Contents

ORAL PRESENTATIONS

Oral presentation
9:10 – 9:25 am

Effect of glucose and insulin on the equine vascular endothelial glycocalyx

Kexin Fang[1], Kile Townsend[1], Lynn Martin[1], Francisco Ramirez-Perez[2], Christopher Foote[2], Philip Johnson[1]

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Background and significance:
Laminitis is a common disease of horses, resulting primarily from either sepsis or glucose/insulin dysregulation (equine metabolic syndrome). Microcirculatory dysregulation is an important part of laminitis pathophysiology. Moreover, endothelial glycocalyx (EG) degradation is increasingly recognized as a pivotal contributor to microcirculatory dysregulation. We previously demonstrated that EG damage is occurring in some horses with sepsis. However, whether EG damage is occurring in horses with glucose/insulin dysregulation, remains unknown.
Objective and hypothesis:
We assessed the role of glucose and insulin on the integrity of the EG in horses. We hypothesized that elevated glucose and/or insulin levels promote EG degradation.

Methods:
Intravenous glucose tolerance tests (IVGTT), oral sugar tests (OST), and insulin tolerance tests (ITT) were performed in 8 adult horses. Blood samples were obtained at zero (T0), 60 (T1) and 120 (T2) mins for laboratory determination of: glucose, insulin, hyaluronan, and neuraminidase-3 concentrations and neuraminidase activity. Data were normally distributed (Shapiro-Wilk normality test). A paired Student's t test was used to assess the effect of performing the IVGTT, OST and ITT at T0, T1, and T2. Statistical significance was set at $p \leq 0.05$. Data have been represented as mean± standard deviation.

Results:
Plasma hyaluronan concentration did not change during either the IVGTT or OST. In the ITT, plasma hyaluronan concentration was decreased at T2 (1.00 ± 0.12 U/l) compared to T1 (1.08 ± 0.12 U/l). Plasma neuraminidase activity at T1 (1.12 ± 0.26 U/l) was decreased compared to T0 (1.19 ± 0.31 U/l) during the IVGTT. Plasma neuraminidase activity at T2 (1.12 ± 0.21 U/l) was increased compared to T0 (1.04 ± 0.25 U/l) in the OST. There was no significant effect on plasma neuraminidase activity during the ITT. Plasma neuraminidase-3 concentration increased over time throughout the IVGTT (T0, 1.17 ± 0.32 U/l; T1, 1.59 ± 0.43 U/l; T2, 1.92 ± 0.44 U/l). Plasma neuraminidase-3 concentration was unchanged during both OST and ITT.

Conclusions:
These results suggest that EG degradation is occurring during hyperglycemic but not hyperinsulinemic conditions in horses.

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Oral presentation

9:25 – 9:40 am

Role of adropin in reducing arterial stiffness in type 2 diabetes

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Adropin is a peptide primarily expressed and secreted by the liver and is known to regulate energy homeostasis. Increasing evidence also indicates that adropin can exert vascular effects and its low circulating levels are associated with increased arterial stiffness, obesity, and type 2 diabetes. However, whether reduced adropin contributes to arterial stiffening in type 2 diabetes remains unknown. Herein, we tested the hypothesis that loss of adropin in non-diabetic mice causes arterial stiffening and that the converse is also true. That is, adropin exposure reduces arterial stiffness in diabetic mice. In alignment with this hypothesis, we found that 1) mesenteric arteries from adropin knockout male mice are stiffer than those from wild-type littermates; 2) exposure of human endothelial cells and mesenteric arteries from diabetic (i.e., db/db) male mice to adropin reduces actin polymerization and stiffness; 3) adropin-induced reduction of actin polymerization and softening of endothelial cells and diabetic arteries is abrogated by inhibition of nitric oxide synthase; and 4) treatment of diabetic male mice with adropin for four weeks reduces arterial stiffness in mesenteric arteries. Collectively, these findings support the notion that reduced adropin may be implicated in arterial stiffening and thus represent a novel therapeutic target to lessen arterial stiffness in type 2 diabetes.
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Oral presentation
1:45 – 2:00 pm

Regulation of endocytic trafficking and VEGFR2 receptor availability by a component of the microtubule motor dynein

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Dynein cytoplasmic 1 light intermediate chain (dync1li1) is a core member of the dynein motor complex, and defects in this gene are predicted to alter dynein motor assembly, Rab binding capacity, and cargo trafficking. The domain structure of Dync1li1 allows it to interact with cargo adaptors while simultaneity being integrated into the dynein motor complex. These adaptors are multifunctional proteins that serve as docking sites for other proteins to control endosome targeting, e.g., Rilp-Rab7a. For instance, binding of Rab7a has been shown to drive VEGFR2 trafficking towards late endosomes promoting degradation, while Rab11a regulates endosomal recycling.

We recently identified a novel zebrafish mutant in the exon 12/13 splice acceptor site of dync1li1 that C-terminally truncates the protein, leading to an increase in blood vessel growth. Mutations in the adaptor rilp1/2 are able to phenocopy this increase in angiogenesis. We hypothesize that Dync1li1 normally constrains blood vessel growth by limiting VEGFR2 activity in endothelial cells by promoting Rab7 meditated endosomal degradation, which is deficient in both novel mutants. Additionally, our preliminary data indicates that dync1li1 and rilp1/2 mutants have slower degradation of VEGFR2 and increased recycling of endosomes compared to siblings. Endothelial cell specific expression of constitutively active (CA)-rab11a in the zebrafish leads to over branching of blood vessels, phenocopying the morphology of dync1li1 and rilp1/2 mutants. These findings support the conclusion that Dync1li1 and/or Rilp1/2 deficiency leads to increased recycling of VEGFR2-containing endosomes back to the cell surface via increased Rab11a activity and a coupled decrease in Rab7a mediated vesicle degradation, thus resulting in excess angiogenesis.

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Oral presentation
2:00 – 2:15 pm

Unilateral Vagotomy Blunts Cardiorespiratory Responses to Vagal Afferent Stimulation and Nucleus Tractus Solitarii (nTS) Glutamate Microinjection

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The nTS of the brainstem is the initial integration site of sensory information from the cardiorespiratory system, including afferents from the vagus nerve carrying input from the heart and lungs. The nTS is an important site of neuroplasticity, which depends on factors including neuronal and astrocytic activity, with the latter regulating glutamate (Glu) signaling. We have shown that unilateral transection of the vagus nerve to decrease afferent input results in morphologic changes in nTS glia. Vagal nerve transection also decreases nTS synaptic transmission studied in vitro. The influence of vagotomy on in vivo cardiorespiratory function via afferent-driven Glu signaling is unknown. We hypothesized that chronic vagotomy blunts cardiorespiratory responses to vagal afferent stimulation via decreased Glu signaling. Male Sprague-Dawley rats (6-wk, N=19) were randomly assigned to vagotomy or sham groups. Right cervical vagus nerve transection caudal to the nodose ganglion or sham surgery was performed. One week after surgery, rats were anesthetized, ventilated, and instrumented to measure mean arterial pressure (MAP), heart rate (HR), splanchnic sympathetic and phrenic nerve activity (SSNA and PhrNA, respectively). Intact vagus nerves were transected acutely to prevent efferent stimulation. Left and right vagus nerves were stimulated (20 sec, 0.2mA, 2-10Hz, 0.5ms pulse width) and responses recorded for 3 minutes. Chronic vagotomy had no effect on baseline cardiorespiratory parameters compared to shams. Vagal nerve stimulation on either side increased MAP, HR, and SSNA and decreased PhrNA in all groups. There were no consistent group differences in MAP, HR, and SSNA responses. However, chronic vagotomy blunted the stimulation-induced decrease in PhrNA frequency and amplitude and decreased apnea duration on the ipsilateral side compared to contralateral stimulation and to shams. To evaluate the mechanism of altered respiratory responses, Glu was nanoinjected (30nL, 1-10mM) into the left and right nTS of chronic vagotomy and sham rats. Glu decreased MAP, HR, SSNA, and PhrNA in all groups and on both sides. Chronic vagotomy had minimal effects on MAP and SSNA responses, but blunted the decrease in HR and PhrNA frequency and amplitude during ipsilateral Glu injection compared to contralateral. The blunted responses to vagal afferent stimulation and nTS glutamate suggest that chronic vagotomy alters Glu signaling in part via postsynaptic mechanisms. Additional experiments are needed to determine the mechanisms (including the role of astrocytes) of altered nTS Glu signaling to changes in afferent input in cardiorespiratory health and disease.

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Oral presentation
2:15 – 2:35 pm

Effect of obesity on the vascular response to sympathetic activation during hypoxia in female participants

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Background: Activation of the sympathetic nervous system causes vasoconstriction and an acute reduction in peripheral blood flow. We have shown previously that premenopausal female study participants with normal weight attenuate sympathetically-mediated vasoconstriction during steady-state hypoxia, whereas male participants do not. Herein we hypothesized sympathetically-mediated vasoconstriction during hypoxia – previously shown to be absent in female participants with normal weight – would be present in females with obesity.

Methods: Blood pressure (BP, finger photoplethysmography) and forearm blood flow (FBF, venous occlusion plethysmography) were assessed in premenopausal female participants of normal weight (n=16, 23±4 yrs; 23±2 kg/m2) and those with obesity (n=5, 25±7 yrs; 37±3 kg/m2). Participants completed two trials consisting of a 2-min cold pressor test (CPT) during baseline normoxia (0.21 FiO2, 98±1% SpO2) and following 3-5 min of steady-state hypoxia (0.10±0.01 FiO2, 82±3% SpO2). FBF was normalized for BP (forearm vascular conductance, FVC). A change in FVC from steady-state during the last 1-min of CPT was calculated (ΔFVC) and the difference between ΔFVC under normoxic and hypoxic conditions (ΔFVCHypoxia – ΔFVCNormoxia) was compared between groups.

Results: In female participants of normal weight, ΔFVC during normoxia was attenuated during hypoxia (-0.56±0.91 vs +0.22±1.40 mL/dL/100mmHg/min; p=0.04). In female participants with obesity, ΔFVC during normoxia did not differ from the response during hypoxia (-0.31±1.02 vs -0.87±0.77 mL/dL/100mmHg/min; p=0.43). The difference between ΔFVC under normoxic and hypoxic conditions was group-specific (Normal weight: +0.78±1.38 vs Obesity: -0.57±1.46 mL/dL/100mmHg/min; p=0.07).

Conclusion: Sympathetically-mediated vasoconstriction is attenuated during steady-state hypoxia in premenopausal females of normal weight, whereas this response is not observed in female adults with obesity. These data advance our understanding of the impact of obesity on hypoxic vascular control.

Funding: NIH HL130339

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POSTER PRESENTATIONS

Poster

#1 - Mechanical Deadspace Physiology: Ventilation and Oxygenation Effects of Wearing Surgical Masks in Adults and Children

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Background: Surgical masks remain a focal part of the CDC guidelines to decrease COVID-19 transmission. Evidence refuting significant effects of masking on ventilation is mostly limited to small studies, with a relative paucity of studies on children.

Methods: 116 subjects were enrolled in a prospective interventional study with each subject serving as their own mask-free control (70 with ages ranging 18 - 66 years old, 46 with ages ranging 2 – 14 years old). End tidal CO2 (ETCO2), inspired CO2 (ICO2), and respiratory rate were measured by nasal cannula attached to an anesthesia machine D-fend module. Pulse oximetry and heart rate were also followed. Five minutes of mask-free data and 15 minutes of mask-worn data were collected.

Results: Mask free ICO2 levels were not statistically different between age groups. Final masked mean ICO2 levels for the 2- to 7-year-old group, 7.67(6.67 – 8.67), were significantly higher (p<.001) than the masked mean ICO2 levels for both children >7 years old, 5.33 (4.63 – 6.03) and adults 4.57 (4.18 – 4.95). Surgical masking resulted in a statistically significant (p<0.01) rise in ETCO2 levels of 1.30 mmHg in adults and 1.36 mmHg in children. The final respective ETCO2 levels, 34.35 (33.55 – 35.15) and 35.07 (34.13 – 36.01), however, remained within normal limits. In the pediatric subgroup, the respiratory rate increased early after masking, yet remained in the normal range throughout the observed period and improved during the last 5 minutes of masking. Therefore, after the full 15 minutes of masking, the differences in the respiratory rate for the youngest age group were not statistically significant compared to the unmasked period (p = .241): 18.48 (16.93 – 20.03) to 19.97 (16.87 – 23.07). Pulse oximetry and heart rate were not significantly affected.

Discussion: Even triple layer surgical masks like the models used in this study have high breathability, as measured by the low differential pressure of < 5 mm H20/cm2. Neither oxygen nor carbon dioxide will be obstructed in its flow across a surgical mask, yet some amount of expired carbon dioxide may remain behind the mask in the form of a mechanical deadspace at the end of the expired breath. An increase in mechanical deadspace has a larger effect on younger subjects, due to the higher deadspace to tidal volume ratio. In children, this results in a brief but clinically insignificant increase in respiratory rate. ETCO2 levels correlate very well with arterial CO2 partial pressure levels, and are the standard of care for continuous assessment of ventilation in patients under sedation or general anesthesic in the operating room. Despite occasional reported concerns regarding rebreathing CO2 while wearing a surgical mask, and the confirmation in this study of increased ICO2 after masking, the wearers' CO2 levels are the important end point.

Conclusions: The wearing of an ASTM Level 3 surgical mask results in a significant rise in inspired CO2 and a smaller rise in ETCO2. Because ETCO2 and other variables remain within normal limits, these changes are clinically insignificant.

Registration ClinicalTrials.org identifier: NCT05114993

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Poster

#2 - Diet-Induced weight loss improves vascular insulin sensitivity in patients with type 2 diabetes

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Background: Insulin-stimulated blood flow plays a key role in glucose and insulin delivery to target tissues, such as skeletal muscle. With the development of obesity and Type 2 Diabetes (T2D), there are impairments in vascular insulin signaling, blunting insulin-induced vasodilation. We have previously shown that vascular insulin resistance precedes insulin resistance in other metabolically active tissues in healthy humans. However, once vascular insulin resistance fully manifests in a setting of obesity and T2D, it is unknown if diet-induced weight loss attenuates vascular insulin resistance in both men and women.

Methods: Nine women (aged 61.9±2.8 yrs; BMI 36.1±1.8) and seven men (aged 54.9±6.0 yrs; BMI 37.6±2.6) with T2D were recruited (IRB #2012106) to undergo a six-month diet to induce weight loss by reducing sugar intake to <5% of energy and total daily energy intake by 500 kilocalories. Anthropometrics, brachial artery flow-mediated dilation (FMD), superficial femoral artery blood flow, and biochemical parameters were assessed at baseline (month 0) and at the end of the intervention (month 6). Dietary records were analyzed using Nutrition Data System for Research software.

Results: The low-energy diet was effective at reducing body weight (Fig. 1A), as well as other metabolic outcomes including markers of liver function and inflammation (Table 1). These improvements were accompanied with an increase in flow-mediated dilation, but not a reduction in pulse wave velocity (Fig. 1B). Lastly, vascular insulin sensitivity and skeletal muscle perfusion (Fig. 2A) in response to insulin infusion were improved following the dietary intervention, an effect primarily driven by women ($P<0.05$).

Conclusions: Our results indicate that diet-induced weight loss enhances vascular insulin sensitivity in patients with T2D and that these effects are primarily driven by women.

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Poster

#3 - APE1 mediated cleavage of methoxyamine-capped abasic sites, a proposed inhibitor of APE1 for Chemotherapeutics

Don Pivithuru Liyanarachchi, Tanhaul Islam, Nathan E. Price, Kent S. Gates

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APE1 processes Abasic sites (AP sites), which are formed as an intermediate in the base excision repair (BER) pathway. APE1 cleavage of the AP site is a crucial step in the DNA repair process. Overexpression of APE1 is associated with higher mortality rates in cancer patients; therefore, inhibition of APE1 may have a remedial importance as a potential enhancer for chemotherapeutic treatment of solid tumors. Methoxyamine is currently undergoing clinical trials as a potential APE1 inhibitor to be used as a combination drug with DNA-damaging chemotherapeutic agents. The proposed mode of action involves formation of a stable oxime with AP sites that blocks the action of APE1. Here we show that AP sites derivatized with methoxyamine do not completely block the action of APE1 at biologically-relevant enzyme concentrations. This suggests a need to explore the structure-activity relationships within this class of chemical agents, in pursuit of more effective APE1 inhibitors.

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Poster

#4 - Glutamatergic Signaling in a Rat Model of Alzheimer's Disease (AD)

Cassadi Forman[1], Alicia Koogler[1], Brooke Diehl[1], Robert Tipton[2], Chuma Humphrey[2], Tim Ostrowski[2], Daniela Ostrowski[1]

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Alzheimer's disease (AD) is the most prevalent form of dementia, affecting memory, thinking, and behavior in humans. AD patients also experience other symptoms related to cardiorespiratory function, including sleep-disordered breathing and orthostatic hypotension. While brain atrophy and neuronal loss within the hippocampus have been associated with the memory impairment in AD, cellular changes resulting in cardiorespiratory symptoms are not understood.

The streptozotocin-induced AD rat model (STZ-AD) mimics many symptoms found in AD patients. We have previously shown that the STZ-AD model comprises impaired spatial learning and memory, blunted respiratory responses to hypoxic challenges, and baroreflex dysfunction. The cardiorespiratory symptoms may be caused by alterations in brainstem nuclei. The nucleus tractus solitarii (nTS) integrates signals about changing blood pressure from baroreceptors located at the heart and about blood oxygen level from chemoreceptors at the carotid body. Altered function of nTS neurons may therefore contribute to STZ-AD symptoms.

Hyperexcitability, an increase in neuronal activity, is one of the early events in AD pathogenesis and known to contribute to memory loss in AD patients. Glutamate is the main excitatory transmitter in the central nervous system and alterations within glutamate signaling may cause this hyperexcitability. The aim of this study was to analyze potential changes in glutamate signaling at the synaptic level within the hippocampus and brainstem (nTS) using immunohistochemistry and/or Western Blot techniques.

STZ-AD was induced by intracerebroventricular injection of 2 mg/kg STZ in 6-week-old male Sprague Dawley rats. After two weeks, rats were sacrificed and brain tissue was collected. For immunohistochemistry, rats were transcardial perfused with paraformaldehyde, brains removed, and cut into 30 µm thin sections. For Western Blots, rats were deeply anesthetized, decapitated, and brain samples (hippocampus, brainstem) were flash-frozen using liquid nitrogen.

While the number of neurons (anti-NeuN) within the hippocampus and nTS did not change in STZ-AD, synaptic density (anti-synaptophysin) was significantly reduced in both brain areas. Astrogliosis (anti-GFAP) was found within the nTS, but not the hippocampus. When analyzing components of synaptic glutaminergic signaling, vesicular glutamate transporter 1 and 2 (anti-vGluT1, anti-vGLut2) and Glutamate receptor 2 (anti-GluR2) were not differently expressed in the hippocampus or nTS. Glutamine synthetase, an essential enzyme for glutamate metabolism, was not changed within the hippocampus, but significantly increased in the calamus-region of the nTS. Recently, we analyzed the expression of excitatory amino acid transporters 1 and 2 (anti-EAAT-1, anti-EAAT2) using Western Blot. A slight increase of EAAT2 was observed in the brainstem, but not in the hippocampus. Future studies using immunohistochemistry will analyze their expression specifically within the nTS.

In summary, our model of AD depicts the early changes of glutamatergic signaling likely involved with the previously observed hyperexcitability. These changes seem to primarily target astrocytes before changes at neuronal structures or neuronal death becomes apparent. Altered astrocyte function may thus play an initial role leading to the cardiorespiratory dysfunction observed in STZ-AD.

Funding: NIH R15AG065927 (TO&DO)
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Poster

#6 - Stretch and hypokalemia-induced atrial arrhythmia in isolated hearts of aged C57BL/6 mice

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Atrial Fibrillation (AF) is the most common sustained arrhythmia, and more than 2.7 million people are living with AF in the United States. The elderly are at greatest risk for AF with prevalence estimated at ~10% in individuals over the age of 80. The aged myocardium is subjected to chronic stretch, and atrial muscle stretch is an established risk factor for atrial arrhythmogenesis. Low potassium in humans has also been associated with increased risk of atrial arrhythmia and increased cardiovascular mortality by up to 10-fold. The goal of this laboratory investigation was to test the hypothesis that increases in right-atrial pressure while under hypokalemic conditions lead to atrial arrhythmogenesis in a mouse model of advanced age. To test this hypothesis, we used isolated hearts (excised hearts in a modified Langendorff perfusion technique) of aged (25-29 month) C57BL/6 male (n=5) and female (n=7) mice subjected to atrial preloads of 0 cm and 12 cmH20 (i.e., increasing stretch) and normokalemic (6 mM K+) and hypokalemic (2mM K+) conditions. Atrial arrhythmias were monitored using left-atrial placement of an intracardiac electrocardiogram. Arrhythmias were defined as transient (premature atrial contractions and salvos of <3 premature atrial contractions) or sustained (salvos of >3 premature atrial contractions, atrial tachycardia, or AF). At 0 cmH20 and normokalemic conditions, the average arrhythmia score per 5 minute time period was 0.083 (n=12). With elevation in right-atrial preload to 12 cmH2O pressure under normokalemic conditions, the average arrhythmia score was 0.125 (n=4). However, at 12 cmH2O and hypokalemic conditions, the average arrhythmia score was 1.02 (n=8). In conclusion, the aged mouse atrium exhibits sustained arrhythmia in response to increases in right-atrial preload pressure in hypokalemic conditions. The aged C57BL/6 mouse model with this technique may therefore be useful for pre-clinical studies of age- and stretch-induced atrial arrhythmias.

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Poster

#7 - Dissecting Vagal-Brainstem Mechanisms Underlying Opioid-Induced Respiratory Depression

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Respiratory depression is the primary cause of death in opioid overdose, but the mechanism is not well understood. One brain structure associated with opioid-induced respiratory depression (OIRD) is the nucleus of the solitary tract (nTS). The nTS is a key regulatory site of cardiorespiratory afferent information. The nTS receives inputs from the vagus nerve, which relays some of this cardiorespiratory sensory afferent inputs to this region. Vagal afferent fibers that terminate within the nTS express mu opioid receptors (MORs). Therefore, the goal of this study was to further characterize the role of the nTS in OIRD by evaluating mu opioid receptor expression and neuronal activation in the nTS in rats that receive an administration of fentanyl before and after undergoing a unilateral vagotomy. Immunohistochemical analysis was used to identify MOR in the nTS, activated neurons, and confirm successful vagotomy of the vagus nerve. Unilaterally vagotomized animals showed a significant reduction in motor neurons in the dorsal brainstem as well as an elimination of presynaptic MOR in the nTS. However, these changes did not alter cardiorespiratory responses to intravenous fentanyl injections. The contralateral nTS showed higher activation in vagotomized rats, suggesting a possible compensatory respiratory response mechanism to OIRD. In the future, a chemogenetic will be used to bilaterally inhibit vagal afferents rather than sever the nerve so the efferent pathway can remain intact while the role of the afferent pathway is examined in OIRD.

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Poster

#8 - Orexin facilitates the peripheral chemoreflex via activation of nucleus tractus solitarius-projecting corticotrophin-releasing hormone neurons

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Orexin neurons in the hypothalamus contribute to cardiorespiratory and autonomic homeostasis. Previously we showed that orexin facilitates the peripheral chemoreflex (PCR)-mediated hypoxic ventilatory response (HVR), especially in the active phase of the circadian cycle. This effect was associated with increased Fos-immunoreactivity (IR) in orexin neurons (indicating activation). Orexin neurons project to the paraventricular nucleus of the hypothalamus (PVN) and nucleus of the solitary tract (nTS), two nuclei integral to the HVR. Many PVN neurons that are activated by hypoxia project to the nTS and are immunoreactive (IR) for corticotropin-releasing hormone (CRH). It is unknown whether orexin facilitates the HVR via the activation of the CRH neurons in the PVN or, alternatively, via direct excitation of nTS neurons. Based on our previous data showing that orexin receptor blockade reduces the activation of CRH neurons by hypoxia, here we hypothesized that orexin facilitates the hypoxia-induced activation of the nTS via the excitation of nTS-projecting CRH neurons in the PVN. To test this hypothesis, we microinjected fluorescent retrobeads bilaterally into the PVN to label projecting orexin neurons. In a separate group of rats we microinjected beads into the nTS to label nTS-projecting orexin neurons, and nTS-projecting CRH neurons. Seven days after microinjection of beads, rats were acclimated to the chamber for 2 hrs/day over 2 days. The following day rats were randomly assigned to breathe hypoxia (%O2=0.11; n=4) or normoxia (%O2=0.21; n=4) for 2 hours in the active phase. Immunohistochemistry (IHC) was performed to quantify the number of Fos-IR (i.e., activated) orexin neurons projecting to nTS or PVN. To test whether orexin facilitates the hypoxia-induced activation of nTS-projecting CRH neurons, separate groups of rats were injected with suvorexant (an orexin receptor (OxR) antagonist; 20 mg/kg i.p.), or vehicle (DMSO) prior to being exposed to 2 hrs of hypoxia (n=4) active phase. IHC was performed to quantify the number of activated (i.e., Fos-IR) nTS-projecting CRH neurons. Compared to normoxia, hypoxia increased the number of activated PVN-projecting orexin neurons by ~35 % (O2 level: $p=0.0015$). There was no significant effect of hypoxia on the number of activated nTS-projecting orexin neurons (O2 level: $p=0.1161$). OxR blockade reduced the number of activated nTS-projecting CRH neurons (by ~32%; drug: $p<0.0001$). Thus, orexin may facilitate the peripheral chemoreflex response to hypoxia via a pathway that involves CRH neurons in the PVN.

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Poster

#9 - A Novel BioInk To Fabricate Human Organoids

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In tissue engineering, 3D scaffolds are focused on utilizing growth factors and a combination of primary cells to create clinically relevant 3D tissues. However, some growth factors have been shown to have negative effects in patients, especially in pediatric populations. An alternative approach is to utilize dissolvable bioactive glasses doped with therapeutical relevant ions. Borate bioactive glass has recently: (i) helped speed the healing of dermal wounds in > 90% of elderly patients in the clinic, (ii) provided no signs of inflammation or infection surrounding the scaffolds, (iii) resulted in little to no scarring, (iv) demonstrated hair regrowth, (v) had complete healing of the dermatological wounds, and (vi) been administered safely in multiple applications. The goal of this study was to create a bio-ink with borate bioactive glass and adipose stem cells (ASCs). Scaffolds measuring 10x10x1 mm3 were 3D bioprinted using our novel bio-ink. Results show that ASCs can be printed in conjunction with borate bioactive glass and will survive for ≥ 2 weeks.

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Poster

#10 - Hypothalamic Neuroendocrine neurons are involved in elevated sympathetic outflow in hypertension

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The corticotrophin-releasing hormone (CRH) is a neuropeptide synthesized in hypothalamic paraventricular (PVN) CRH-producing neurons that critically regulates neuroendocrine and automatic function. CRH is involved in regulating stress response through the hypothalamic-pituitary-adrenal axis. It has been shown that the CRH mRNA and the total number of CRH-producing neurons are significantly increased in the PVN in primary hypertension patients, suggesting that CRH-producing neurons are critically involved in the pathogenesis of primary hypertension. In our study, we determined the role of CRHPVN neurons in regulating blood pressure and sympathetic outflow using optogenetic approaches. We found that firing activity of PVN-CRH neurons was significantly increased in spontaneously hypertensive rats (SHRs) than those in normotensive control Wistar-Kyoto (WKY) rats. Inhibitory light-sensitive channel soma-targeted Guillardia theta anion-conducting channelrhodopsins (stGtACR2s) or excitatory light-sensitive channel ChR2s were selectively expressed in CRHPVN neurons guided by CRH-Cre. Optical inhibition of CRHPVN neurons significantly decreased the firing activity of these neurons, blood pressure measured by telemetry approach in conscious SHRs, renal sympathetic nerve discharge (RSND) and hemodynamics in anesthetized SHRs. In WKY rats, inhibition of CRHPVN neurons induced smaller decreases in blood pressure and RSND than those in SHRs. On the other hand, optical stimulation of CRHPVN neurons significantly increased the firing activity, blood pressure in conscious rats, RSND and blood pressure in anesthetized WKY rats. In brain slice from SHRs, we found that optical inhibition of CRHPVN neurons suppressed the firing activity of RVLM-projecting PVN neurons, an effect was abolished by bath application of CRH receptor antagonist astressin. Optical stimulation of CRHPVN neurons in brain slices from WKY rats increased the firing activity of RVLM-projecting PVN neurons, which was blocked by bath application of astressin. These results suggest that the CRHPVN neurons play a pivotal role in regulating sympathetic outflow and blood pressure in hypertension through CRH receptors on the RVLM-projecting PVN neurons.

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Poster

#11 - Lysine Acetylation Regulates Mitochondrial Pyruvate Carrier Activity and Cardiac Pyruvate Oxidation

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The normal adult heart is metabolically flexible and can switch preferred fuel sources based on delivery. While this is beneficial in physiological scenarios, this flexibility is often lost in cardiac pathologies. During fasting, blood glucose levels drop and increased free fatty acid delivery to the heart causes the heart to shut off glucose/pyruvate oxidation and increase fat oxidation. This balance, known as the Randle cycle, has mainly been attributed to regulation of pyruvate dehydrogenase (PDH) activity via PDH phosphorylation. However, we hypothesized that decreased pyruvate entry into the mitochondria via the mitochondrial pyruvate carrier (MPC) could also play a role. Wildtype mice were allowed to consume normal chow ad libitum or were fasted for 24 hours. As expected, 24 h fasting reduced blood glucose and insulin concentrations, and increased circulating free fatty acids. After euthanasia, hearts were excised, and mitochondrial oxidation of pyruvate/malate was assessed in permeabilized cardiac muscle fibers. As expected, pyruvate oxidation rates were decreased in fasted hearts compared to fed, while oxidation of fatty acids (palmitoyl-CoA plus carnitine) was increased in fasted hearts. Cardiac lysates were prepared for western blotting analyses which showed the expected increase in phosphorylation of PDH-E1alpha known to decrease PDH activity. Immunoprecipitating cardiac lysates with anti-acetyl lysine beads and then blotting for MPC2 suggested increased acetylation of MPC2 in fasted hearts. Using a model structure of the MPC, we have recently proposed and validated lysine 49 (K49) of MPC2 as a critical pyruvate binding site within the MPC. To assess the possible importance of K49 acetylation we mutated K49 to glutamine to mimic acetylation and observed that this MPC mutant was no longer able to bind pyruvate or competitive inhibitors in a bioluminescent resonance transfer (BRET) assay. We next created MPC2 knockout H9C2 cardiomyocyte cells by CRISPR-Cas9, and observed complete loss of MPC2 and MPC1 expression in these cells which resulted in decreased pyruvate respiration compared to wildtype H9C2 cells. Overexpression of wildtype MPC1 and MPC2 constructs increased pyruvate respiration in these MPC knockout cells, while overexpression of the MPC2-K49Q mutant was unable to improve pyruvate respiration. Altogether, these results suggest that physiologic conditions such as fasting result in decreased cardiac oxidation of pyruvate in part by acetylation of the MPC and decreased mitochondrial pyruvate transport. Future work will investigate if MPC acetylation plays a role in limiting cardiac pyruvate oxidation in pathological scenarios such as ischemia, heart failure, or diabetes.

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Poster

#12 - Mutations in Ighmbp2: Investigating what differentiates SMARD1 from CMT2S

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Mutations in the Immunoglobulin-μ-Binding Protein 2 (IGHMBP2) gene give rise to two different diseases, the more severe spinal muscular atrophy with respiratory distress type 1 (SMARD1) and the milder disease Charcot Marie Tooth Type 2S (CMT2S). SMARD1 is an infantile motor neuron disease characterized by progressive distal muscle atrophy, respiratory impairment, autonomic nervous system, and sensory defects. Without artificial ventilation patients die within the first 13 months of life. CMT2S is an axonal neuropathy that leads to slowly progressive muscle weakness, wasting, and sensory loss. IGHMBP2 is a SF1 helicase with several proposed roles including translation.
We have developed Ighmbp2 mouse models based on six patient mutations that represent a broad clinical spectrum and result in SMARD1 or CMT2S. Our goal is to use these mice to understand the biological processes that IGHMBP2 functions within and to discern why one mutation results in SMARD1 while another CMT2S. We recently published the characterization of the first SMARD1 mouse model with respiratory distress. This mouse model, called Ighmbp2-D564N, is based on the patient mutation D565N. D564N mutant mice have significantly reduced lifespan, motor function defects, motor neuron degeneration, NMJ denervation and respiratory distress.
We are currently examining disease progression and pathology in two mutant mouse models Ighmbp2D564N/H922Y and Ighmbp2H922Y/H922Y that significantly differ from each other and D564N mutant mice. We hope to use these models to understand what pathology is similar or different in each of these mutants and which molecular pathways are altered that result in these different disease outcomes.

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Poster

#13 - Impact of tongue exercise on hypoglossal neuron survival and glial density after induced neuronal cell death

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Motor neuron diseases (e.g., amyotrophic lateral sclerosis) result in life-threatening alterations in upper airway function primarily due to degeneration of hypoglossal (XII) motor neurons, which leads to ventilator and/or feeding-tube dependence. Despite its critical importance, upper airway function has seldom been studied in motor neuron diseases; thus, effective treatments remain to be discovered. Since genetic rodent models of motor neuron loss develop global symptoms (e.g., limb dysfunction, etc.), we have developed an inducible model of only XII motor neuron death (adult male rats intralingually injected with cholera toxin B conjugated to saporin; CTB-SAP) in order to study targeted therapeutic interventions to enhance the functional capacity of spared XII motor neurons to improve functional outcomes. We hypothesize that deficits in upper airway function are reversed by tongue exercise that slows XII motor neuron death and decreases glial density in CTB-SAP rats. To test our hypothesis, we studied XII motor neuron survival and glial density (microglia and astrocytes) using immunohistochemistry in control and CTB-SAP rats +/- tongue exercise; n=5-8/group. Confocal microscopy and ImageJ were utilized to capture and analyze images. However, our preliminary data suggest that glial density is unaffected in CTB-SAP rats in the absence and presence of tongue exercise, and that tongue exercise does not slow XII motor neuron death (n=3/group; $p>0.05$). Although tongue exercise appears to not affect hypoglossal motor neuron survival or glial density in CTB-SAP rats thus far, this does not rule out a beneficial impact on neuronal output and swallowing function. If successful, this work will ultimately identify a behavioral (tongue exercise) strategy for future translational studies to mitigate upper airway deficits in patients with motor neuron diseases.

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Poster

#14 - Unilateral overexpression of NAMPT in the brain produces asymmetric improvement in SOD1G93A ALS mice

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Amyotrophic lateral sclerosis (ALS) is an adult on-set neurodegenerative disease that causes the degeneration of upper and lower motor neurons. This degeneration results in widespread muscle weakness, muscle atrophy, progressive paralysis, and, eventually, death. Nicotinamide adenine dinucleotide (NAD+) is one of the most abundant metabolites in cells and is involved in hundreds of reactions, including energy metabolism, oxidative stress, and DNA repair. The majority of NAD+ in mammalian cells is produced via the salvage pathway, with nicotinamide phosphoribosyltransferase (NAMPT) serving as the rate-limiting enzyme. Previously it has been found that NAD+ levels are decreased in ALS. To determine if NAMPT in the cortex or hippocampus on one side of the brain confers any behavioral benefits, NAMPT was over-expressed by AAV transduction in neurons or astrocytes in the motor cortex, or in hippocampal astrocytes on one side of the brain of SOD1G93A mice. Unilateral NAMPT overexpression did not improve performance on behavior tests. However, during behavior testing, we observed asymmetric hindlimb impairment, with the limb contralateral to the AAV injection having reduced functional decline. To investigate this, we assessed walking gait, the morphology of spinal cord motor neurons, and the structure of semitendinosus neuromuscular junctions (NMJs). Gait analysis did not reveal any asymmetrical effects but did indicate that NAMPT over-expression improved stride length and stride width in ALS mice. NAMPT overexpression in ALS mice ameliorated the reduction of spinal cord motor neuron area and ChAT staining. NAMPT overexpression also increased the area, length and breadth of NMJs in ALS mice. Importantly, the neurons and NMJs contralateral to the AAV injection tended to be more similar to wild-type neurons and NMJs than the injection side. In summary, NAMPT overexpression improved walking gait and morphology of both spinal cord motor neurons and NMJs in the limb contralateral to the AAV injection.

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Poster

#15 - Role of astrocytic G6PD in bioenergetics and oxidative stress in astrocytes

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Focal ischemic stroke (FIS) is a serve neurodegenerative disorder. Astrocytes are the predominant glia cell type in the central nervous system. Astrocytes are activated and experience notable changes in proliferation, gene expression and metabolism after focal ischemic stroke (FIS). Increasing evidence suggest that these reactive astrocytes play important roles in neuronal survival and brain recovery after FIS. In previous study, we found that glucose-6-phosphate dehydrogenase (G6PD), the rate limiting enzyme in pentose phosphate pathway (PPP), is highly unregulated in reactive astrocytes after FIS. PPP is a parallel pathway to glycolysis for glucose catabolism. PPP is vital for the production of NADPH which is essential for the regeneration of reduced glutathione (GSH), the primary antioxidant for combating oxidative stress in disease condition. To study the effect of G6PD on metabolic reprogramming and oxidative stress after ischemic stress, we overexpress G6PD in primary astrocytes through DNA transfection. We found G6PD overexpression significantly reduced astrocytes' glycolysis while did not affect mitochondrial respiration. Overexpression of G6PD upregulated the production of NADPH, but reduced NAD+. Moreover, G6PD overexpression promoted regeneration of GSH with concurrent decrease in reactive oxygen species (ROS) after OGD. Our results suggest G6PD overexpression causes metabolic shift from glycolysis to PPP without affecting mitochondrial function, and increases anti-oxidant capacity. Moreover, G6PD overexpression can promote cell survival after oxygen-glucose deprivation (OGD) of astrocytes. In summary, our research suggests that G6PD is a potential therapeutic target for FIS.

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Poster

#16 - Oxytocin and corticotropin-releasing hormone augment synaptic activity in the nTS of rats subjected to chronic intermittent hypoxia

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Hypertension is a consequence in individuals with obstructive sleep apnea. Chronic intermittent hypoxia (CIH) in rodents mimics the hypoxia-induced hypertension seen in these individuals. Alterations in synaptic activity within the nucleus tractus solitarii (nTS), the central integration site of sensory afferents, are involved in the CIH-induced hypertension. The paraventricular nucleus of the hypothalamus (PVN) has a modulating role in controlling nTS activity to influence blood pressure. The PVN sends direct neuronal projections containing oxytocin (OT) and/or corticotropin-releasing hormone (CRH) to the nTS, and these neuropeptides alter nTS synaptic signaling. During hypoxia, there is an increase in the production of these neuropeptides, yet their contribution to nTS activity after CIH is not understood. We hypothesized OT and CRH will individually increase nTS synaptic activity, their influence will be enhanced after CIH, and co-application of OT and CRH will further magnify synaptic transmission in CIH. Male Sprague-Dawley rats (3-4 weeks) were exposed to either 10 days normoxia (21% O2) or CIH (30-40 sec of 6% oxygen, 10 episodes/h, 8 h/day). Horizontal brainstem slices were generated, and using whole-cell patch-clamp recordings, we examined synaptic neurotransmission of monosynaptic nTS neurons. nTS neurons were also acutely dissociated and calcium influx was examined via Fura-2 imaging to isolate postsynaptic effects. Spontaneous (s) and afferent (TS)-evoked excitatory postsynaptic currents (EPSCs) in slices or Ca2+ fluorescence in isolated cells were examined during sequential aCSF control, OT (600 nM) alone, CRH (300 nM) alone, and the combination of OT (600 nM) and CRH (300 nM). Vehicle (aCSF) application during the equivalent time period served as controls. In nTS normoxic slices, vehicle application (1 hr) did not change sEPSC frequency or amplitude, or TS-evoked EPSC amplitude. During neuropeptide application (n=9), OT alone did not alter s- or TS-EPSCs. CRH alone increased sEPSC and TS-EPSC amplitude, and elevated overall currents in response to 20 Hz stimulation. Co-application of OT and CRH did not further alter TS- or sEPSCs. After CIH, as in normoxia rats, vehicle did not alter synaptic currents. OT and CRH elevated TS-EPSC amplitude after CIH (n=9), although sEPSCs were unaffected. Co-application of OT and CRH significantly augmented TS-EPSC amplitude evoked in response to 0.5 and 20Hz stimulation. Comparing the OT+CRH responses in both groups demonstrated the relative increase current amplitude during 20 Hz stimulation was greater in CIH than Norm. In isolated nTS neurons, individual OT and CRH application increased Ca2+ relative to initial baseline and vehicle in normoxia and CIH. The co-application of OT and CRH enhanced the increase of cytosolic Ca2+ in CIH rats compared to their Norm controls. In summary, these data show that following CIH, OT and CRH enhanced afferent-driven neurotransmission to nTS neurons via an increase in postsynaptic responses. The data predicate an increase in their receptors and a shift in CRH receptors after CIH.
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Poster

#17 - Mesenchymal stem cells in autoimmune disease: a systematic review and meta-analysis

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The original paradigm for mesenchymal stem cells (MSCs) therapy was cellular replacement. However, that paradigm has shifted due, in part, to the ability of MSCs to modulate inflammation and direct immune responses. These features are of particular interest in patients that suffer from autoimmune disease (AD). Currently, there are discrepancies in literature if MSCs from AD-patients are as therapeutic as MSCs from normal, healthy persons. We performed a meta-analysis on studies evaluating MSCs from psoriasis, lupus, multiple sclerosis, rheumatoid arthritis, and systemic sclerosis. By quantitatively summarizing information and data from independent studies, our objective is to determine if MSCs in AD have been altered and, if so, what are the biomolecules altered.

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Poster

#18 - A Mechanistic Role for RECK in the Regulation of Hepatocellular Inflammation

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RECK (Reversion Inducing Cysteine Rich Protein with Kazal Motifs), a membrane-anchored glycoprotein, modifies the extracellular matrix involved in various cancers, including hepatocellular carcinoma (HCC). Its role in regulating inflammatory and fibrogenic processes has also been postulated. Both inflammation and fibrosis contribute to progression of nonalcoholic steatohepatitis (NASH) to HCC. Here, we tested the hypothesis that inducing or sustaining RECK expression will inhibit proinflammatory amphiregulin and epidermal growth factor receptor (EGFR) signaling, progression of NASH and the development of HCC using an in vitro cell culture model employing isolated adult mouse primary hepatocytes. Ectopic RECK overexpression (gain-of-function) was achieved by the adenoviral (Adv) transduction of murine RECK cDNA. Adv.GFP served as a control. RECK expression was silenced (loss-of-function) by transducing RECK-specific siRNA using lipofectamine. Nonspecific siRNA served as a control. Hepatocytes were exposed to TNFα in the presence or absence of inhibitors against ADAM 10 (A Disintegrin and Metalloproteinase Domain-Containing Protein 10) and/or ADAM17 – two well-established pro-inflammatory sheddases. Results show that RECK overexpression inhibited TNFα-induced ADAM10/17 activity, and amphiregulin (an EGFR ligand) and EGFR expression. In the presence of ADAM10/17 inhibitors, no further reduction was observed in amphiregulin and EGFR activity, suggesting a maximal inhibitory effect of RECK on EGFR activity. In contrast, silencing RECK significantly increased amphiregulin secretion, and this effect was reversed by inhibition of ADAM10/17, indicating RECK signals via regulation of these sheddases. Thus, RECK not only regulates sheddase activity of ADAM10 and 17, but also downstream amphiregulin and EGFR signaling under pro-inflammatory stimuli. Because increased and sustained EGFR activity contributes to progression of NASH to HCC in preclinical models, our results indicate that inducing RECK has the potential to inhibit hepatocellular inflammation in the setting of NASH and HCC.

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Poster

#19 - Oxidative Damage to Cellular Components in a Respiratory Brainstem Area of an Alzheimer's Rat Model

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Alzheimer's disease (AD) is a progressive neurodegenerative disease affecting more than 50 million individuals worldwide. Sporadic AD comprises 95% of all cases and is most commonly known for its hallmark symptoms of cognitive decline and memory degradation. In addition, respiratory dysfunction is found in up to 80% of AD patients and often manifests as sleep-disordered breathing. In the early stages of AD, increased reactive oxygen species cause widespread oxidative stress, which can damage cellular components and severely impact neuronal activity. While the respiratory dysfunction and oxidative stress is well known, there is little known about the underlying mechanisms and their potential intersection in AD patients.

This study used the Streptozotocin (STZ)-AD rat model, which is the only well-established model mimicking sporadic AD with its multifactorial presentations including AD hallmark symptoms and respiratory dysfunction. Two weeks after induction of the STZ-AD rat model (2 mg/kg STZ), we analyzed targets of oxidative stress in a critical respiratory control area in the brainstem, the nucleus Tractus Solitarii (nTS). Immunohistochemistry was used to fluorescently stain brainstem sections in order to analyze for oxidation of lipids (4-hydroxynoneal, 4-HNE), DNA (8-hydroxyguanine, 8-OHG), and protein sulfhydryl groups (dimedone-tagged sulfenic acid).

While there was no pronounced lipid peroxidation, the STZ-AD group had significantly increased levels of DNA oxidation when compared to the control (CTL). In addition, sulfhydryl groups, which are present on many cellular proteins, also had a significantly increased baseline oxidation in the STZ-AD group when compared to CTL. Oxidation of these sulfhydryl groups is known to be responsible for altering ion channel function and thus neuronal activity. Reducing agent Dithiothreitol (DTT) was able to revert sulfhydryl oxidation in STZ-AD to its reduced form, while CTL rats did not change from baseline. On the other hand, addition of the oxidizing agent hydrogen peroxide increased sulfhydryl oxidation in CTL, but not in STZ-AD.

Overall, our results show that STZ-AD causes increased oxidation throughout a key brainstem area of respiration. This oxidation also affects protein components important for neuronal activity, which could be an underlying cause for the respiratory dysfunction in AD.
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Poster

#20 - Phagocytosis of LM-MEL-45 and Jurkat Cells by M0 and M1 Macrophage Phenotypes

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Background: Macrophages can display different phenotypes based on environmental signals. M1-like macrophages are pro-inflammatory, but how this phenotypic switch affects phagocytic capacity toward cancer cells like melanoma remains uncertain. Here, we compare the M1-like macrophage phenotype to the undifferentiated M0 macrophage phenotype, with respect to its phagocytic activity on apoptotic LM-Mel-45 melanoma (LM45) cells. To evaluate the overall susceptibility of melanoma to phagocytosis, we also compared the ability of the two macrophage phenotypes to phagocytize Jurkat cells, an immortalized T lymphocyte cell line.

Methods: We used THP-1 monocyte derived macrophages for this protocol. Macrophage differentiation to the M0 phenotype was induced by exposing THP-1 monocytes to 50 ng/ml PMA for two days, followed by four days of rest in RPMI 1640 10% heat-inactivated FBS. Further differentiation to an M1-like phenotype was induced by exposure to 20 ng/ml IFN-γ for 24 hours. Unpolarized M0 macrophages continued to be rested in RPMI. M1 differentiation was confirmed by an increase in CCR7 expression. The target bait cells (LM45 or Jurkat) cells were prepared for the assay by treatment with 0.5 mM staurosporine for 24 hours to induce apoptosis. To allow cell type differentiation following the phagocytic process, bait cells were stained with CellTrace® Violet (stock 250 □M, @1:100) and macrophages were stained with CellTracker® Orange CMRA (stock 2.5 mg/ml, @1:100). The phagocytic assay consisted of plating 5 x 105 stained macrophages in 35 mm cell culture dishes and allowing them to adhere. After 24 hours, stained bait cells (5 x 105, LM45 or Jurkat) were added to the dishes and phagocytosis was allowed to proceed for 16 hours. Flow cytometry on a Bio-Rad S3e Cell Sorter was used to quantify phagocytosis. We corrected for spillover, gated out debris, and analyzed cells for orange and violet fluorescence. Gating cutoffs were set such that 99% of pure macrophage or pure bait control samples were below their respective cutoff. Positive phagocytosis was expressed as the percent of orange macrophages that had violet staining above the violet cutoff.

Results: Phagocytic rate averaged 16.1 ± 2.2% for M0 macrophages and averaged 19.1 ± 2.8% for M1-like macrophages. This difference did not reach statistical significance. Phagocytic rate averaged 13.1 ± 1.3% for LM45 bait and averaged 23.3 ± 3.4% for Jurkat bait. Thus, macrophages were significantly less effective at phagocytizing melanoma bait than Jurkat bait ($P<0.001$).

Conclusions: This work suggests that polarizing macrophages toward the M1-like phenotype does not increase the amount of phagocytosis of LM45 cells. The M0 and M1-like macrophages both showed significant increases in the phagocytosis of Jurkat cells, suggesting that the LM45 cell line is resistant to phagocytosis. This increased phagocytic resistance may help promote the survival of melanoma and suggests that chemotherapy designed to increased phagocytic vulnerability might have potential in melanoma treatment.

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Poster

#21 - Furosemide-induced dilation of pulmonary veins as a prophylactic for exercise-induced pulmonary hemorrhage

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Horses undergoing intense exercise are often diagnosed with exercised-induced pulmonary hemorrhage (EIPH), and Furosemide (Lasix™) is the only pharmacotherapy to demonstrate efficacy in reducing the severity of EIPH. Therefore, as a preventative, veterinarians routinely administer furosemide (Lasix™), a diuretic which inhibits Na+, K+ Cl- cotransporter, NKCC2 in the kidney, prior to exercise. The use of Lasix™ is controversial since its mechanism of action in the pulmonary system is incompletely understood. Our research is aimed at elucidating pulmonary mechanisms by which furosemide reduces the severity of EIPH. We hypothesized that furosemide induces dilation of pulmonary veins through inhibition of the Na+, K+, Cl- cotransporter, NKCC1. Pulmonary veins (2-4mm outer diameter) were isolated from the caudodorsal (CD) and cranioventral (CV) right lung lobe regions of nine horses (5 Thoroughbreds and 4 other breeds). Each vessel was subjected to wire myography to assess dilation to furosemide (1e-6 to 1e-3 [logM]). As hypothesized, furosemide induced dilation of equine pulmonary veins taken from both Thoroughbreds (CD, 92 ±11%; CV, 83 ±10%) and other breeds (CD, 106 ± 12%; CV, 96 ±12% relaxation at 1e-3 [logM]). qPCR was used to determine mRNA expression of NKCC1. Veins isolated from both CV and CD portions of the lung had mRNA expression similar to positive control tissue (kidney, lung, spleen) in both Thoroughbreds and other breeds. In future, histology will be used to determine the protein location of the NKCC1 transporter in horse lung veins. In conclusion, preliminary findings indicate furosemide dilates isolated equine pulmonary veins in vitro, and that NKCC1 is expressed in pulmonary tissue. These data are the 1st evidence to demonstrate furosemide has a direct effect on equine pulmonary vasculature, and that NKCC1 mRNA exists in the lung. These data suggest NKCC1 inhibition is a plausible pulmonary vein-mediated mechanism underlying the efficacy of furosemide for prophylaxis of EIPH in horses.

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Poster

#22 - Hypoxia Augments TRPM3-mediated Calcium Influx in Vagal Sensory Neurons

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Transient receptor potential melastatin 3 (TRPM3) channels contribute to nodose afferent and brainstem nucleus tractus solitarii (nTS) activity. Short, sustained hypoxia (10% O2, 24-hr) enhances nTS activity, although the mechanisms are unknown. We hypothesized TRPM3 may contribute to increased neuronal activity in nTS-projecting nodose ganglia viscero-sensory neurons, and its influence is elevated following hypoxia. Rats were exposed to either room air (normoxia) or 24-hr of 10% O2 (hypoxia). A subset of neurons from normoxic rats were exposed to in vitro incubation for 24-hr in 21% or 1% O2. Intracellular Ca2+ of dissociated neurons was monitored via Fura-2 imaging. TRPM3 was activated via Pregnenolone sulfate or CIM0216, which augmented Ca2+. Pregnenolone responses were eliminated by the TRPM3 antagonist ononetin confirming drug specificity, as well as removal of extracellular Ca2+ further confirming Ca2+ influx via membrane-bound channels. Incubating dissociated cultures in 1% O2 (24 hrs) enhanced the Pregnenolone Ca2+ responses compared to their normoxic controls. In neurons isolated from hypoxic-exposed rats, the TRPM3 elevation of Ca2+ was greater than normoxic-exposed rats. This increase was reversed following normoxic exposure following the initial hypoxic exposure. RNAScope imaging demonstrated TRPM3 expression in sliced sections of both 24-hr Norm and 24-hr Hx rat NPG. Taken together, these results suggest a hypoxia-specific increase in TRPM3 function, driven by the influx of extracellular Ca2+.

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Poster

#24 - Drosophila Cyp1 Regulates Larval Behavior and Toxin Sensitivity

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Human CypD opens a mitochondrial pore to initiate neuronal apoptosis in several neurologic diseases. The CypD chaperone folds an unknown component of the ATP synthase complex, converting it into a large-conducting channel (the mitochondrial permeability transition pore; mPTP). Cell death signaling results from the sustained opening of the mPTP, though we don't know CypD's protein partners, and this lack of knowledge limits our ability to design therapeutics that halt neuronal loss after a traumatic brain injury, during Parkinson's Disease, etc. Drosophila Cyp1 and human CypD are highly identical (87%), and both proteins localize to mitochondria. Nevertheless, very little is known about Cyp1 and its relation to apoptotic signaling or other Drosophila phenotypes. To strengthen the homology between Cyp1 and CypD, we examined the sensitivity to cyclosporine A (CsA), a drug that binds cyclophilins in a tripartite complex with Calcineurin. CsA-containing food becomes lethal to control flies starting at 10uM, with few if any flies emerging from 20uM food vials. However, a significant number of Cyp1 mutant flies grew on even 30uM food, presumably because Cyp1 (the major cyclophilin in flies) is missing and thus Calcineurin signaling is less affected. Molecular biology experiments are beginning to test the protein folding capabilities of Cyp1, using small peptide targets whose conformation is detectable with spectrophotometry. We do not detect abnormal sized flies, larvae, or larval brains in Cyp1 mutants, but Cyp1 mutations do alter larval crawling behavior. Transposon-induced mutations and RNAi transgenes affecting Cyp1 all cause excessive pausing and head swinging, followed by frequent changes in crawling direction. This behavioral phenotype was observed in larvae with either neuron- or glial-specific Cyp1 knockdown. We are currently identifying the Cyp1-expressing glial populations that regulate larval crawling and have ruled out wrapping glia, which loosely myelinate peripheral axons. Cyp1 mutations increase the number of boutons at the larval neuromuscular junction, the synapse between motoneurons and bodywall muscles. Given this phenotype, we have begun looking at mitochondrial behavior in larval nerves, focusing on the kinetics of their anterograde and retrograde axonal transport. These early studies on Cyp1 function are starting to identify phenotypes that can be used to examine and screen for Cyp1 target proteins. They are also setting the stage for structure-function studies in which enzymatically dead Cyp1 flies can be made and tested, with the potential for examining specific interactions with ATPase components.

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Poster

#25 - Regulation of endocytic trafficking and VEGFR2 receptor availability by a component of the microtubule motor dynein

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Dynein cytoplasmic 1 light intermediate chain (dync1li1) is a core member of the dynein motor complex, and defects in this gene are predicted to alter dynein motor assembly, Rab binding capacity, and cargo trafficking. The domain structure of Dync1li1 allows it to interact with cargo adaptors while simultaneity being integrated into the dynein motor complex. These adaptors are multifunctional proteins that serve as docking sites for other proteins to control endosome targeting, e.g., Rilp-Rab7a. For instance, binding of Rab7a has been shown to drive VEGFR2 trafficking towards late endosomes promoting degradation, while Rab11a regulates endosomal recycling.

We recently identified a novel zebrafish mutant in the exon 12/13 splice acceptor site of dync1li1 that C-terminally truncates the protein, leading to an increase in blood vessel growth. Mutations in the adaptor rilp1/2 are able to phenocopy this increase in angiogenesis. We hypothesize that Dync1li1 normally constrains blood vessel growth by limiting VEGFR2 activity in endothelial cells by promoting Rab7 meditated endosomal degradation, which is deficient in both novel mutants. Additionally, our preliminary data indicates that dync1li1 and rilp1/2 mutants have slower degradation of VEGFR2 and increased recycling of endosomes compared to siblings. Endothelial cell specific expression of constitutively active (CA)-rab11a in the zebrafish leads to over branching of blood vessels, phenocopying the morphology of dync1li1 and rilp1/2 mutants. These findings support the conclusion that Dync1li1 and/or Rilp1/2 deficiency leads to increased recycling of VEGFR2-containing endosomes back to the cell surface via increased Rab11a activity and a coupled decrease in Rab7a mediated vesicle degradation, thus resulting in excess angiogenesis.

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Poster

#26 - Role of adropin in reducing arterial stiffness in type 2 diabetes

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Adropin is a peptide primarily expressed and secreted by the liver and is known to regulate energy homeostasis. Increasing evidence also indicates that adropin can exert vascular effects and its low circulating levels are associated with increased arterial stiffness, obesity, and type 2 diabetes. However, whether reduced adropin contributes to arterial stiffening in type 2 diabetes remains unknown. Herein, we tested the hypothesis that loss of adropin in non-diabetic mice causes arterial stiffening and that the converse is also true. That is, adropin exposure reduces arterial stiffness in diabetic mice. In alignment with this hypothesis, we found that 1) mesenteric arteries from adropin knockout male mice are stiffer than those from wild-type littermates; 2) exposure of human endothelial cells and mesenteric arteries from diabetic (i.e., db/db) male mice to adropin reduces actin polymerization and stiffness; 3) adropin-induced reduction of actin polymerization and softening of endothelial cells and diabetic arteries is abrogated by inhibition of nitric oxide synthase; and 4) treatment of diabetic male mice with adropin for four weeks reduces arterial stiffness in mesenteric arteries. Collectively, these findings support the notion that reduced adropin may be implicated in arterial stiffening and thus represent a novel therapeutic target to lessen arterial stiffness in type 2 diabetes.
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Poster

#28 - Effect of glucose and insulin on the equine vascular endothelial glycocalyx

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Background and significance:
Laminitis is a common disease of horses, resulting primarily from either sepsis or glucose/insulin dysregulation (equine metabolic syndrome). Microcirculatory dysregulation is an important part of laminitis pathophysiology. Moreover, endothelial glycocalyx (EG) degradation is increasingly recognized as a pivotal contributor to microcirculatory dysregulation. We previously demonstrated that EG damage is occurring in some horses with sepsis. However, whether EG damage is occurring in horses with glucose/insulin dysregulation, remains unknown.
Objective and hypothesis:
We assessed the role of glucose and insulin on the integrity of the EG in horses. We hypothesized that elevated glucose and/or insulin levels promote EG degradation.

Methods:
Intravenous glucose tolerance tests (IVGTT), oral sugar tests (OST), and insulin tolerance tests (ITT) were performed in 8 adult horses. Blood samples were obtained at zero (T0), 60 (T1) and 120 (T2) mins for laboratory determination of: glucose, insulin, hyaluronan, and neuraminidase-3 concentrations and neuraminidase activity. Data were normally distributed (Shapiro-Wilk normality test). A paired Student's t test was used to assess the effect of performing the IVGTT, OST and ITT at T0, T1, and T2. Statistical significance was set at $p \leq 0.05$. Data have been represented as mean± standard deviation.

Results:
Plasma hyaluronan concentration did not change during either the IVGTT or OST. In the ITT, plasma hyaluronan concentration was decreased at T2 (1.00 ± 0.12 U/l) compared to T1 (1.08 ± 0.12 U/l). Plasma neuraminidase activity at T1 (1.12 ± 0.26 U/l) was decreased compared to T0 (1.19 ± 0.31 U/l) during the IVGTT. Plasma neuraminidase activity at T2 (1.12 ± 0.21 U/l) was increased compared to T0 (1.04 ± 0.25 U/l) in the OST. There was no significant effect on plasma neuraminidase activity during the ITT. Plasma neuraminidase-3 concentration increased over time throughout the IVGTT (T0, 1.17 ± 0.32 U/l; T1, 1.59 ± 0.43 U/l; T2, 1.92 ± 0.44 U/l). Plasma neuraminidase-3 concentration was unchanged during both OST and ITT.

Conclusions:
These results suggest that EG degradation is occurring during hyperglycemic but not hyperinsulinemic conditions in horses.

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