.e., Nkcc1in mice impairs insulin secretion in response to nutrients leading to metabolic dysregulation.Accordingly, wegenerated mice lacking Nkcc1exclusivelyβ-cells ($Nkcc1^{\beta KO}$)by using the Cre/LoxP strategy. Our findings demonstrate impaired insulin secretion in response to food in 10w old $Nkcc1^{\beta KO}$ mice, which associate with hyperlipidemia and hyperglycemia. Notably, these mice are normotolerantto exogenous glucose and insulin indicating compensated fuel homeostasis. However, older Nkcc1βKOmiceshowed increased body weight gain, hyperinsulinemia and hyperglycemia accompanied by glucose and insulin intolerance and signs of adipose tissue inflammation and steatohepatitis. Therefore, our results suggest that elimination of Nkcc1from insulin-secreting β-cells results in age-dependent deterioration of metabolic health associates with early impairments in insulin secretion.

2. **Elevated Bile AcidsAlters Erythrocyte CompositionAnd FunctionIn Dysbiotic Mice**

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Bile acids (BA) are amphipathic molecules with detergent-like properties, whose canonical function is to emulsify dietary lipids and fat-soluble vitamins and facilitate their absorption. . Recently, new insights delineate an assortment of physiologic roles for BA in various organ systems. Systemically elevated quantities of BA is commonly associated as a concomitant outcome of liver disease, however, rarely measured in healthy individuals due to its asymptomatic nature. Upon generating a novel strain of dysbiotic C57BL/6 mice using Tlr5-/- microbiota (WTdys), we observed significantly elevated BA in their sera (>40 μM/L). Based on these findings, we hypothesized that, as BA have been studied extensively in literature to be cytotoxic, chronic exposure to BA in sera may induce significant physiological changes to circulating erythrocytes in dysbiotic mice. Complete blood count analysis of WTdys mice displayed a significant reduction of hematocrit and hemoglobin levels. Correspondingly, analysis for mature and immature erythrocytes (alias reticulocytes) via flow cytometry revealed an increased percentage of immature peripheral $CD71^+Ter119^+$ erythrocytes in WTdys mice. As increased reticulocyte presence is common in individuals suffering from anemia, we decided to determine whether these erythrocytes were more susceptible to lysis. To test this, we used the osmotic hemolytic fragility assay to induce lysis of erythrocytes at various osmotic concentrations, and interestingly observed erythrocytes from WTdys mice were significantly resistant to osmotic fragility-induced hemolysis. Additionally, erythrocytes incubated in the presence of either WTdys plasma or exogenous BA (e.g.cholate, deoxycholate) also displayed protection against hemolysis. Next, we generated erythrocyte membranes (aliaserythrocyte

ghosts) through repeated lysis and centrufigation in hypotonic Tris buffer. We observed erythrocyte membrane physiology differed between the groups with WTdys mice exhibiting reduced Na^+/K^+-ATPase expression and activity, ferroportin expression, phospholipids, and membrane protein glycosylation. Furthermore, cholemic mice were hypercapnic, displaying increased blood CO_2 levels. Overall, our findings highlight a novel, non-canonical role of BA on membrane physiology. Future studies may uncover how these striking effects of gut microbiota-systemic BA impact on red blood cell membranes would affect vascular functions, O_2 transport and erythrocyte-specific pathogens.

3. Enhanced Holographic Optical Trap

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Optical traps have been used for the past 40 years to manipulate micron-sized biological objects. Our laboratory has successfully used a single beam optical trap to study the mechanical properties of 0.2 micron-diameter primary cilia, but the obtained information was limited. By creating two (or more) traps, we can expand the capabilities of our apparatus. We use a spatial light modulator (SLM) to construct a so-called Holographic Optical Trap (HOT) capable of generating multiple simultaneous and moveable traps. We present our HOT design and discuss the initial performance capabilities. For example, one result shows the generation of two optical traps, each holding a 2-micron diameter polystyrene sphere. One particle is held in the diffracted trap and can be moved while the other trap is static. The relative trapping force between the two traps can be varied by the experimenter. We conclude with a discussion of upcoming experiments involving primary cilia and also discuss broader capabilities of our HOT.

4. Effects of Adenine on Renal Salt and Water Handling

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Adenine is a purine nucleobase that has been used extensively to induce chronic kidney disease in animal models. A recent study on rats showed that adenine when given orally in high dose as 0.5% (wt/wt) has a massive water depletion effect in rats during the first 24 hours of treatment and that effect is accompanied by a downregulation of aquaporine-2 (AQP-2) water transporters in the collecting ducts of kidney. In our study, we investigate the dose-response and the time-response of Adenine in doses of 0.15, 0.20 and 0.25%.
Method: Adenine feeding: Male Sprague-Dawley rats were placed in metabolic cages for two days for adjustment and were freely fed with powdered rodent chow. On the 3rdday, rats were randomly divided into four groups (n = 5 rats in each) and were allowed free access to rodent chow alone (control) or powdered supplemented with 0.15%, 0.20% or 0.25% (wt/wt) of adenine with free access to distilled water. Powdered diet was made freshly every day and we measured body weight, food intake, water intake, urine volume and urine osmolality daily for 6 days and for two days on each of the 3rdand the 7thweek. Membrane protein isolation and immunoblotting: Membrane proteins were prepared from the three kidney regions; cortex, inner and outer medulla. Immunoblotting experiments were performed for AQP-2, NKCC2 detection. Actin was used as a control protein.
Results: Effects of Adenine Feeding on Water Balance, and Urine osmolality: Adenine feeding with doses 0.20% and 0.25% during the first week is associated with a significant increase in urine volume, which is followed by a significant increase in water intake and a significant decrease in urine osmolality. No significant change in the previous parameters was noticed with 0.15% adenine. Urine volume and urine osmolality in 0.25% fed rats were 25.31 ± 2.30 ml and 821 ± 40 mOsm compared to 11.60 ± 1.72ml and 1793 ± 134 mOsm in control group on the third week, and 33.18 ±3 .50ml and 647 ± 62 mOsm compared to 11.45 ± 1.21 ml and 1738 ± 126 on the seventh week. Effect of adenine feeding on AQP2 expression in the collecting duct: with 0.20% adenine, AQP2 protein in outer medulla is significantly downregulated for both the native (29kDa, -50%) and the glycosylated forms (35kDa, -50%) of AQP2 protein in Inner Medulla.

Also, with 0.25% adenine, AQP2 protein in outer medulla is significantly downregulated (-50% for 29 kDa protein and -60% for 35 kDa protein) compared to control group.
Conclusion: Adenine in doses 0.20% and 0.25% produces an increase in water excretion, reduction in urine osmolality and a decrease in AQP2 expression in a dose and time dependent manner without affecting salt excretion, food intake or body weight in rats. Thus, Adenine could be used as an aquaretic agent for cases such as hyponatremia.

5. **ΔNp63α Suppresses Cell Invasion through Downregulating Rac1 Activity**

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ΔNp63α, a member of the p53 family of transcription factors, is overexpressed in a number of cancers and plays a role in proliferation, differentiation, migration and invasion. We showed previously that ΔNp63α suppresses cell invasion by positively regulating miR-320a, resulting in the downregulation of its direct target protein kinase C gamma (PKCγ) anda corresponding reduction in phosphorylated Ras-related C3 botulinum toxin substrate 1 (Rac1). Here, we further show that ΔNp63α inhibits Rac1 GTPase activity. Using a Rac1 pull down assay to measure GTP-Rac1 levels, we showed that silencing ΔNp63α led to a significant upregulation of GTP-Rac1 in multiple SCC cell lines. Silencing ΔNp63α in SCC cell lines also led to an increase in p21-activated kinase (PAK1) phosphorylation, confirming the negative regulation of ΔNp63α on Rac1. Inhibiting GTP-Rac1 levels using EHop-016led to a significant reduction in both pRac1 and pPAK1 levels. Treatment with EHop-016 reversed the increase in cell invasion observed upon silencing ΔNp63α. RT-PCR analysis performed on an array of 43 Rac guanine exchange factors (Rac-GEFs) showed that 6 GEFs are significantly upregulated by silencing ΔNp63α in A431 cells. Taken together, our data suggest that ΔNp63α negatively regulatesGTP-Rac1 activity via downregulation of a Rac-GEF, thereby reducing GTP-Rac1 levels and inhibitingcancer cell invasion.

6. **Interactionof Chloride Intracellular Channels CLIC4 and CLIC5 in Cardiac Mitochondria and Their implications in Cardiac Function**

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Chloride channels are importantin regulating cellular and organellar membrane potential, cell volume, pH and various physiological conditions. Recently, we have identified two of the paralogs of chloride intracellular channel (CLIC) proteins, CLIC4 and CLIC5 in the mitochondria of cardiac cells. CLIC4 being enriched in the outer mitochondrial membrane while CLIC5 is exclusively localized tothe inner mitochondrial membrane. Further, blocking of CLIC4 and CLIC5 either pharmacologically or by using knock out (KO) mouse model resulted in decreased cardiac mitochondrial function and increased myocardial infarction (MI) upon ischemia-reperfusion (IR) injury. Therefore, it is necessary to reveal their biophysical and functional relevance in mitochondria, their protein interactome and the downstream signaling pathways regulated by them to understand their role in such complex pathology. Our recent findings, provide a strong evidence for the interaction of CLIC4 and CLIC5 in cardiac mitochondria. A high degree of colocalization of CLIC4 and CLIC5 with each other was observed in both cardiomyocytes (38±3%) and isolated cardiac mitochondria (42±1%). In addition, HEKcells transfected with recombinantFlag-tagged CLIC4 and Myc-tagged CLIC5 exhibited 58±1% colocalization with each other. In support, our pull-down and mass spectrometry analysis further confirmed the interaction of CLIC4 and CLIC5 with each other in both crude cardiac tissue as well as cardiac mitochondrial lysates. Furthermore, cardiac mitochondria isolated from clic4 and clic5 double-KO (DKO) mice exhibit decreased calcium retention capacity of the mitochondria resulting in early onset of mitochondrial permeability transition pore (mPTP)

opening. This could be attributed to their interaction with mPTP components Cyclophilin D and ATP synthase in our pull down experiments. Furthermore, left ventricular function as determined by echocardiography was severely compromised in DKO mice in comparison to wt, clic4$^{-/-,}$ and clic5$^{-/-}$ mice suggesting that these proteins possibly work in consortium to maintain cardiac function. Thus, our results for the first time decipher the molecular identity of the CLIC channel, their role in mitochondrial function and also highlight the possible role of hetero-oligomerization of CLICs in modulating cardiac function.

7. **Proteomics Analysis of Beta Cell Glucotoxicity and Role of DnaJC3 in Proinsulin Folding**

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Glucose is an essential regulator of insulin secretion in beta cells; however chronic high glucose (glucotoxicity) can greatly impair beta cell function. Folding of proinsulin, the precursor to insulin, is monitored by BiP and its co-chaperones inside the endoplasmic reticulum (ER).When the demand for insulin outmatches the ER's folding capacity, ER stress ensues which has been identified in several diabetic models. In this study, we systematically characterize the beta cell response to glucotoxicity using Stable Isotope Labeling with Amino acids in Cell culture (SILAC)-based quantitative proteomics in INS-1 832/13 cells treated with high (25mM) glucose for 48 hours. Additionally, we utilized the CRISPR-Cas9 system to KO JC3, an ER protein that serves as a co-chaperone to BiPand study the impact on proinsulin folding and ER export. In four separate proteomics experiments, a total of 5007beta cell proteins were identified among which 3634were identified in all 4 experiments. 806proteins were significantly different between normal andhigh glucose treatment involving major biological processes: Carbohydrate metabolism, lipid metabolism, ketone metabolism, protein folding, glycosylation, alcohol metabolism and fat-soluble vitamin metabolism. We identified key alterations to beta cell specific genes such as PDX1 (0.39, p=.04), INS1 (0.34, p=.03), and CPE (0.44, p=.01) to be downregulated. We also identified that the ER was the compartment that yielded the most significant changes in glucotoxic conditions. Moreover, we identified proinsulin misfolding correlates with JC3 expression, and KO of JC3 causes ER stress, and delayed proinsulin ER export. Overall, this study identified glucotoxicity results in beta cell identity loss and impaired ER homeostasis; and we also identified JC3 as a critical protein involved in proinsulin maturation in the ER.

8. **MCT Inhibition As a Potential Mechanism to Overcome Enzalutamide-Resistance in Prostate Cancer**

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Enzalutamide is a second-generation anti-androgen used for the treatment of prostate cancer. However, almost all patients develop resistance to this drug, thereby creating an urgent need of identifying new approaches that can overcome said resistance. Using parental and Enzalutamide-resistant C4-2B and 22RV1 cells, we targeted cellular metabolism to overcome therapeutic resistance. Prostate cancers are more heterogeneous in their use of energy sources compared with other solid tumors and the most common metabolic end product is lactate, which is toxic to cancer cellsupon accumulation. Hence, cancer cells upregulate the expression of monocarboxylate transporters (MCTs) that play a pivotal role in lactate efflux. Different MCT isoforms are differentially expressed across prostate cancer progression and presumably according to cellular demands at different stages. Gradual progression from benign prostate, to prostatic intraepithelial neoplasia, to in-situ carcinoma shows an increase in the expression of MCT2 and MCT4, with MCT4 being expressed in invasive prostate cancers. Based on our preliminary data that the expression of MCTs is higher in prostate cancer cells resistant to enzalutamide, we hypothesized that MCT inhibition may be an attractive therapeutic strategy against enzalutamide-resistant prostate cancer cells. We found that the suppression of MCT activity using the MCT1/2

antagonists AR-C155858 or AZD3965 and the MCT 4 antagonist Syrosingopine not only diminishes the proliferation and survival of prostate cancer cells, but also re-sensitizes resistant cells to treatment with enzalutamide. We also found that treatment with AR-C155858 or AZD3965 either singly, or in combination with Enzalutamide suppressed prostate cancer cell xenograft growth in SCID mice bearing subcutaneously injected parental or Enzalutamide-resistant C4-2B cells. These findings imply that the MCTs have an intrinsic role in the acquisition of resistance to Enzalutamide based therapy in prostate cancer.

9. *In vivo* Force Experiments Suggest Calcium Handling Defects during Repetitive Activity in Huntington's Disease Skeletal Muscle

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We have previously shown that skeletal muscle from the transgenic R6/2 model of Huntington's disease is hyperexcitable due to decreases in chloride and potassium currents. To assess whole muscle function in R6/2 mice, in vivo isometric contractions were recorded from the plantar flexor muscles (medial and lateral gastrocnemius, soleus, and plantaris) by stimulating the sciatic nerve. The force-frequency relationship revealed a slightly higher specific force and a shift toward a lower activation frequency in the R6/2 muscle compared to wild type muscle from age-matched littermates (WT). Because R6/2 muscle appeared more prone to fatigue, we examined the responses to repeated stimulation at 30 Hz (approximate midpoint of force-frequency relationship) in a long exercise protocol. Early in the protocol, WT muscle force slowly potentiated and then began to fatigue. In contrast, R6/2 began the exercise protocol at potentiated force levels and very quickly developed fatigue. Additionally, R6/2 muscle exhibited a higher and less changing fusion index (degree of fused, steady-state force) during trains of stimulation compared to WT. We also assessed the intra-train force profiles, R6/2force begins to sag throughout the 30 Hz stimulation, whereas the WT intra-train force does not. These data strongly suggest calcium handling issues during repetitive activity in R6/2 skeletal muscle. The altered force profiles during exercise are expected to significantly impact motor function, consistent with symptoms of Huntington's disease.

10. Loss of Intestinal Retinoic Acid Receptor Alpha (RARα) Exacerbates Diet Induced Hyperlipidemia

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Intestine plays an important role in maintaining whole body lipid levels by mediating intestinal absorption of dietary and biliary lipids. Vitamin A metabolism contributes significantly to maintaining the intestinal homeostasis. Vitamin A metabolites like retinoic acids mediates its effects via retinoic acid receptors. Previous studies have shown that retinoic acid receptor alpha (RARα) signaling in the intestinal epithelial cells (IEC) participates in the establishment of intestinal mucosal homeostasis. Deletion of RARα in the IEC alters differentiation and function of epithelial barrier while changing the microbial composition. However, the role of IEC-specific RARα in maintaining lipid homeostasis is yet to be elucidated. We hypothesized that IEC-specific RARα may play a role in intestinal lipid absorption. To test this hypothesis, we conducted *in vivo* studies using floxed Rarα ($Rar\alpha^{fl/fl}$) mice crossed with vilin-cre mice to generate IEC-specific Rarα knockout mice ($Rar\alpha^{vilin}$). $Rar\alpha^{fl/fl}$(control) mice and $Rar\alpha^{vilin}$ mice were given a high fat/high cholesterol (HFHC) diet for 16 weeks. H&E staining showed differences in epithelial cellular composition. Analysis of plasma lipids showed significant increases in plasma triglycerides, total cholesterol, free cholesterol, cholesteryl esters and low-density lipoprotein cholesterol whereas high density lipoprotein cholesterol lwas significantly reduced. In addition, hepatic cholesterol

levels were significantly increased. Analysis of gene expression of the distal small intestine showed a significant induction of the genes involved in cholesterol absorption and transport, including Npc1l1, Acat2, Mttp, and Apob. We then determined whether IEC-specific RARα regulated cholesterol absorption using the plasma dual isotope ratio method and the results showed that loss of Rarα in IECs significantly increased cholesterol absorption. Loss of Rarα in IECs also increased chylomicron secretion of triglycerides. Thus, our study has shown that IEC-specific RARα protects from hyperlipidemia by regulating cholesterol and fat absorption. Research Support –R01DK118805.

11. Fast *in vivo* Detection of Myocardial Norepinephrine Levels in the Beating Porcine Heart

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The sympathetic nervous system modulates cardiac function by controlling key parameters such as chronotropy and inotropy. Sympathetic control of ventricular function occurs through extrinsic innervation arising from the stellate ganglia and thoracic sympathetic chain. In the healthy heart, sympathetic release of norepinephrine (NE) results in positive modulation of chronotropy, inotropy, and dromotropy, significantly increasing cardiac output. However,in the setting of myocardial infarction or injury, sympathetic activation persists, contributing to heart failure and increasing the risk of arrhythmias, including sudden cardiac death. Methodologies for detection of norepinephrine in cardiac tissue are limited. Present techniques rely on microdialysis for analysis by high-performance liquid chromatography coupled to electrochemical detection (HPLC-ED), radioimmunoassay, or other immunoassays, such as enzyme-linked immunosorbent assay (ELISA). Although significant information about the release and action of norepinephrine has been obtained with these methodologies, they are limited in temporal resolution, require large sample volumes, and provide results with a significant delay after sample collection (hours to weeks). In this study, we report a novel approach for measurement of interstitial cardiac norepinephrine, using minimally invasive, electrode-based, fast-scanning cyclic voltammetry (FSCV) applied in a beating porcine heart. The first multispatial and high temporal resolution, multichannel measurements of NE release in vivo are provided. Our data demonstrate rapid changes in interstitial NE profiles with regional differences in response to coronary ischemia, sympathetic nerve stimulation, and alterations in preload/afterload.

12. Role of AdipokineLipocalin-2 (LCN2) and HepatokineInter-Alpha-Trypsin Inhibitor Heavy Chain 3(ITIH3) on Cellular Bioenergetics

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Background: Metabolic syndrome is a collection of metabolic traits such as obesity, insulin resistance and dyslipidemia that increase and advance the development of cardiovascular disease, type 2 diabetes, and fatty liver disease. To determine what mechanism(s) have had a role in diet-induced metabolic complications, our lab has used a valuable multidimensional omics dataset on different metabolic traits such as obesity, insulin resistance, heart function, plasma, and liver lipids. For this, a diverse mouse population of over ~100 inbred mice strains, termed the hybrid mouse diversity panel also known as HMDP, was used. When coordinated with natural genetic variation, these multi-omics analyses can be utilized to demonstrate biologic pathways and reveal regulatory mechanisms, also known as Systems Genetics. This approach has simplified studies of cross-tissue communications, along with gene-by-environment interactions. With these approaches, along with tissue-specific loss-of-function and/or gain-of-function models, our lab

has demonstrated that adipose-derived lipocalin-2 (LCN2) causes obesity, liver and heart complications and liver-derived inter-alpha-trypsin inhibitor heavy chain 3 (ITIH3)causes liver complications. Our current objective is to understand what cellular pathways are regulated by these two secreted proteins in their respective target tissues. Since both liver and heart are metabolically active tissues, we wanted to focus on mitochondrial bioenergetic pathways using cell culture models.
Methods: To test the direct roleofLCN2or ITIH3oncellular bioenergetics, multiple cell culture models were utilized namely mouse AML12 (liver), human HEK293 (kidney)and neonatal rat ventricular myocytes (heart).These cells were treated with either recombinant human rhLCN2 or rhITIH3 proteins for 24 hours after which both gene expression via quantitative PCR and cellular bioenergetics via Seahorse bioanalyzer were analyzed.
Results: We observed that both rhLCN2 and rhITIH3 proteins increased the expression of multiple mitochondrial electron transport chain (ETC) genes (Ndufv2, Ndufs4, Sdhc and Atp5f1) and concomitantly, there was an increase in both the rates of mitochondrial respiration and glycolysis as measured by Seahorse bioanalyzer. Interestingly, we also observed that rhLCN2 was able to regulate its own expression in the target tissues, while rhITIH3 was not.
Conclusion: We show here the direct role of both LCN2 and ITIH3 on cellular bioenergeticsvia increasing the expression of ETC genes, and in case of LCN2, along with their own expression. In the future, we aim to dissect how these two proteins modulate the transcriptional status of the ETC genes. Given the need for promising targets for drug development against metabolic complications involving liver and heart, we feel that our findings will be of considerable interest to the scientific community.

13. **Structure-function Analysis of defective proventriculus (dve) in Drosophila melanogaster Eye Growth and Development**
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During development, axial patterning is required to establish Antero-posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD) axes, which is crucial for the generation of a 3-dimensional organ from a monolayer organ primordium. Of the three axes, DV axis is the first lineage restriction event during eye development and any deviation results in developmental birth defects. In our study, we have used Drosophila melanogaster (Fruit fly) eye as a model system to understand the role of different domains of a new dorsal eye fate selector gene, defective proventriculus (dve, an ortholog of SATB1) in growth and development. In humans, SATB1, functions as a transcriptional regulator and chromatin organizer and requires tetramerization by the ULD domain. In Drosophila eye, dve regulates expression of wingless (wg), a negative regulator of eye. In the genetic hierarchy, dve acts downstream of GATA-1 transcription factor pannier (pnr) and upstream of wg. Loss-of-function of dve results in dorsal eye enlargement while gainof-function results in eye suppression. We performed structure function analysis of Dve protein to elucidate the role of various domains in patterning, growth and development. We have developed several transgenic lines, which will allow us to induce expression of the specific domains of Dve protein and assay their effect on Drosophila eye growth and development. Dve has a ULD domain for tetramerization, HOX domains for DNA binding and PPP4R2 domain for H2AFX dephosphorylation. Here we present our results on ectopic induction of these domains alone or in combination with other domains, their effect on eye phenotype and wg expression in the developing eye.

15. Structure of Dopaminergic Brain Networks Following Postnatally-Occurring Hypoxic Insults
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Objective: Up to 10% of children are prematurely born in the United States. Their immature brain respiratory control systems and lungs are unable to adequately maintain ventilation and acid

base physiology. Consequently, these preterm infants experience persistent periods of apnea with bradycardia accompanied by frequent reductions in arterial blood oxygenation (hypoxemia). We developed a rodent model emulating that clinical condition which revealed persistent reductions in synaptic brain dopamine levels, impaired working memory, and hyperactivity that emerged during adolescence. The objective of this study was to determine the extent that findings in our rodent model would accurately predict related structural neuropathology in school aged children who had been born preterm and experienced apnea, bradycardia and hypoxemia.
Methods: The 16 study participants included term-born children as well as school-aged survivors of preterm birth with birth gestations ranging 23-28 weeks and birth weights appropriate for gestational age. Continuous oxygen saturation data were collected during their neonatal hospitalization. We recruited and studied 3 male and 6 female prematurely born children (current Mean age 11.13 ± 0.94 years; Mean gestational age 26.14 ± 1.96 weeks) and 4 male and 3 female children born at term (current Mean age 12.12 ± 1.90 years; Mean gestational age 39 ± 0.92 weeks).Participants were placed in a 3 Tesla magnetic resonance imaging (MRI) system. Foam blocks were placed on either side of the head to reduce movement; earplugs were inserted to reduce MRI noise. Brain structure was quantified with T1 and T2 structural scans. Brain white and gray matter volumes were quantified in 105 specific brain regions using BrainSuite.
TM Results: Structural differences in brain volume emerged between preterm and term born children in several brain regions including the basal forebrain, the site of convergence of sleep and thermoregulatory function. Other brain regions mediating memory (hippocampus, mammillary body, middle temporal gyrus, precuneus), visual processing (inferior occipital gyrus, lingual gyrus, cuneus, etc.), motor function (paracentral lobule), spatial orientation (superior colliculus), and processing and integration of the senses (middle occipital gyrus, post-central gyrus, superior colliculus, transverse temporal gyrus) were also different between groups. In contrast with our model, we also observed ventricular enlargement and leukomalacia in several prematurely born children.
Conclusions: Our findings suggest that prematurely born children experiencing repetitive apneas with hypoxemia exhibit neuropathologic changes that persist into middle childhood. Those structural changes are found across multiple brain regions mediating executive performance and sensory integration. Many, but not all, of the structural differences that we observed in prematurely born children paralleled those first described in our rodent model.

16. Characterizing the Dose Response Curve of Hyperoxia-Induced Reductions in Brain Perfusion

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Objective: High altitude aviators require continuous administration of inspired oxygen concentrations (FiO_2) of 80-100% (hyperoxia) to reduce risk of hypobaric hypoxia and decompression injuries. Hyperoxia is not without consequence; cerebral perfusion (CBF) is reduced by up to 35%. That decrement may confer increased vulnerability towards hypergravity-induced reductions in CBF, which can lead to hypergravity-induced loss of consciousness (GLOC). To minimize the deleterious, additive effect of hyperoxia and hypergravity upon CBF, an immediate need exists to establish the maximum FiO_2 delivered to aviators without leading a reduction in CBF. Our objective was to characterize CBF during escalating levels of hyperoxia, with the intent to define the specific FiO2at which CBF dropped significantly.
Methods: This study was conducted in Cincinnati, OH, located 482′ above sea level with an average barometric pressure of 30.6 inches. Healthy participants (13 males and 13 females) were randomized to receive either low dose FiO_2 of 30%, 40%, 50% and 100% followed by a return to 21% or else high dose FiO_2 of 60%, 70%, 80% and 100% followed by a return to 21%. Participants were placed onto the gantry of a 3 Tesla MRI scanner equipped with pseudocontinuous arterial spin labeling software (PCASL) to measure CBF. A non-breather oxygen delivery mask was securely placed over the nose and mouth; an oxygen sensor validated inspired and expired FiO_2. A 20 channel head coil was placed over the head to enhance signal

acquisition. As 21% FiO_2 was delivered to the non-rebreather mask, a scout scan established anatomical landmarks, followed by 192-slice T1and 30 slice T2 anatomical scans. This was followed by a baseline PCASL. FiO_2 was then increased to each predetermined level by use of an oxygen-air blender where the next PCASL scan was obtained. Upon completing the 100% FiO_2 PCASL scan, we returned the participant to 21% FiO_2 for a final PCASL scan following a 5-minute pause. In total, each person received two anatomical scans and six PCASL scans, requiring ~ 1.5 hours in the MRI scanner.
Results: Baseline CBF levels (ml/min/100 grams of tissue) at 21% FiO_2 were not different ($p = 0.46$) between the low dose FiO_2 group (49.23 ± 10.73) and the high dose FiO_2 group (46.16 ± 10.11). Low dose FiO_2 exposure did not significantly reduce CBF. In contrast, each high dose FiO_2 exposure significantly reduced CBF from baseline. Exposure to 100% FiO_2 led to a 24.7% ± 10.0 mean reduction in CBF in the low dose group and 29.5% ± 7.3 mean reduction in CBF in the high dose group. Following the return to 21% FiO_2, CBF returned to initial baseline values in both groups. The groups did not differ in response to 100% FiO_2 ($p = 0.18$) or to 21% FiO_2 after hyperoxic exposure ($p = 0.53$).
Conclusions: Our findings suggest that the neurovascular system responds to increasing FiO_2 levels in a dose dependent manner, with significant reductions in CBF beginning at 60% FiO_2 for study participants maintained sea level. Future studies are needed to characterize the extent that hyperoxia influences CBF when study participants are maintained at ambient environmental pressures paralleling those in the cockpits of high performance aircraft.

17. Mechanism by which Progesterone Affects Immune Cells at the Human Maternal-Fetal Interface

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Throughout pregnancy, modulation of the maternal immune system occurs to prevent immunologicrejection of the developing conceptus. The steroid hormone progesterone is essential for establishing pregnancy and maintaining uterine relaxation until parturition and is also thought to affect maternal immune cells to maintain tolerance of the allogeneic conceptus. Maternal immune activation is a risk factor for preterm labor and E.coli-derived lipopolysaccharide (LPS) is used to model inflammation-induced preterm labor in mice. Coupling the necessity of progesterone during gestation with the known labor-inducing effects of inflammation, we hypothesize that parturition involves loss of progesterone immune-modulatory activity that causes inflammation in the chorion-decidua that transforms the adjacent myometrium to the labor state. To test this hypothesis, methods to isolate immune cells from the chorion-decidua compartment were developed. With patient consent, fetal membrane tissues, containing the chorion-decidua including resident maternal immune cells, were obtained from deliveries at the University Hospitals MacDonald Women's Hospital. Resident immune cells were isolated and characterized using flow cytometry and qPCR. Our data suggest that resident immune cells do not express nuclear progesterone receptors (PRs). Our hypothesis is therefore that progesterone affects resident immune cells at the maternal-fetal interface indirectly via decidual stromal cells that contain high levels of PR. We have therefore developed a fetal membrane explants model system in which explanted tissues are responsive to progesterone and inflammatory stimuli, including IL-1β. Preliminary data from our explants model system suggest that progesterone/PR signaling inhibits IL-1β induced IL8 expression. Taken together, these data suggest that in the chorion-decidua, progesterone acts through PGR-expressing decidual stromal cells to elicit anti-inflammatory (i.e., anti-labor) activities at the tissue-level.

18. Elastin Insufficiency Impairs Renal hemodynamics and Accelerates Aging-Associated Structural and Functional Remodeling of Preglomerular Arterioles

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Elastin degradation and fragmentation are hallmarks of arterial stiffness and renal dysfunction associated with aging. However, it is unclear whether elastin insufficiency contributes to the changes in the structure and function of the resistance vasculature of the kidney during aging. Here we determined how increased vascular stiffness due to elastin insufficiency alters renal hemodynamics and mechanical properties of preglomerular arterioles. We assessed renal hemodynamics under anesthesia in 14–16-month-old female wildtype (WT) and elastin heterozygous (Eln+/-) mice. Renal autoregulation was assessed by a stepwise increase in renal perfusion pressure (RPP) by simultaneously occluding the superior mesenteric and celiac arteries. Myogenic constriction and arteriolar stiffness were assessed by pressure myography of isolated renal interlobar arteries. Baseline renal vascular resistance (RVR) was elevated in Eln+/-mice (13.8 ± 2.9 vs 11.2 ± 1.4 mmHg/μL/min/g left kidney weight), while systolic blood pressure (SBP; 75.1 ± 7.4 vs 91 ± 4.2 mmHg), renal blood flow (RBF; 6.3 ± 1.2 vs 7.4 ± 1.7 μL/min/g left kidney weight), renal plasma flow (RPF; 3.4 ± 0.8 vs 5 ± 1.2 mL/min/g/ left kidney weight) and urine flow rate, all trended lower in Eln+/-mice compared to WT mice. Glomerular filtration rate (GFR) and filtration fraction (FF) were similar between the two groups. A step increase in RPP caused a slower decline and rise in RBF and RVR, respectively, in Eln+/-relative to WT mice. The maximal changes in RBF (5 ± 1.1 vs 4.7 ± 0.8 μL/min/g left kidney weight), RVR (17.6 ± 7.3 vs 22.5 ± 2.1 mmHg/μL/min/g left kidney weight), urine flow rate, GFR, and FF were less robust in Eln+/-mice. RPF decreased in WT mice in response to raising RPP, whereas it remained unchanged in Eln+/-mice. Myogenic response and increases in elastic modulus and wall tension following stepwise changes in intraluminal pressure were all augmented in interlobar arteries from Eln+/-relative to WT mice. However, there was no difference in kidney weight/tibia length ratio between the two genotypes. We conclude that elastin insufficiency impairs renal hemodynamics by exacerbating age associated increase in vascular stiffness.

19. Analysis of Structural Alteration in the Kidney Resulting from Thoracic Spinal Cord Injury in Mice

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Thoracic spinal cord injury (SCI) disrupts supraspinal control of autonomic input to peripheral organs below the level of injury, including the kidney. Preliminary examination of kidneys isolated from mice with T3 and T10 spinal transection revealed impairment of renal hemodynamics and autoregulation of blood flow that are observable 24 hr after injury and worsens 4 weeks (wk) later during the chronic stage of SCI. However, it is unknown whether impaired renal function resulting from SCI is also associated with structural changes in the kidney. In this study, we used mouse models of high (T3tx) and low (T10tx) thoracic-level SCI to determine 1) whether thoracic SCI causes structural changes in the kidney and 2) how the level of injury affects the extent of structural changes. Kidneys from uninjured, T3tx and T10tx SCI mice with a CBL57/6 genetic background were harvested 24 hr and 4 wk after spinal cord transection (tx) surgery. Formalin-fixed tissue samples were paraffin-embedded, sectioned and processed for histological (MOVAT pentachrome and Masson trichrome) staining for interstitial and perivascular collagen deposition, and vascular elastic lamina organization. Additionally, collagen content, red blood cell (RBC) count, and glomerular size were evaluated. FIJI (ImageJ) software was used for quantitative analysis of blindly acquired images of histological sections. We observed markedly enhanced levels of perivascularcollagen deposition in samples from T3tx SCI mice at 24 hr post injury compared to T3tx SCI mice at 4wk and T10tx mice at both time points. Medial elastin fragmentation was observable in both T3tx and T10tx SCI mice at 24hrs and 4 wk;

however, it was most apparent in T3Tx SCI mice at 24 hr post injury. Indicated by increased RBC within the glomerulus, T3tx SCI kidneys isolated 24 hr and 4 wk post injury displayed glomerular congestion, appearing most severe at 4 wk. Together, these results indicate high thoracic-level SCI leads to structural damage to the kidney interstitium and vasculature in addition to impaired renal hemodynamics, both exacerbated by high-level injury.

20. Multiple Freeze-thaw Cycles Induce Delayed Recovery of Function Despite Enhanced Circulating Cryoprotectant Accumulation in the Freeze Tolerant Anuran Cope's Gray Treefrog Dryophytes chrysoscelis

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The freeze tolerant anuran Dryophytes chrysoscelis accumulates cryoprotectants including glycerol to mitigate the osmotic stress generated by freezing and thawing as much as 65% of its extracellular fluid. Though D. chrysoscelis likely freezes and thaws many times seasonally in northern habitats, previous work in freeze tolerant anurans has almost exclusively focused on single freeze-thaw events. The objective of this study is to evaluate and compare physiological responses to single versus multiple freeze-thaw cycles. It is hypothesized that the efficiency of post-freeze recovery will be affected by multiple freeze-thaw cycles, and will correlate with changes in circulating cryoprotectant levels and cell damage relative to a single freeze-thaw event. All animals were cold acclimated over a period of 7 weeks in which the ambient temperature was reduced to 5°C with 8.5 hours daylight. A control group of animals was maintained at 5°C ("cold-acclimated"). The "single freeze-thaw" experimental group was frozen and thawed once by chilling the animal to -2.5°C, inoculating with ice, maintaining in the frozen state for 24 hours, and thawing for 24 hours at 5°C. A second experimental group ("repeated freeze-thaw") underwent three rounds of freezing and thawing. Frog skin color, gross morphology, and changes in body position were documented by digital photography during post-freeze recovery. Blood and tissues were collected for analysis. Animals that were repeatedly frozen and thawed were significantly delayed in recovery of spontaneous respiration ($P<0.05$, determined by abdominal width expansion) and physical body thawing ($P<0.05$, quantified by increase in body surface area). Though variation in skin color during freezing is unstudied to date, the degree of blue or green color saturation varied in both initial skin color at time of thawing and throughout post-freeze recovery in single freeze-thaw animals versus repeated freeze-thaw animals ($P<0.0001$, see figure). Plasma osmolality and glycerol were elevated in repeated freeze-thaw animals compared to the control cold-acclimated animals ($P< 0.05$). Plasma hemoglobin (a measure of erythrocyte hemolysis) was elevated in repeated freeze-thaw animals compared to both single freeze-thaw and cold-acclimated animals ($P<0.05$); however, plasma LDH (a general indicator of cell/tissue damage) was not ($P=0.7$). Our finding that extracellular cryoprotectant levels were progressively elevated with repeated freeze-thaw cycles suggests that cryoprotectant depletion did not explain the progressive delay in recovery from repeated freeze-thaw events. Possible explanations include shifts in distribution of cryoprotectants (intra-vs. extracellular) or accumulating cryodamage (e.g. hemolysis).Overall, results of this study indicate that this ecologically relevant repeated freeze-thaw protocol can provide novel insights into the phenomenon of natural freeze tolerance in D. chrysoscelis.

21. Disrupted Cell Polarity and Small GTPase-regulated Focal Adhesion Properties are Associated with Morphological and Migratory Impairment, during Rbpj-Mediated Brain Arteriovenous Malformation

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Brain arteriovenous malformations (BAVM) are characterized by abnormally enlarged blood vessels,which direct blood flow through arteriovenous (AV) shunts, bypassing the normal artery-

capillary-vein network. These high-pressure, low-resistance AV shunts disrupt healthy blood flow and can result in rupture or hemorrhage in the cerebrovasculature. Clinically, current BAVM treatments are highly invasive and not applicable to all BAVM cases; thus, there is critical need to understand molecular and cellular BAVM pathologies and develop molecular-and cellular-based therapies. We previously generated a genetic mouse model of BAVM, by deleting Rbpj –a downstream effector of canonical Notch signaling –from endothelial cells (ECs), from birth. In this *in vivo* system, clinical features of BAVM are seen by postnatal day 14, demonstrating that intact Rbpj is required in brain ECs to ensure proper cerebrovascular development during this early postnatal period. Using our *in vivo* Rbpj-BAVM model, here we show that Rbpj-deficient (mutant) brain ECs acquired an abnormally rounded morphology, with no change to cell area, and increased EC density along AV shunts, as compared to controls. Mutant ECs also showed disrupted cell polarity and focal adhesion properties along abnormal vessels. Using isolated brain ECs, we found altered small GTPase activity in mutant vs. control ECs, suggesting that Rbpj regulates small GTPase-mediated focal adhesion in brain ECs. Together, these data suggested that motility/rearrangement of mutant ECs, along brain AV shunts, may be impaired during early postnatal cerebrovascular remodeling. To test EC motility, we used an *in vitro* cell migration assay. We used siRNA to knockdown (kd) RBPJ in human brain microvascular cells, and we found altered migration of RBPJ-kd cells as compared to control cells. Our data suggest that endothelial Rbpj is required to maintain healthy brain EC morphology and brain EC movement/rearrangement by regulating cell polarity and small GTPase-mediated focal adhesion properties. Thus, endothelial Rbpj promotes normal cerebrovascular remodeling and prevents BAVM during early postnatal development. Such mechanistic insight is critical to understand BAVM pathogenesis and to develop novel therapeutic treatments for BAVM patients.

22. Circadian Disruption in 3xTg Alzheimer's Disease Mice

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Background: Circadian rhythms are generated by a transcriptional-translational feedback loop (TTFL) in the suprachiasmatic nucleus of the hypothalamus and occur with a repeating period of 24 hr. Rhythms generated by the brain are transmitted neuronally and hormonally to the periphery to ensure that internal physiology is synchronized to the external environment. Disruption to circadian rhythms is associated with the development of metabolic syndrome and is also a hallmark clinical symptom of Alzheimer's disease. The triple transgenic Alzheimer's disease mouse model (3xTg) display features of metabolic syndrome, but rhythms in this disease model are not well-characterized and the effects of sleep deprivation on peripheral metabolism in the mice are unknown. Here, we implemented a long-term circadian disruption protocol to determine the effects of rhythm dysregulation on peripheral metabolism.
Methods: Long-term circadian disruption was induced in female C57Bl/6J control wild type mice (WT) and 3xTg-AD mice using automated sleep disruption machines (6 hrs daily, 5 days per week, for 3 months). Bile acid and lipid metabolism were investigated, qPCR was performed to characterize clock and metabolism signaling pathways, and histology was performed to assess liver morphology.
Results: Liver TTFL core clock gene expression was altered by circadian disruption. *Clock* mRNA expression was significantly suppressed in both WT and 3xTg mice, while *Bmal1*was significantly suppressed in 3xTg mice and trended decreased in WT mice. Interestingly, the core clock genes *Per1* and *Per2* were significantly induced by circadian disruption only in 3xTg mice. Bile acid content was significantly reduced in the intestine of 3xTg mice compared to WT mice and serum bile acids trended towards reduced, while expression of bile acid synthesis genes remained unchanged. Liver triglycerides and free fatty acids were significantly upregulated in 3xTg mice, and surprisingly, were reduced following long-term circadian disruption.
Conclusion: 3xTg Alzheimer's mice have altered bile acid and lipid metabolism and core circadian clock genes are differentially affected by chronic circadian disruption in these mice.

23. High-Fat, High-Sugar Diet and the Bile Acid Receptor TGR5

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Background: High-fat and high-sugar diets can lead to symptoms of metabolic syndrome, including obesity, fatty liver disease and dyslipidemia. Development of non-alcoholic steatohepatitis (NASH) and fibrosis from fatty liver disease is a major health concern. Bile acids aid in nutrient absorption and xenobiotic processing; they also mediate metabolic and inflammatory pathways in the liver and gut through the activation of receptors. One such receptor, G protein-coupled bile acid receptor 1 (Gpbar1, aka TGR5) is expressed in cholangiocytes and Kupffer cells of the liver, and in the small intestine and colon. Activation of TGR5 induces glucagon-like peptide 1 secretion from enterocytes to improve insulin sensitivity, and in the gut it suppresses inflammation. Here, we used a high-fat, high-fructose, and high-sucrose diet to determine how bile acid signaling through TGR5 may regulate hepatic and gut metabolism during progression to NASH/fibrosis.
Methods: Female C57Bl/6J control wild type mice (WT) and TGR5 knockout mice (Tgr5 KO) were fed a high fat (40% kcal), high fructose diet + 20% sucrose water (HF) for 20 weeks. Metabolic phenotypes of were characterized through examination of bile acid synthesis pathways, lipid and cholesterol metabolism pathways, and fibrosis and inflammation pathways.
Results: Tgr5 KO mice were more glucose intolerant when fed HF diet for 20 weeks, despite gaining the same amount of weight as WT mice. Tgr5 KO mice accumulated significantly more hepatic cholesterol and triglycerides on HF diet compared to WT mice on the same diet, and gene expression of *Acc* and *Fasn* were significantly upregulated. Hepatic Cyp7a1gene expression was consistently elevated in Tgr5 KO mice, while *Cyp7b1, Cyp27a1, Fxr,* and *Shp* were downregulated by HF diet. Despite these changes in bile acid synthesis pathways, bile acid content remained stable, with increased fecal excretion of bile acids in both WT and Tgr5 KO mice on HF diet. Interestingly, serum bile acids were significantly induced by HF diet only in WT mice, while Tgr5 KO mice had reduced serum bile acids. Hepatic expression of genes involved in inflammation and fibrosis were also significantly reduced in Tgr5 KO mice fed HF diet.
Conclusion: Tgr5 KO mice may be protected from the progression of steatosis to NASH/fibrosis induced by a high fat, high sugar diet.

24. The Effect of Global Sertad4 KO on Post-MI Cardiac Remodeling

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Heart failure (HF) affects millions of adults in the United States and its prevalence is projected to increase 46% by 2030. HF is characterized by the heart's inability to sufficiently perfuse the body, resulting in edema, pulmonary congestion, and multi-organ dysfunction. One common cause of HF is myocardial infarction (MI). At the ischemic site of damage, activated fibroblasts help establish a stabilizing scar. However, fibroblast activation can extend beyond the damaged area and cause excess deposition of extracellular matrix in previously healthy areas, leading to HF and possible death. The nuclear protein, Sertad4 (SERTA domain-containing protein 4) is a member of the SERTAD family of proteins, characterized by a conserved SERTA domain. Other members of this family have been identified as cell cycle regulators and transcriptional co-factors, but little is known about the role of Sertad4. In 2019, cell culture experiments identified Sertad4 as a potential regulator of cardiac fibroblast activation and we have observed increased protein expression of Sertad4 in human ischemic heart failure samples. We sought to determine the effect of a global Sertad4 knockout (KO) on post -MI cardiac remodeling in mice. After 4 weeks of permanent ligation of the left anterior descending artery (LAD), echocardiography was performed to measure systolic function. Relative to wild-type controls, the Sertad4 KO mice showed preserved systolic function as evident by improved ejection fraction and fractional shortening. β-

Gal staining in the Sertad4/LacZ reporter also showed robust Sertad4/LacZ expression in the infarct scar which extended into non-ischemic tissue. This data supports the notion that Sertad4 has a key role in cardiac remodeling in response to ischemic injury. Future directions include investigating Sertad4 in cardiac inflammation, investigation of Sertad4 molecular mechanisms, and cell-type specific disruption of Sertad4.

25. Investigating the Essential Molecular Components of the Defense Against Whole-Animal Respiratory Acidosis: A Novel Hypercapnia Assay

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Respiratory acidosis (RAc) developswhen the lungs are unable to eliminate sufficient CO_2 from the body. The impaired lung function caused by scoliosis, chronic obstructive pulmonary disease(COPD), asthma, pulmonary fibrosis, myasthenia gravis, narcotic overuse, severe obesity or obstructive sleep apnea usually causes RAc. As a consequence, arterial pH (pH_a), the pH of interstitial fluids and intracellularpH falls <7.35 ($\uparrow[CO_2]\rightarrow\downarrow$pH), which ultimately leads to debilitating consequences for the patient. During chronic RAc, the kidney attempts to compensate the $\downarrow pH_a$ by excreting the excess H^+ and generating new HCO_3^- to replace that lost in the blood due to H^+ titration. The renal proximal tubule (PT) normally handles ~80% of the kidney-secreted H^+ and in response to RAc, the PT epithelia appropriately adjusts their rate of H^+ secretion (J_H) into the tubule lumen and HCO_3^-reabsorption (J_{HCO3}) into the interstitium to compensate for the $\downarrow pH_a$. The acidosis is sensed at the basolateral (BL) side of the PT, but not directly as ΔpH, but rather as $\Delta[CO_2]_{BL}$ and $\Delta[HCO_3^-]_{BL}$. However, the molecular mechanism by which the PT transduces the $\Delta[CO_2]_{BL}$ and $\Delta[HCO_3^-]_{BL}$ signals into apical ΔJ_H and BL ΔJ_{HCO3} is poorly understood. Our lab previously established that the BL CO_2-evoked increase in J_H requires endogenously secreted ANG II to bind to apical AT_{1A} receptors, and is blocked by inhibitors of the BL localized ErbB receptor tyrosine kinases. Furthermore, knocking out receptor protein tyrosine phosphatase γ(RPTPγ), a novel extracellular CO_2/HCO_3^- sensor localized in the PT BL membrane, eliminates the ΔJ_H produced by $\Delta[CO_2]_{BL}$ or $\Delta[HCO_{3-}]_{BL}$, and significantly reduces the ability of the mouse to regulate pH_a during metabolic acidosis (MAc: $\downarrow[HCO_3^-]\rightarrow\downarrow$pH). We hypothesize that RPTP$\gamma$and its downstream effectors (ErbB1, ErbB2, ACE, and AT_{1A}) are also essential in the whole-body responses to RAc. Here we describe a novel assay in which we expose mice to hypercapnia (inspired CO_2= 8%) to impose RAc. We cannulate the carotid arteries of wild-type (WT) or knockout (KO) mice, and after recovery, sequentially sample arterial blood from conscious mice subjected to control (inspired 0.04% CO_2) or RAc conditions at 5 min, 4h, 24 h, 48 h and 7 days post-initiation to determine their ability to defend against the acid load. In WT mice, after 5 min of 8% CO_2, $pH_a\downarrow$from 7.39 to 7.23, arterial $pCO_2\uparrow$ from 29 to 65mmHg, and $[HCO_3^-]_a\uparrow$ from 18 to 27mM. pH_a recovers to 7.28 by 4 h post hypercapnia onset, arterial $pCO_2\uparrow$ to 71mmHg, and [HCO3−]a↑to 34 mM. At 48 h, pH_a plateaus at 7.33 (i.e. without returning to baseline values).While previous preliminary data from our lab showed that $AT_{1A}^{-/-}$mice have a limited capacity to defend pH_a during MAc, our hypercapnia assay shows that the AT1Areceptor does not appear to be essential for defending pH_a during RAc. Ongoing work with conditional ErbB1 and global RPTPγKO mice will produce major insights into whether these proteins are essential for transducing the signals necessary to elicit a whole-animal response to RAc-induced changes in $[CO_2]$.

26. Early Onset Cholestasis Sustains Bone Growth in Aged Mice

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Hepatic Osteodystrophy is a metabolic bone disease (i.e.,osteoporosis) that can later occur in patients with cholestatic liver disease. There is no suitable cholestasis animal model that allows a long-term study to examine hepatic osteodystrophy. Notably, we have generated a subset of wild-type mice to exhibit early onset anicteric cholestasis (WTAC) as categorized by significantly elevated serum total bile acids and absence of hyperbilirubinemia at the time of weaning. The objective of this study was to investigate how early onset cholestasis impacts liver and bone health after aging WTAC mice to 8months. As expected, WTAC mice showed indicators of steatosis and mild liver cirrhosis at the gross, histological, and transcriptlevels. Unexpectedly, micro-computed tomography imaging and histological staining of the tibia revealed improved bone morphology in WTAC mice. Changes in trabecular bone mass and structure included an increase of bone volume and connectivity density, switch from a rod to plate shape, and a decrease in thickness and spacing. WTAC mice also had slightly denser cortical bone at the diaphysis but displayed less bone marrow area. Osmium tetroxide staining confirmed WTAC mice to have lower bone marrow adiposity. Interestingly, putative genes that negatively regulate osteoclast differentiation (e.g., Rankl, Opg) were highly expressed in WTAC mice. The significant upregulation of osteoblast (e.g., Runx2, Osx, Wnt10b) and bone mineralization (e.g., Alpl) genes in WTAC mice further explains the potential mechanism for greater bone mass. Overall, this study delineates a novel finding that early onset cholestasis sustains bone growth even after mice reach peak bone mass.

27. TIP60 Regulation of ΔNp63αis Associated with Cisplatin Resistance

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About 5.4 million basal and squamous cell skin cancers are diagnosed each year in the US. Cisplatin, a chemotherapeutic drug is often used to treat squamous cell carcinoma (SCC) patients. However, 55% of cancers fail to respond leading to lower response rates and higher rates of disease re-occurrence. ΔNp63α, a member of the p53 transcription factor family is overexpressed and considered oncogenic in non-melanoma skin cancer where it regulates cell survival and proliferation. TIP60 (Tat-interacting protein 60kDa), a histone acetyltransferase has been shown to mediate cellular processes such as transcriptional regulation and the DNA damage response (DDR). We recently reported that TIP60 positively regulate ΔNp63αprotein levels in a catalytic-dependent manner to promote SCC proliferation. Since ΔNp63α is known to transcriptionally regulate several DDR genes and promote resistance to cisplatin, its stabilization by TIP60 may contribute to the failure of platinum-based drugs in SCC. We hypothesize that TIP60 mediated acetylation of ΔNp63α regulates its transcriptional activity thereby modulates chemoresistance. In this study, we showed that silencing of endogenous TIP60 in multiple SCC cell lines led to a decrease in ΔNp63α transcript and protein levels confirming that TIP60 positively regulates ΔNp63α. Increased levels of TIP60 positively correlated with increased ΔNp63α expression and contributes to cisplatin resistance. Further, stable expression of TIP60 or ΔNp63α individually promoted resistance to cisplatin and reduced cell death, whereas loss of ΔNp63α and TIP60 sensitized cells to cisplatin. Higher acetylation of ΔNp63α and TIP60 were seen in cisplatin resistant cell line. Taken together, our data suggests that TIP60-mediated stabilization of ΔNp63α increases cisplatin resistance and has potential implications for SCC cancer treatment and drug design. Additionally, since ΔNp63α confers cisplatin resistance

through regulation of genes involved in DNA damage repair, our findings provide critical insights into the mechanism by which the genes involved in chemoresistance are regulated and may lead to strategic treatment for resistant SCC tumors and other epithelial cancers with increased efficacy.

28. Long-term Regulation of Protein Concentration in HeLa cells at Variable Osmotic and Ionic Conditions

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The concept of cell volume (CV) regulation is not applicabletoprolonged exposure to a hypoosmotic or hyperosmotic environment, as the cells continue to grow or divide. To enable a study of long-term CV regulation, intracellular protein density (PD) instead of CV was focused on. To measure PD, a combination of a transmission-through-dye microscopic technique for CV measurement and transport-of-intensity equation imaging for quantification of the total protein content was used. It was observed that after 24 h of incubation in media with osmolarities ranging from 0.1 to 0.6 osm/L, HeLa cells restore their initial PD of approximately 0.2 g/ml. Unexpectedly, at extreme dilutions of the media (0.075 osm/L), PD increased. A similar response was observed in the presence of cation ionophore gramicidin that causes initial swelling. The PD was restored after 24 h at moderate gramicidin concentrations or increased at high gramicidin concentrations. The molecular nature of PD maintenance was investigated. Initial data indicate that PD may be regulated by mTOR (the protein kinase complex that controls the balance between anabolism and catabolism) and by the volume-regulated anion channel VRAC.

29. Studying Intestinal Epithelial Cell Differentiation using Human Intestinal Organoid (HIO) derived Enteroids

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Intestinal enteroids are epithelial-only 3D structures that have revolutionized the study of intestinal tissue *in-vitro*. Traditionally, intestinal enteroids were obtained from human biopsies. However, enteroids can also be generated from pluripotent stem cell-derived human intestinal organoids (HIOs). HIOs are grown *in-vitro* for 35 days and can then be transplanted into immuno-compromised mouse kidneys for further morphological maturation and growth allowing cells like the enteroendocrine cells to form and become functional. Enteroendocrine cells (EECs) are specialized nutrient-sensing cells that regulate many aspects of metabolism and are found along the GI tract, but due to their rarity, they can be difficult to study. The objective of this project was to study and compare two published EEC differentiation driving mechanisms: a doxycycline-inducible NEUROG3 construct (genetic induction) vs chemical induction approach. The findings from this study can potentially enhance studying the intestine if it is shown that one technique results in more EECs and their subtypes than the other. Results from these experiments can also illustrate if genetic induction has any differential results compared to the less invasive chemical induction technique. We generated enteroids from *in-vivo* HIOs, compared the proportions of epithelial cell types by immunofluorescence staining and quantitative PCR, and focused on the EEC lineages by analyzing specific hormones expressed at the different time points. Findings indicate that genetic induction yielded more EECs and its subtypes than chemical induction from both quantitative PCR and immunofluorescence staining data. However, some subtype hormones were induced equally well with both inductions rather than one prevailing. In the future, we will use enteriods to study how EECs control nutrient homeostasis and might be targeted as a therapeutic to treat patients with malabsorption.

30. Structural Investigation into Gating and Modulation of Alpha1 Glycine Receptor in Lipid Nanodiscs

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Glycine receptors (GlyR) mediate fast chemical transmission of synaptic signals in the central and peripheral nervous system. GlyR are anionic members of pentameric ligand gated ion channels and are central players in motor coordination and pain perception. Potentiation of GlyR activity results in dampening pain signals in the spinal cord making these receptors an attractive target for pain therapy. Here, we present Cryo-EM structures of the full-length alpha1 GlyR reconstituted into lipid nanodiscs that are captured in multiple functional states that include, the unliganded state (closed) and glycine-bound conformations (open and desensitized). In addition, we present conformations of GlyR in various ligand-bound forms that provide insights into the mechanisms underlying allosteric modulation. The functional state assignments were validated by molecular dynamics simulations. A comparison of these states reveals global conformational changes underlying GlyR channel gating and modulation.

31. Altered Creatine Kinase Activity and Mitochondrial Oxidative Capacity in Muscular Dystrophic mdx Mice after Repeated Muscle Contraction

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This study investigated the creatine kinase (CK) activity and the effects of repeated muscle contraction on muscle metabolism in mdx mice, a mouse model for Duchenne Muscular Dystrophy (DMD) at young (10-12 weeks) and adult (20-22 weeks) ages. DMD is caused by the absence of dystrophin protein which is essential in maintaining sarcolemma integrityand stability. The loss of dystrophin leads to muscle degeneration which results in poor muscle function[1–3]. Abnormal mitochondrial function is also a prominent characteristics of DMD[1,2,4]. Molecular analysis on the skeletal muscle of mdx mouse have reported reduced mitochondria DNA derived transcript and proteins, as well as reduced substrate activity in oxidative phosphorylation, suggesting reduced mitochondrial content[5–7]. Exercise training can improve muscle strength as well as improve mitochondrial function via mitochondrial biogenesis. Phosphorous-31 magnetic resonance spectroscopy (^{31}P-MRS) provides the opportunity to assess various aspects of muscle metabolism *in vivo*[8]. In particular, ^{31}P-magnetic resonance spectroscopic fingerprinting (^{31}P-MRSF) allows rapid quantification of CK reaction rate constant. Dynamic ^{31}P-MRS in combination with a metabolic perturbation protocol (such as exercise), can evaluate mitochondrial oxidative capacity in skeletal muscle through monitoring changes in high-energy phosphate metabolites such as adenosine triphosphate and phosphocreatine (PCr). Figure 1 shows the implementation of ^{31}P-MRSF and dynamic ^{31}P-MRS to study muscle metabolism on mice hindlimb. In this study the skeletal muscles were subject to two bouts of stimulation-induced muscle contraction using two needle electrodes inserted subcutaneously over the third lumbar vertebrae and the greater trochanter, respectively[9]. Using ^{31}P-MRSF, we observed a significant increase in CK rate constant after muscle stimulation for both mice at same age groups (young and adult) as shown in figure 2 A and C. However, CK rate constant was significantly lower in mdx mice compared to the control for both age groups. There was no change in PCr recovery rate in mdx mice at young age between first and second round of muscle contraction as shown in figure 2B. In control mice the PCr recovery rate increased significantly after second round of stimulation. Nevertheless, both control and mdx mice display no changes in PCr recovery rate at adult age as shown in figure 2 D. These *in vivo* observations suggest a positive acute change in mitochondrial function in response to muscle stimulation, which diminished with age and pathological changes.

32. Rapid Measurement of Cardiac Neuropeptide Dynamics by Capacitive Immunoprobe in the Porcine Heart

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Sympathetic control of regional cardiac function occurs through postganglionic innervation from stellate ganglia and thoracic sympathetic chain. Whereas norepinephrine (NE) is their primary neurotransmitter, neuropeptide Y (NPY) is an abundant cardiac cotransmitter. NPY plays a vital role in homeostatic processes including angiogenesis, vasoconstriction, and cardiac remodeling. Elevated sympathetic stress, resulting in increased NE and NPY release, has been implicated in the pathogenesis of several cardiovascular disorders including hypertension, myocardial infarction, heart failure, and arrhythmias, which may result in sudden cardiac death. Current methods for the detection of NPY in myocardium are limited in their spatial and temporal resolution and take days to weeks to provide results [e.g., interstitial microdialysis with subsequent analysis by enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), or mass spectrometry]. In this study, we report a novel approach for measurement of interstitial and intravascular NPY using a minimally invasive capacitive immunoprobe (C.I. probe). The first high-spatial and temporal resolution, multichannel measurements of NPY release in vivo are provided in both myocardium and transcardiac vascular space in a beating porcine heart. We provide NPY responses evoked by sympathetic stimulation and ectopic ventricular pacing and compare these to NE release and hemodynamic responses. We extend this approach to measure both NPY and vasoactive intestinal peptide (VIP) and show differential release profiles under sympathetic stimulation. Our data demonstrate rapid and local changes in neurotransmitter profiles in response to sympathetic cardiac stressors. Future implementations include real-time intraoperative determination of cardiac neuropeptides and deployment as a minimally invasive catheter.

33. Progress in the Development of Pre-clinical Animal Models for Mechanistic Study of Health and Disease

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There is strong evidence for the protective power of higher levels of fitness against a wide variety of complex diseases. Yet the mechanisms responsible for the protective effects of high fitness; Or conversely, the mechanisms responsible for the extraordinary risk associated with low fitness are various. In addition, commonly used animal models of disease are too simplistic to enable progress in understanding related conditions such as cardiovascular disease, diabetes, Alzheimer's disease, and aging. Examples include the use of genetically manipulated mouse models to reveal "disease-related" phenotypes for diseases that are not single gene disorders but are rather driven by complex polygenetic and environmental interactions. The long-term objective of this work is to develop more realistic animal models for the study of health and disease that are accurate, predictive of mechanism(s), and most importantly, based on consistent epidemiological findings of biological factors that clinically impact health. For this project, we initiated the Energy Transfer Hypothesis (ETH): Variation in capacity for energy transfer (i.e., exercise capacity) is the central mechanistic determinant of the divide between disease and health. To test this hypothesis, we artificially selected rats for divergent, intrinsic low and high treadmill running capacity over several generations. Consistent with the ETH, we find numerous disease risks co-segregated with selection for low exercise capacity including diminished longevity (~ 6 months),

manifestation of metabolic syndrome and obesity, and increased susceptibility to cancer and neurological disease. The selection experiment was powerful because it both tested ETH and simultaneously provided contrasting models —Low-Capacity Runners (LCR) vs High-Capacity Runners (HCR) — for mechanistic studies. The results of our study are in line with the suggestion that disease risk is driven by genes that mediate energy transfer capacity that is cryptically concealed by the miniscule scale of each accumulative genetic change that occurs with evolution. Such a path is consistent with the resultant high complexity and relatedness of life. Results from our large-scale selection experiment—now at generation 46 of selection—is organized around a consortium of 50+ investigators and several collaborative teams formed to address disease causation, energy metabolism, diabetes, vascular lipid biology, cancer susceptibility, fatty liver disease, obesity, Alzheimer's disease, musculoskeletal disease, and cardiac function. Using our novel rodent model based on ETH and disease susceptibility driven by genes for exercise capacity, we provide a more unified approach that is different from mainstream concepts for understanding the ostensibly "intractable" nature of complex disease.

34. **Postnatal Exposure to Brief Hypoxia Alters Brain VEGF Expression and Capillary Density in Adult Mice**

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Background: Perinatal hypoxia leads to changes in cerebral angiogenesis and persistent structural and functional changes in the adult brain. Perinatal hypoxia may also result in greater vulnerability to subsequent challenges in adulthood.
Objective: We investigated the effect of postnatal day two (P2) hypoxic preconditioning on adult brain capillary density and brain vascular endothelial growth factor (VEGF) expression in mice.
Materials and Methods: P2 mice were exposed to hypoxia (5% O_2) in a normobaric chamber for two hours and then returned to normoxia while their littermates remained in normoxia (normoxic control). After 2-6 months, they were euthanized and their brains were removed for capillary density determination. Another set of animals (P2 hypoxic mice and normoxic controls) were euthanized at different time points (2, 10, 23, and 60 days after birth) and brain VEGF expression was assessed by Western blot analyses. Statistical analyses were performed using SPSS V27.0 for Windows. The comparison between any two groups was analyzed with ANOVA and one-tailed t-test, and significance was considered at the level of $p < 0.05$.
Results: Adult brain capillary density was significantly increased in the P2 hypoxic mice when compared to the P2 normoxic control mice. Additionally, VEGF expression appeared to be elevated in the P2-hypoxia mice when compared to the P2-normoxia mice at all time points, and VEGF levels in P2-hypoxia mice declined with age similarly to P2-normoxia mice.
Conclusions: These data demonstrate that transient early-postnatal hypoxic stress leads to an increase in capillary density that persists in the adult, possibly due to increased VEGF expression. These results might be explained by epigenetic factors in the VEGF gene.

35. **Stimulatory Effect of Phosphomimetic Mutants of IRBIT on the Electrogenic Na/HCO_3 Transporter NBCe1-B**

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In 2006, Shirakabe and colleagues reported that (1) the soluble protein IRBIT [inositol trisphosphate (IP_3)-receptor (IP_3R) binding protein released with IP_3] markedly stimulates the electrogenic Na/HCO_3 cotransporter NBCe1-B when co-expressed in *Xenopus* oocytes; and (2) the first 62 amino acids of the -NH_2 terminus of NBCe1-B are essential for this stimulatory effect of IRBIT on NBCe1-B (i.e., interaction). Studies of IRBIT led to the conclusion that serine (S) phosphorylation of IRBIT—specifically at pS68, pS71, pS74, ±pS77 within the PEST domain of IRBIT—is required for IRBIT activity. One of the studies identified a protein phosphatase-1 (PP1) binding site in the upstream region of the PEST domain. The optimization of this PP1-

binding site renders the construct nonfunctional (sub-IRBIT), presumably because the strong binding of PP1 dephosphorylates nearby S and threonine (T) residues in the PEST domain. Conversely, the elimination of this PP1-binding site yields an IRBIT construct (super-IRBIT) that is more powerful than wild-type IRBIT in IP_3R assays, presumably because blockade of dephosphorylation (in the presence of background kinase activity) leaves the S and T residues in the PEST domain highly phosphorylated. We quantified the contribution of super-IRBIT and sub-IRBIT on NBCe1-B activity, finding that super-IRBIT increases NBCe1-B activity by ~30% more than wild-type IRBIT, whereas sub-IRBIT does not stimulate at all. Here, we hypothesize that, on the background of sub-IRBIT, introduction of phosphomimetic mutations—conversion of S or T to aspartate (D) in the PEST domain—will restore the stimulatory action on NBCe1-B. To test our hypothesis we use two-electrode voltage-clamping (2eVC) of oocytes either co-expressing NBCe1-B with phosphomimetic mutants of IRBIT, or expressing NBCe1-B alone. 2eVC provides a precise measure of electrogenic transporter activity. We found that phosphomimetic mutations—as many as 15 individual conversions of S®D or T®D, from S64 to S97 within the PEST domain—produce IRBIT constructs that are powerful stimulators of NBCe1-B activity. Moreover, on the background of the most active phosphomimetic IRBIT mutant (i.e., 15 mutations), the triple dephosphomimetic mutant S68A/S71A/S74A is still a powerful activator of NBCe1-B activity. Our findings are consistent with the hypotheses that [1] multiple negative charges within the PEST domain—produced either by natural phosphorylation or by phosphomimetic mutations—are essential for IRBIT's stimulation of NBCe1-B; and [2] contrary to a previously proposed model, IRBIT phosphorylation at residues S68/S71/S74 per se is not necessary for IRBIT activation. We suggest that, in a natural environment, phosphorylation of S68/S71/S74 is a prerequisite for phosphorylation of downstream S and T residues in the PEST domain, and that it is the phosphorylation of these downstream residues that is actually essential for the action of IRBIT on NBCe1-B.

36. Putative Role of Renal (Malpighian) tubules in Regulating Calcium Homeostasis in the Mosquito *Aedes aegypti*

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The goal of our research is to investigate renal mechanisms of calcium (Ca^{2+}) homeostasis in mosquitoes *Aedes aegypti*. In other insects, the renal (Malpighian) tubules play a key role in regulating extracellular fluid (hemolymph) Ca^{2+} homeostasis by storing excess Ca^{2+} in intracellular vesicles and/or secreting excess Ca^{2+} into the urine for excretion. However, the renal regulation of Ca^{2+} balance in mosquitoes has not been previously examined. To understand the role of Malpighian tubules in regulating Ca^{2+} homeostasis, the distribution of Ca^{2+} in adult female *Ae. aegypti* was determined in response to various dietary Ca^{2+} loads. Groups of adult females were fed on diets (10% sucrose) containing varying amounts of $CaCl_2$ (5, 10, 20 and 40 mM) after emergence; controls were fed 10% sucrose without $CaCl_2$. After a week, Ca^{2+} content was measured in homogenates of the whole body and isolated Malpighian tubules using a Ca^{2+}-selective sensor. We found that the Ca^{2+} contents in both whole bodies and Malpighian tubules increased significantly with all the Ca^{2+}-containing diets compared to controls. However, the sensitivity of the responses differed between the whole bodies and Malpighian tubules. That is, the percent increase of Ca^{2+} content in Malpighian tubules was concentration-dependent and increased by ~88% relative to controls at the highest dietary Ca^{2+} concentration (40 mM). In contrast, the percent increase of Ca^{2+} in whole bodies was not concentration-dependent and only changed by 25% relative to controls at the highest dietary Ca^{2+} concentration (40 mM). Among the dietary treatments, ~40-80% of the total change in whole body Ca^{2+} increase could be attributed to Malpighian tubules. Taken together, these results suggest that the Malpighian tubules of adult female *Ae. aegypti* are a major site of Ca^{2+} regulation in response to dietary Ca^{2+} loads. This is consistent with what has been found in the other insects, such as adult blowflies, where Malpighian tubules function mainly by secreting and sequestering extra dietary Ca^{2+}. Future studies will examine the molecular mechanisms that contribute to the changes in Malpighian tubules Ca^{2+} content and whether similar changes occur when mosquitoes are fed blood.

37. UPR^{mt} activation protects against MPP+-induced toxicity in a cell culture model of Parkinson's disease

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The pathogenesis of Parkinson's disease (PD) remains elusive, but mitochondrial dysfunction is believed to be one crucial step in its pathogenesis. The mitochondrial unfolded protein response (UPR^{mt}) is an important mitochondrial quality control strategy that maintains mitochondrial function in response to disturbances of mitochondrial protein homeostasis. Activation of the UPR^{mt} and the beneficial effect of rescuing mitochondrial proteostasis have been reported in several genetic models of PD. However, the pathogenic relevance of the UPR^{mt} in idiopathic PD is unknown. The present study examined the link between the UPR^{mt} and mitochondrial dysfunction in 1-methyl-4-phenylpyridinium (MPP+)-treated SH-SY5Y cells. Treatment with MPP+ induced activation of the UPR^{mt}, reflected by an increase in the expression of UPR^{mt}-related chaperones, proteases, and transcription mediators. UPR^{mt} activation that was induced by overexpressing mutant ornithine transcarbamylase significantly reduced the production of mitochondrial reactive oxygen species (ROS) and improved cell survival in SH-SY5Y cells following MPP+ treatment. Moreover, the overexpression of activating transcription factor 5 (mammalian UPR^{mt} transcription factor) conferred protection against MPP+-induced ROS production and against cell death in SH-SY5Y cells. Overall, our results demonstrate the beneficial effect of UPR^{mt} activation in MPP+ - treated cells, shedding new light on the mechanism of mitochondrial dysfunction in the pathogenesis of PD.

38. The Role of C-Terminus and Nuclear Localization Sequence of Parathyroid Hormone-Related Protein (PTHrP) in Pancreatic Islet Morphology and Glucose Homeostasis

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Parathyroid Hormone-related protein (PTHrP) is an important polyhormone with multiple functions in development and cell regulation. PTHrP is expressed by numerous cell types including pancreatic beta cells, which are the cell type responsible for insulin secretion. Previous studies have focused on the ability of N-terminal PTHrP to stimulate proliferation in beta cells. We have developed a knock-in mouse model lacking the C-terminus and nuclear localization sequence, retaining N-terminal PTHrP. These mice die at ~day 5, are severely stunted in growth (weigh 54% less than control mice), have chondrodystrophy, hypoinsulinemia, hypoglycemia (~40-60mg/dL), hypotriglyceridemia, and lack body fat. Percentage stomach weight (including ingested milk) measurements in relation to body weight were consistent among all mouse genotypes indicating that inanition was not the cause of death. To characterize the pancreatic islets in these mice, islets (~10-20) were isolated from 2-5-day-old-mice using collagenase digestion and a glucose-stimulated intracellular calcium assay was conducted at 0, 4, 8, 12, 16 and 20mM glucose. Results showed higher intracellular calcium response (135.8 ± 0.9nM) in knock-in islets compared to control islets (85.2± 43.90nM) at 0mM glucose and a similar trend in 20mM glucose stimulation (768.3 ± 120.4nM and 422.3 ± 131.7nM for knock-in and control mice, respectively). This study revealed that the C-terminus and nuclear localization sequence of PTHrP are crucial to life, including regulation of glucose homeostasis and islet response to glucose, energy and fat metabolism. Control of islet cell PTHrP may be useful for preventing or treating Type 2 diabetes mellitus, including regulation of beta cell mass.

39. **Med12 is Essential for Skeletal Muscle Development**
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Tight regulation of tissue- and cell-specific gene expression is essential for healthy physiological development. The Mediator Complex links basal transcriptional machinery, transcription factors, and epigenetic modifiers to gene targets to coordinate transcriptional regulation during development. MED12, a Mediator complex subunit, is a protein that controls tissue- and cell-specific gene expression and has been studied in the context of developmental disorders and cancers. In addition to these diseases, *MED12* mutations have been linked to muscle related phenotypes such as skeletal muscle hypotonia and congenital heart defects, but the function of MED12 in muscle and muscle cell development has not been elucidated. This study seeks to define the molecular role of MED12 in skeletal muscle development. Our lab has created a conditional skeletal muscle-specific *Med12* knockout mouse model (*Med12mKO*) driven by a Myogenin-Cre transgene that is expressed during embryonic muscle development. *Med12mKO* mice have a runted phenotype, smaller muscles, disorganized muscle architecture, and suffer premature death. Gene expression analysis by RNA-sequencing (RNA-seq) of muscle from 1-day old *Med12mKO* mouse muscle demonstrated that *Med12* deletion dysregulates metabolic and structural genes. Upstream regulator analysis (URA) of these dysregulated genes predicted transcription factors upstream of these genes, including SRF and PPARg. Additionally, we employed Immunoprecipitation-Mass Spectrometry (IP-MS) to identify protein binding partners of MED12 in the C2C12 mouse myoblast cell line. This experiment identified transcription factors, epigenetic histone modifiers, and RNA binding proteins as MED12 protein interactors. We hypothesize that MED12 interacts with epigenetic histone modifiers and transcription factors to coordinate structural and metabolic gene expression during muscle development and that dysregulation of this process results in impaired myogenesis. Using primary isolated myoblasts from the *Med12mKO* mouse and C2C12 myoblast cell lines, we are investigating which aspects of myogenesis are disrupted by *Med12* knockout. Mechanistically, we are assessing MED12 interaction with transcription factors and histone modifiers identified in our preliminary data sets and using ChIP-seq and RNAseq to understand the transcriptional and epigenetic consequences of *Med12* loss throughout myogenesis. Our findings will elucidate a function for MED12 and the Mediator complex in transcriptional control of muscle development and offer insight to pathology of MED12 associated developmental diseases.

40. Unraveling the Function of Protein Interactions with the Nuclear Polyadenosine Binding Protein (PABPN1), an RNA Binding Protein Associated with a Late-onset Muscular Dystrophy
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Polyadenosine binding protein nuclear 1 (PABPN1) is an mRNA binding protein that facilitates multiple steps in RNA processing including poly-adenylation, alternative polyadenylation regulation of 3’ terminal intron retention, export, decay, and translation. The PABPN1 protein contains an amino terminal poly-alanine tract with no known function. Expansion of the PABPN1 polyalanine tract by 1-8 alanine residues leads to a late onset and progressive muscular disease known as oculopharyngeal muscular dystrophy (OPMD). Few studies have systematically explored the role of protein binding partners in PABPN1 function the context of skeletal muscle, the tissue affected by OPMD. Considering that proteinbinding partners dictate function of a given RNA binding protein, understanding interaction networks of PABPN1 may provide clues into the pathology of OPMD. We recently reported several protein-protein interactions of PABPN1 in the context of mature mouseskeletal muscle, some of which further implicate PABPN1 in intron retention and alternative splicing. However, these studies relied on overexpression of exogenous tagged PABPN1 in skeletal muscle. Thus, our objective is to better understand the mechanism of PABPN1 involvement in splicing by probing the protein-protein interactions with endogenous PABPN1. We sought to identify PABPN1 interaction candidates from our previously published

data and validate their interaction with endogenous PABPN1. These candidates, HNRNPH2, SRSF2, FAM120A, and RAB14, were selected based on high confidence data from our published mass-spectrometry studies. Using immunoprecipitation of endogenous PABPN1 in the immortalized C2C12 myoblast cell line, we were able to reproduce our published data by capturing both HNRNPH2 and FAM120A. Given its known function in splicing and alternative polyadenylation, we focused on HNRNPH2 as a candidate PABPN1 binding partner. Attempts to precipitate PABPN1 using antibodies to HNRNPH2 have not been successful due to poor specificity for both antibodies. To circumvent these issues, we have generated a construct containing flag-tagged Hnrnph2 under the control of an inducible promoter. We will use this construct to evaluate if HNRNPH2 can precipitate PABPN1, and whether interaction with PABPN1 is RNA dependent. To determine the functional relevance of the PABPN1 interaction with HNRNPH2, we will knock down of Hnrnph2 or Pabpn1 to assay the effects on known PABPN1 and HnRNPH2 targets, respectively. Our future studies will focus on further defining the interaction between PABPN1 and HNRNPH2 and how this interaction is affected by alanine expansion of PABPN1. These studies will lead to a deeper understanding of how PABPN1 functions in splicing and intron retention and the molecular mechanisms of OPMD pathology.

41. **O_2 Fluxes via RhAG and NtPIP1;3 Quantified in a Neutral-buoyancy Assay**

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Three key discoveries contradict the simple central dogma of transmembrane gas flux, that all gases cross all membranes by dissolving in the lipid phase of the membrane: (1) CO_2-impermeable membranes; (2) CO_2-permeable membrane proteins or “gas-channels”, namely, certain aquaporins (AQPs) and rhesus (Rh) proteins; and (3) decreased CO_2-permeability when the CO_2-impermeable *Nicotiana tabacum* AQP NtPIP2;1 is incorporated into artificial membranes. Characterization of the CO_2 and NH3 permeabilities of AQPs 0-9 and several rhesus (Rh) proteins shows that they can exhibit gas selectivity and during O_2-offloading, ~55% of O_2 exits mouse red blood cells (RBCs) via AQP1 and the Rh complex (i.e., RhAG + mRh). We modified of our Neutral Buoyancy Assay (NBA), originally developed to assess transmembrane N_2 fluxes, to characterize the mechanism by which RhAG and other proteins conduct O_2, and to screen for novel O_2 channels in a heterologous expression system. We inject a precise volume of N_2 gas (number of gas molecules = nGas) into a *Xenopus* oocyte, which we place into a saline-containing pressure-resistant tube. Sufficient pressure is then imposed in the air phase above the air-water interface to collapse the injected bubble enough to maintain the oocyte at a depth of 5 cm. As N_2 gas escapes the bubble and dissolves in the cytosol and ultimately diffuses across the plasma membrane into the extracellular fluid (ECF), the bubble shrinks, cell density increases, and the oocyte starts to sink. A camera/computer combination detects the sinking and decreases air-phase pressure enough to maintain neutral buoyancy at 5 cm depth. Calibration exercises allow us to compute the time course of ΔnGas, and thus gas efflux. Here we modify the NBA to quantify gas influx. When we raise $[N_2]$ in the ECF from [N2]o = 0.56 mM (room air at 1×ATA) to $[N_2]o$ = 2.06 mM (pre-equilibrating saline with 93% N2/7% O_2 at 3×ATA) at constant $[O_2]o$ = 0.26 mM, nGas rises as N2 enters the cell during the NBA. nGas rises faster when we selectively increase $[O_2]o$ from 0.26 mM to 0.91 mM (pre-equilibrating saline with 78.96% N_2/21% O_2/0.04% CO_2 at 3.5×ATA) while holding $[N_2]o$ constant at 2.06 mM. RhAG-expressing oocytes exhibit no significant difference in ΔnGas over 1000 s when $[N_2]o$ = 2.06 mM/$[O_2]o$ = 0.26 mM vs. control oocytes (injected with H2O rather than cRNA encoding RhAG). Thus, RhAG is not a N_2-selective channel. However, ΔnGas over 1000 s is significantly greater in RhAG vs. control oocytes when [N2]o = 2.06 mM/$[O_2]o$ = 0.91 mM. Thus, RhAG exhibits selectivity for O_2 over N_2. We repeated the low-to-high $[O_2]o$ experiment with oocytes expressing NtPIP1;3 (reported to facilitate O_2 flux in a spectrophotometric assay when expressed in yeast protoplasts). NtPIP1;3 expressing oocytes show substantial increases in ΔnGas over 1000 s vs control oocytes. These data are consistent with our previous findings in RBCs that the Rh complex constitutes a major pathway for O_2 flux across the plasma membrane, and also supports the report from another

laboratory that NtPIP1;3 is an O_2 channel. Although we specifically developed the NBA for N_2 fluxes, here we demonstrate the versatility of the assay for measuring transmembrane fluxes of other gases.

42. Exploring Oxygen Permeability of Red Blood Cell Membranes with a Computational Model of Oxygen Off-loading from Red Blood Cells

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Red blood cells (RBCs) perform the vital task of carrying oxygen (O_2) from the alveolar air to the systemic tissues, and carbon dioxide (CO_2) in the opposite direction. An important step in O_2 delivery and CO_2 removal is the transport of these gases across the plasma membrane (PM) of various cells, including the PM of RBCs. From the time of Overton, common belief had been that all gases cross all cell membranes by dissolving in and diffusing through the membrane lipids. However, various studies have now challenged this view by identifying (1) the apical membrane of gastric gland cells as the first CO_2-impermeable membrane and (2) the aquaporins (AQPs), the rhesus (Rh) proteins, and urea transporters (UTs) as families of integral membrane proteins that can augment transmembrane gas permeability (i.e., "gas channels"). In human RBCs, AQP1 and RhAG are responsible for~90% of membrane CO_2 permeability (PM, CO_2). Following these findings, Zhao and colleagues (Zhao et al, 2016 FASEB J) hypothesized that AQP1 and RhAG can also be responsible for enhancing the O_2 permeability (PM, O_2) of RBC membranes. These authors employed stopped-flow (SF) absorbance spectroscopy to measure the rate constant of hemoglobin (Hb) deoxygenation ($kHbO_2$) during O_2 off-loading from oxygenated mouse RBCs exposed to a solution containing an O_2 scavenger. They found that $kHbO_2$ decreases by (i) ~9% in RBCs genetically deficient in AQP1, (ii) ~17% in RBCs genetically deficient in RhAG, and (iii) ~30% in RBCs genetically deficient in both AQP1 and RhAG (dKO). Moreover, they found that in WT RBCs, the amino-reactive agent 4,4'-diisothiocyanatostilbene-2,2'-disulfonate (DIDS) reduces $kHbO_2$ by ~31%,and pCMBS by ~61%;in dKO RBCs, DIDS reduces kHbO2 by ~53%, and p-chloromercuribenzenesulfonate (pCMBS) by ~78%. Neither DIDS nor pCMBS react with membrane lipids or cross the PM to enter RBCs. Therefore, the KOs and inhibitors presumably all act by reducing the number or activity of PM proteins. To examine the extent to which the observed decreases in $kHbO_2$ correspond to decreases in PM, O_2, we developed a reaction-diffusion computational model of O_2 off-loadingfrom a spherical RBC, with diameter equal to the RBC thickness. The model describes the changes in time and space of the concentrations of O_2, oxyhemoglobin (HbO_2), and Hb as O_2 diffuses from the RBC intracellular fluid, across the PM, throughout the extracellular unconvected fluid, and to the bulk extracellular fluid. Informed by hematology, flow cytometry and SF hemolysate data, the model can simulate the $kHbO_2$ data with high accuracy, confirms that decreases in $kHbO_2$ correspond to decreases in PM, O_2, and allows quantification of such decreases. Specifically,the model predicts that PM,O_2 decreases by ~22% in $AQP1^{-/-}$, ~36% in $RhAG^{-/-}$, ~55% in dKO, ~56% in WT+DIDS, ~82% in WT+pCMBS, ~76% in dKO+DIDS, and ~91% in dKO +pCMBS. In conclusion, our SF data and mathematical simulations suggest that: (1) ~91% of O_2 exits RBCs via gas-channel mediated pathways; (2) ~55 percentage points of this 91% exits RBCs via AQP1 and RhAG; (3) the remaining 36 percentage points (= 91% −55%) exits via not-yet-identified gas channels.

43. High-Phosphate Diet Induces Exercise Intolerance in Mice

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Inorganic phosphate (Pi) is used extensively as a preservative and a flavor enhancer in the western diet. Physical inactivity is associated with increased cardiovascular morbidity and mortality and frequently accompanies consumption of a western diet. It is not known whether excess dietary Pi contributes to exercise intolerance and physical inactivity in otherwise healthy subjects. To investigate the relationship between excess dietary Pi and exercise we used clinical data and human blood samples from Dallas Heart Study phase 2 (DHS-2) participants. We found that

higher serum Pi was independently associated with reduced time spent in moderate to vigorous physical activity and increased sedentary time, but there was no association between serum Pi and left ventricular ejection fraction or volumes. To test the hypothesis that high phosphate diet effects exercise capacity we fed mice high-Pi diet (HP) for 12 weeks and performed exercise tolerance tests. HP diet did not alter body weight or left ventricular function, but reduced maximal oxygen uptake, treadmill duration, and spontaneous locomotor activity in mice. In skeletal muscle, high-Pi diet led to downregulation of genes involved in fatty acid synthesis, release, and oxidation, and upregulation of genes involved in glucose metabolism. Our data demonstrate a detrimental effect of excess dietary Pi on skeletal muscle fatty acid metabolism and exercise capacity that is independent of obesity and cardiac contractile function. Future studies will investigate the mechanisms by which Pi regulates gene expression, and whether the negative effects of high-Pi diet are reversible. Dietary Pi may represent a novel and modifiable target to reduce physical inactivity associated with the Western diet.

44. Regulation of Uterine Artery Myogenic Tone by cAMP Signaling

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Deficiency in regulator of G protein signaling 2 (RGS2), a GTPase activating protein for Gq/11 and Gi/oclass G proteins, is implicated in impaired uterine blood flow in pregnant and non-pregnant states. Previously, we showed that RGS2 negatively regulates Gi/o signaling to modulate uterine artery myogenic constriction, suggesting that Gi/o signaling is involved in the development of myogenic tone in the uterine vascular bed; however, the mechanisms are poorly understood. In this study, we tested the hypothesis that Gi/opromotes myogenic constriction by inhibiting cAMP-dependent uterine artery vasodilation. Using video microscopy and isolated vessel preparation, we examined the myogenic tone of uterine arteries from WT and Rgs2 KO mice in the presence and absence of isoproterenol, forskolin, or exogenous cAMP. Consistent with previous studies, myogenic tone was augmented in uterine arteries from Rgs2 KO relative to WT mice. Incubation with cAMP (10 μM) or forskolin markedly inhibited myogenic response in both WT and Rgs2 KO arteries. However, isoproterenol had no significant effect on myogenic constriction in WT arteries. Our preliminary data suggest that cAMP triggers the inhibition of uterine artery myogenic constriction independently of receptor-mediated regulation of adenylyl cyclase activity. Funding Sources: This study is funded in part by a grant from the National Institutes of Health –NHLBI (R01 HL139754) to Patrick Osei-Owusu. The funding agencies had no role in the study design or execution.

45. Air Pollutants During Pregnancy Associated with Methylation Changes in Neonates

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DNA methylation (DNAm), one process by which the transcription of DNA is reduced, is affected by environmental factors, including particulate matter smaller than 2.5 microns (PM2.5). DNAm regulates gene expression and can have functional consequences to biological processes and, therefore, health. PM2.5 is associated with fetal development and other physiological pathways such as inflammation (Fu et al., 2020; Yue et al., 2020). The goal of this project is to identify specific loci of the epigenomeat which DNAmat birth is associated with ambient PM2.5 levels during pregnancy. From a cohort of 91 neonates, salivary samples were collected to be processed on an Infinium Methylation EPIC array designed to measure the methylation state of over 850k methylation sites (CpG sites) on the epigenome. Daily ambient PM2.5concentrations were estimated at a 1x1 km resolution using a previously validated exposure assessment model based on the geocoded address of primary residence during pregnancy (Brokamp et al., 2018). For each mother-infant dyad, PM2.5was averaged over the first two trimesters, separately and combined, and epigenome-wide associations (EWA) were used to test the association between

those PM2.5averagesand methylation variation across the 850kCpGsites, adjustingfor sex, race, maternal age at date of conception, study batch, and sample position on the methylation array. Prior to the association, surrogate variable analysis was performed to identify variation not attributed to measured biological or technical sources and adjust for that in the model. Correction for multiple comparisons in the EWA was performed using the false discovery rate procedure, and corrected p-values were compared to a critical significance level of 0.05.For each predictor, every site with a $p < 0.0001$ was selected to undergo pathway and network analysis with the Ingenuity Pathway Analysis (IPA) software to identify the biological pathways and systems affected by the regulation of genes controlled by the selected CpG sites. Through EWA analysis, cg11845050was identified as significant in the association with second trimester PM2.5, and cg18705808was identified as significant in the association with the average PM2.5of both trimesters (Figure 1). TMEM184A, the gene regulated by cg18705808, has a putative role in inflammatory pathways, which makes our findings consistent with previous research into PM2.5 (Fu et al., 2020). PI4KAP2, the gene regulated by cg11845050, has no such link to inflammation.IPA analysis revealed little similarity between the first and second trimesters in regard to the pathways and networks implicated by selected CpG sites (Table 1).These differences may indicate trimester-specific effects of PM2.5on DNAm during pregnancy. Further analysis with greater temporal resolution would be valuable to fully characterize the effect of PM2.5 on DNAm and child development.

47. Satiation Responses to Food are Deteriorated in Mice Deficient in Insulin Secretion

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Insulin is an anabolic hormone involved in the regulation of energy homeostasis by controlling glucose uptake and metabolism in insulin-sensitive peripheral tissues and by restricting food intake at the central level. Accordingly, relative deficiency of insulin secretion secondary to obesity and other insulin resistant states is usually accompanied by hyperglycemia, hyperlipemia and increased energy intake, a vicious cycle involved in the pathogenesis of type 2 diabetes. However, it remains unknown if early primary deficiencies in insulin secretion alters feeding behavior prior to the development of obesity and hyperinsulinemia. We addressed this question by testing age-dependent behavioral feeding responses to food in an animal model of deficient insulin secretion i.e., mice lacking the products of the *Slc12a2* gene i.e., Na^+, K^+, Cl^- cotransporters-1 (*Nkcc1*) exclusively in insulin-secreting β-cells ($Nkcc1^{\beta KO}$) of the islets of Langerhans in the pancreas. The feeding behavior of $Nkcc1^{\beta KO}$ mice was assessed in the context of the two variables determining energy intake i.e., satiation and satiety. To determine satiation and satiety responses to food, we measured ad libitum mean meal size and the time spent not eating between meals during 3 weeks of continuous monitoring of their feeding microstructure. Our results demonstrate age-dependent increase in the nocturnal meal size of 10w, 20w and 30w old $Nkcc1^{\beta KO}$ mice, thus suggesting reduced satiation responses to food. Further, 20w and 30w old mice spent less time between meals or were unable to spend enough non-eating time commensurate with their significantly increased meal size, indicating that older $Nkcc1^{\beta KO}$ mice have impaired satiety responses to food as well. These behavioral abnormalities were related to nocturnal hyperphagia in younger $Nkcc1^{\beta KO}$ mice and obesity in older ones. Therefore, our results suggest that early deficiency in insulin secretion promote hyperphagia, which antecedes the development of obesity.

48. Structure-Function Relationship Investigations of Primary Cilia

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We investigate the hypothesized flow-sensing function of primary cilia. Primary cilia are slender membrane-encased microtubule-based structures protruding from the cell body into the extracellular space. We use a variety of experimental and computational methods to examine the

hypothesis that renal primary cilia function as flow sensors used by the cell to maintain tissue and organism homeostasis. Our primary experimental methods used are optical trapping and perfused tissue culture. Here, we focus on results of using an optical trap to characterize the mechanical response of primary cilia to an externally applied force and also discuss different methods we use to alter the mechanical response of primary cilia. Our primary result to date is strong evidence that the existing mechanical model of primary cilia is insufficient, and we will present our improved model based on anisotropic tubes. This result was obtained by a combination of experimental data and numerical modeling. We also found that application of compounds that stabilize Hypoxia Inducible Factor-1 (HIF-1) result in longer and more flexible cilia. We will present our new Holographic Optical Trap (HOT) and discuss the use of silencing RNA (siRNA) to alter the protein composition of primary cilia as ongoing novel methods to explore the structure-function relationship of primary cilia. In conclusion, we have begun to systematically explore how the structure of primary cilia impact the physiological function of primary cilia.

49. Effects of TRPM7 Kinase Inactivation in Murine Macrophages

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Transient Receptor Potential Melastatin 7 (TRPM7) is a unique protein that containsan ion channel anda functional kinase. TRPM7 is highly expressed in immune cells such as lymphocytes and macrophages. Complete deletion ofTRPM7 or the kinase domain is embryonic lethal in mice. Global kinase inactivation in viable mice is achieved by introducing a kinase-dead (KD) point mutationK1646R. KD mice display moderate splenomegaly and ectopic hemopoiesis. We have previously demonstrated that T cells from the spleen of KD mice have a defect in proliferation and reduced store-operated calcium entry (SOCE).In the present study, we have investigated the consequences of TRPM7 kinase inactivation in macrophages. Specific cell populations were characterized by flow cytometry. Macrophages isolated from KD mice expressed F4/80, a marker found on splenic red pulp macrophages, high levels of leukocyte adhesion and migration marker CD11b, and C-type lectin receptor marker CD209b.Compared to WT littermates, KD macrophages expressed lower levels of lipopolysaccharide (LPS) binding protein CD14 and the marginal metalophillic marker CD169.The altered expression levels of these markers may indicate that KD macrophages differ in their phagocytic properties. We therefore characterized phagocytosis in splenic macrophages using latex beads, pHrodo fluorescent bioparticles and opsonized red blood cells and compared to WT mouse cells. Furthermore, we measured intracellular calcium levels since Ca^{2+} signaling is required for Fc-receptor-mediated phagocytosis. We used the ratiometric dye FURA-2 to measure SOCE. We found that SOCE in macrophages was significantly smaller than in T cells. We also found no obvious differences in SOCE in macrophages isolated from KD mice compared to their WT littermates, unlike lymphocytes from the same mouse model. KD macrophages were more alkaline than WT cells. Basal TRPM7 channel activity was measured by patch-clamp electrophysiology and found to be higher in KD mouse macrophages. Reversing alkalinization by blockade ofthe sodium hydrogen exchanger (NHE1), phagocytosis in KD macrophagesas well as basal channel activity was also reduced. We hypothesize that higher basal TRPM7 current in KD macrophages is likely due to alkalinization. In summary, we have identified a novel role for TRPM7 kinase as a suppressor ofbasal phagocytosis and a regulator of cellular pH. We are currently investigating the relationship between TRPM7 channel activity and phagocytosis.

50. Magnesium Deficiency Increases Cortisol Production in Liver Cells: Implications for Inflammation and Hormonal Dysmetabolism

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Mg^{2+} deficient hepatocyte exhibit an increased production of intrahepatic cortisol due to increased expression and activity of reticular H6PD/11β-HSD1 tandem enzymes. The increased production of cortisol, in turn enhances gluconeogenesis, intrahepatic fatty acid synthesis, and cholesterol production thus increasing intrahepatic triglyceride levels. Returning cellular Mg^{2+} content to its physiological levels decreases cortisol production and renormalizes expression and activity of H6P, 11β-HSD1, and cortisol-responsive genes. Preliminary observation suggests that 11β-HSD1 expression and activity increase following translocation of NF-kB to the nucleus and activation of TNFα. These results can be relevant for the onset of metabolic syndrome and its complications.

51. Enterobactin, an Archetype Bacterial Siderophore Promotes a Pro-inflammatory Response in Intestinal Epithelial Cells Via the Formyl Peptide Receptor

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Intestinal epithelial cells (IECs) have pleiotropic functions in the regulation of luminal microbiota and the host immune system. Defects in barrier integrity increases intestinal permeability and uncontrolled passage of harmful luminal agents, which can contribute to the development of inflammatory diseases. Iron is an indispensable nutrient for the survival of not only mammals, but also for the microbiota. To thrive under iron limiting conditions, bacteria synthesize siderophores to acquire iron from their host. One such catecholate-type siderophore known to have the strongest affinity for iron is enterobactin (Ent). Considering that IECs are the first cell-type in contact with luminal microbial products, including Ent, we hypothesized that Ent is apro-inflammatory danger signal promoting FPR activation for interleukin (IL)-8 secretion from human IECs. HT29, DLD-1 and Caco-2/BBe human IECs were cultured *in vitro* in the presence of Ent and after 24h, quantitated for the pro-inflammatory chemokine, IL-8 by ELISA. Ent induced the secretion of IL-8, from HT29 and DLD-1, but not Caco-2/BBe cells. The ability of Ent to potentiate IL-8 from IECs was dependent on its iron-chelating property, where IL-8 secretion could be abrogated by pre-saturating Ent with an equimolar concentration of iron. Additionally, we observed that Ent also impeded the generation of reactive oxygen species (ROS) from IECs without affecting cell viability. By the calcein-AM method, we confirmed that Ent chelated the intracellular labile iron pool (LIP) in IECs, which could explain the reduction of iron-dependent ROS generation. Interestingly, we observed that secretion of Lipocalin2 (Lcn2; Ent-binding protein) in IECs could prevent Ent from hindering their LIP. The higher Lcn2 expression in Caco-2/BBe cells may explain their lack of IL-8 response to Ent, when compared to HT29 and DLD-1cells.Of note, inhibiting formylpeptidereceptor (FPR) using its antagonists abrogated Ent-induced upregulation of IL-8, implicating that such IEC response could be, in part, dependent on FPR activation. Moreover, in dextran sulfate sodium-induced colitis mice, we observed that Ent provided survival advantage to E. coli in the inflamed gut, which could be due to its higher iron chelation activity. Taken together, our results suggest that excess Ent may serve as a danger signal to instigate FPR activation for IL-8 secretion. The interaction between Ent and FPR could be further studied to gain mechanistic insight on host innate immune responses toward bacterial siderophores, especially in the context of infection and/or inflammation. Funding: This work was supported by a grant from the National Institutes of Health R01 (CA219144) to Matam Vijay-Kumar.

52. **Predicting Transmembrane (TM) Domain Dimer Structures using Martini 3**

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Determination of the structure and dynamics of transmembrane (TM) domains of single-transmembrane receptors is key to understanding their mechanism of signal transduction across the plasma membrane. Though many studies have been performed on isolated soluble receptor domains in aqueous solutions, limited knowledge exists on the lipid embedded TM region. In this study, we predict the assembly of functional and inhibitory receptor TM domain dimers using the refined Martini 3 force field for coarse-grain (CG) molecular dynamic simulations. This new version of Martini 3 has new bead types and an improved interaction balance, which allows more accurate predictions of molecular packing and interactions compared to the previous Martini 2 force field. Our results from the CG simulations with Martini 3 force field show good agreement with *ab initio* predictions using PREDDIMER and with available NMR derived structures. We also utilized umbrella sampling with the coarse grain system using Martini3.0 to calculate the binding free energy between TM dimer associations, finding that these compare well with the available experimental results. Studying the mechanism of TM domain association may help us to better understand the signaling mechanism of these receptors, in turn providing the opportunity for development of new and advanced pharmaceuticals, some of which are peptide based.

53. The Feasibility and Safety of using an EMG Controlled Arm Orthosis in the Acute Rehabilitation of People with Severe Motor Deficit after Stroke: A Clinical Case Report

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Background: Almost 30 % of stroke survivors with initial severe arm impairment fail to achieve sufficient recovery of arm function to enable them to independently perform activities of daily living. Functional deficits can be improved by combining rehabilitation with promising new technologies. EMG controlled exoskeletons sense and amplify the weakened EMG signals generated by the muscles of the affected limb and use these signals to activate a motorized orthosis that support the arm to complete a task that cannot be completed otherwise.
Purpose: This study aims to investigate the feasibility and safety of using an EMG controlled arm orthosis as an adjuvant motor recovery tool in acute rehabilitation of patients with severe deficit after stroke. The second aim is to identify the functional brain changes that drive improvement in motor abilities in response to practice with EMG controlled orthosis during acute rehabilitation. Participant: A 47-year-old female Veteran with severe arm deficit (< 2 months post stroke) participated in treatment utilizing the MyoPro™ myoelectric orthotic device. Method: Therapy was provided at a frequency of 3sessions per week (60–90 minutes per session) for 6 weeks. Therapy sessions were divided into in-device motor learning-based training and out-of-device motor learning-based training. Patient was instructed to perform out-of-device motor learning-based training on non-therapy days. Clinical outcome measures included Fugl-Meyer Upper Limb Assessment (FM), modified Ashworth Scale (MAS), and Arm Recovery Action Test (ARAT). Neurophysiological outcome measures included Transcranial magnetic stimulation (TMS), electroencephalography (EEG), and near infrared spectroscopy (NIRS).
Results: Our experience with the first participant suggests that it is feasible and safe to use an EMG controlled arm orthosis as an adjuvant motor recovery tool in acute rehabilitation of patients with severe deficit after stroke. The results also showed a remarkable reduction in arm impairment where the participant demonstrated clinically important improvement of 34points on a measure of motor control impairment (FM). Muscle tone measured with MAS improved by 2.5points compared to baseline. The participant also demonstrated a remarkable improvement of 54 points in the arm function measure ARAT compared to baseline. The motor threshold measured by TMS was also improved and changes in task-specific brain activation patterns measured by NIRS and EEG were observed.
Discussion: The safety and feasibility of using the EMG controlled orthoses in acute rehabilitation is promising. These devices provide individuals with stroke who have limited arm function a unique movement training paradigm that allows them to volitionally practice isolated arm movements that they otherwise cannot practice on their own. The improvement in arm function and the reduction in motor deficit are encouraging.

54. Novel Role Of Exosomal Bk Channel in Cardioprotection

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Extracellular vesicles (EVs) specifically exosomes are important in mediating intracellular communications, are capable of transferring genetic information between cells, and are markers for various diseases. Exosomes are used as a drug delivery vehicle to carry cargo for targeted therapy and have emerged as one of the most promising candidate for treating cardiovascular diseases. Exosomes get packaged inside the cell, excise out to the extracellular environment, and deliver the cargo to the target cells. However, the precise mechanism of how exosomes handle the differential ionic environment and the physiological role of their ion channels is not determined. Given that potassium (K^+) ions has the largest gradient, we focused on identifying the presence and physiological relevance of K^+ channels in exosomes. Using the in silico approach, several ion channel candidates were identified, the most prominent ion channel being large conductance Ca^{2+} and voltage-activated potassium channel (BK). To record BK in exosomes, we incorporated a novel electrophysiology approach called near field electrophysiology (NFE), as the canonical patch-clamp methods are not feasible due to the size of EVs. Our NFE indicates a presence of K^+ channels in intact exosomes and 45% of them are sensitive to IbTX. Since IbTX specifically blocks, BK channels, we estimated 2 functional channels (single-channel conductance of 300 pS with 50% open probability) in a single exosome. Plasma-derived exosomes from $BK^{+/+}$ and $BK^{-/-}$ mice subjected to differential K^+ gradient indicated that functional BK channels exist in exosomes, and help in maintaining their structural integrity. Furthermore, plasma derived exosomes from $BK^{+/+}$ mice showed cardioprotection from ischemia-reperfusion injury whereas exosomes from BK-/-did not. Thus, the presence of BK determines the packaging as well as cardioprotective function of exosomes .Overall, the study elucidates the novel role of exosomal BK channel in cardioprotection.

55. Voltage-Dependent Protonation of the Calcium Pocket Enable Activation of TMEM16A byVoltage

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Background: TMEM16A is a Ca^{2+} activated Cl^- channel, participates in physiological processes like epithelial fluid transport, smooth muscle contraction, block of polyspermy, insulin release, among others. The activation mechanism of TMEM16A is a complex cross taking between increments of intracellular Ca^{2+} concentration and membrane depolarizations. Though TMEM16A can also be activated by voltage in absence of intracellular Ca^{2+}, the voltage-activation mechanism is yet not clear since TMEM16A does not have a canonical voltage sensor. Therefore, in this work, we focused on the study of voltage-gating of TMEM16A in absence of intracellular calcium**.**
Methods: We used the patch-clamp technique in the whole cell and inside out configurations and site-directed mutagenesis to record TMEM16A activity and to mutate residues of the Ca^{2+}-binding pocket, respectively.
Results: We obtained that TMEM16A activation by voltage in absence of intracellular Ca^{2+} shows an anion currents smaller than those obtained by calcium, also are strong outwardly rectifying, and constants through the stimuli, with fast or absent tail currents. The current obtained by voltage-activation was inhibited by tannic and 9-Anthracenecarboxylic acids. Since the intracellular protons compete with Ca^{2+} for binding sites in the pocket, we hypothesized that voltage-dependent titration of these sites would induce voltage-gating. Indeed, intracellular

acidification enabled activation of TMEM16A by voltage-dependent protonation, which enhanced the open probability of the channel. Mutating Glu/Asp residues in the Ca^{2+}-binding pocket to glutamine (to resemble a permanent protonated Glu) yielded channels that were easier to activate at physiological pHi. Notably, the response of these mutants to intracellular acidification was diminished and became voltage independent.
Conclusion: We concluded that voltage-dependent protonation of glutamate/aspartate residues (Glu/Asp) located in the Ca^{2+}-binding pocket underlines TMEM16A activation in the absence of intracellular Ca^{2+}. This activation of TMEM16A to induce fast and sustained chloride currents that are large enough to regulate electrical excitability of the cell.

56. Expression and Purification of the ADAM10 Prodomain for Structure Determination

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The human genome encodes 21 different a disintegrin and metalloproteinase (ADAM) proteins that function in ectodomain shedding and cellular adhesion (Edwards et al., 2008). Despite the abundance of ADAM family proteins, ADAM10 stands apart from other members for its essential roles in neuron development and disease pathogenesis. ADAM10 is a multidomain type one transmembrane protein the utilizes an extracellular protease domain to release Notch receptors (Kovall & Blacklow, 2010), amyloid precursor protein (Lammich et al., 1999), and neuroligin-3, a promoter in glioma proliferation (Venkatesh et al., 2017). Historically, the development of therapeutic molecules targeting ADAM10 activity has proven difficult, with many compounds suffering from undesirable pleotropic effects that likely arise from low selective properties. However, the ADAM10 prodomain specifically inhibits ADAM10 activity (Moss et al., 2007) and represents a potential for biotherapeutic development. Unfortunately, the structure of the ADAM10 prodomain as well as the atomic details of its selective inhibition of ADAM10 is currently unknown. We aim to crystalize the ADAM10 prodomain and solve its X-ray structure. Previous efforts to crystalize the entire ADAM10 ectodomain were successful, but yielded crystals that weakly diffracted to 3.7 Å. Single particle, negative stain electron microscopy of the zymogen ectodomain revealed that the prodomain causes substantial conformational rearrangements, separating the catalytic domain from the ancillary domains, compared to the compact structure of the mature ADAM10 ectodomain (Seegar et al., 2017). Therefore, we have designed a more compact expression construct of the pro-and catalytic domains for use in a crystallography workflow. To date, we have successfully expressed and isolated the ADAM10 pro-cat domains to homogeneity and are working toward identifying suitable crystallization conditions. We expect the X-ray structure of the ADAM10 prodomain to reveal the atomic details surrounding the regulation of ADAM10 activity and provide a model for the further development of therapeutic intervention.

57. **Structural and Functional Studies of the Effects of Phosphorylation on Ephrin Receptor Tyrosine Kinase, Epha2, and the Relationship with Its Sam Domain as an Autoinhibitor**

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Eph receptors are the largest subfamily of membrane-bound RTK family. Eph receptors have significant roles during embryonic development, cell maturation, and adulthood. The role of EphA2 in axon guidance and synaptogenesis is well established. While normally a repulsive signal, non-canonical cell migration promoting activity has been observed by unliganded

EphA2signaling mechanism. Here we probed the effects of phosphorylation on intracellular region (ICR) interactions of EphA2 in solution and bound to membranes. Results from this study indicate that deletion of the sterile α motif (SAM) domain leads to an increased binding between kinase domains. Interestingly, upon oligomerization, reduced kinase activity is observed, compared to that of monomeric state of EphA2 ICR. Thus, intriguingly, while deletion of the SAM domain increases oligomerization in case of phosphorylated ICR, it appears that such persistent protein-protein interactions are not required for kinase activity in vitro. Mutation studies of the linker region between kinase domain and SAM domain give insight into its regulatory role for EphA2 activity. Also docking and all atom simulations only support a weakly bound kinase dimer in solution. The activation likely involves an allosteric mechanism by disrupting SAM domain-kinase domain and/or SAM domain-membrane interactions.

58. A Novel Protein Tagging System, BONCAT to Decipher Intercellular Interactions

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Background: Heart failure after myocardial infarction (MI) occurs in almost 20-30% of MI patients. Stem cells, mainly human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (hiCMs) are promising candidates for cardiac regeneration after MI. However, their use is impeded by poor survival and engraftment post-transplant in the ischemic myocardium. Despite these limitations, an improvement in cardiac function after hiCM transplantation has been reported, which is mainly attributed to underlying paracrine mechanisms. However, the precise identity of the hiCM proteome and secretome in vivo remains largely unknown.
Hypothesis: We hypothesize that in the ischemic microenvironment, transplanted hiCMs secrete proteins that promote cardiomyocyte survival and angiogenesis and reduce fibrosis. Experimental Design: We adapted a novel protein tagging system, bio-orthogonal non-canonical amino acid tagging (BONCAT) by expressing the mutant methionyl tRNA synthetase (L274GMmMetRS, or 'L274G') in hiPSCs. The L274G will enable tagging of newly-synthesized nascent proteins with the non-canonical amino acid, azidonorleucine (Anl) in L274G hiPSCs and their derived hiCMs.
Results: The L274G hiPSCs obtained by the transduction of WT hiPSC line, CYS0105, with the mCh-L274G-Puro lentivirus, expressed the pluripotency markers, OCT4, SOX2, SSEA4 and TRA-1-60 as shown by immunostaining, flow cytometry and qPCR. These cells could also differentiate into cells of the ectoderm, endoderm and mesoderm, including cardiomyocytes. Furthermore, the L274G showed a dose-dependent incorporation of Anl into the proteins when cultured in varying concentrations of Anl and methionine. Additionally, our preliminary results showed of selective corporation of Anl into L274G hiCMs when cultured with WT HUVECs, in vitro.
Discussion and future directions: Our data shows proof of concept for identification of cell-specific secreted proteome using BONCAT. This system will establish an innovative approach to study the paracrine mechanisms underlying transplanted stem cell-derived secretome post-transplantation and will help identify novel candidates for a cellular therapies for myocardial repair and regeneration.

59. The Role of High Phosphate Diet on Muscle Metabolism

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Background: Inorganic phosphate (Pi) is used in high amounts as a preservative in the western diet, but the effects of Pion metabolism gene expression has not been investigated. We previously observed that high serum phosphate levels are associated with low physical activity in humans, and we demonstrated that high phosphate diet decreases exercise capacity in male mice. However, the mechanisms responsible for high phosphate-impaired exercise remain unknown.

Objective/Hypothesis: Our hypothesis is that high phosphate diet alters gene expression in skeletal muscle, which affects muscle function and metabolism, and contributes to decreased exercise capacity.
Methods: We used C2C12 myoblasts and myotubes as an *in vitro* model system to study the effects of high phosphate on gene expression inmuscle cells. During C2C12 myoblast differentiation, we grew cells with normal phosphate (NP, 1mM) or high phosphate (HP, 3mM) media. Myoblasts and myotubeswere collected at day 0, 1, 3, 5, and 7 of differentiation with NP or HP media, RNA was isolated and muscle differentiation and metabolism genes were quantified using qPCR.
Results: We observed a downregulation of genes involved in fatty acid synthesis, release, and oxidation such as mPgk1 andmFabp4. In addition, glucose metabolism genes such as mLdha were also downregulated. However, we did not observe changes in differentiation of C2C12 myoblasts or expression of muscle differentiation genes.
Conclusions: The data suggests that high phosphate has a detrimental effect on metabolic gene regulation in myotubes, which could potentially lead to decreased exercise capacity. Future studies will determine whether the negative effects of inorganic phosphate are reversible or time dependent. We will also investigate the mechanisms regulating high phosphate-induced changes ingene expression.
Acknowledgements: Funding: National Institute of Diabetes and Digestive Kidney Diseases (NIDDK), The Ohio State University, Wexner Medical Center (OSUWMC)

60. Defining Post-Transcriptional Regulation of the Copper Transporter ATP7A in Skeletal Muscle Cells

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Copper is an important trace nutrient that functions as an enzyme cofactor and signaling molecule. However, Cu is also toxic, so intracellular copper must be regulated by transporter and chaperone proteins. Cu export is mediated by the trans-Golgicopper transporter, ATP7A, which provides copper to secreted cupro-enzymes and can also localize to the cell membrane to export excess copper. Loss of ATP7A causes Menkes disease, wherein dietary copper absorption is impaired. One symptom of Menkes disease is muscle weakness, which is largely unexplained. Skeletal muscle is a post-mitotic tissue comprised of multi-nucleated myofibers and can be regenerated by a closely-associated pool of stem cells. Few studies have focused on ATP7A in skeletal muscle. Our previous work showed that total cellular copper, ATP7A levels, and *Atp7a* mRNA stability increase during muscle stem cell differentiation. Here, we used an immortalized muscle cell line, C2C12, to demonstrate that ATP7A is necessary for proper muscle stem cell differentiation. The change in Atp7a mRNA stability indicates that it is post-transcriptionally regulated during muscle stem cell differentiation. RNA binding proteins mediate post-transcriptional regulation, so we used a candidate-based approach to identify key RNA binding proteins. Candidates were chosen based on previously described interaction with *Atp7a* mRNA and other copper homeostasis genes and their potential ability to bind copper. With these criteria, we identified PTBP1 as a candidate. Using RNA immunoprecipitation, we confirmed that PTBP1 binds *Atp7a* mRNA in C2C12 cells. Additionally, we used siRNA to knock down PTBP1 in C2C12 myoblasts and found that knocking down PTBP1 increases *Atp7a* mRNA and protein levels. Our work identifies ATP7A as an important protein in muscle development, and further elucidates the post-transcriptional regulation of *Atp7a*.

61. Differential modulation of Aquaporin-1in Chronic Lung Toxicity Models

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Background. Aquaporins (AQPs), commonly referred to as water channels, are known to play a role in lung homeostasis and pathophysiology. Of the four aquaporins expressed in lung (AQP1, 3, 4, and 5), AQP5 and AQP1 are primarily expressed in alveolar epithelia and vasculature endothelia respectively to allow for movement of the fluid between the air space and the associated vasculature in the alveoli (Yadav et al 2020).While the lung AQPs have been shown to be responsive to acute lung injury (ALI) particularly that associated with oxygen levels (ventilator use, high altitude)and infections, little is known on regulation of lung aquaporins intoxicant-induced lung injuries(acute or chronic). Inhalation of toxic chemicals or particulates disrupts lung homeostasis causing pulmonary edema, inflammation, and/or other lung toxicity effects. The objective was to investigate how chronic exposure to toxic chemicals (Cigarette smoke chemicals) versus toxic nanoparticles (Carbon nanotubes) or co-exposure modulates aquaporin (s) in relation to other phenotypic endpoints of lung injury in the impacted lungs.

Experimental. Mouse models of chronic toxicity based on C57BL/6 mice (5-7 weeks old) were used in this study. The toxicant treatment regime involved oropharyngeal exposure to carbon nanotube particles (33 ug suspension once) for a duration of 4 weeks or to Cigarette smoke Extract (CSE)@ a daily dose of 30µl/mouse for 4 weeks, or a co-exposure using the combination of the regimes. Vehicle control mice followed the same dosing schedule. The following homeostasis changes in the lung lumen and tissue were measured to assess pulmonary toxicity/injury: protein infiltration and expression of AQPs, surfactant protein A, and Mucin 5b.Gene expression analysis was performed using qRT-PCR based on gene-specific primers. DC protein estimation kit (BioRad) was used for estimation of total protein content in bronchoalveolar lavage (BAL) fluid. Data analyses was performed using GraphPad Prism 5.0 (La Jolla, CA, USA).

Results. Mice exposed to nanoparticles or coexposed to nanoparticles plus smoke chemicals showed increased protein content in the BAL fluid implying a compromised membrane integrity and cellular infiltration in the lung alveoli. AQP1 was significantly upregulated in nanoparticle-exposed lungs as against other exposure types which showed either down regulation (smoke-exposed) or intermediate level of expression (co-exposure). Both exposure types (nanoparticles and smoke) showed significant downregulation of Muc5b and SP-A expression and the co-exposure showed either significant downregulation (SP-A)or no significant effect (Muc5b); these effects implied a disrupted homeostasis with an increased propensity to infections in the toxicant impacted lungs.

Conclusion. Both toxicant types, nanoparticles and smoke chemicals, caused similar downregulation of lung innate defense targets (SP-A, Muc5b) and largely a summative effect of the co-exposure. In contrast, the two toxicant types showed differential induction of aquaporin-1 coinciding with the corresponding differential impact on vascular permeability/alveolar injury. This implies potential of AQP1 as a differential marker of toxicant type-specific pulmonary injury. Citation: Yadav E, Yadav N, Hus A, Yadav JS. 2020. Aquaporins in Lung Health and Disease: Emerging Roles, Regulation, and Clinical Implications. Respiratory Medicine174 (2020) 106193*Presenting author: Ekta Yadav currently with St. George's University School of Medicine is an alumnus of the Medical Physiology program at the Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA(Phone: 513-376-5065; email: eyadav@sgu.edu).

62. Base Attachment Stress Analysis for Primary Cilia in a Double Renal Model

Nerion Zekaj, Andrew Resnick, and Shawn Ryan

The study objective of the project is to analyze the in-plane stresses of a primary cilia attached to a inner wall of a renal tube (the primary tube), with a neighboring renal tube (secondary tube) in close proximity of the primary tube. We hypothesize that the stress at the base of the primary cilia will defer depending on if there is tube-tube interplay near the base of the cilia versus if there is no secondary tube near the primary tube of the cilia. We use a numerical software COMSOL® to model the fluid-structure interaction of the pulsatile flow and renal wall interaction, and we place a boundary load across the face of the primary cilia during this simulation to displace the primary cilia which in turn produces a stress at the base of the primary cilia, we obtain our data through COMSOL® data evaluation methods. We confirm our hypothesis by observing that on average the in-plane stresses are greater at the base of the cilia when there is a neighboring renal tube versus if there is no neighboring tube at all. These results however, may be limited in their interpretation due to the model set up, and further improvements to the model may potentially improve the results obtained in this current model.

63. Hepatocyte miR-34a is a key regulator in the development and progression of non-alcoholic fatty liver disease

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Objective: Hepatic miR-34a expression is elevated in diet-induced or genetically obese mice and patients with non-alcoholic steatohepatitis (NASH), yet hepatocyte miR-34a's role in the progression of non-alcoholic fatty liver disease (NAFLD) from non-alcoholic fatty liver (NAFL) to NASH remains to be elucidated.
Methods: Mice overexpressing or deficient in hepatocyte miR-34a and control mice were fed a diet enriched in fats, cholesterol, and fructose (HFCF) to induce NASH. C57BL/6 mice with NASH were treated with an miR-34a inhibitor or a scramble control oligo. The effect of miR-34a on the development, progression, and reversal of NAFLD was determined.
Results: The hepatocyte-specific expression of miR-34a aggravated HFCF diet-induced NAFLD. In contrast, germline or adult-onset deletion of hepatocyte miR-34a attenuated the development and progression of NAFLD. In addition, pharmacological inhibition of miR-34a reversed HFCF diet-induced steatohepatitis. Mechanistically, hepatocyte miR-34a regulated the development and progression of NAFLD by inducing lipid absorption, lipogenesis, inflammation, and apoptosis but inhibiting fatty acid oxidation.
Conclusions: Hepatocyte miR-34a is an important regulator in the development and progression of NAFLD. MiR-34a may be a useful target for treating NAFLD.