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Thank you to all who are participating

APPLIED HUMAN PHYSIOLOGY FITNESS TRAIL PROJECT: BENEFITS FOR LOCAL RESIDENTS AND UNDERGRADUATE STUDENTS

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The Clinical and Applied Human Physiology Laboratory at Michigan Technological University has created a science outreach program with the Chassell Trails Club beginning in the spring of 2019. Based on the data from our local health assessment nearly 30% of all Houghton County residents are obese and 21% are below the poverty line. More than 55% of our local residents stated that lack of affordable facilities and programs for year-round physical activity is significant priority. The primary aim of this project is to provide free access to trails that allow for quality aerobic, resistance, and balance exercise training. There were three major goals established at the beginning of the project that were, and will be, collaboratively completed with local residents and members from our research laboratory. First, we worked with a representative of the Trails Club to secure funding from the Portage Health Foundation to replace a bridge on the 4-kilometer fitness trail, and to purchase and install ten pieces of equipment from Outdoor-Fitness. Second, we confirmed plans to make exercise technique videos (to be posted on the township's website) and laminated posters for each exercise station so that residents will know how to safely and effectively use all equipment. Third, laboratory members will complete an energy expenditure study on the course in the future. We will utilize our new Cosmed K5 expired air analysis system to quantify energy expenditure and exercise intensity around the course while walking only, and while adding the additional ten resistance and agility exercises. Outcomes already achieved include: 1) the bridge replacement in conjunction with the Chassell High School shop class, 2) installation of the equipment to target all major muscle groups, 3) pictures posted on the township's website and social media sites to increase awareness, and 4) purchase of a new Cosmed K5 system for our exercise intensity and energy expenditure portion of this project through the Portage Health Foundation Infrastructure Enhancement Research Excellence Fund at Michigan Tech. Collaboration between faculty, undergraduate students, and the community has brought awareness to the trail system, increased motivation to use the trails on a regular basis, and provided tips on how to safely engage in regular exercise. Specifically, this outreach program has been working to promote accessibility and to provide free educational resources to community members for safe and effective exercise.

Category: Other

KRILL OIL ADDITION TO 8-WEEKS OF HIGH-FAT DIET ENHANCES MTORSER2448 PHOSPHORYLATION IN RAT HEARTS

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Krill oil (KO) derived from shrimp-like crustaceans, has been marketed as a substantial source of dietary long chain omega-3 (LC n-3) polyunsaturated fatty acids, particularly rich in eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3). Consumption of EPA and DHA has been linked to the reduction of cardiovascular disorders including coronary heart disease and myocardial infarction, however the cellular mechanism for these cardioprotective benefits is unclear. The mechanistic target of rapamycin (mTOR) is a Ser/Thr kinase that acts as a nutrient sensor, subsequently controlling various key cellular processes including cell growth, cell survival, and protein synthesis. Diets high in saturated fat content have been associated with myocardial dysregulation through alteration of the mTOR signaling pathway, suggesting that interventions that ameliorate mTOR signaling may counter these deleterious effects in the heart. The goal of the current study is to determine the effect of krill oil supplementation to a high saturated fat diet on mTOR signaling in cardiac muscle. Male Sprague-Dawley rats (approximately 12-months old), were assigned by mass to one of three isocaloric dietary groups for 8 weeks: control diet group (CON; 17% saturated fat, 54% carbohydrate, 29% protein; n=8), a high saturated fat group (HF; 42% saturated, 43% carbohydrate, 15% protein; n=12), and a high fat group with krill oil supplementation (KO; 34% saturated fat, 8% krill oil, 43% carbohydrate, 15 % protein; n=12). All rats were given ad libitum access to food and water. Body weights and food consumption was recorded weekly. After 8 weeks of dietary intervention and an overnight fast, blood and hearts were collected from anesthetized rats during terminal surgery. Western immunoblot analysis was used to determine phosphorylation levels of key mTOR signaling proteins including mTORSer2448, p70S6KThr389, 4E-BP1Thr37/46, eIF4ESer209, eIF2αSer51. There was an increase in body mass of the HF and KO groups when compared to the control following 8 weeks of treatment. There was a significant increase ($p < 0.05$) in phosphorylation of mTORSer2448 in the KO group when compared to the HF and CON groups. Phosphorylation of downstream targets of mTOR was not different between groups. Eight weeks of KO supplementation can enhance phosphorylation of mTORSer2448, however the cardioprotective impact of this remains unknown. Further research is needed to examine how KO consumption can affect other key downstream mTOR-related targets associated with improved cardiovascular health..

Category: Cardio

FEMALE MICE ARE RESISTANT TO INWARD REMODELING OF PARENCHYMAL ARTERIOLES OBSERVED IN MALE MICE DURING ANGIOTENSIN II-INDUCED HYPERTENSION

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Hypertension increases risk of vascular cognitive impairment and dementia (VCID) by impairing the structure and function of cerebral arteries. These arterial changes result in chronic hypoperfusion of the brain parenchyma and, eventually, cognitive impairment. Epidemiological studies suggest a sex difference in development of VCID, with women being protected from its development until late in age. We have previously shown that angiotensin II (AngII)-hypertension led to inward hypotrophic remodeling and decreased wall stress in the parenchymal arterioles (PAs) of male mice. This PA remodeling in AngII-hypertension was associated with reduced cerebral perfusion, increased myogenic tone, and cognitive dysfunction. Female mice, however, were protected from memory impairments despite a similar increase in blood pressure and decrease in cerebral perfusion measured by scanning laser Doppler. We hypothesized that AngII-hypertension in female mice will cause a decrease in cerebral perfusion measured by laser speckle contrast, as well as a decrease in outer diameter, lumen diameter, and wall thickness in the PAs. Sixteen to eighteen-week-old female C56BL/6 mice were treated with AngII (1200 ng/kg/min via osmotic mini-pump) for four weeks. This dose was selected to produce a similar increase in blood pressure to the previously studied male mice. Sham mice served as control. Blood pressure was measured by tail-cuff plethysmography and pial blood flow was measured. After euthanasia, brains were collected and PAs were isolated and mounted on a pressure myograph for assessment of spontaneous myogenic tone generation and structure. PA structure was assessed by increasing intralumenal pressure from 3mmHg to 120mmHg in 20mmHg increments. Results are presented as means ± SEM and PA data are reported at an intralumenal pressure of 40mmHg (n=5-12). Systolic blood pressure was increased (Sham: 141 ± 7, AngII: 179 ± 7mmHg; $p<0.05$) and cerebral perfusion was reduced in AngII-hypertension (Sham: 328 ± 6, AngII: 288 ± 11 perfusion units; $p<0.05$). AngII infusion slightly decreased lumen (Sham: 49 ± 3, AngII: $42 \pm 4\square$m; $p=0.17$) and outer (Sham: 57 ± 4, AngII: $48 \pm 4\square$m; $p=0.11$) diameters, although not significantly. AngII-hypertension reduced wall thickness (Sham: 4 ± 0.3, AngII: $3 \pm 0.2\square$m; $p<0.05$) and wall area (Sham: 691 ± 83, AngII: $441 \pm 52\square$m2; p0.05) or generate more myogenic tone in response to hypertension (Sham: 27 ± 4, AngII: $22 \pm 2\%$; $p>0.05$). While AngII-hypertension resulted in reductions in lumen diameter, outer diameter, wall thickness, and wall stress in the PAs of male mice, PAs of female mice had only reduced wall thickness with modest reductions in lumen and outer diameters. Preliminary data suggest that females may be resistant to inward PA remodeling during AngII-hypertension, despite similar reductions in cerebral blood flow as male mice. This potential protection of the cerebrovascular structure and myogenic tone generation could explain the absence of cognitive dysfunction in response to hypertension in female mice.

Category: Cardio

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Lower-body aerobic exercise with blood flow restriction (BFR) (e.g., walking, cycling), is an effective mode to improve both aerobic capacity and muscular function. Physiological responses to upper-body aerobic exercise with BFR have not been well documented. The purpose of this study was to evaluate cardiorespiratory (heart rate, VO2, RER, ventilation), metabolic (tissue saturation, deoxyhemoglobin (HHb) concentration), and perceptual (effort, pain) responses to submaximal arm cranking with BFR at varying arterial occlusion pressures (AOP). METHODS: Ten adults performed 4 intermittent arm cranking protocols (6x2-min, 1-min recovery): 1) low load (LL) – 40% VO2peak and 0% AOP; high load (HL) – 80% VO2peak and 0% AOP; (BFR50) – 40% VO2peak with 50% AOP; (BFR70) – 40% VO2peak with 70% AOP. Variables were measured using a variety of techniques, including open-circuit spirometry, near-infrared spectroscopy, and doppler ultrasound. Changes in dependent variables were assessed using repeated measures ANOVA. Relationships between HHb and pain were explored with correlations. RESULTS: Compared to LL, heart rate, RER, and ventilation increased with BFR (all $P<0.05$) but were less than HL (all $P<0.05$). During exercise, BFR decreased tissue saturation (~6%) and increased deoxyhemoglobin concentrations (~10) compared to LL ($P<0.05$) and increased further with pressure. BFR increased effort (9 ± 2 vs 7 ± 1) and pain (2 ± 1 vs 0 ± 0) compared to LL and were not different from HL. There was a moderate relationship between HHb and pain during arm cranking ($P<0.01$). These results suggest arm cranking with BFR may increase metabolic stress necessary for improvement in physical function, without excessive cardiorespiratory strain or exertion. Continued research to strike the ideal balance between metabolic stress and pain could influence future BFR studies. The next step should be to evaluate the chronic adaptations to arm cranking BFR to elucidate its efficacy as a training modality for a variety of populations.

Category: Other

MICROBIAL METABOLITE SIGNALING IS REQUIRED FOR SYSTEMIC IRON HOMEOSTASIS

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Iron is a central micronutrient that is needed for all living organisms. We harbor a diverse group of microbial populations in our intestines that also rely on host diet for their iron. It is unclear if and how our gut commensal microbiota compete with the host intestinal iron absorption pathways. Germ free (GF) and control mice fed with iron-sufficient (350 ppm), moderately iron-containing (35 ppm) and iron-deficient (<5 ppm) diets for 2 weeks demonstrated a significant resistance to iron deficiency anemia in the GF compared to the controls. This provides the first evidence of a reciprocal competition between host and commensals for limiting dietary iron. The GF mice on all three iron diets (350, 35 and <5 ppm) exhibited significant induction of duodenal iron transporters, divalent metal transporter 1(DMT1), duodenal cytochrome ferric reductase (Dcytb1), and ferroportin (Fpn1). The expressions of DMT1, Dcytb1 and Fpn1 are maximally induced during iron deficiency (<5 ppm diet), but GF mice on iron deficient diet showed even higher level of duodenal expression of these three transporters. Systemic iron homeostasis is tightly regulated via three distinct yet integrated systems: hepcidin, a liver-derived peptide hormone, controls iron mobilization through ferroportin (FPN), the only known mammalian iron exporter; hypoxia-inducible factor (HIF)-2 regulates the intestinal absorptive response and intracellular iron storage is mediated by ferritin (FTN). We demonstrate that gut microbiota possess an active iron-dependent mechanism that inhibits host iron transport and storage. Microbial community analysis by 16sRNA sequencing of fecal and duodenal isolates from wild-type mice revealed that iron deficient diet favored growth of Lactobacillus species, L. johnsonii and L. reuteri being the most abundant. Using a high-throughput microbial metabolite screen, we demonstrate that gut microbiota produce metabolites that suppresses HIF-2 and increase FTN to decrease intestinal iron absorption. Specifically, we identified 1,3-diaminopropane (DAP) and reuterin as inhibitors of HIF-2. These bacterial metabolites interact with the pseudo-ligand binding pocket of HIF-2 to inhibit heterodimerization with aryl hydrocarbon receptor nuclear translocator (ARNT) and modulate the expression of host iron absorptive machinery. Furthermore, DAP and reuterin effectively ameliorated systemic iron overload, whereas the gut-specific antibiotic Rifaximin improved anemia in mouse models. This work provides evidence of intestine/microbiota metabolic crosstalk that is essential for host systemic iron homeostasis, and suggest the utility of a probiotic approach to treat iron-related disorders.

Category: Cell and Molecular

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INTRODUCTION

Threshold concepts are those ideas that learners often find difficult, but must understand in order to master the discipline. In order for a concept to be defined as a threshold concept, it must meet at least three out of five characteristics: transformative, irreversible, integrative, bounded, and troublesome. In the realm of threshold concepts research, there has been very little conducted in regards to medical school curriculum specifically. Medical physiology has often been a challenging core topic for medical students. The goal of this study is to better understand what students considered to be threshold concepts in physiology so medical school curricula can better address the needs of students and ensure that they have a strong foundation.

METHODS

Volunteers from a medical school were recruited and three focus groups were conducted during the preclinical years. Each focus group consisted of 7-8 students. The focus groups began with a brief introduction of what threshold concepts are followed by the presentation of a brief clinical case. The students were given a case that integrated knowledge that had been taught in organ system courses prior to the focus groups. Students were asked to come up with a differential diagnosis and suggestions for underlying pathophysiology. After this, they were allowed to ask questions to gather a history and explain the underlying physiological concepts that led them to their differential diagnoses. A fourth focus group was conducted in the early weeks of the clinical years and after USMLE Step 1 examination. Following all focus groups, the audio was transcribed and thematic analysis was conducted.

RESULTS

Based on the four focus groups conducted, several potential candidate threshold concepts emerged including secondary messenger pathways, Starling forces, preload and afterload, and cell membrane potentials.

CONCLUSION

This study identified a set of candidate threshold concepts in medical physiology from the students' perspective. Further characterization of these candidate threshold concepts can be used as a foundation for designing an effective and powerful physiology curriculum.

Category: Educational

AN ALGORITHM FOR CLASSIFYING ECG WAVEFORMS AND DETECTING ATRIAL FIBRILLATION USING PERSISTENT HOMOLOGY

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Accurate computer-aided diagnosis of electrocardiogram (ECG) recordings can reduce physician workload, especially on cardiac units and in emergency departments where there are an abundance of ECGs to be analyzed. Most arrhythmia detection algorithms currently rely on a combination of machine learning methods and Fourier analysis to extract properties of ECG features by decomposing waveforms into their constituent components. The algorithm presented here was written using the topological data analysis (TDA) package in R and uses persistent homology (an algebraic method to quantify topological features of data) to study shapes that arise in lead-II of ECGs. To further preprocess denoised ECG data with time intervals of 10 seconds from Shaoxing Hospital Zhejiang University School of Medicine, a solid baseline connecting the isoelectric segments was added so that P-waves and T-waves form loop-like structures and the data were normalized such that the largest datum of the tallest R-wave is one. This algorithm detects P-waves, Q-waves, S-waves, and T-waves based off of persistence thresholds of their dimension one homological features (H1 features), intuitively thought of as equivalence classes of noncontractible loops, along with the centers of mass of their representative cycles within the data. Between each RR-interval, the most persistent H1 feature with a persistence in [0.03,0.2] and a center of mass above the isoelectric line was identified as a T-wave, and all other H1 features with a persistence in [0.026,0.1] and a center of mass above the isoelectric line were identified as P-waves. For a given R-wave, the preceding Q-wave was determined as the H1 feature with a persistence in [0.002,0.05] with the right-most center of mass lying below the isoelectric line. Similarly, the subsequent S-wave was determined as the H1 feature with a persistence in [0.002,0.06] with the left-most center of mass lying below the isoelectric line. Within each 10 second recording, atrial fibrillation was said to be present if one of the following three criteria were satisfied: 1) the number of P-waves is less than or equal to 70% of the number of R-waves , 2) the number of RR-intervals with no detected P-waves is greater than or equal to 30% of the number of RR-intervals, 3) the number of P-waves is less than or equal to 85% of the number of R-waves and the number of RR-intervals with no detected P-waves is greater than or equal to 15% of the number of RR-intervals. Using this set of criteria and the P-wave detection methods described above, atrial fibrillation was successfully identified in 10/10 ECGs from patients with atrial fibrillation. Eleven non-atrial fibrillation ECGs from patients with either normal sinus rhythm or a first-degree atrioventricular block were tested, and ten of these were not classified as atrial fibrillation, thus giving a roughly 9% false positive rate for atrial fibrillation.

Category: Cardio

DEVELOPING PREDICTIVE MODELS OF VOLUNTARY PHYSICAL ACTIVITY IN MALE AND FEMALE ACTIVE RATS

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Regular physical activity promotes healthy living by improving respiratory, cardiovascular and brain function, while also helping to reduce the risk of many diseases. Cardiovascular disease, the leading cause of death in the United States, is lower in young women than age-matched men, possibly due to protection afforded by female reproductive hormones. In addition, men commonly have better exercise performances than women due to several biological factors such as muscle mass, bone length and total body mass. However, these assumptions may not always hold universally constant since numerous factors determine how, and to what extent individuals benefit from regular physical activity. For example, seemingly straightforward parameters such as body weight, which differ between males and females, may be a reflection as to why female rats run more in studies from our laboratory. The purpose of this investigation is to determine whether sex-differences in voluntary physical activity (24-hour access to running wheels) relate to the progression of weight gain over time. Conversely, we tested the creation of a possible function of prediction to hypothesize future running patterns. We hypothesized that female rats run longer distances than males at similar speeds when accounting for body weight gains occurring naturally over time. In addition, we predicted that females will have lower body weights than male rats and a greater negative association with running distances but a less negative association with running speeds. Body weight, the explanatory variable, was correlated to average speed (km/hr) and trip distance (daily running distance, km), the response variables, using bicycle computers following acquisition in male and female Sprague Dawley rats (initially four weeks old) for four consecutive weeks. In comparing positive linear regressions for body weight and weekly distance in both males and females, the mean distance females traveled modeled a slope of 0.184 +/- 0.0343 g/km and in males a slope of 0.0342 +/- 0.0121 g/km (P = 0.002). In contrast, the correlation between body weight and average speed were not statistically significant between sexes (P = 0.462), with mean slopes in female animals of -0.00558 +/- 0.00155 g/km/h and males of -0.00792 +/- 0.00263 g/km/h. In conclusion, sex differences have been determined when examining the relationship between relative weight gain and trip distance, supporting the hypothesis that females run farther per day than males at roughly the same speed. For every gram of weight gained, females have a larger increase in trip distance, which highlights biological factors needed for exploration in future studies. With regular physical activity a healthier lifestyle can be achieved, and risks of cardiovascular disease may be decreased.

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Category: Cardio

THE ASSOCIATION BETWEEN PRE-DIABETES AND SEPSIS MORTALITY

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Background/Objective: Sepsis is a leading cause of mortality in the intensive care unit. In recent years, both sepsis complications and diabetes continue to grow, which asks for a further investigation of the association between the two. To date, the relationship between diabetes and sepsis outcomes has been heavily investigated but results remain inconclusive. However, pre-diabetes has not been stratified as a cohort group in these studies. Thus, the objective of this paper was to address this gap in knowledge by studying the relationship between pre-diabetes and sepsis outcomes as well to elucidate any patterns that exist here. Our hypothesis maintained that septic patients with diabetes have worse outcomes than patients without diabetes or with pre-diabetes.

Methods: We conducted a retrospective study of all intensive care unit inpatients meeting Sepsis-3 criteria at a large academic center between January 2001 and May 2014. We stratified the patients into three groups: non-diabetics (HbgA1c 6.4). To assess sepsis outcomes, we measured acute kidney injury (AKI), acute cardiac injury, vasopressor use, and in-hospital mortality. We also captured the patients' mean age, BMI, HbgA1c, and APACHE course.

Results: We had 2367 in the non-diabetic patient group, 850 patients in the pre-diabetic group, and 1490 patients in the diabetic group. The mean hospital stay for these groups were 15, 15, and 13 days, respectively (P-value of 0.0011). The median post-sepsis hospital stay was 3, 3, and 3 days, respectively (P-value of >0.05). The mean peak troponin was 5.5, 5.9, and 6.1, respectively (P-value of >0.05). The mean and median of the number of vasopressors used was 0.4 (0), 0.5 (0), and 0.4 (0), respectively (P-value of <0.0001). The post-sepsis AKI rate was 26.5%, 27.5%, and 20.2%, respectively (P-value of 0.005). The in-hospital mortality rate was 17.2%, 22.7%, and 15.5%, respectively (P-value of 0.0040).

Discussion/Conclusion: We found an unexpected association between pre-diabetes and mortality despite no significant difference in APACHE scores. This could be due to the fact that the pre-diabetic patient cohort and diabetic patient cohort usually have the same co-morbidities, but only the diabetic patient group is reaping the potential protective effect of diabetes treatment and insulin. There was also an unexpected protective effect of diabetes against AKI. This could represent the fact that the diabetic patient cohort is more likely to transition from AKI to acute renal failure or it could provide insight into the fact that diabetic therapy and insulin is playing a protective role in sepsis outcomes. Moving forward, we need to further analyze our results to characterize the co-morbidities. Standard retrospective review limitations apply to this study. Our results provide valuable insight into the necessity of stratifying the diabetic and pre-diabetic patient groups when investigating the association between diabetes and sepsis outcomes.

Category: Other

DIFFERENTIATION OF CORTICAL NEURONS BASED ON THE ROLE OF AUTISM SPECTRUM DISORDER GENE ASXL3

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ASXL3 has been identified as a high confidence gene for Autism Spectrum Disorder (ASD) which affects 1 out of every 59 children in the United States. Abnormal development and incorrect organization of the cerebral cortex are believed to lead to ASD. Proper neuronal cell differentiation of the distinct 6-layer cortex is vital for healthy neurodevelopment. The expression of ASXL3 coincides with the early stages of neuronal differentiation required for the production of the unique neuronal subtypes, each with its own function and morphology. Additionally, ASXL3 is involved in regulating the chromatin mark H2A monoubiquitination (H2AUb1). H2AUb1 is a repressive histone mark, which has repressive effects on nearby genes. Regulation of H2AUb1 governs the expression of genes needed for the proper production of the neurons that comprise the cortex. We hypothesize that loss of ASXL3 results in altered H2AUb1 which can lead to improper development of neuronal subtypes in the cortex. To test this hypothesis, we performed Western blotting, immunohistochemistry, cresyl violet staining, and EdU birthdating analysis with our Asxl3 mice of different genotypes. The results showed elevated levels of H2AUb1, a delay in the differentiation of layer 5 neurons, and an increase in the layer 6 excitatory neurons. Additionally, we observed defects in axonal projections for ASXL3 null mice consistent with a reduction of layer 5 neurons. These findings indicate that loss of Asxl3 leads to changes in H2AUb1 which may contribute to defects in the production of layer 5 neurons. In the future, we will be investigating the role of H2AUb1 during the generation of layer 5 neurons. In summary, these findings provide us with a greater understanding of the possible mechanism in which ASXL3 might lead to ASD.

Category: Neuro

EXPRESSION OF THE PRO-FORM OF BRAIN-DERIVED NEUROTROPHIC FACTOR AND THE P75 RECEPTOR IN THE ROSTRAL VENTROLATERAL MEDULLA OF PHYSICALLY ACTIVE VERSUS SEDENTARY RATS

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The rostral ventrolateral medulla (RVLM) is an integrative region in the brainstem containing sympathoexcitatory neurons that regulate sympathetic nerve activity; controlling blood pressure. The regulation of sympathetic activity is crucial in maintaining long-term cardiovascular health. Structural and functional forms of RVLM neuroplasticity adapt to sedentary versus physically active conditions. For example, published studies from our laboratory show that sedentary rats have increased dendritic branching and greater sympathoexcitation in response to microinjections of glutamate in the RVLM compared to active rats, suggesting that sedentary rats have increased RVLM excitability. Brain-derived neurotrophic factor (BDNF) plays an important role in the formation and modulation of dendritic branching and synaptic connections, respectively. BDNF is initially synthesized in its pro-form, proBDNF, and undergoes proteolytic cleavage to release its mature form, mBDNF. In other brain regions, proBDNF has a high affinity for binding with the p75 neurotrophin receptor, which inhibits dendritic branching. In contrast, mBDNF has a high affinity for binding with TrkB receptors, which promotes dendritic branching. Preliminary and published studies from our laboratory indicate that compared to active animals, sedentary animals exhibit decreases in both proBDNF ($p=0.013$, $n=6$) and mBDNF ($p=0.024$, $n=6$), and increases in dendritic branching (Mischel et al., 2014). However, the relationship between proBDNF and mBDNF, along with expression of the p75 and TrkB receptors in the RVLM and their effect on dendritic branching remains unknown. The purpose of this study is to test the hypothesis that proBDNF and the p75 receptor are upregulated in the RVLM of active rats, resulting in less dendritic branching. To test this hypothesis male Sprague-Dawley rats will be divided into two groups: Physically Active (24-hour access to in-cage running wheel, $n=6$) and Sedentary (no running wheel, $n=6$), and will be housed for 10-12 weeks. Rats will be sacrificed for fresh tissue removal, and the brainstems will be cryosectioned at 80 Î¼m. Serial sections will be collected on uncoated slides. Bilateral tissue punches will be retrieved from cryostat sections and placed in centrifuge tubes containing lysis buffer and protease inhibitors. Post-punched sections will be stained with cresyl violet to determine the location of the RVLM relative to the caudal pole of the facial nucleus. Punches will be pooled for western blotting to examine sub-regional distribution pro-BDNF and p75 using validated antibodies. If, as our preliminary data indicate, proBDNF is higher in active rats, we will predict that the p75 receptor will also be increased. An increase in proBDNF signaling could contribute to less dendritic branching observed in active animals. Since dendritic branching is associated with synapse formation and maturation, less dendritic branching could decrease the number of excitatory synapses in the RVLM. In response to microinjections of glutamate, a decrease in the number of excitatory synapses would be expected to decrease sympathoexcitation produced by RVLM neurons. A decrease in the activity of RVLM neurons could also lower resting blood pressure and reduce the incidence of cardiovascular disease. These results could contribute to understanding the incidence of increased cardiovascular diseases among sedentary individuals.

Category: Neuro

SECRETED FACTORS FROM M2-LIKE MACROPHAGES ARE RESPONSIBLE FOR PROFIBROTIC CHANGES IN FIBROBLASTS AND ALVEOLAR EPITHELIAL CELLS IN IN VITRO MODELS

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a poorly understood, progressively lethal lung disease with no known cure. IPF is characterized by alveolar epithelial cell (AEC) injury, dysregulated wound repair, and increased inflammation. During fibrogenesis, recruited myeloid cells accumulate and are believed to harbor a "M2-like" phenotype more than a "M1-like" phenotype. We sought to confirm this observation in vivo and to answer the question of whether secreted factors from M1 or M2 in vitro polarized macrophages could cause profibrotic effects in AECs and fibroblasts?

Methods: We used a PrimeFlow RNA assay to determine relative expression of heparin-binding epidermal like growth factor (HB-EGF), an M2-like marker, in pulmonary myeloid cells of mice that had been treated with either saline or bleomycin for 21 days. Additionally, we isolated bone marrow derived macrophages (BMDMs), polarized them to an M1-like or M2-like phenotype, then used the supernatant to investigate its potential profibrotic effects on AECs and fibroblasts through apoptosis assays, proliferation assays, and migration scratch wound assays.

Results:Our work showed that the majority of HB-EGF+ macrophages in the fibrotic mouse lung after bleomycin were monocyte-derived alveolar macrophages (moAMs), the cell type thought to be primarily responsible for the development of fibrosis. Additionally, we discovered that while secreted factors in M2 supernatant induces fibroblast proliferation, there is no change in overall fibroblast migratory capacity between fibroblasts treated with M2 supernatant and those treated with the BMDM-polarizing media alone. Intriguingly, administration of M2 supernatant to AECs induced significant and drastic increases in overall AEC apoptosis compared to AECs treated with M1 supernatant, HB-EGF, or polarizing cytokines in media alone. Preliminary results from RNAseq analysis suggest that while mRNA expression in M1-like BMDMs tends to have high-enrichment clusters in categories of immunity and anti-viral responses, in M2-like BMDMs, the clusters with highest enrichment are more prominently related to cell signaling and proliferation.

Implications:Although previous work in the field has suggested a skew to M2 phenotypes during fibrosis, a precise mechanism of action for how M2 macrophages may promote fibrogenesis has not been described. In this work, we show that soluble factors secreted by M2-like BMDMs are sufficient to induce AEC apoptosis and fibroblast proliferation, two hallmark traits of lung fibrosis. We confirm a robust M2-like phenotype in the accumulating fibrotic lung macrophages through a previously uncharacterized marker (HB-EGF) and technique. Additionally, our PrimeFlow analysis demonstrates that the majority of HB-EGF+ cells in the lung are moAMs, perhaps implying the necessity of HB-EGF expression/signaling for the recruitment and/or profibrotic character of these macrophages. Our ongoing studies will seek to further uncover differences between M1/M2 gene expression and generate targeted hypotheses to determine the primary genes responsible for increased AEC apoptosis with M2 supernatant.

Category: Other

RE-INVENTING HOW WE TEACH VENOUS RETURN

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Guyton's venous return curve describes venous return in terms of right atrial pressure, mean circulatory filling pressure, and sympathetic activity (1). Guyton's work revolutionized the understanding of cardiovascular physiology at the time it was published. However, experience shows that venous return curves are difficult to present or understand, and have limited direct clinical application (2,3). Venous return remains of central importance to the pathophysiology underlying many clinical disorders (4,5).

We propose a simple, intuitive model to explain venous return that can be progressively presented to explain both venous filling and circulatory mobilization of blood. The latter concept encompasses venoconstriction as well as other mobilizing factors.

We begin with a heart that stopped beating, so that central venous pressure (CVP) and mean arterial pressure (MAP) are equal, and yet are not zero due to circulatory filling pressure. Restarting the heart we illustrate how CVP and MAP develop, explaining that circulatory filling pressure prevents CVP from falling below that needed to maintain a preload sufficient to support normal cardiac output.

External filling sources that control filling pressure include the urinary, gastrointestinal, respiratory, integumentary systems. Internal filling results from the balance of total body water between plasma and interstitial fluid, as controlled by transcapillary forces (Starling forces).

In addition to filling, CVP is controlled by the redistribution of blood within the circulatory system. This can include sympathetic mobilization of blood from systemic venous circulation and the gastrointestinal tract (including the liver and spleen). Metabolic control of precapillary sphincters, such as the opening of muscular capillary beds during exercise, can also contribute to CVP.

The model is qualitative, while the venous return curve is quantitative. However, we anticipate it being more easily understood, more comprehensive, and more easily applied to clinical medicine.

1 Guyton, A. C. (1955). Physiol rev, 35(1), 123-129.

2 Beard, D. A., & Feigl, E. O. (2011). Am J Physiol, 301(3), H629-H633.

3 Henderson, W. R., Griesdale, D. E., Walley, K. R., & Sheel, A. W. W. (2010). Critical care, 14(6), 243.

4 Bressack, M. A., & Raffin, T. A. (1987). Chest, 92(5), 906-912.

5 Funk, D. J., Jacobsohn, E., & Kumar, A. (2013). Critical care med, 41(1), 255-262.

Category: Educational

PHENOTYPING PATHOPHYSIOLOGY OF HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFPEF) USING MODEL-BASED ANALYSIS OF PATIENT RECORD DATA

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Diagnosis and treatment of heart failure are guided through the analysis of transthoracic echocardiography (TTE) and right heart catheterization (RHC) measures made in the clinic. To uncover underlying mechanisms affecting individual patients with heart failure with reduced ejection fraction (HFrEF, ejection fraction 50%) we used a closed loop model of the cardiovascular system coupled with patient specific TTE and RHC measures to identify key parameters governing mechanical cardiovascular function. These parameters include stiffness of the systemic arteries along with the systolic contractility and diastolic relaxation of the left and right ventricles. Thirty-one patient records (ten HFrEF, twenty-one HFpEF) were obtained from the Cardiovascular Health Improvement Project (CHIP) database at the University of Michigan. Model simulations were tuned to match RHC and TTE measures of systolic and diastolic pressures in the right ventricle, pulmonary artery, and aorta along with cardiac output, pulmonary capillary wedge pressure, and left ventricular volumes in each patient with average error between data and model of $6.0 \pm 2.3\%$. Results confirm that the main mechanistic parameter driving HFrEF is reduced contractility, while for HFpEF greater phenotypic variability is observed. Principal Component Analysis (PCA) in addition to k-Means and hierarchical clustering on the tuned model parameters reveals two distinct groups of HFpEF patients, one with a cardiovascular phenotype more similar to the HFrEF cohort than to the other HFpEF group. Analysis of model simulations representing individuals from these two HFpEF groups show significant differences between these groups in terms of left ventricular active contractility, left ventricular relaxation, right ventricular relaxation and systemic arterial stiffness. Using personalized simulations to phenotype heart failure patients provides mechanistic insight that is not available by analyzing the clinical measures alone. Using this approach we are able to sub-categorize HFpEF into two distinct HF phenotypes. The mechanistic insights afforded by application of cardiovascular systems modeling to patient data have the potential to provide guidance for patient-specific treatment strategies.

Category: Cardio

IMPACT OF SERUM CONCENTRATION AND GLUT5 SPECIFIC FRUCTOSE MIMICS ON VIABILITY OF BREAST CANCER CELLS

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Differential carbohydrate metabolism is a distinguishing characteristic for malignant breast cancer cells. In cancer cells, dysregulation of glucose transporters, GLUT1 and GLUT2, facilitates the increased metabolic requirement by increasing their glucose uptake. In addition, the increased uptake of fructose via GLUT5, a fructose specific transporter, and its key role in cancer development and progress has been identified (1). However, very little is known about the viability of cells supplemented with fructose. We have previously reported, the rapid uptake of fructose measured using fluorescently labeled fructose mimics (ManCou) in breast cancer cell lines in vitro (2, 3). In this study, we present our findings on the relative cell viability of cancer cells grown in complete media with different concentrations of fetal bovine serum (FBS) and ManCou following starvation. Breast adenocarcinoma cells MCF7(ATCC® HTB-22™) and MDA-MB-231(ATCC® HTB-26™) were starved in serum free media for 24 hours and reintroduced into their growth in media with different concentrations of FBS (0,1,5 or 10 % by volume) and ManCouH (0µM, 10µM, 20µM, 50µM, 100µM). CellTiter-Blue® Cell Viability Assay was used to estimate the viability of cells after 24,48 and 72 hours. While MCF7 cells showed similar relative viability after 48 and 72-hours, the malignant MDA-MD-231 exhibited a increasing trend in cell viability with increasing concentrations of ManCouH after 48 hours followed by drop after 72hours. It appears that the metabolic activity is associated with the metastatic potential of cells. Thus, further research is warranted in studying the roles of fructose and glucose transporters in cancer progression.

References:

1. Zamora-León SP, Golde DW, Concha II, Rivas CI, Delgado-López F, Baselga J, et al. Expression of the fructose transporter GLUT5 in human breast cancer. Proceedings of the National Academy of Sciences. 1996;93(5):1847-52.

2. Kannan S, Begoyan VV, Fedie JR, Xia S, Weseliński ŁJ, Tanasova M, et al. Metabolism-Driven High-Throughput Cancer Identification with GLUT5-Specific Molecular Probes. Biosensors. 2018;8(2):39.

3. Begoyan V, Weseliński Ł, Xia S, Fedie J, Kannan S, Ferrier A, et al. Multicolor GLUT5-permeable fluorescent probes for fructose transport analysis. Chemical Communications. 2018;54(31):3855-8.

References:

1.   Zamora-LeÃ³n SP, Golde DW, Concha II, Rivas CI, Delgado-LÃ³pez F, Baselga J, et al. Expression of the fructose transporter GLUT5 in human breast cancer. Proceedings of the National Academy of Sciences. 1996;93(5):1847-52.

2.   Kannan S, Begoyan VV, Fedie JR, Xia S, WeseliÅ„ski Å• J, Tanasova M, et al. Metabolism-Driven High-Throughput Cancer Identification with GLUT5-Specific Molecular Probes. Biosensors. 2018;8(2):39.

3.   Begoyan V, WeseliÅ„ski Å• , Xia S, Fedie J, Kannan S, Ferrier A, et al. Multicolor GLUT5-permeable fluorescent probes for fructose transport analysis. Chemical Communications. 2018;54(31):3855-8.

Category: Cell and Molecular

ACUTE AND OVERNIGHT ARTERIAL STIFFNESS RESPONSES TO BINGE ALCOHOL CONSUMPTION

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Acute binge alcohol consumption is known to increase blood pressure, whereas chronic heavy alcohol use and alcoholism is associated with hypertension. A known contributor to hypertension development is arterial stiffness, or the elastic nature of the arterial division of the cardiovascular system. We tested the hypothesis that arterial stiffness would be elevated the morning after binge drinking. Participants (n=21; age 21-38 years; mean 24.5 years) arrived to the laboratory at 4:00pm for baseline arterial stiffness measures, and then were provided a standardized meal according to their body mass. In a randomized order, separated by one month, participants received a binge alcohol dose or fluid control. Two alcohol or fluid control doses were administered between 8:00pm and 8:15pm, and 9:00pm and 9:15pm. The beverage consisted of a 1:3 mixture of 190 proof grain ethanol and fruit juice (i.e., Orange or Cranberry Juice). 30 minutes post-consumption of each beverage, arterial stiffness was recorded via applanation tonometry using a SphygmoCor device. Briefly, a tonometer was placed at the radial artery for pulse wave analysis, and, while gated to a three-lead electrocardiogram, at the carotid and femoral arteries for pulse wave velocity. Fifteen minutes following 8-hours of sleep acquisition, arterial stiffness was reassessed. Statistical analysis was performed using repeated measures ANOVA with a condition level of 2 (i.e., alcohol vs. fluid control) and 4 time points (i.e., baseline, post drink 1, post drink 2, and morning). Means were considered significantly different when $p<0.05$. Mean arterial pressure was not different between alcohol and fluid control. However, aortic augmentation index normalized to 75 heart beats (AIx@75) was significantly different from baseline following the alcohol condition (time: $p = 0.006$). Post-hoc paired t-tests revealed AIx@75 was significantly reduced after the first dose of alcohol consumption (-8±2%), but significantly increased in the morning (6±2%) compared to baseline ($p = 0.005$ for both). Alcohol consumption also exhibited a significant time effect ($p = 0.028$) on carotid-femoral pulse wave velocity (cfPWV), often considered the gold-standard assessment of aortic arterial stiffness. Post-hoc paired t-tests revealed that cfPWV was increased in the morning after fluid control ($\Delta 0.28 \pm 0.12$ m/s; $p = 0.035$) and alcohol ($\Delta 0.36 \pm 0.14$ m/s; $p = 0.021$), when compared to the pre-supper baseline. In summary, our results indicate binge alcohol consumption depressed AIx@75 after the first dose in the evening, but increased the morning after alcohol consumption. cfPWV was also increased the morning after alcohol consumption and fluid control. Increases in AIx7@75 and cfPWV may increase the risk for cardiovascular complications the morning after binge drinking.

Category: Cardio

ELECTROSPUN PCL SCAFFOLDS OF DIFFERENT MORPHOLOGIES AS 3D CELL CULTURE PLATFORMS

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Cell culture in vitro on 2D platforms, such as tissue culture plates have been traditionally used to understand the cellular mechanics, physiological processes, material interactions and organ formations. The simplicity and cost effectiveness have made it a staple method before ex vivo and in vivo experiments. However, the unreliability and lack of replicating complex in vivo conditions such as hypoxia, differential proliferation of cells and biomechanics has shifted the focus to 3D platforms. Hence, there is a need for 3D platforms which are easy to use and replicable, while at the same time mimicking the complex microenvironment present in vivo. In this study, electrospun nanofiber scaffolds composed of polycaprolactone of various morphologies (honeycomb, aligned and mesh) were used as a 3D platform to investigate differences in cell behavior and protein expression in normal breast epithelial cells, 184B5, and adenocarcinoma MCF7 and MDA-MB-231. The scaffolds have the same surface properties but different topographies and mechanical properties. Cells were seeded on the scaffolds and analyzed after 24 and 48 hours. The cells on scaffolds (3D platform) and culture plates (2D platform) were immunofluorescent labelled against a glucose transporter overexpressed in cancer cells (GLUT5) and KRT18, a type of cytokeratin commonly expressed in single layer of epithelial cells was used for analysis. Statistically significant differences in cell morphology and protein expression was observed amongst the different culture platforms. The morphologies and protein expression were compared to the in vivo behavior of cells based on prior literature. The current platform provides flexibility in designing experiments for understanding tissue biomechanics, tumor engineering and cancer progression in an easy and reproducible manner.

Category: Other

THE EFFECT OF SCAFFOLD MORPHOLOGY ON CELL-MATRIX INTERACTIONS OF RENAL, LUNG AND BLADDER CANCER CELLS

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Cell-matrix interactions are a major regulator of phenotypic features such as cell migration, adhesion, proliferation, apoptosis and controls several downstream signaling cascades. Three-dimensional (3D) microenvironments to mimic in vivo conditions are currently being used to understand cell-matrix interactions. In this study, we report 3D scaffold systems with different morphologies (topography and mechanical properties), but with similar surface chemistry. Three scaffold morphologies, the honeycomb (porous structure with highest mechanical strength), aligned (stacks of aligned fibers) and mesh (interconnected network of fibers with lowest mechanical strength) were fabricated and tested. The cell-viability, cell interactions including nuclear alignment and shape on scaffolds, and cell alignment were analyzed for cells seeded on the scaffolds. Clear cell renal carcinoma, renal cell adenocarcinoma, bladder grade IV transitional cell carcinoma, bladder transitional cell papilloma, bladder transitional cell carcinoma, normal renal epithelial cells, and lung carcinoma were used. The stiff matrices were favored by bladder grade IV transitional cell carcinoma that penetrated through the scaffolds and spread uniformly. The mesh like architecture with low mechanical strength were favored by renal and lung carcinoma and adenocarcinomas. The bladder transitional cell papilloma showed distinct preference to 3D architecture with increased alignment and spreading in the aligned architecture. The normal epithelial renal cells showed no preferences to architecture. The nuclear shape and alignment also differed between certain cell lines demonstrating the effect of down scale signaling cascades. This study establishes the scaffolds as a tool to analyze the material interactions affecting tumorigenesis, cancer progressions and migration behavior of malignant and metastatic cancer cells.

Category: Other

A NOVEL 3D MICROFLUIDIC DEVICE FOR STUDYING CANCER DYNAMICS

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The use of microfluidic devices for mimicking biochemical and biofluid dynamics in the microscale has opened up new avenues of research. The recent trends in device fabrication allow the incorporation of intricated designs, reducing resource consumption during experimentation. We fabricated microfluidic devices using Polydimethylsiloxane (PDMS) affixed to conventional culture plates to form a platform that can be used to monitor migration of cells in real-time. We have previously reported a microfluidic device for reproducing the metastatic phenomenon of extravasation of cancer cells, in vitro. Under static flow conditions, we have monitored the chemotactic migration of cancer cells in response to differential gradient in the concentration of nutrients and growth factors across microchannels. However, the microfluidic platform, by itself does not account for the complex tissue biomechanics and cell-matrix interactions. Artificial scaffolds have previously been used for understanding the role of biophysical properties in cell proliferation, migration and death. In this study, we use electrospun polycaprolactone (PCL) scaffolds embedded in the wells of a microfluidic device to stimulate the 3D environment present in vivo. The current microdevice incorporates the required 3D microarchitecture needed for modelling hypoxia, scaffold-cell interactions and can potentially be used for growth of tumors, rapid diagnosis, tumor modelling and rapid drug testing.

Category: Other

SEX-RELATED DIFFERENCES IN NEURAL CONTROL OF BLOOD PRESSURE VIA THE ROSTRAL VENTROLATERAL MEDULLA

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Cardiovascular disease (CVD) is the most prominent cause of death globally, with numbers surpassing various forms of cancer, respiratory illnesses, or organ failure. Young women are at a lower risk of developing CVD than males, potentially due to reproductive hormones. Reproductive hormones, such as progesterone, offer protection in the nervous system. Hypertension can lead to development of CVD. In addition, control of blood pressure is located in a brain region known as the rostral ventrolateral medulla (RVLM), through sympathetic nerve activity. However, the mechanisms by which female reproductive hormones lower risk of CVD are unknown. We want to investigate sex-related differences on nerve activity between female rats and male rats. We hypothesized that inhibition of the ongoing neural activity in the RVLM would produce greater decreases in mean arterial pressure (MAP) and sympathetic nerve activity in females versus males. Inactin anesthetized rats, both male and female, will be configured to record MAP and splanchnic SNA (sSNA). Microinjections of the inhibitory neurotransmitter GABA (30 nl, 0.3â€"600 mM) will be given in the RVLM region at five-minute intervals to allow for recovery of parameters. We will also measure the level of progesterone in the female rats because these levels positively correlate with decreases in SNA, such that for a similar dose of GABA we would predict greater decreases in SNA when progesterone is highest. Lavages will be performed to determine what part of the estrous phase the rats were at during GABA injection. Based on previous studies in males only, we predict GABA will suppress nerve activity and decrease blood pressure. In both males and females, we also expect that greater concentrations of GABA will result in greater decreases in sSNA. We predict that injection of GABA will decrease sSNA activity to a greater extent in female rats than the male rats. Lastly, GABA injected just after the estrus phase of the estrous cycle, when there are peak progesterone levels, will be expected to yield a greater decrease in SNA in females. From these studies, we conclude that the decrease in MAP and SNA will confirm previous studies that suggests the RVLM plays a significant role in neural regulation of arterial blood pressure. More specifically, the larger decrease in SNA to GABA after the estrus phase suggests that progesterone (or perhaps an active metabolite) plays a protective effect over sympathetic nerve activity and contributes to the lower risk of cardiovascular disease in young females compared to males. (HL096787).

Category: Neuro

HIF-2A MEDIATES VULNERABILITY TO OXIDATIVE CELL DEATH IN COLON CANCER

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Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the US. Hypoxia is a hallmark of solid tumors which promotes tumor cell growth, survival, metastasis and confers resistance to chemo and radiotherapies. Targeting hypoxic cells has been difficult. Moreover, inhibitors for the major transcription factors, hypoxia inducible factor (HIF)-1a and HIF-2a have not shown long-term efficacy in most cancers. We have previously shown that HIF-2a is essential for colon tumorigenesis. Using an unbiased screen, we show a significant increase in synthetic lethality of HIF-2a overexpressing tumor enteroids to oxidative cell death activators. The treatment with hypoxia mimetic FG4592 (Roxadustat), led to a robust increase in erastin-, RSL3-, and dimethyl fumarate-induced cell death in a dose- and time-dependent manner. Further, our in-vitro data shows that HIF-2a knock-down cells are completely resistant to these drugs. HIF-2a activation promotes upregulation of oxidative stress and cell death of colon epithelial cells in vivo. The FG4592 treatment itself is sufficient to activate intracellular ROS, which is attenuated by PT2385 compound, a HIF-2a specific inhibitor. RNA-sequencing data confirms the upregulation of genes associated with ROS generation in HIF-2a overexpressed mice, suggesting role of HIF-2a in priming the cells vulnerable to oxidative cell death. Moreover, the treatment of DMF and hypoxia mimetic FG4592 promoted apoptosis and greatly reduced tumor weight and volume in colon cancer xenograft mouse model. Taken together, our results suggest that this intrinsic sensitivity towards oxidative cell death could be utilized as a persistent and dynamic form of cell death for colon cancer treatment.

Category: Other

AMINO ACID SENSING IS AN ESSENTIAL MECHANISM OF MTORC1 ACTIVATION IN COLORECTAL TUMORIGENESIS.

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Colorectal cancer (CRC) is the third leading cause of cancer related deaths in the United States. Colorectal cancer cells mutate numerous components of cell growth signaling pathways to survive, grow, proliferate and metastasize. mTOR (mammalian target of rapamycin) pathway is a 'master' regulator of cell growth and is found to be dysregulated in more than 50% of all cancers including CRC. Thus, determining molecular components regulating mTORC1 pathway is essential to identify potential pharmacological targets that may lead to better therapeutic control of disease.

In most cancers, constitutive activation of the growth factor pathway (PI3K/AKT) is thought to be the major mechanism by which mTORC1 activity is chronically sustained at high levels. However, cellular amino acid levels play an important role in activation of mTORC1. Discoveries of amino acid sensors of GATOR1/2 (GAP activity towards Rags) complexes has shed new light into how amino acids are sensed leading to mTORC1 activation. However, the contribution of amino acid sensing pathways in colon cancer growth is unclear.

Our preliminary findings demonstrate that amino acids (but not PI3K/Akt) are the essential mechanism leading to high mTORC1 activity in human colon cancer cells. DEP domain containing protein 5 (Depdc5, GATOR1 complex) is a negative regulator while WD Repeat Domain 24 (Wdr24, GATOR2 complex) is a positive regulator of mTORC1 activity. Remarkably, under amino acid rich conditions, we observed that knockout of Depdc5 or Wdr24 was sufficient to increase or repress mTORC1 activity, respectively. Moreover, loss of intestinal-specific Depdc5 resulted in hyperactive mTORC1 activity and increased tumor burden in a mouse model of colon cancer. We also found reduced expression of Depdc5 in human colon cancer samples when compared to adjacent normal colon tissue and found mutations in GATOR1/2 complexes in colon cancers. Further, data mining from the Cancer Genome Atlas (TCGA) revealed a strong association between reduced expression of Depdc5 and reduced survival of CRC patients. These findings suggest a critical role of amino acid sensing pathways in CRC.

Category: Digestive

PRIMARY AND SECONDARY STRUCTURAL PATTERNS IN PROTEIN GLYCATION

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Protein glycation is the stochastic post-translational addition of a reducing sugar to a protein, most commonly on the N-terminal amino group or lysine epsilon amino groups of a protein. Previous attempts to elucidate patterns in protein glycation have focused on identifying amino acids overrepresented in glycated sequences and have been met with limited success with observed patterns conflicting with experimental results and/or not being able to offer a potential explanation for their role in glycation. Free lysine residues and potential glycation sites in small peptides readily glycate, but in larger proteins this ability is largely lost. Based upon this, the hypothesis tested here was that identifying amino acids over- and under-represented surrounding nonglycating lysine residues would demonstrate the patterns underlying the lost ability for these residues to glycate. Using sequences obtained from the Compendium of Protein Lysine Modifications (CPLM) glycation database, tests of proportion were run for each position in a 31 residue window (15 upstream and 15 downstream of the central lysine) against known average frequencies of amino acids in humans. A number of statistically significant ($p < 0.05$) over- and under-represented residues were identified around the nonglycating lysine residues. The most apparent pattern was the overrepresentation of glycine and proline repeats which are indicative of tight coiling. Additionally, tryptophan was overrepresented adjacent to the central lysine residue suggesting the possibility of steric interference. Commonly underrepresented amino acids included cysteine, histidine, serine, and tyrosine likely indicating their role in forming favorable secondary structures, but these residues are also common members in catalytic triads which means there could also be inadvertently catalyzation of the glycation reaction. To investigate this further, secondary structures for the proteins in the CPLM glycation database were predicted using SPOT1D according to Dictionary of Protein Secondary Structure (DSSP) classifications. It was found that α-helices were commonly associated with glycated sequences while coils and β-pleated sheets were associated with non-glycated sequences, suggesting that steric constraints imposed by secondary structure are a major factor in determining whether or not a lysine is able to glycate. However, while the primary and secondary patterns identified here explain the glycation status of many lysine residues, they don't explain them all. It seems likely that these unexplained cases are the results of nuanced interplay between primary and secondary structure wherein the particular physicochemical characteristics in a local environment prevent glycation where it would otherwise be predicted to occur and vice versa. Consequently, future research will focus on training machine learning algorithms to aid in the identification of these subtle differences.

Category: Cell and Molecular

EFFECTS OF 8-WEEK ACTIVE MINDFULNESS AND STRESS MANAGEMENT ON ANXIETY AND MENTAL HEALTH DURING THE COVID-19 PANDEMIC.

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Introduction: Over the last 6 months, the novel coronavirus (COVID-19) has crossed international borders and affected millions globally. The rate of infection has increased in the United States of America since January 2020. This forced people in Michigan, and other states, to follow stay at home orders which could influence psychological well-being and anxiety. There may be increased anxiety among people because of staying inside their homes for long duration, losing their jobs, lack of interaction with friends and family, or even potentially getting infected. Increased anxiety has negative impact on psychological health and physical health. Long term anxiety can cause elevated blood pressure which is a major cause for cardiovascular diseases. To study the effects of a pandemic on anxiety levels, we conducted a study in alignment with our current research on effects of mindfulness-based stress reduction (MBSR) and stress management education (SME).

Aims: To analyze the effects of ongoing pandemic on mental state, we compared a group of individuals which were affected by the pandemic (PG) and a group which was not affected by the pandemic (NPG). The aim of the study was also to compare the effects of active interventions in the form of MBSR and SME to no intervention (CG). The study analyzed the impacts of COVID-19 on state- trait anxiety, decentering, and five facets of mindfulness.

Methods: 48 adults participated in the study. The PGs had 27 and the NPG had 19 participants. From the PGs, 16 completed an active intervention (8 MBSR and 8 SME) and the other 11 were in the CG. Intervention group underwent an 8-week MBSR or SME training. The MBSR group practiced meditation, body scanning and light yoga once a week for 2h and SME had sessions on stress, nutrition, exercise and sleep for the same duration. Participants in both groups also averaged ~45 minutes/ day of at home practice. The first four weeks of the training was conducted in person and the last four weeks were online. All the participants filled out a questionnaire for 20 items on state anxiety, 20 on trait anxiety. In addition, the active intervention participants completed 11 items for decentering and 39 for five facets of mindfulness before and after the 8-weeks.

Results: The results showed that during the pandemic, there was a difference in trait anxiety between CG and intervention group ($p=0.043$). The CG had a significant increase in trait anxiety during the pandemic, while the intervention group did not change. It was also noted that the participants who underwent interventions, either MBSR or SME had improved ability to decenter during the pandemic vs. the intervention participants that completed the study before the pandemic ($p=0.05$, $\Delta4$).

Conclusion: The results conclude that the pandemic has a negative impact on the anxiety levels of people not actively involved in MBSR or SME. But when the participants received an intervention in the form of MBSR or SME they had increased ability to decenter and did not note any significant changes in trait or state anxiety.

Category: Other

THE ROLE OF SUMOYLATION OF HEPATIC E4BP4 IN NONALCOHOLIC FATTY LIVER DISEASE

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Background: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the US with a prevalence of 30-40%. Characterized as simple steatosis due to excess of lipid accumulation at the early stage, 3-12% of NAFLD cases progress to steatohepatitis, cirrhosis, and eventually liver failure and hepatocellular carcinoma. Since currently there are no effective preventives or treatments, NAFLD poses as a great threat to public health and a huge burden on the health care system.

We previously reported that E4 promoter-binding protein 4 (E4BP4) is an insulin-induced stabilizer of the lipogenic factor SREBP-1c and promotes SREBP-1c-mediated lipogenesis in hepatocytes, indicating a potential role of E4BP4 in lipid accumulation during the pathogenesis of NAFLD.

Our most recent data uncovered that E4BP4 undergoes SUMOylation, a type of post-translational modification that regulates protein localization, stability, and activity. So far, its role in regulating de novo lipogenesis and liver steatosis remains largely unknown. Here we assessed how SUMOylation of E4BP4 affects its subcellular localization and lipogenic action in hepatocytes and the mouse liver.

Results: Based on the presence of five highly conserved SUMOylation motifs in E4BP4, we generated an E4BP4 mutant containing five lysine to arginine (5KR) substitutions, namely E4BP4-5KR. In vitro SUMOylation assay confirmed that E4BP4-WT but not E4BP4-5KR mutant was indeed directly SUMOylated. Both E4BP4-WT and E4BP4–5KR mutant showed no differences in subcellular localization in 293AD cells. We further discovered that Hepa1 cells transfected with pNTAP-E4BP4-5KR showed higher level of lipid droplet formation compared to those transfected with pNTAP-E4BP4-WT. Lastly, our immunoprecipitation assay revealed that SUMOylated E4BP4 was reduced in the liver of high-fat diet-fed mice.

Conclusion: Our study has demonstrated that E4BP4 is a direct target of SUMOylation, which tends to enhance lipid droplet formation in hepatocytes and is reduced in the liver of high-fat diet-fed mice. Our study suggests that targeting E4BP4 SUMOylation could be a novel avenue for treating NAFLD.

Category: Other

EXERCISE IS MEDICINE: STAYING ACTIVE DURING THE COVID-19 PANDEMIC

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The COVID-19 pandemic poses a major threat to the health and wellness of Michigan residents as more than 5,500 people have died. Staying at home and maintaining social distancing are helping, however, individuals still need to remain physically active. Recent data have demonstrated a considerable reduction (7-38%) in individual's average step count, suggesting that the pandemic is decreasing physical activity levels. Exercise provides numerous protective health benefits such as increased immune function and is also recently linked to reduce risk of acute respiratory distress, a major cause of death in COVID-19 patients. Additionally, exercise can prevent and treat a wide range of chronic illnesses, such as heart disease, diabetes, and cancer, all of which place individuals infected with coronavirus at high risk of severe illness and death. Exercise also offers mental health benefits that can help to cope with additional life stresses that come with social isolation, financial insecurity, and health threat. However, direct access to fitness facilities (e.g., gyms, community centers, parks, trails) and professional care (e.g., clinics) is extremely limited for the entire population, making regular exercise difficult. In accordance with the American College of Sports Medicine national call to "Stay Active" during COVID-19, it is imperative to find creative and effective ways to encourage physical activity while adhering to the current social guidelines. The goal of this project is to develop and implement an 8-week online "Exercise is Medicine" program. The specific aims are to: 1) promote participation in physical activity, 2) evaluate the effectiveness of the program to increase activity levels, and 3) assess the effect of exercise on health and well-being during social isolation. The Exercise is Medicine program is currently being developed and will consist of weekly live and recorded at-home exercise sessions delivered through an online platform 2x per week (Facebook Live, Zoom, YouTube). This program will include a variety of aerobic and muscle strengthening exercises led by graduate and undergraduate kinesiology students. Additional educational resources related to exercise, nutrition, and sleep will also be provided. Using an online survey tool, individual levels of participation, physical activity, health, wellness, and quality of life will be assessed before, during, and after the program. A repeated measures ANOVA will be used to assess change in each of the measured variables during the course of the program. This project will have a direct impact on the health of Michigan residents and provide valuable insight into the use of an online exercise program to counteract both the COVID-19 and physical inactivity pandemics simultaneously.

Category: Other

ER STRESS-INDUCED HEPATIC E4BP4 PROMOTES LIVER STEATOSIS BY SUPPRESSING AMPK SIGNALING

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Prolonged ER stress has been known to be one of major drivers of impaired lipid homeostasis during the pathogenesis of NAFLD. However, the downstream mediators of ER stress pathway in promoting lipid accumulation remain poorly understood. Here we present data showing that either the pharmacological ER stress inducer tunicamycin treatment or chronic feeding of high-fat, low-methionine and choline-deficient (HFLMCD) diet potently induces the b-ZIP transcription factor E4BP4 in both hepatocytes and the mouse liver. We showed that such an induction is partially mediated by the PERK-ATF4-CHOP pathway. Furthermore, tunicamycin promotes large lipid droplet formation and alters lipid metabolic gene expression in primary mouse hepatocytes from E4bp4flox/flox but not E4bp4 liver-specific KO (E4bp4-LKO) mice. In vivo, compared with E4bp4flox/flox control mice, E4bp4-LKO female mice exhibit reduced liver steatosis and partially improved liver function after 10-week HFLMCD diet. Mechanistically, we observed elevated AMPK activity in the liver of E4bp4-LKO mice. Inhibition of AMPK pathway by acute depletion of the Ampk-ï• ¢1 subunit partially restores lipid droplet formation and lipid metabolic gene expression in E4bp4-LKO primary mouse hepatocytes. Taken altogether, our study highlighted hepatic E4BP4 as a key factor linking ER stress, lipid accumulation, and the development of NAFLD. Targeting E4BP4 in the liver may be a novel therapeutic avenue for the treatment of NAFLD.

Category: Cell and Molecular

E4BP4 IS A NOVEL REGULATOR OF STELLATE CELL ACTIVATION

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Background: Liver fibrosis is a common final stage in the progression of NAFLD to NASH. Activation of hepatic stellate cells (HSCs) has been shown to be a critical step towards liver fibrosis. TGF-β, ER stress, and palmitic acid have been identified as potent activators of HSCs. Our lab's preliminary data showed that hepatic ER stress induces E4 promoter–binding protein 4 (E4BP4) in the liver during diet-induced liver fibrosis. In this study, we investigated the function of E4BP4 in HSCs activation.

Results: We found that genes of cell cycles, proliferation, and liver fibrosis decreased in human stellate LX2 cells with knockdown of E4bp4, whereas overexpression of E4BP4 induces those genes. The ER stress inducer tunicamycin induces the protein abundance of E4BP4 in human stellate LX2 cells while increasing the mRNA level of E4bp4 in both LX2 cells and primary HSCs. HSCs activation inducer TGF-β1 and palmitic acid also potently induce the protein levels of E4BP4 in LX2 cell and HSCs. Further, PERK is critical for such an induction of E4BP4 by ER stress in LX2 cells. Our wound healing assay showed that E4BP4 promotes the migration of LX2 cells. Lastly, E4BP4 overexpression and palmitic acid treatment have a synergistic effect on inducing fibrotic genes in LX2 cells.

Conclusions: Our study uncovered a novel function of E4BP4 in the process of HSCs activation, highlighting that E4BP4 could be targeted for treating liver fibrosis.

Category: Other