The 2022 John H. Lawrence Biomedical Symposium
AND
The Midlands Society of Physiological Sciences Annual Meeting

PROGRAM BOOKLET

October 7-8, 2022
Lee Medicine & Science Hall
University of South Dakota
Vermillion, South Dakota
NO PARKING 2AM TO 6AM IN LOTS: 1, 6, 6A, 9A, 14, 18, 20W, 21, 23, 26, 27, AND THE 'A' PERMIT PARKING AREAS IN LOT 8.

METER PARKING AVAILABLE IN LOTS: 3, 7, 16, 18, 20C, 21, 22, 30, 37

MOTORCYCLE PARKING AVAILABLE IN LOTS: 2, 4, 6A, 8, 13, 15, 16, 20D, 35, 37

FOR MORE INFORMATION: www.usd.edu/administration/university -police/parking.cfm

FOOD/DINING
Dear Colleagues:

On behalf of the Organizing Committee for the 2022 John H. Lawrence Biomedical Symposium and MSPS Annual Meeting, it is my great honor and pleasure to welcome you. As reflected by the name, this conference is sponsored jointly by University of South Dakota (USD) Sanford School of Medicine (SSOM) via the John H. Lawrence Science Symposium & Research Endowment Fund and by the Midlands Society of Physiological Sciences (MSPS), an official chapter of the American Physiological Society (APS).

MSPS has membership from primarily the States of South Dakota and Nebraska. The goal of MSPS is to foster advances in physiology-related research, training, and public awareness. MSPS annual meeting is the most important part of the endeavor to fulfill this goal.

The John H. Lawrence Science Symposium & Research Endowment Fund administered by USD Foundation was established in 1982 by John H. Lawrence, a USD graduate who subsequently studied medicine at and graduated from Harvard University in 1930. Dr. Lawrence was a faculty member at Yale and subsequently UC Berkeley. He was regarded as the father of nuclear medicine and his brother Ernest O. Lawrence, also a USD graduate, won the Nobel Prize in Physics in 1930 for his creation of the cyclotron.

To honor Dr. John H. Lawrence’s interdisciplinary spirit, it is our intention to present you with an exciting festival of biomedical sciences that covers disciplines beyond the boundaries of traditional physiology. You will find the interdisciplinary nature in Keynote Lectures by an academic cardiologist, a vascular/neural scientist, and a development biologist who are all world-renowned, in the two invited lectures from local faculty who represent recent additions to MSPS, and in the rather diverse topics of submitted cutting-edge works from trainees and faculty that will also be featured in this conference. Thus, we expect highly productive and stimulatory exchanges among the participants during this meeting as interdisciplinary collaboration is a key to success in current biomedical sciences.

I would like to take this opportunity to express my sincere appreciation to Dr. Tim Ridgway (Dean of the SSOM) and Dr. William Mayhan (Dean of USD Basic Biomedical Sciences) for their guidance and unparalleled support, to the Keynote Speakers and Local Faculty Speakers for accepting our invitation, to judges of abstracts and posters to select the best sciences for trainee awards, to faculty and trainees for their active participation, and to the APS and other sponsors for their support. I also would like to thank other members of the Organizing Committee for their time and efforts devoted to preparation of this conference and especially Dr. Mark McGlynn for his tireless and timely efforts to update the MSPS website with all relevant information.

May you have a joyful and productive conference!

With warmest regards,

Xuejun “XJ” Wang, M.D., Ph.D.
Chair, Organizing Committee
President, MSPS
The 2022 John H. Lawrence Biomedical Symposium and MSPS Annual Meeting
Organizing Committee

Advisors: William Mayhan, Dean, Basic Biomedical Sciences (BBS), USD
          John Dudley, Dean, College of Arts & Sciences, USD
          Harold D Schultz, UNMC (APS Chair of Chapter Committee)

Chairs: Xuejun Wang, BBS, USD (MSPS President; Chair)
        So-Youn Kim, UNMC (MSPS President-elect; Co-Chair)
        Hong Zheng, BBS, USD (MSPS Councilor; Co-Chair)

Members: James Hoefelmeyer, Chemistry, USD (USD Chemistry Chair)
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         Seok-Yeong Yu, UNMC (Postdoc Representative to MSPS Council)
         Mark McGlynn, UNO (Student Representative to MSPS Council)
         Wendy Pederson, (USD BBS Graduate and Education Manager)
AGENDA
The 2022 John H. Lawrence Biomedical Symposium and MSPS Annual Meeting

Friday, October 7th, 2022

11:45 am – 12:25 pm Registration & Check-in (Atrium of Lee Medical Science Building [LMS])
Address for GPS Navigation to USD Visitor Parking Lot #36:
912 N Dakota St, Vermillion, SD 57069 (“Pizza Ranch” restaurant, right across lot #36)

12:25 pm – 1:20 pm Lunch in LMS Atrium

1:20 pm – 4:35 pm Scientific Session #1 (LMS 107)
Moderator: XJ Wang, M.D., Ph.D.

1:20 – 1:30 Opening Remark by Dr. XJ Wang, President of MSPS

1:30 – 1:35 Welcoming Remark by Tim Ridgway, M.D., USD VP/Dean, Sanford School of Medicine

1:40 – 2:40 Opening Keynote Lecture: David A. Kass, M.D.
Introduced by XJ Wang, M.D., Ph.D.
Speaker: David A. Kass, M.D.
Abraham and Virginia Weiss Professor of Cardiology
Professor of Biomedical Engineering, Pharmacology and Molecular Sciences
Director, Institute of CardioScience
Johns Hopkins University School of Medicine, Baltimore, MD
Title: “Obesity and Heart Failure with Preserved Ejection Fraction – A big problem”

2:40 – 3:15 Invited Local Faculty Speaker #1:
Speaker: Pooneh Bagher, PhD
Associate Professor, Dept of Cellular & Integrative Physiology
University of Nebraska Medical Center (UNMC), Omaha, NE
Title: “Effects of Spaceflight on Coronary Function: Lessons from Rodent Research Missions”

3:15 – 3:35 Postdoc oral presentation #1: Seok-Yeong Yu, Ph.D. (P2: Submission ID 17)
“UNCOVERING TUMOR-PROMOTING ROLES OF ACTIVIN A IN Pancreatic DUCTAL ADENOCARCINOMA”

3:35 – 3:55 Grad student oral presentation #1: Liuqing Yang (G4: Submission ID 07)
“GENETIC MIMICRY OF THE ACTIVATION OF 26S PROTEASOMES BY CAMP-DEPENDENT PROTEIN KINASE PROTECTS AGAINST PROTEOTOXICITY”

3:55 – 4:15 Grad student oral presentation #2: Shane Boomer (G6: Submission ID 09)
“PRESSOR RESPONSE AND PVN ACTIVITY IN CAPSAICIN-INDUCED EXCITATORY RENAL REFLEX IN NORMOTENSIVE AND SHR RATS”

4:15 – 4:35 Undergrad student oral presentation: Wyatt Windhorst (U2: Submission ID 13)
“CARDIOMYOCYTE-RESTRICTED OVEREXPRESSION OF RPN6 INCREASES MYOCARDIAL PROTEASOME PEPTIDASE ACTIVITIES IN MICE”

4:35 – 5:05 Poster Setup in LMS Atrium

6:00 pm – 9:00 pm Social and Dinner at Old Lumber Company Grill and Bar (15 Court St, Vermillion, SD 57069)
Saturday, October 8th, 2022

8:00 am – 8:55 am  Breakfast in LMS Atrium and 107

9:00 am – 3:45 pm  **Scientific Session #2**

**Moderators:**  Hong Zheng, M.D.; Co-Chair of the Organizing Committee
So-Youn Kim, Ph.D.; Co-Chair of the Organizing Committee

09:00 – 10:00  **Keynote Lecture #2:** Frank M. Faraci, Ph.D.
Introduced by William Mayhan, Ph.D., Dean of BBS, USD Sanford School of Med
Speaker: Frank M. Faraci, Ph.D.
Professor of Internal Medicine-Cardiovascular Medicine
Professor of Neuroscience and Pharmacology
Carver College of Medicine, University of Iowa Health Care, Iowa City, IA
Title: “Cerebral Circulation and Dementia”

10:00 – 10:35  Invited Local Faculty Speaker #2:
Speaker: Yohaan Fernandes, Ph.D.
Assistant Professor, Dept of Biology and Division of Basic Biomedical Sciences
University of South Dakota, Vermillion, SD
Title: “Use of zebrafish to model fetal alcohol spectrum disorders (FASD)”

10:35 – 10:55  Grad student oral presentation #3: Mingqi Cai
“SUSTAINED BUT DECOYED ACTIVATION OF THE JAK1-STAT PATHWAY BY
EXPRESSION OF MISFOLDED PROTEINS EXACERBATES PROTEOTOXICITY”

10:55 – 11:20  Break & Poster Viewing

11:20 – 11:40  Grad student oral presentation #4: Samiksha Giri
“THE COP9 SIGNALOSOME PROMOTES NEOINTIMAL HYPERPLASIA THROUGH
CULLIN DENEDDYLAATION DEPENDENT AND INDEPENDENT MECHANISMS”

11:40 – Noon  Postdoc oral presentation #2: Mark Bouska, Ph.D.
“RNA SEQUENCING ACROSS LIFESPAN OF THE CARDIAC SPECIFIC
CRYABR120G PROTEOTOXIC STRESS MOUSE MODEL”

Noon – 2:30  **Lunch and Poster Presentation** (LMS Atrium)
12:30 pm – 1:20 pm  **Poster Session #1** (Odd Number)
1:30 pm – 2:20 pm  **Poster Session #2** (Even Number)

2:30 – 3:30  **Keynote Lecture #3:** Patrick Seale, Ph.D.
Introduced by So-Youn Kim, Ph.D.
Speaker: Patrick Seale, Ph.D.,
Professor, Department of Molecular and Developmental Biology
Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
Title: “Mesenchymal progenitor cells in fat tissue development and maintenance”

3:30 pm – 4:00 pm  **Award Ceremony and MSPS Business Meeting**

3:30 – 3:45  Award Ceremony and Sponsors Acknowledgement
3:45 – 4:00  MSPS Business Meeting
    APS offers Chapter Award
    Financial Report: Cindy Norton
    Election Results: Dr. XJ Wang
    Closing Remark by Outgoing President: Dr. XJ Wang
    Remark by Incoming President: Dr. So-Youn Kim
Accepted Abstracts
**THE RELATIONSHIP BETWEEN CEREBROVASCULAR DYSFUNCTION AND BEHAVIORAL ABNORMALITIES IN MALE AND FEMALE RATS EXPOSED TO ALCOHOL IN UTERO**

**Tiffany M. Knecht**, Partha S. Saha, Jamie L. Scholl, Michael J. Watt, Denise M. Arrick, and William G. Mayhan

University of South Dakota, Division of Basic Biomedical Sciences, Sanford School of Medicine, Vermillion, SD

**Background/hypothesis of the study:** The purpose of this study was to determine a relationship between cerebrovascular dysfunction and behavioral abnormalities in rats exposed to alcohol in utero using a preclinical rat model. Maternal consumption of alcohol during pregnancy is an established risk factor for fetal alcohol spectrum disorders (FASD), which has been estimated to impact approximately 1% of children in the United States. While studies have shown impairment in functional responses of cerebral resistance arterioles in rats exposed to prenatal alcohol, no studies have examined the association between impaired cerebrovascular function and contributory behavioral abnormalities. We hypothesize that the cerebrovascular dysfunction caused by in utero alcohol exposure results in a decrease in blood flow to the brain and, hence, a decrease in oxygen delivery, which may contribute to behavioral abnormalities.

**Methods and Results:** We fed Sprague-Dawley dams a liquid diet with or without alcohol (3% ethanol) for the duration of their pregnancy (21-23 days). We then performed short-term memory (Novel Object Recognition [NOR]) and coordination and balance (Rotarod) assessments on the offspring during adolescence (4-6 weeks) and adulthood (14-16 weeks). Following the behavioral evaluations, we examined the reactivity of cerebral arterioles to nitroglycerin (NOS-independent), adenosine diphosphate (ADP; eNOS-dependent), N-methyl-D-aspartate (NMDA; nNOS-dependent), and iloprost (BK channel-dependent). All tests were examined in control male and female and prenatal alcohol male and female. We found impairment in eNOS-, nNOS-, and K-channel-dependent dilation of cerebrovascular arterioles in rats exposed to alcohol in utero. Associated with this impaired vascular function, we found that short-term memory was impaired in rats exposed to alcohol in utero. Further, we found modest learning differences in the coordination/balance assessment.

**Conclusions:** Based on the findings, we suggest that impaired cerebral vascular function may contribute to behavioral abnormalities seen in fetal alcohol spectrum disorders.
CARDIOMYOCYTE-RESTRICTED OVEREXPRESSION OF RPN6 INCREASES MYOCARDIAL PROTEASOME PEPTIDASE ACTIVITIES IN MICE

Wyatt Windhorst¹, Jack Sternburg¹, Daniel Finely², Xuejun Wang¹

¹Division of Basic Biomedical Sciences, University of South Dakota Sanford School of Medicine, Vermillion, SD; ²Department of Cell Biology, Harvard Medical School, Boston, MA.

Background and Aims: In healthy conditions, the proteasome degrades unneeded and unwanted proteins in the cell, but in most heart diseases, there is an increase in misfolded proteins that overwhelms the proteasome, contributing to cardiac pathogenesis. One method to deal with this problem is to increase proteasome proteolytic function. Research in C. Elegans has found that increasing the expression of the proteasome subunit Rpn6 causes increased proteolysis by the proteasome. This study aimed to determine whether Rpn6 overexpression can be achieved in mammals and, if so, whether that increases proteasome activities.

Methods and Results: The mouse model was created by targeting a transgenic construct capable of conditional overexpression of Rpn6 into the Rosa26 (R26) locus of the mouse genome. In the transgenic construct, a loxP flanked blocker was inserted between the promoter and the Rpn6 cDNA so that Rpn6 overexpression can be induced in a tissue when coupled with transgenic Cre expression in the tissue. We crossed the R26-Rpn6 mice with a cardiomyocyte-restricted Cre overexpressing mouse (Myh6-Cre) and examined both male and female littermate R26-Rpn6::Myh6-Cre and My6-Cre mice at 4 weeks of age. Western blot analyses reveal that myocardial Rpn6 and 20S α3 subunit were increased respectively by 73% [100.0±5.620(N=8) vs. 173.1±11.20(N=7), p< 0.0001] and 31% [100.0±8.372(N=8) vs. 131.2±8.012(N=7), p=0.0192] in R26-Rpn6::Myh6-Cre mice compared with littermate control groups. The myocardial proteasomal peptidase activity assay showed a 37% increase in 26S chymotrypsin-like activity in R26-Rpn6::Myh6-Cre mice compared with the control group [100.0±1.64 N=6 vs 136.9±14.15(N=5), p=0.0720], although the difference did not reach statistical significance yet due to large variation.

Conclusions: These results show that RPN6 can be overexpressed in mouse hearts and this overexpression likely increases proteasome abundance and activity, producing a potentially new proteasome gain of function model for further investigation.

*SD BRIN Undergraduate Scholar
ROLES OF CYTOSKELETAL INHIBITORS ON THE METASTASIS OF DIABETIC TRIPLE NEGATIVE BREAST CANCER CELLS

Stephanie Vielmas-Duarte*, Osaira R. Ovando*, and Surabhi Chandra

Department of Biology, University of Nebraska at Kearney, Kearney, NE; *Both authors contributed equally to the study.

Background and Hypothesis: Patients with concomitant diabetes and breast cancer have increased mortality due to chemoresistance and metastasis. Metastasis of cancer cells is highly dependent on the reorganization of the cell cytoskeleton and is responsible for the high mortality associated with cancer. Hence, it is hypothesized that cytoskeletal inhibitors prevent metastasis of breast cancer cells in hyperglycemic conditions.

Methods and Results: To test this hypothesis, scratch wound healing assays and trans-well migration assays were performed with MDA-MB-231 cells (late-stage metastatic triple negative breast cancer cells). Cells were treated with normal glucose (5mM) or high glucose (25mM) conditions in the presence of Rho kinase (Y-27632, 10 μM) or focal adhesion kinase (FAK, 10 μM) inhibitors. It was observed that while glucose treatments completely covered the scratch after 24h, inhibition of Rho kinase did not have any protective effect. Interestingly, FAK inhibition markedly suppressed the migration of cells under both normal (mean area change 17.3±6.74% of 5mM glucose control at 0h) and high glucose conditions (mean area change 0.3±0.33% of 5mM glucose control at 0h) after 24h in the scratch wound healing assay. These results are being corroborated using transwell migration assay.

Conclusions: The data suggests that focal adhesion kinase inhibition can prevent the metastasis of triple negative breast cancer cells in diabetic as well as normal conditions and can be further explored as a chemotherapeutic option for resistant triple negative breast cancer.
Graduate/Med/Postbac Category

G1: Submission ID 03 - Oral Presentation

THE COP9 SIGNALOSOME PROMOTES NEOINTIMAL HYPERPLASIA THROUGH CULLIN DENERDDYLATION DEPENDENT AND INDEPENDENT MECHANISMS

Samiksha Giri, Chao Suo, Douglas S. Martin, Xuejun Wang

Division of Basic Biomedical Sciences, University of South Dakota Sanford School of Medicine, Vermillion, SD.

Background: Neointimal hyperplasia (NH) is a common pathological response to vascular injury. NH is caused primarily by vascular smooth muscle cell (VSMC) proliferation and migration. The COP9 signalosome (CSN) is formed by 8 canonical subunits (CSN1 through CSN8) with its deneddylation activity residing in CSN5. Despite strong evidence linking the CSN to cell cycle regulation, the role of the CSN in vascular biology remains obscure. To fill this critical gap, this study tests the hypothesis that the CSN promotes NH through Cullin deneddylation dependent and independent mechanisms.

Methods and Results: We performed left common carotid artery (LCCA) ligation in adult mice to produce NH and analyzed the LCCA segment proximal to the ligation. LCCA ligation markedly increased the mRNA and protein levels of CSN subunits in wild type mice (p<0.05). VSMC proliferation was suppressed in mice with smooth muscle-restricted CSN5 knockout (CSN5-SMKO) (0.102±0.07 vs. 1.00, p<0.0001), but NH was significantly exacerbated by CSN8 hypomorphism (CSN8-hypo) (2.36±0.5 vs. 1.00±0.3, p<0.0001). In vitro, isolated, and cultured VSMCs from CSN8-hypo mice also showed increased proliferation in response to PDGF-BB compared to littermate non-hypomorphic (CTL) VSMCs (p<0.05). Further, we detected smaller nuclear to cytoplasmic ratios for both p27 and CSN5, as well as increases in the cytoplasmic CSN5 mini-complex in CSN8-hypo mouse tissues and cultured CSN8-hypo VSMCs (p<0.05). Nuclear export inhibition with leptomycin attenuated the increases in PCNA and cytoplasmic p27 induced by CSN8 hypomorphism (p<0.05). Interestingly, treatment with the CSN denerddylase inhibitor (CSN5i-3) did not alter the proliferation of CSN8-hypo VSMCs but suppressed the PDGF-BB induced proliferation in CTL VSMCs. Additionally, genetically disabling CSN5 nuclear-export but not disabling CSN5 denerddylase activity suppressed the hyperproliferation and restored p27 nuclear localization in the hypomorphic VSMCs (p<0.05).

Conclusions: Both CSN denerddylation activity and CSN5-mediated nuclear exclusion of p27 promote VSMC proliferation and NH in injured vessels.
**SCL01B1**

**ALLELE IS ASSOCIATED WITH ATORVASTATIN DISCONTINUATION AND ADVERSE MUSCLE SYMPTOMS IN THE CONTEXT OF ROUTINE CARE**

Anna Myrmoe¹, Deepak Voora², Jordan Baye¹, Adam McDermaid¹, Smitha Narayana Gowda¹, Russell A. Wilke¹, Catherine Hajek¹, Eric A. Larson¹

¹Sanford Imagenetics and Sanford School of Medicine, Department of Internal Medicine, University of South Dakota, Sioux Falls, SD; ²Center for Applied Genomics & Precision Medicine at Duke University School of Medicine, Department of Medicine, Duke University, Durham, NC.

**Background/hypothesis:** SCL01B1 genotype is known to influence patient adherence to statin therapy, in part by increasing the risk for statin-associated musculoskeletal symptoms (SAMS). For simvastatin specifically, the SCL01B1*5 allele has previously been associated with SAMS. For atorvastatin, prior studies assessing the impact of SCL01B1*5 on SAMS have yielded inconclusive findings, due to insufficient power.

**Methods and Results:** This study quantifies the impact of SCL01B1*5 on atorvastatin discontinuation and SAMS in a large observational cohort using electronic medical record (EMR) data from a single health care system. In the study cohort (n = 1,627 patients exposed to atorvastatin during the course of routine clinical care), 56% (n = 912 of 1,627 patients) discontinued atorvastatin, and 18% (n = 303 of 1,627 patients) developed SAMS. A univariate analysis revealed that SCL01B1*5 increased the likelihood that patients would stop atorvastatin during routine care (Odds Ratio 1.2, 95% confidence interval [C.I.]: 1.1 - 1.5, \( p = 0.04 \)). Further analyses using a multivariate Cox proportional hazards model demonstrated that this same variant was associated with time to atorvastatin discontinuation (Hazard Ratio 1.2, C.I. 1.1 - 1.4, \( p = 0.004 \)). Additional time-to-event analyses also revealed that SCL01B1*5 was associated with SAMS (Hazard Ratio 1.4, C.I. 1.1 - 1.7, \( p = 0.02 \)).

**Conclusions:** Atorvastatin discontinuation was associated with SAMS (Odds Ratio 1.67, \( p = 0.0001 \)) in this cohort. It is plausible that SCL01B1-informed statin therapy with the aim of reducing muscle side effects may lead to improved adherence for this important class of drugs.
A PRELIMINARY PROBE INTO THE MYOCARDIAL UBIQUITIN-PROTEASOME SYSTEM
PERFORMANCE OF A MOUSE MODEL OF HEART FAILURE WITH PERSERVED EJECTION FRACTION

Jose R. Lira, Andrew L. Guymon, Liuqing Yang, Jack O. Sternburg, Samiksha Giri, Xuejun Wang

University of South Dakota Sanford School of Medicine, Vermillion, SD.

Background: Heart failure with preserved ejection fraction (HFpEF) is a leading cause of death and disability with a prevalence surpassing that of heart failure with reduced ejection fraction. HFpEF can be modelled through simultaneous metabolic and hypertensive stresses in male mice provoked by a combination treatment of a high fat diet (HFD) and constitutive nitric oxide synthase inhibition by Nω-nitro-L-arginine methyl-ester (L-NAME). Ubiquitin-proteasome system (UPS) dysfunction was detected in many forms of cardiomyopathy, but whether it occurs in HFpEF remains unknown.

Methods and Results: Adult littermate male FVB/N mice transgenic and non-transgenic for GFPdgn (an inverse UPS reporter) were randomly assigned to the CHOW group (a standard chow diet) or the HFD+L-NAME group [HFD and L-NAME in drinking water (0.5g/L)] throughout the study. Glucose tolerance tests (GTT) and echocardiography were performed at multiple timepoints. The HFD+L-NAME group developed HFpEF by 7-week as evidenced by reduced glucose tolerance measured by GTT [AUC: 37714±3922(6)* vs. 23885±1039(6); P=0.007], reduced exercise tolerance as revealed by reduced running distance to fatigue [168.1±17.04(6) vs. 268.6±27.81(6); P=0.011], cardiac hypertrophy as revealed by increased LV end-diastolic wall thickness [0.82±0.036(6) vs. 0.72±0.021(6); P=0.037], but comparable ejection fraction [55.61±1.66(6) vs. 52.96±1.27(6); P=0.234] compared with the CHOW group. These changes were also detected at 15- or 17-week. Tail-cuff blood pressure measured at 17-week showed increases in systolic pressure [128.9±3.2(6) vs. 118.5±3.1(5); P=0.047]. Terminal experiments at 18-week revealed increased body weight to tibial length ratio [2.15±0.098(6) vs. 1.80±0.061(5); P=0.017], and a tendency of increases in ventricular myocardial Nppb mRNA levels [1.78±0.352(6) vs. 1.00±0.219(5); P=0.098] and in GFPdgn protein [1.84±0.373(n=4) vs. 1.00±0.205(n=3); P=0.137] but not mRNA levels in the HFD+L-NAME group, compared with the CHOW group.

Conclusions: The HFD+L-NAME treatment can induce HFpEF in FVB/N male mice; myocardial UPS functioning is likely impaired during HFpEF and warrants further comprehensive investigation.

*Mean±SEM(n)
G4: Submission ID 07 - Oral Presentation

GENETIC MIMICRY OF THE ACTIVATION OF 26S PROTEASOMES BY CAMP-DEPENDENT PROTEIN KINASE PROTECTS AGAINST PROTEOTOXICITY

Liuqing Yang¹, Nirmal Parajuli¹, Jinbao Liu², Xuejun Wang¹

¹Division of Basic Biomedical Sciences, University of South Dakota Sanford School of Medicine, Vermillion, SD, USA; ²Department of Pathophysiology, Guangzhou Medical University, Guangzhou, Guangdong, China

Background: A cell culture study shows cAMP-dependent protein kinase (PKA) activates 26S proteasomes by phosphorylating Ser14 of Rpn6, but this discovery and its physiological significance remain to be established in vivo. Here we tested the hypothesis that Ser14-Rpn6 phosphorylation (pS14-Rpn6) mediates the activation of proteasomes by PKA and reduces proteotoxicity in vivo.

Methods and Results: We generated two mouse models, in which Ser14 of endogenous Rpn6 was mutated to either Ala (S14A) or Asp (S14D) to respectively block or mimic pS14-Rpn6. In cultured neonatal rat cardiomyocytes, either S14D or activating adenylate cyclase with forskolin enhanced proteasome-mediated degradation of CryAB-R120G, a disease-causing misfolded protein. Forskolin increased cardiac pS14-Rpn6 and proteasome activities in wild-type but not S14A mice, establishing that pS14-Rpn6 is required for PKA-mediated proteasome activation. Proteasome activities were increased in S14D mouse hearts; crossbreeding GFPdgn (a proven proteasome substrate) into S14D mice revealed that S14D decreased cardiac GFPdgn protein (0.81±0.08 vs. 1.0±0.08, p<0.05) via post-transcriptional mechanism, suggesting S14D suffices to enhance 26S proteasomes. Crossbred into the CryAB-R120G mice, a classic model of cardiac proteotoxicity, S14D increased cardiac proteasome activities and reduced aberrant CryAB-R120G protein aggregation (p<0.05) in CryAB-R120G hearts. The reactivation of fetal genes (ANF, BNP, Myh7) in CryAB-R120G hearts was blunted by S14D (p<0.05). Echocardiography revealed that S14D attenuated the decreases in left ventricular ejection fraction (52.66±10.63 vs. 38.88±13.59%, p<0.001), stroke volume (39.34±6.75 vs. 28.84±8.38μl, p<0.001), and cardiac output (18.05±2.87 vs. 12.01±3.32ml/min, p<0.001) of CryAB-R120G mice at 6 months. Moreover, S14D extended the lifespan of both male and female R120G mice (224 vs. 201 days, p<0.001). These data compellingly demonstrate that genetic mimicry of pS14-Rpn6 ameliorates cardiac proteotoxicity.

Conclusions: We established pS14-Rpn6 mediates the activation of 26S proteasomes by PKA in vivo and provided the first and unequivocal genetic evidence that PKA-induced proteasome activation protects against proteotoxicity in animals.
SUSTAINED BUT DECOYED ACTIVATION OF THE JAK1-STAT PATHWAY BY EXPRESSION OF MISFOLDED PROTEINS EXACERBATES PROTEOTOXICITY

Mingqi Cai, Bo Pan, Peng Xiao, Megan T. Lewno, Xuejun Wang

Division of Basic Biomedical Sciences, University of South Dakota Sanford School of Medicine, Vermillion, SD.

Background: Aberrant protein aggregation and increased proteotoxic stress (IPTS) are major pathogenic factors of heart failure. The JAK-STAT pathway mainly mediates cytokine signaling and is implicated in cardiac pathogenesis. A recent study using genome-wide siRNA screening in cultured cardiomyocytes identified JAK1 as a key effector of protein aggregation in cultured cardiomyocytes, but whether this happens in vivo and if JAK1 kinase activity is required remain unknown. Here we have tested the hypothesis that JAK1 kinase activity is required for the dysregulated JAK1-STAT pathway to exacerbate aberrant protein aggregation, ubiquitin-proteasome system (UPS) impairment, and proteotoxicity during IPTS.

Methods and Results: Western blot analyses revealed striking increases in phosphorylated (p-STAT1) and total STAT1 in the insoluble fraction of myocardium of mice overexpressing CryABR120G or ΔR172-E178-Desmin, two well-characterized models of cardiac IPTS. However, neither protein nor mRNA levels of SOCS1/2, the JAK-STAT target genes for negative feedback, were increased. Immunofluorescence staining showed markedly increased STAT1 in the cytoplasmic protein aggregates but not in cardiomyocyte nuclei, indicative of a decoyed activation of the JAK-STAT pathway in IPTS hearts. Consistently, global RNA sequencing did not detect an activation of the JAK-STAT pathway in CryABR120G mouse hearts. In cultured neonatal rat cardiomyocytes, adenovirus-mediated overexpression of CryABR120G increased p-STAT1 and STAT1 proteins in the insoluble protein fraction (1.03±0.03 vs. 2.57±0.23, p=0.007; 1.00±0.18 vs. 2.59±0.36, p=0.008); inhibition of JAK1 kinase with Itacitinib reduced protein levels of CryABR120G (1.00±0.13 vs. 0.37±0.06, p=0.004) and UPS surrogate substrate GFPu (1.00±0.09 vs. 0.53±0.07, p=0.016), as well as cytotoxicity (1.59±0.10 vs. 1.17±0.06, p=0.022).

Conclusions: Sustained but decoyed activation of the JAK1-STAT pathway is induced by misfolded proteins in cardiomyocytes. JAK1 kinase inhibition attenuates aberrant protein aggregation and UPS impairment, thereby protects against IPTS.
PRESSOR RESPONSE AND PVN ACTIVITY IN CAPSAICIN-INDUCED EXCITATORY RENAL REFLEX IN NORMOTENSIVE AND SHR RATS

Shane Boomer, Xuefei Liu, Mohammad Amir Afjal, and Hong Zheng

Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, SD.

Background: The kidneys play an influential role in sympathetic activity and blood pressure homeostasis. The hypothalamic paraventricular nucleus (PVN) is important in mediating sympathetic activity to regulate blood pressure. In this study, we hypothesized that enhanced excitatory renal reflex contributed to sympathoexcitation and elevated blood pressure in hypertensive rats.

Methods and Results: We used normotensive (SD) and spontaneously hypertensive rats (SHR) to examine the pressor responses and PVN neuronal activation by renal afferent stimulation with capsaicin. The subjects were outfitted with an arterial catheter to monitor blood pressure and heart rate. A pelvic catheter was placed within the ureter to allow for administration of saline control or capsaicin (5, 10, 20µM). Brain tissues were fixed and processed for c-Fos staining within the PVN subregions. Findings showed that pelvic capsaicin injection significantly increased mean arterial pressure (MAP), but not heart rate in both SD and SHR rats compared to the saline control. The MAP response was significantly enhanced in SHR rats (18.5 +/- 3.6 mmHg) compared to the normotensive rats (7.5 +/- 2.4 mmHg, P<0.05) at the dose of 20µM. Capsaicin stimulation significantly increased c-Fos positive staining cells in the PVN in both normotensive (dpPVN: 9.0 +/- 2.3 vs 44.8 +/- 5.8, vpPVN: 17.8 +/- 0.8 vs 58.7 +/- 3.8, mPVN: 8.2 +/- 2.0 vs 48.6 +/- 10.3, P<0.05) and SHR rats (dpPVN: 12.7 +/- 0.7 vs 36.4 +/- 3.8, vpPVN: 27.0 +/- 5.3 vs 51.5 +/- 7.2, mPVN: 12.2 +/- 2.2 vs 35.9 +/- 5.3, P<0.05) compared to the saline control. There was no significant difference in c-Fos staining between the normotensive and SHR rats with capsaicin stimulation.

Conclusions: Our studies indicate that renal afferent stimulation with capsaicin causes a renal excitatory reflex, leading to pressor response, and PVN neuronal activation. The PVN is an important central nucleus in the pathway of the renal excitatory reflex. This may contribute to over-sympathetic activation and the development of hypertension.
MOLECULAR REGULATION OF CARDIAC FERROPTOSIS IN T1DM

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Background/hypothesis of the study: Diabetes mellitus (DM) increases the risk of heart failure by promoting myocardial cell death. A recent study shows that ferroptosis, an iron-dependent cell death mechanism, is essential for DM-induced cardiomyopathy. However, molecular regulation of ferroptosis in the DM heart remains unclear. Reduced glutathione peroxidase-4 (GPX4), iron overload, and reactive oxygen species (ROS) accumulation altogether promote lipid peroxidation leading to ferroptosis. Antioxidants (cytosolic - superoxide dismutase (SOD) and mitochondrial - catalase) reduce ROS accumulation. We hypothesize that increased iron import through transferrin receptor (TFR), decreased GPX4 activity due to reduced glutathione disulfide reductase (GSR) - essential for GPX4 activity, and increased ROS accumulation due to decreased antioxidants altogether promote ferroptosis in the T1DM heart.

Methods and Results: Following the Guidelines on Models of Diabetic Heart Disease, we used Akita mouse as the model of T1DM. We randomly selected 12-week-old male Akita and its littermate normoglycemic WT mice and evaluated ferroptosis in the heart (left ventricle) following the Guidelines for Evaluating Myocardial Cell Death. Proteomic analysis of WT and Akita hearts showed decreased (-2.49-fold) levels of GSR and increased (+2.44-fold) levels of TFR in the Akita heart. Furthermore, antioxidants were decreased (SOD1: -1.10-fold, SOD2: -1.58-fold, catalase: -1.56-fold) in the Akita heart. Immunoblotting showed a decrease in GPX4 (-0.56-fold) levels in the Akita heart.

Conclusions: Our results revealed that T1DM induces cardiac ferroptosis by three combined mechanisms: increased iron import, decreased GPX4 expression and activity, and reduced antioxidants.
TAp63 DETERMINES THE FATE OF OO CYTES AGAINST DNA DAMAGE INDUCED BY CYCLOPHOSPHAMIDE

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Background/Hypothesis of the study: Although advanced cancer therapies have significantly improved the life expectancy of cancer survivors, the treatment itself increases the risk of reproductive insufficiency. Maintaining ovarian function against cancer therapies is an unmet need for female cancer patients. Cyclophosphamide (CPA), an alkylating chemotherapeutic agent, forms DNA crosslinks to induce apoptosis in rapidly proliferating tumor cells. However, the mechanisms of CPA-induced oocyte death remain speculative. ABL inhibitor has been proposed to prevent primordial follicle loss by CPA, while TAp63 was phosphorylated by other chemotherapeutic agents in the oocytes. Here, we hypothesize that CPA induces ovarian follicle depletion through TAp63-related signaling pathways.

Methods and Results: Oocyte-specific Abl1 and Trp63 knockout mice (n ≥ 4) were utilized to delineate the mechanism of primordial follicle loss by CPA. GNF2 and CHK2 inhibitor were tested for follicle preservation against CPA. GNF2, the ABL allosteric inhibitor, did not impede the primordial follicles loss induced by CPA (p>0.05), and the number of primordial follicles from oocyte-specific Abl1 knockout mice dramatically declined after CPA administration (p<0.001). Instead, CHK2 was time-dependently expressed in the oocytes exposed to CPA metabolite (p<0.0001), and TAp63 was hyperphosphorylated by CPA. Trp63 deletion in oocytes significantly preserved primordial follicles from CPA (p<0.0001). CPA-treated oocyte-specific Trp63 knockout females delivered comparable numbers of litters and pups to solvent-treated females (p>0.05). The absence of Trp63 evaded the apoptosis with undetectable levels of BAX and cleaved PARP. CHK2 inhibitor significantly preserved the primordial follicles from CPA (p<0.0001).

Conclusions: TAp63 is fundamental in determining the signaling of oocyte death against DNA damage. CHK2-TAp63 pathway rather than ABL is the main pathway in oocyte death of primordial follicles against CPA. Therefore, TAp63 is potentially an effective target to prevent cancer treatment-induced primordial follicle loss without compromising the efficacy of chemotherapy in female cancer patients.
DIETARY MOLYBDENUM COFACTOR AND SUPPLEMENTAL MOLYBDATE PROMOTE METABOLIC FITNESS IN THE NEMATODE C. ELEGANS

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Background/hypothesis of the study: Molybdenum Cofactor Deficiency (MoCD) is a rare and lethal disease caused by the inability to endogenously synthesize Molybdenum cofactor (Moco). Unfortunately, Moco readily degrades when exposed to air and cannot be supplemented to MoCD patients. Sulfite oxidase (SUOX-1), one of four Moco-requiring enzymes in animals, is responsible for sulfite detoxification. Many symptoms of MoCD are caused by sulfite accumulation due to compromised SUOX-1 function. Molybdate, the bioavailable form of molybdenum, is essential for Moco synthesis. Uniquely, Caenorhabditis elegans can use endogenously synthesized Moco and Moco acquired through its microbial diet. This phenomenon is unique and obtaining Moco was previously considered a cell-autonomous, de novo synthesis process. Leveraging this natural phenomenon, we use C. elegans as an inducible model of MoCD to understand the transport and stability of Moco and molybdate in vivo.

Methods and Results: We sought to biochemically evaluate Moco homeostasis in C. elegans. To quantify SUOX-1 activity and Moco content in C. elegans, we developed a colorimetric SUOX-1 activity assay and an HPLC protocol for measuring Moco. C. elegans deprived of dietary Moco (Moco-) and mutant animals unable to synthesize Moco [moc(-)] had reduced SUOX-1 activity and Moco content. Loss of either source of Moco caused; decreased Moco content, reduced SUOX-1 activity, and sensitivity to toxic sulfite. Interestingly, supplemental molybdate rescues C. elegans development when SUOX-1 activity is compromised in Moco- environments.

Conclusions: We have demonstrated that SUOX-1 activity and Moco content in C. elegans are decreased when endogenous or dietary Moco is removed. Both Moco sources are essential for sulfite tolerance and animal development when SUOX-1 activity is compromised. Also, we show that supplemental molybdate can rescue animals with decreased SUOX-1 activity. Together, our results further characterize the physiological importance of dietary Moco, endogenous Moco, and molybdate for Moco homeostasis in C. elegans.
THE ROLE OF THE SUPERIOR CERVICAL GANGLION IN THE CHRONIC SENSITIZATION OF THE CAROTID BODIES POST-ACUTE LUNG INJURY

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Background/hypothesis of the study: Acute lung injury (ALI) increases respiratory rate (RR), induces hypoxemia. This stimulates carotid body (CB) chemoreflex to maintain oxygen homeostasis. Our previous study indicates a sensitized chemoreflex during the recovery of ALI. Chronic chemoreflex hyperactivity negatively affects the overall quality of life. The neural mechanisms underlying the sensitized CB chemoreflex during the recovery from ALI remain a gap in knowledge. Electrical stimulation of the superior cervical ganglion (SCG) sensitizes the CB chemoreflex in rats. We hypothesized that the SCG causes the CB chemoreflex sensitization post-ALI.

Methods and Results: We performed a bilateral SCG ganglionectomy (SCGx) or sham-SCGx (Sx) in our male SD rats 2 weeks before inducing ALI (W-2). After 2 weeks of recovery, ALI was induced using a single intra-tracheal instillation of bleomycin (day 1) and measured the resting respiratory variables - RR, TV (Tidal Volume), and MV (Minute Ventilation). The chemoreflex response to 10% hypoxia and 5% normoxic hypercapnia were measured on W(-3) (pre-SCGx), W0 (post-SCGx/pre-ALI) and W4 (post-ALI) using whole-body plethysmography (WBP). Ganglionectomy did not alter the resting lung parameters and the chemoreflex response to either gas challenge in normal, healthy rats. An increase in resting RR at W1 post-ALI was not significantly different between Sx and SCGx rats. At W4 post-ALI, there were no significant differences in resting lung parameters between Sx and SCGx rats. Consistent with our previous study, the CB chemoreflex response to hypoxia and normoxic hypercapnia in Sx rats at W4 post-ALI was sensitized (p<0.01). However, the chemoreflex sensitivity significantly lowered in SCGx rats in response to hypoxia (p=0.01) and normoxic-hypercapnia (p=0.002).

Conclusions: The CB chemoreflex sensitization during the recovery from the ALI may be mediated by the SCG. Further understanding of the underlying mechanism will provide important information to develop therapeutic approaches to pulmonary disease to improve clinical outcomes.
HEAT SHOCK-DERIVED EXOSOMES AS A TREATMENT FOR ALZHEIMER’S DISEASE

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Background/hypothesis: By 2050, 13.8 million Americans over 65 are projected to be diagnosed with Alzheimer’s Disease (AD). While much work has been done on AD in the last hundred years, no effective therapies can stop or reverse the progression of the disease. Exosomes, 50 – 150 nanometers in diameter extracellular vesicles, have gained recent popularity as a potential therapeutic agent due to their ability to cross the blood-brain barrier (BBB), biodegradability, strong protection of cargo, and low immunogenicity following systemic injection. The low yield from cultured cells, mainly stem cells, has limited their application as a therapeutic agent. This study aims to test the hypothesis that heat shock (HS) will increase the production of exosomes and provide therapeutic efficacy in an AD mouse model.

Methods and Results: Exosomes were collected from the media of neural stem cells in non-heat shock (NHS) and HS conditions. Nanoparticle tracking analysis confirmed that HS-derived exosomes exhibit significantly increased concentration and larger diameter compared to NHS-derived exosomes (p-value <0.05). Mass spectrometry analysis identified fewer diverse proteins in HS-derived exosomes compared to NHS-derived exosomes. Next, we tested the therapeutic efficacy of NHS- and HS-derived exosomes in the AβPPswe/PS1dE9 AD mouse model. Mice treated with HS-derived exosomes exhibited a significant increase in learning and memory compared to control mice in the novel object recognition test (p-value <0.01). Further, HS-derived exosomes significantly decreased the neuritic plaque load in male AD mice (p-value <0.05).

Conclusions: In response to HS, neural stem cells can increase exosome production and alter exosome morphology and cargo to provide improved neuroprotection in AD. These findings have implications for utilizing HS as a method to increase the production of exosomes without hindering their therapeutic efficacy.
Skeletal Muscle UCHL1 Regulates P62 Expression and Secretion

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Background: Ubiquitin C-terminal hydrolase L1 (UCHL1) is a deubiquitinating enzyme that was originally found in neurons. We found that UCHL1 is also expressed in skeletal muscles, but its functions remain to be fully understood. P62, also known as Sequestosome-1 (SQSTM1), is a multifunctional protein which primarily functions as a receptor of autophagy.

Methods and Results: Here we reported that UCHL1 regulates p62 protein levels and potentially p62 release from skeletal muscle. First, we observed that skeletal muscle specific knockout (smKO) of UCHL1 reduced p62 level in skeletal muscle. Consistent with this in vivo result, siRNA knockdown of UCHL1 or overexpression of UCHL1 in C2C12 myotubes respectively decreased and increased p62 level in the cells. Immunoprecipitation was able to co-pull down UCHL1 and p62, indicating the direct interaction of these two proteins. Moreover, in UCHL1 smKO mice, while p62 level in skeletal muscle was reduced, the plasma p62 level was increased.

Conclusions: Given the recent evidence that p62 can be released as an extracellular inflammatory mediator, this data suggests that UCHL1 may function as an inhibitory regulator of p62 release from skeletal muscle. The functional significance of this novel mechanism in skeletal muscle functions and diseases remain to be fully studied.
EXPRESSION AND FUNCTION OF SKELETAL MUSCLE DERIVED BDNF

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Background/hypothesis: Brain derived neurotropic factor (BDNF), originally discovered in the brain, has been shown to be expressed and released from skeletal muscle as a myokine. In the brain BDNF is translated as proBDNF, which can be cleaved into mature BDNF or remain as proBDNF eliciting effects distinct from those of mature BDNF. While myokine BDNF has been shown to promote fatty acid metabolism and skeletal muscle regeneration, the expression and function of proBDNF in skeletal muscle is unknown. In the present study, we aim to determine the expression and function of pro and mature BDNF in skeletal muscle ischemia reperfusion (IR) injury, a major health concern in many populations. We hypothesize proBDNF may be necessary in facilitating the inflammatory response, while mature BDNF may be necessary in promoting inflammation resolution, myogenesis, and angiogenesis.

Methods and Results: Western blot analysis comparing the BDNF protein levels in brain and skeletal muscle revealed that skeletal muscle expressed more proBDNF than mature BDNF. In skeletal muscle with IR, proBDNF expression increased significantly (p=0.013), and its signaling pathways were upregulated. Furin, the enzyme responsible for the conversion of pro to mature BDNF, was downregulated in early recovery from IR and later upregulated, suggesting the requirement for the balance between pro and mature myokine BDNF throughout the course of recovery from IR. Mice with skeletal muscle specific knockout of BDNF showed that in the absence of BDNF perfusion of the IR limb was increased, while muscle regeneration was slowed compared to wildtype mice.

Conclusions: Myokine proBDNF is necessary to promote inflammation and clearance of damaged tissue, while mature BDNF is necessary to promote skeletal muscle regeneration. By determining their functional role and underlying mechanism, BDNF and proBDNF may be potential targets for treatment of skeletal muscle IR injury.
DIFFERENTIAL FATTY ACID UPTAKE IN HUMAN TROPHOBLAST CELLS IS ATP-DEPENDENT AND MITOCHONDRIA-MEDIATED

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**Background:** Physiologic long chain polyunsaturated fatty acid (LCPUFA) biomagnification in the fetus during the third trimester suggests placental fatty acid (FA) transport is ATP-dependent, but its mechanism at a cellular level is poorly understood. This study aimed to determine whether differential FA uptake in human trophoblasts is mitochondria-mediated and consequently drives the poor outcomes associated with gestational diabetes.

**Methods and Results:** Using MitoTracker fluorescent tagging of mitochondria and 4,4-difluoro-3a,4a-diaza-s-indacene, BODIPY, a fluorophore attached to FAs of variable lengths, we tracked live-cell uptake in BeWo and isolated cytotrophoblasts from consenting mothers. Experiments were repeated in the presence of drugs that target mitochondria-governed metabolic processes (oligomycin, 2-deoxyglucose, etomoxir, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP)) and cytochalasin D (CytD), that perturbs the cellular actin. Statistical analysis was conducted using Student's T-test per drug or 2- and 1-way ANOVA to determine FA-specific, drug-based, and interaction effects. Significance was set at p<0.05. Live-cell imaging of BeWo and CTB shows rapid uptake of both BODIPY-C12, which mimics a 16-carbon saturated FA like palmitate, and BODIPY-C16, which mimics a 20-carbon LCPUFA. The time to reach peak uptake was longer for BODIPY-C16 than BODIPY-C12 (p<0.0001). Both FAs colocalize with mitochondria with predominantly perinuclear accumulation. Treatment with oligomycin, 2-deoxyglucose, etomoxir, or CytD significantly decreased ATP levels in BeWo while FCCP did not (p=0.017, 0.002, 0.012, 0.458 respectively; n=3). Mitochondria-targeting ATP-inhibitors, slowed the rate of BODIPY-C16 uptake (p<0.0001 with 2-deoxyglucose and etomoxir; n=4) but tended to increase the rate of BODIPY-C12 uptake (p=0.011 with 2-deoxyglucose; n=4). Primary cytotrophoblasts had a similar trend (n=3). CytD increased rate of uptake for both BODIPY-C12 and C16 (p=0.003, 0.045 respectively).

**Conclusions:** The differential fatty acid uptake demonstrated in human trophoblasts is exacerbated by processes that perturb mitochondria function and ATP production which may explain both placenta lipotoxicity and fetal LCPUFA deficiency associated with gestational diabetes. Funded by NIH/NIGMS 2P20GM103620-06.
Systemic Mapping of Organ Plasma Extravasation Post Myocardial Infarction

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Background: Chronic Heart failure (CHF) is a prevalent disease leading to significant morbidity and mortality. Diffuse vasculopathy is a common feature of the morbidity associated with CHF. Increased vascular permeability leads to plasma extravasation (PEx) that can develop into edema, inflammation, and multi-organ dysfunction. However, a systemic examination of PEx in vital organs among different time windows of CHF has not been performed.

Methods and Results: In the present study, we investigated time-dependent PEx in major visceral organs between sham-operated and CHF rats induced by myocardial infarction (MI). Plasma extravasation was determined by evaluating Evans Blue concentrations colorimetrically at fluorescence excitation wavelength of 620 nm (bandwidth 10 nm) and an emission wavelength of 680 nm (bandwidth 40 nm) at 3 days, 8-10 weeks and 4 months following MI. Heart PEx was initially high at day 3 post MI and then gradually decreased but still remained at a moderately high level at 8-10 weeks and 4 months post MI. Other organs, such as the lungs and liver experienced PEx beginning at day 3 and remained significantly high at both 8-10 weeks and 4 months post MI. Spleen PEx was significantly high at 8-10 weeks and 4 months but not day 3 post MI. For the gastrointestinal (GI) organs there was a general trend for PEx to gradually increase following MI and reach a statistically significant level at 4 months post MI. Renal PEx was initially high at day 3 but returned to normal at 8-10 weeks post MI but was significantly elevated again at 4 months.

Conclusions: In summary, we found that MI generally induces a time-dependent PEx of multiple visceral organs; however, the timing of individual organs to an MI challenge was different, suggesting that different mechanisms may be involved in the pathogenesis of PEx in these vital organs during CHF.
CHEMOTHERAPY INDUCES VASCULAR TOXICITY AND IMPAIRS OVARIAN FUNCTION IN MICE OVARIES

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Background/Hypothesis: While chemotherapy has improved the survival rate of patients with varying cancers, it has also produced off-target toxicities. A study with premenopausal breast cancer patients reported significantly reduced ovarian blood flow and volume immediately following chemotherapy. This indicates chemotherapy can induce vascular damage and hinder ovarian function. Therefore, we hypothesize different chemotherapeutic agents variably affect ovarian vasculature and proangiogenic agents might ameliorate vascular toxicity in the ovary caused by chemotherapy.

Methods and Results: 1-month-old, CD-1 mice were injected intraperitoneally with cisplatin, cyclophosphamide (CPA), or doxorubicin. Ovaries from postnatal day 5, CD-1 mice were cultured with either 4-Hydroperoxy-cyclophosphamide (4-HC) only, 4-HC with vascular endothelial growth factor 165 (VEGF165, proangiogenic), or 4-HC with VEGF165b (antiangiogenic) to examine direct effects of chemotherapy and adjuvants. Ovaries from mice treated with cisplatin (N=9), CPA, or doxorubicin exhibited reduced ovary weight and decreased CD31/PECAM-1 (platelet endothelial adhesion molecule) expression compared to control mice. Ex-vivo cultured ovaries with chemotherapy further demonstrated that CD31 expression in the ovarian cortex decreased after chemotherapy treatment, implying diminished blood vessels. VEGF165 treatment appeared beneficial against 4-HC-related vascular toxicity while VEGF165b appeared to have further diminished CD31 expression compared to 4-HC only treatment.

Conclusions: We demonstrate different chemotherapies impair ovarian function and affect vascularization within mice ovaries. This indicates delivery of nutrients and oxygen to growing ovarian follicles decreases and prevents folliculogenesis within the ovary in addition to depletion of ovarian reserve. VEGF165 plays a beneficial role in ovarian vasculature insult following chemotherapy, suggesting the necessity for the quantification of vascular function parameters using in vivo bioimaging and for elucidating mechanisms of ovarian vascular toxicity. The findings from this study will aid in preserving ovarian function from chemotoxicity in female cancer survivors, leading to maintenance of endocrine function and reproductive lifespan.
ADMINISTRATION OF SELECTIVE PDE4B INHIBITOR A-33 AMELIORATES NON-ALCOHOLIC STEATOHEPATITIS IN MICE

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Background/Hypothesis of the Study: There is a high incidence of liver injury (LI) throughout the world, and there are currently no approved pharmacotherapies. Cyclic adenosine monophosphate (cAMP) is a critical intracellular second messenger involved in numerous signaling cascades and pathways associated with LI. cAMP is degraded to AMP by phosphodiesterase (PDE) enzymes found throughout the body. Previous literature has shown that the PDE4B enzyme is upregulated in states of LI, and selective inhibition of this enzyme can be used as a therapeutic target. However, the applicability of PDE4 inhibitors as therapeutics is limited due to adverse gastrointestinal effects. A-33 (2-[4-[[2-(5-Chloro-2-thienyl)-5-ethyl-6-methyl-pyrimidin-4-yl]amino]phenyl]acetic acid) is a commercially available highly selective PDE4B inhibitor that could prove helpful in treating LI. We hypothesize that the administration of free A-33 will ameliorate the signs and symptoms of LI.

Methods and Results: Mice were fed a methionine and choline-deficient (MCD) diet to model non-alcoholic steatohepatitis (NASH) for four weeks before beginning treatment with free A-33 through a daily intraperitoneal injection at a dose of 0.3mg/kg and 1mg/kg. Mice were treated for two weeks before being sacrificed and having their tissue and blood collected. RNA, Protein, and Triglycerides were extracted and analyzed. Tissue was fixed in formalin and used for histological imaging. Blood was analyzed for aminotransferase levels. We observed that mice treated with A-33 had fewer histopathological signs of liver injury and showed a statistically significant decrease in liver-to-bodyweight ratio as well as serum aminotransferase and total bilirubin levels.

Conclusions: Administration of free A-33 to mice chronically fed an MCD diet ameliorated the histopathological signs of NASH and reduced serum aminotransferase and bilirubin levels. Future studies into this line of inquiry will continue to analyze the molecular effects of A-33, examine this molecule’s efficacy in animal models of alcohol-associated liver disease, and explore formulation strategies to make this an effective therapeutic in various liver diseases.
SATURATED FREE FATTY ACIDS INDUCE TROPHOBLAST LIPOAPOPTOSIS AND SUBCELLULAR STRESS

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Background: Maternal obesity, a metabolic condition, has become a major public health concern among the women of reproductive age owing to an increased risk for the development of pregnancy complications, including gestational diabetes, pre-eclampsia, maternal inflammation, intrauterine growth retardation and large-for-gestational-age infants. Studies have also shown that maternal obesity could potentially impact the metabolic health of newborns and could increase the risk of future development of metabolic syndromes like obesity, diabetes and cardiovascular diseases in offspring.

Methods and Results: Studies in our lab showed that increased saturated free fatty acids in maternal circulation as a result of increased adipose tissue lipolysis induced trophoblast apoptosis, commonly known as lipoapoptosis. Trophoblasts exposed to physiological concentration of Palmitic (PA) and Stearic acid (SA) showed a concentration dependent increase in cell cytotoxicity and caspase 3/7 activity. While PA and SA increased trophoblast cytotoxicity, Oleic acid (OA), a monounsaturated fatty acid protected cell against PA and SA induced cell cytotoxicity. We showed increased cleaved caspase 3 and PARP levels in cells treated with PA and SA.

Conclusions: To further characterize the mechanism behind cell cytotoxicity, we hypothesize that upon exposure to trophoblasts, free fatty acids induces organelle stress, in particular endoplasmic reticulum stress (ER stress) and MAPK activation, which could potentially mediate cell cytotoxicity by apoptosis. Initial results show that PA induces MAPK activation (JNK and ERK), phosphorylation of critical mediators of ER stress (IRE1α) and nuclear localization of CHOP in trophoblasts, thereby potentially contributing to lipoapoptosis induction.
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PALMITOLEATE PROTECTS ZIKA VIRUS-INDUCED APOPTOSIS IN NEURONAL CELLS

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Background/Hypothesis: Zika virus (ZIKV) infection during pregnancy leads to the development of major fetal complications such as microcephaly and congenital Zika syndrome affecting fetuses. ZIKV crosses the fetal blood-brain barrier and cause severe neuronal cell apoptosis and damage. We had earlier demonstrated that palmitoleate an omega-7 monosaturated fatty acid protects ZIKV-induced apoptosis in placental trophoblasts. In the present study, we focused on the protective role of palmitoleate against ZIKV-induced neuronal cell apoptosis.

Methods and Results: Neuroblastoma (SHSY5Y) and glioblastoma (A172) cell lines were infected with 0.1 and 1 multiplicity of infection (MOI) of r-MRV (recombinant Ugandan MR766 ZIKV strain). Palmitoleate (200 µM) was treated using 1% BSA containing media post infection. Apoptosis was characterized using biochemical and structural markers such as caspase 3/7 activity (fold change) and changes in nuclear morphology (apoptotic nuclei percentage), respectively. The results show that the 1 MOI of r-MRV infection significantly increased caspase 3/7 activity and percent apoptotic nuclei levels. Treatment of pan-caspase inhibitor to the neuronal cells that are infected with ZIKV (1 MOI) showed significant decrease in ZIKV-induced apoptosis. In addition, treatment of palmitoleate (200 µM) to ZIKV-infected SHSY5Y cells showed a significant decrease in caspase activity and percent apoptotic nuclei compared to ZIKV infected cells alone. These data show that palmitoleate significantly decrease ZIKV-induced neuronal apoptosis. The mechanism of palmitoleate protection is currently underway.

Conclusions: Our findings show that ZIKV induced caspase dependent apoptosis and treatment of palmitoleate dramatically prevents ZIKV-induced neuronal apoptosis.
GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY BLUNTS SKELETAL MUSCLE HYPERTROPHY

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Background and Hypothesis: Skeletal muscle mass and function decline with age. This contributes to reduced quality of life, increased fall risk, and mortality. A comprehensive understanding of the mechanisms controlling skeletal muscle adaptability is therefore of significant clinical and therapeutic importance. While great progress has been achieved in the molecular pathways associated with muscle growth (e.g., ribosome biogenesis and protein synthesis) there is little information of how essential building blocks such as nucleotides contribute to muscle hypertrophy. Our laboratory recently demonstrated that the pentose phosphate pathway (PPP), particularly glucose-6-phosphate dehydrogenase (G6PD), is upregulated during hypertrophy. It has been demonstrated that G6PD is essential for cell growth by facilitating nucleotide synthesis. Therefore, we hypothesize that G6PD deficiency lowers capacity for nucleotide synthesis which would lower transcriptional capacity through impaired ribosome biogenesis, decreasing muscle growth.

Methods and Results: Adult (6-8 mo) male and female Sprague-Dawley rats wild-type or mutant (G6pdS188F) were used in this study. The G6pd mutant rats harbor a nonsynonymous (S 188F) substitution in the coding region of the G6pd gene, near the G6pd catalytic domain, resulting in an 80% decrease in G6pd activity. To induce hypertrophy, rats underwent 10 days of either sham or synergist ablation (SA) surgeries, a well-established rodent model of muscle hypertrophy. G6PD activity was significantly lower in the plantaris muscles of mutant rats (p<0.05). Following SA, reduced G6pd resulted in blunted muscle hypertrophy and total RNA failed to increase, suggesting defective nucleotide synthesis. It is estimated that total RNA approximates ribosome content, given that ~80% of the RNA in the cells is ribosomal RNA. Corresponding with total RNA, c- MYC, a “master regulator” of transcription and growth, and 28s, a large ribosomal subunit, were reduced in mutant rats.

Conclusions: While very preliminary, our results suggest that G6PD has a key role during skeletal muscle hypertrophy, likely due to its role in nucleotide synthesis.
Prenatal Exposure to Alcohol Impairs Responses of Cerebral Arterioles to Activation of Potassium Channels: Role of Oxidative Stress

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Background: Potassium channels play an important role in the basal tone and dilation of cerebral resistance arterioles in response to many stimuli. However, the effect of prenatal alcohol exposure (PAE) on specific potassium channel function remains unknown. The first goal of this study was to determine the influence of PAE on reactivity of cerebral arterioles to activation of $K_{ATP}$ and BK channels. Our second goal was to determine whether oxidative stress contributed to potassium channel dysfunction of cerebral arterioles by PAE.

Methods and Results: We fed Sprague-Dawley dams a liquid diet with or without alcohol (3% ethanol) for the duration of their pregnancy (21-23 days). We examined in vivo responses of cerebral arterioles in control and PAE male and female offspring (14-16 weeks after birth) to activators of potassium channels, iloprost (BK channels) and pinacidil ($K_{ATP}$ channels), before and following inhibition of oxidative stress with apocynin. We found that PAE impaired dilation of cerebral arterioles in response to activation of potassium channels with iloprost and pinacidil, and this impairment was similar in male and female rats. In addition, treatment with apocynin reversed impaired vasodilation to iloprost and pinacidil observed in PAE to that observed in control rats. This effect of apocynin also was similar in male and female rats.

Conclusions: PAE induces dysfunction in the ability of specific potassium channels to dilate cerebral arterioles which appears to be mediated by an increase in oxidative stress. We suggest that these alterations in potassium channel function may contribute to the pathogenesis of cerebral vascular abnormalities and/or behavioral/cognitive deficits observed in fetal alcohol spectrum disorders.
UNCOVERING TUMOR-PROMOTING ROLES OF ACTIVIN A IN PANCREATIC DUCTAL ADENOCARCINOMA

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Background/Hypothesis: Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancer types with a 5-year overall survival of approximately 10%. Excessive tumor fibrosis and involuntary weight loss (cachexia) are recognized as primary causes of poor response to chemotherapy and death. Nonetheless, little is known regarding what mediates tumor fibrosis and cachexia. Activin A is a dimer of Inhibin βA that is encoded by the INHBA gene and is overproduced from PDAC cancer cells. Serum Activin A levels are positively correlated with stage and cachexia in PDAC patients. Thus, we hypothesize that tumor Activin A suppression would improve PDAC prognosis.

Methods and Results: We employed orthotopic models by implanting mouse PDAC cells into the pancreata of C57BL/6 male mice and grouped them into no treatment (NT) and treatment groups with either scramble (Sc) or Inhba siRNA (Inhba) packaged with CPX nanoparticles, which previously showed tumor-targeted delivery. Sham group served as a control for orthotopic groups. We measured body weight daily and harvested blood and tissues after sacrifice. Pancreatic stellate cells were cultured to confirm the in-vivo observations. Implanted cells generated PDAC tumors, elevated serum Activin A levels, and developed cachexia in the NT and Sc groups when compared to the sham group (p<0.05). When compared to the Sc group, Inhba siRNA significantly decreased tumor growth and serum Activin A levels (p<0.05). Body weight and survival rates were significantly improved by Inhba siRNA (p<0.01). Histologically, suppressed cancer cell proliferation, less accumulation of α-SMA-positive fibroblasts, and high cytotoxic T cell infiltration characterized tumors from the Inhba group. Furthermore, Activin A did not influence cancer cell growth but promoted the accumulation of α-SMA-positive fibroblasts in vitro, whose conditioned media promoted cancer cell growth (p<0.05).

Conclusions: Our data indicate therapeutic potentials of targeting tumor INHBA expression which warrant further studies for its application in clinical settings.
ENHANCED EXPRESSION OF SGLT2 IN RATS WITH CONGESTIVE HEART FAILURE IS MEDIATED BY PHOSPHORYLATION OF PKA-ERK AND ENHANCED EXPRESSION OF CAVEOLIN-1

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Background: Recently we demonstrated that enhanced sodium-glucose cotransporter 2 (SGLT2) is an important mediator of sodium reabsorption in rats with congestive heart failure (CHF). Interestingly there was enhanced translocation of SGLT2 expression in the luminal membrane of proximal tubular cells in CHF. However, the molecular mechanism for such overexpression and translocation of SGLT2 during CHF remains to be identified.

Methods and Results: The current study evaluated the involvement of protein kinase A (PKA), extracellular signal-regulated kinases (ERK1/2), exchange protein activated by cAMP (EPAC1) and phosphorylation of PKA-ERK1/2 in this pathway. Caveolin-1, a key regulatory protein for translocation of SGLT2 to the luminal surface within the proximal tubular cells was also evaluated. Male Sprague Dawley rats were subjected to either left coronary artery ligation (n=6) or Sham surgery (n=6) and studied after 4 weeks. Cortical region of kidneys from Sham and CHF rats was harvested and used for the analysis. Western blots were performed to analyze the protein expression levels, whereas qPCR was carried out to evaluate mRNA levels. There was significantly enhanced protein expression of SGLT2 (0.83 ± 0.06 vs. 0.58 ± 0.05, P<0.05) in CHF kidneys compared to Sham as reported previously. Phosphorylated ERK1/2 (0.03 ± 0.01 vs. 0.02 ± 0.01, P<0.05) and PKA (0.05 ± 0.01 vs. 0.01 ± 0.01, P<0.05) were also enhanced significantly in CHF. Concurrently, Caveolin-1 (0.07 ± 0.02 vs. 0.01 ± 0.01, P<0.05) was also enhanced in CHF. Whereas the expression of EPAC1 and ERK1/2 were not altered. In contrast, mRNA level of ERK1 (1.4 ± 0.4 vs. 2.3 ± 1.5, P=0.54, n=6), Caveolin1 (0.5 ± 0.2 vs. 2.2 ± 1.3, P=0.20, n=6), and SGLT2 (0.23 ± 0.06 vs. 0.46 ± 0.24, P<0.05, n=6) were lower in CHF, whereas ERK2 and PKA did not change.

Conclusions: In conclusion, these findings indicate a potential role for the PKA-ERK1/2 pathway in the overexpression of SGLT2 in CHF. These results also purport a role for Caveolin1 in the translocation of SGLT2 to the luminal membrane of proximal tubular cells in rats with CHF.
The Activation of 26S Proteasomes by PKA Protects Against Chronic Kidney Injury Induced by High Fat Diet

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Background: Abnormal 26S proteasome activities are associated with renal pathogenesis. Ser14-Rpn6 phosphorylation is responsible for the activation of 26S proteasomes by cAMP-dependent protein kinase (PKA). This study aimed to investigate the role of Ser14-Rpn6 phosphorylation in regulating kidney function in high-fat diet (HFD)-fed mice.

Methods and Results: Male adult Rpn6^{S14A/S14A} knock-in (S14A) and wild-type (WT) mice were fed with regular chow or HFD (60% of calories from fat) for 14 weeks, resulting in four groups (WT+Chow, WT+HFD, S14A+Chow and S14A+HFD, n=5-6/group). Mice were put in the metabolic cage to collect urine. Kidney tissue was processed to measure proteasome activity, ubiquitinated protein, inflammatory and kidney injury markers. Serum creatinine, urinary sodium excretion and protein concentration were measured. Fourteen weeks of HFD feeding caused significant decreased proteasomal chymotrypsin-like activity (1.50±0.04 vs. 2.90±0.17, \(P<0.01\)) and trypsin-like activity (1.92±0.07 vs. 2.30±0.07, \(P<0.05\)) in S14A mice compared to the WT+Chow mice. Native gel analysis confirmed that HFD decreased doubly-capped 26S proteasome activity (0.030±0.002 vs. 0.060±0.004, \(P<0.05\)) in S14A mice. In the kidney of S14A mice with HFD, there were significant increases in the expression of ubiquitinated proteins and phosphorylation of PKA substrates by western blotting analysis. We also found that HFD induced higher renal inflammatory cytokine IL-6 expression, immune cell infiltration and tubular injury marker NGAL in S14A mice. Further, HFD S14A mice showed significant kidney dysfunction with reduced urine volume (0.12±0.06 vs. 0.87±0.09ml/day, \(P<0.0001\)), and sodium excretion (66.1±13.1 vs. 111.9±9.9µEq/day, \(P<0.05\)), increased serum creatinine (4.06±0.38 vs. 2.60±0.40ng/µl, \(P<0.05\)) and urinary protein concentration (119.6±16.6 vs.70.9±8.4µg/µl, \(P<0.05\)) compared to the normal diet WT mice.

Conclusions: The results suggest that proteasome activation by PKA protects against HFD-induced kidney injury. Blocking Ser14-Rpn6 phosphorylation impairs renal proteasome function and accumulates ubiquitinated proteins in renal tubules, thereby exacerbating inflammation and kidney injury induced by HFD.
RNA SEQUENCING ACROSS LIFESPAN OF THE CARDIAC SPECIFIC CRYAB$^{R120G}$ PROTEOTOXIC STRESS MOUSE MODEL

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**Background/hypothesis**: Over 23 different mutations in the CRYAB gene have been associated with human cardiomyopathies. One of the mutations CryAB$^{R120G}$ expressed exclusively in mouse cardiomyocytes causes aberrant protein aggregation in cardiomyocytes and recapitulated desmin-related cardiomyopathy (DRC), which progresses to congestive heart failure and premature mortality. We therefore hypothesized that transcriptional profiling of the CryAB$^{R120G}$ mutant would reveal transcriptional responses to increased proteotoxic stress and cardiomyopathy progression to identify novel targets and pathways that would lend themselves to genetic or pharmaceutical manipulation to improve outcomes from proteotoxic cardiomyopathies.

**Methods and Results**: RNA sequencing of wild type (WT) vs. CryAB$^{R120G}$ hearts revealed only ~20 significantly differentially expressed genes at 1 month. Seven of these genes are related to Nrf2 mediated Oxidative Stress or Nitric Oxide production. At 3 months there is an increase to ~500 differentially expressed genes overrepresented by myogenesis, fibroblast, and inflammatory signaling genes. By 6 months, ~2000 genes become differentially expressed with mitochondrial dysfunction becoming the most significant differentially expressed group. Looking strictly at genes with the largest copy number changes reveals a different cellular landscape in which ubiquitin ligases, autophagy/aggrephagy, and sarcomere RNAs are highly upregulated at all ages as well as hypertrophic and dilated cardiomyopathy genes. However, only 10 genes have significant differential expression at all three ages measured, including the heart critical kinase SBK2, which has been shown to localize to the sarcomere but its kinase function is unknown.

**Conclusions**: RNA sequencing of the CryAB$^{R120G}$ hearts indicate gene expression becomes more extremely diverged from WT hearts with age with increased stress genes and protein clearance genes. The SBK2 kinase is differentially expressed at all ages and our future work will establish SBK2's kinase function in the heart and determine if overexpression can be protective from proteotoxic cardiomyopathy.
REGULATION OF TAp63 BY CHEMOTHERAPEUTIC AGENTS IN THE OOCYTES OF PRIMORDIAL FOLLICLES

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Background/Hypothesis: Women are born with a finite number of oocytes, which form the ovarian reserve. Advances in cancer therapy have significantly increased the survival rate of cancer patients but have also caused long-term, off-target effects in other organs including the ovary. Depletion of the ovarian reserve by cancer therapy causes premature ovarian insufficiency (POI), leading to endocrine dysfunction and infertility. TAp63, detected as an inactive and dimeric form in oocytes, is considered a critical factor for quality control of germ cells. Double-strand breaks (DSBs) caused by irradiation and chemotherapy hyperphosphorylate TAp63 by activating CHK2 and CK1. The active and tetrameric p-TAp63 acts as a transcription factor and induces apoptosis via transcription of puma and noxa in oocytes in an irreversible way. However, the correlation between TAp63 intensity and apoptosis in oocytes is not well-known. We hypothesize that TAp63 expression is translationally regulated by DNA damage in oocytes.

Methods and Results: Ovaries collected from postnatal day 5 (PD5) CD-1 mice were cultured with 4-hydroperoxy CPA (4-HC), a cyclophosphamide metabolite, for 6 hours at the presence and absence of translational elongation inhibitor, cycloheximide to compare the expression level of TAp63 through immunoblotting and immunofluorescence assay. The number of γH2AX and cleaved PARP positive oocytes were also measured. Our results showed that the intensity of TAp63 was significantly higher in oocytes with chemotherapy treatment in vitro. However, γH2AX and cleaved PARP were not highly expressed in the oocytes. Therefore, translation of TAp63 occurred prior to turning on downstream markers of the apoptotic pathway by chemotherapy in the oocytes.

Conclusion: The expression level of TAp63 as a key, quality-control factor in oocytes is controlled by cancer therapy. Thus, it is critical to understand the regulatory mechanism of TAp63 to protect oocytes in the ovary of cancer patients.
SKELETAL MUSCLE-RELEASED EXTRACELLULAR VESICLES CONTRIBUTE TO POST-EXERCISE HYPOTENSION

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Background and Hypothesis: Post-exercise hypotension (PEH) is a phenomenon of a sustained reduction in blood pressure (BP) after a single bout of exercise that underlies amelioration of hypertension by chronic exercise training (ExT). Although several neuro-humoral factors were considered as potential contributors, the exact mechanism(s) remain to be elucidated. Extracellular vesicles (EVs) are cell-derived lipid bilayer-delimited particles, which play a critical role in intercellular communication. While EVs have been suggested to mediate ExT-benefits, their implications in BP regulation are not well defined. New mouse proteomic data from our laboratory demonstrated that 3-week ExT upregulated a large group of antioxidant enzymes in muscle and plasma, leading us to hypothesize that contracting muscle can release antioxidant-enriched EVs into blood and play a role in PEH.

Methods and Results: Experiments were carried out in 32 isoflurane-anesthetized male SD rats, prepared for electrical stimulation of the sciatic nerve, hindlimb muscle contraction, femoral arterio-venous blood collection, and BP recording. Plasma and muscle EVs were isolated using ultracentrifugation, followed by NanoSight300 measurement of concentration and Western blot assay of antioxidant protein cargo. We found that (1) stimulation of intact sciatic nerve evoked muscle contraction accompanied by a biphasic change in BP, with an initial hypertension followed by a later hypotension. (2) Stimulating the peripheral end of the sciatic nerve induced muscle contraction with only the hypotensive phase. (3) Femoral venous blood contained more antioxidant (SOD2 and GSTA2)-enriched EVs than arterial blood after 1-hour of contraction of muscle where antioxidant-enriched EVs also increased. (4) Intravenous injection of muscle-EVs (100ug cargo proteins) significantly reduced BP with a maximal change in -14±3.2mmHg compared with baseline (p=0.003; n=5).

Conclusions: The above results demonstrate that contracting muscle releases antioxidant-EVs, leading to a reduction of BP. This study reveals a novel mechanism underpinning PEH and provides a potential strategy to treat hypertension.
AMELIORATIVE EFFECT OF PIOGLITAZONE ON ISOPROTERENOL INDUCED HEART FAILURE IN WISTAR RATS

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Background: Heart failure (HF) is a global pandemic with an estimated prevalence of >37.7 million individuals worldwide and confers a substantial burden to the health-care system. Our previous study has indicated that agonist of transcription factor PPAR-α ameliorated the Isoproterenol (ISO) induced heart failure in Wistar rats via its ability to improve cardiac metabolism and anti-oxidative property. In the present study we aimed to evaluate the efficacy of PPAR-γ agonist, Pioglitazone in ISO induced heart failure in rats. Pioglitazone is a well-known member of thiazolidinediones (TZDs) class of drug and selective agonist of PPAR-γ. We hypothesize that ability of Pioglitazone to regulate inflammatory and immune response may confer cardioprotective effects.

Methods and Results: To induce heart failure, rats were administered with Isoproterenol (85 mg/kg, s.c.) for two consecutive days and kept for one week. In the treatment group, after ISO administration, rats were given Pioglitazone (3mg/Kg) orally by gavage daily for one week. ISO treated rats showed no change in hemodynamic parameters however, left ventricle functions and baroreflex sensitivity (BRS) were significantly (p<0.01) decreased. Serum levels of CK-MB, MDA, TNF-α, ICAM-1 along with cardiac expression of NADPH oxidase and NF-κB were increased (p<0.01) significantly. Treatment with Pioglitazone significantly improved the ISO induced oxidative stress, cardiac injury, inflammation, lipid profile, left ventricular functions and consequently restored the BRS.

Conclusions: Hence, present study highlights that Pioglitazone exerts cardioprotective efficacy possibly due to blockade of NF-κB pathways and its antioxidant property.
EFFECT OF HIGH-FAT DIET AND TLR7 AGONIST ON THE DEVELOPMENT OF METABOLIC SYNDROME AND LUPUS IN FVB/N MICE

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Background/hypothesis of the study: Lupus patients have a high incidence of metabolic syndrome. Toll like receptor-7 (TLR7) activation is an important driver of autoimmunity in lupus. A six-week study from our lab demonstrated hyperinsulinemia in TLR7 agonist imiquimod (IMQ)-high fat diet (HFD) treated female C57BL/6 mice. Since mouse strains differ in their susceptibility to metabolic syndrome and target organ damage, the present study investigated whether prolonged exposure (12 weeks) to HFD and IMQ can promote metabolic syndrome and lupus in female FVB/N mice.

Methods and Results: Female FVB/N mice were untreated or treated with IMQ and fed a HFD or control diet for 12 weeks. Supporting early-stage induction of autoimmunity, spleen weights were significantly increased in IMQ-treated mice (P_{Treatment} <0.05), an effect largely driven by an increase in the IMQ-HFD treated group (P<0.05 vs untreated HFD mice). Body weights and gonadal fat pad mass were significantly increased by HFD in both IMQ and untreated groups (P_{Diet}<0.001), with the effect on gonadal fat pad mass accentuated by co-treatment with IMQ (P<0.05). Left ventricle weight to tibia length ratio was significantly greater in HFD than control animals (P_{Diet}<0.001) but not affected by IMQ. Fasting blood glucose was not significantly different between the four groups at 6 or 12 weeks. Untreated HFD mice and control diet mice displayed similar responses to oral glucose tolerance testing at 12 weeks. However, IMQ-HFD treated mice displayed glucose intolerance compared to IMQ-treated control diet mice (P<0.05).

Conclusions: Together with our previous study findings, the effect of HFD on fasting blood glucose appears strain dependent, however there is evidence of TLR7 activation promoting HFD-induced dysregulation of glucose handling in both strains. HFD also accentuated IMQ-induced splenomegaly in FVB/N. Accordingly, additional studies are underway to characterize further effects of these combined insults on target organ damage.
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REPEATED SOCIAL DEFEAT STRESS INDUCES AN INFLAMMATORY GUT MILIEU BY COMPROMISING THE MUCOSAL BARRIER INTEGRITY AND ALTERING GUT MICROBIOTA COLONIZATION

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Background: Post-traumatic stress disorder (PTSD), is a mental health condition, triggered by terrifying events in an individual life which affect human health and promote the risk for comorbidities. However, underlying mechanisms remain ill-understood. Nevertheless, PTSD patients harbor a proinflammatory milieu and dysbiotic gut microbiota. Importantly, the gut barrier plays a critical role in maintaining the normal (gut) luminal environment and its dysregulation promotes inflammation. The dysregulated gut barrier function can also modulate the gut luminal environment and thus microbiota colonization and dysbiosis.

Aim: In this study, we examined the effects of PTSD on gut barrier functions in causal association with gut dysbiosis and proinflammatory signaling using the animal model of repeated social defeat stress (RSDS).

Method and Results: Using WB, Immunostaining, qPCR, and DNA sequencing, we found that the RSDS mice gut showed heightened proinflammatory signaling with increased infiltration of immune cells, NF-kB and STAT3 signaling, and gut dysbiosis where Bacteroidetes/Firmicutes ratio was significantly altered compared to control mice. These alterations were associated with significant changes in intestinal antimicrobial responses and mucus secretion. Further, Ussing Chamber analysis showed a significant reduction in trans-epithelial resistance (TEER) which was accompanied by remarkable changes in the expression of multiple tight junction proteins including claudin-2. Treating Caco-2 cells with norepinephrine, a neurohormone upregulated in RSDS mice, inhibited TEER, and upregulated claudin-2 expression.

Conclusions: We here demonstrate a causal link between PTSD, gut barrier dysregulation, Inflammation, and gut dysbiosis, which may affect the health and the risk for comorbidities in PTSD patients in an interdependent manner.
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Abstract Submission Deadline 05 December 2022
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This collection is led by Guest Editors Dr. William C.W. Chen, Hong Zheng, and Yi-Fan Li at the University of South Dakota, Dr. Surabhi Chandra at the University of Nebraska at Kearney, and Dr. Harold D. Schultz at the University of Nebraska Medical Center. Prospective authors are encouraged to identify major challenges to their field of interest, summarize recent developments and major accomplishments in their field, explore the underlying molecular and cellular mechanisms, provide new methods or solutions to those challenges (with a translational emphasis if applicable), or share their thoughts about the future.

Keywords: molecular physiology, cellular physiology, integrative physiology, tissue physiology, systems physiology, mechanism, translation, genetics, molecular, cellular, tissue, organ, physiology

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