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14. ABSTRACT

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RPPR Final Report
as of 26-Jan-2023

Agency Code: 21XD

Proposal Number: 75715BBRIP **Agreement Number: W911NF-20-1-0155**

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Report Date: 31-Aug-2021 Date Received: 25-Jan-2023
Final Report for Period Beginning 01-Jun-2020 and Ending 31-May-2021
Title: Multi-Well Extracellular Flux Analyzer for Mitochondrial Stress Arrays
Begin Performance Period: 01-Jun-2020 **End Performance Period:** 31-May-2021
Report Term: 0-Other

Submitted By: PhD Natalie Gassman Email: nrg2@uab.edu
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STEM Degrees: **STEM Participants:**

Major Goals: Project 1: Metabolic reprogramming induced by environmental exposures
Project 1A: Modulation of metabolism by environmental toxicants
BPA induces a wide variety of cellular effects, though two common mechanisms have been found across tissues and organisms. These mechanisms are epigenetic reprogramming and oxidative stress. Our interest has been in oxidative stress induced by BPA. As part of this work, we have noted BPA induces oxidative stress, specifically within the mitochondrial compartment. This mitochondrial-specific stress could damage mitochondrial DNA and initiate bioenergetic changes that contribute to obesity and cancer development. Importantly, while other studies have noted mitochondrial stress in the presence of physiologically relevant concentrations of BPA (10-100 nM), no mechanistic study of bioenergetic changes has been conducted. The dedicated Seahorse instrument will support our investigations into the metabolic-specific effects of BPA and their impact on mitochondrial function.
In addition to studies involving BPA, our laboratory has begun investigating DHA, the active ingredient in sunless tanning agents. DHA is also produced by electronic cigarette (e-cigarette) vapor. Similar to tobacco cigarettes, e-cigarettes can have long-term health consequences for users. However, there are additional unknown health effects from the flavoring agents used in these products. DHA is a three-carbon sugar in e-cigarette vapor produced by the combustion of other flavoring agents. DHA can be phosphorylated by cells to DHAP (dihydroxyacetone phosphate) and enter glycolytic pathways.
We previously determined that doses of DHA, consistent with consumer exposures to sunless tanning products, induced senescence and apoptosis a melanoma cell line. Given that e-cigarette use could result in micromolar to millimolar systemic exposure, we are examining the metabolic changes induced by DHA exposure in lung, liver, and kidney model cell lines. Using fluorescent sensors, we found that millimolar DHA doses induce mitochondrial dysfunction and alter mitochondrial morphology in kidney and liver cells. We have also observed loss of ATP production and decreased lactate production using indirect luminescence assays. These results indicate that mitochondrial stress and glycolytic changes are being induced by DHA exposure, but the nature of these changes

and their dynamic behavior need to be observed with the requested Seahorse instrument.

Project 1B: Screening environmental chemicals for metabolic changes – In parallel with our specific studies of BPA and DHA, we have been developing high throughput assays to examine DNA damage and epigenetic changes induced by environmental agents. In a collaboration with the National Toxicology Program, we have received a library of known and unknown environmental agents to screen for their ability to induce DNA damage and epigenetic changes. Part of our analysis will also examine the induction of mitochondrial DNA damage specifically. With the XFe96, we can extend this analysis to include metabolic analysis. This would provide the most comprehensive analysis of the consequences of exposure to environmentally relevant agents. Together, this information will improve our ability to protect cells against exposure injury, mitigate exposure related disease through therapeutic intervention and improve the outcomes of exposed individuals.

Project 2: Metabolic reprogramming induced by imbalances in energy-related B-vitamins
Project 2A. Modulation of cellular metabolism by manipulation of vitamin B3 metabolism
Using a combination of synthetic chemistry and the preparation of isotopically labeled NAD precursors and liquid chromatography coupled mass spectrometry or nuclear magnetic resonance, we have identified and characterized novel precursors to NAD in mammals and humans, evidenced NAD systemic metabolic fluxes and inter-organelle biosynthetic pathway specificity. More recently, we have engaged in establishing a novel biosynthetic pathway to NAD, which uses the reduced form of NR, NRH, and by-passes the conventional NAD biosynthetic pathways. This particular source of NAD could bring new light on the relationship between cellular metabolism, organelle-specific NADH catabolism and metabolic dysfunction. Ultimately, this orally bioavailable NAD precursor could be used as a nutritional supplement during deployment. However, an in-depth understanding of the mode of action of this NAD precursor and its impact on cellular metabolism is required.
Here, an XeF96 system would quantify mitoATP and glycoATP production rates following specific pathway modulation. These new assays allow mitochondrial stress due to B-vitamin availability changes to be measured specifically, which is critical to the flux assays being performed in project 1. Additionally, it allows the glycolytic rate to be calculated separately from the mitochondrial oxidative phosphorylation rate (OXPHOS), providing a more physiologically relevant measurement of the bioenergetics of a cell and how vitamin B1 and B2 supplementation and biosynthetic pathway regulations alter these bioenergetics.

Project 2B: B-vitamin dietary over-abundance and Glucose 6-Phosphate Dehydrogenase (G6PD) Deficiency
Glucose-6-phosphate dehydrogenase (G6PD) deficiency is associated with the reduced stability of the catalytically competent multimeric form of G6PD. G6PD activity is critical to the production of the sugar moiety used in the generation of all nucleic acids, including NAD and NADP from Nam and in maintaining NADPH levels. G6PD-deficient cells often over-express the monomeric G6PD to compensate for the reduced life-time of the multimer, with mutant [G6PD] complexes often functioning at Vmax to maintain G6P oxidation rates and NADPH production. We have synthesized biosynthetic precursors, intermediates and NAD(P) catabolites, with the view of supplementing cultured human hepatic and nephrotic cells expressing G6PD variants. We will establish whether the bioavailability of NAD(P) precursors and catabolites impact glycolysis and oxidative respiration status differently in G6PD deficient cells using the requested XeF96 instrument.

Project 3: The role of vitamin B3 in ovarian cancer recurrence
We are investigating whether a shift in NAD abundance promotes ovarian cancer (OC) cell survival while acting as a switch between glycolysis and oxidative phosphorylation. This work requires the requested XeF96 instrument to investigate the metabolic shifts induced in OC and their dependence on NAD. In addition, with the increasing reliance on PARP inhibition (PARPi) in the treatment of OC, it is necessary that the shifts in cellular energetics induced by this blockade be evaluated in the context of the development of PARPi resistance. Since NAD is generated from supplementation or dietary sources, understanding how NAD bioavailability can be manipulated to increase drug sensitivity and minimize the emergence of aggressive phenotype is critical to develop nutritional interventions in OC patients.
In cisplatin-exposed mice, we observe that the cisplatin-promoted loss of renal NAD and NADH could be remediated by oral supplementation with NRH, indicative of the effective biodistribution of this NAD precursor. Since NAD loss has been shown to subvert nuclear DNA damage repair mechanisms, change the cellular capacity for oxidative phosphorylation and promote glycolysis in several cell based-assays, in cisplatin-exposed OC cells, NAD depletion is likely to globally alter metabolic functions, DNA repair and gene expression and regulation promoting cell survival. To examine this, the effects of supplementing with NAD precursors such as NR and NRH will be measured using the XeF96 seahorse measurements upon exposure to cisplatin. This work will show whether NAD modulation and re-routing of NAD biosynthetic pathways act as drivers of chemo-resistance phenotypes.

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as of 26-Jan-2023

Accomplishments: Accomplishments: Project 1: Metabolic reprogramming induced by environmental exposures
The equipment purchased under W911NF-20-1-0155 allowed us to monitor the metabolic changes induced by environmental toxicants and oxidized metabolites. The 96-well format of the instrument allows highly accurate technical replicates and numerous cell treatment conditions to be tested. We have leveraged the power of this instrument to examine metabolic changes in two projects. The first examined metabolic changes induced by the ubiquitous environmental toxicant bisphenol A (BPA) and a chemical found in electronic cigarettes, dihydroxyacetone (DHA, Project 1A). Using our knowledge of the mechanism of action of bisphenol A, we have been confirming the oxidative stress induced by BPA induces mitochondrial stress and dysfunction. We recently published a review of the importance of BPA's effects on the mitochondria, and the results generated with the instrument from W911NF-20-1-0155 will increase our understanding of BPA exposure and co-exposure effects on the mitochondria. We have also examined the mitochondrial dysfunction and nutrient-sensing changes DHA induces in liver cells. Our work on the changes induced by DHA was recently published in PLoS ONE.
More recently, we have been screening a library of compounds from the National Toxicology Program to determine if environmental toxicants are also mitotoxicants, altering metabolism as well (Project 1B). This work is still developing, but several persistent environmental chemicals show specific mitochondrial dysfunction that reduces oxidative phosphorylation and energetics in cells. We are continuing this work and beginning to utilize advanced mitochondrial-specific fuel flex analysis to understand metabolic switches induced by environmental exposures, which likely contribute to cell senescence, reduced cell function, and contribute to various diseases, including neurotoxicity, obesity, and cardiovascular disease.

Project 2: Metabolic reprogramming induced by imbalances in energy-related B-vitamins
The equipment purchased under W911NF-20-1-0155 allowed us to monitor the metabolic changes induced by supplementation strategies that seek to increase intracellular NAD (Project 2A). NAD intracellular abundance varies greatly due to age, disease, and diet. Using equipment purchased under W911NF-20-1-0155, we also observed that basal bioenergetics were primarily under the control of the riboflavome in hepatocytes through mitochondrial respiration, and nicotinamide and thiamine abundance for glycolysis in epithelial kidney cells. We have developed a novel biosynthetic pathway to NAD, which uses the reduced form of NR, NRH, and by-passes the conventional NAD biosynthetic pathways. Creating NAD through these pathways has highlighted the need for balanced supplementation that doesn't create imbalances in NAD pools leading to increased reductive and mitochondrial stress. We have published a detailed analysis of the effects of NRH on liver and kidney models (PMID: 33166357). Further, we identified cell-specific metabolic consequences of nicotinamide-derived pyridines, which are consequences of oxidative NAD pools in cells (PMID: 33498933). Our novel chemical strategies have identified the existence of oxidized NAD pools that act as pathogenic molecules within cells, altering enzymatic reactions and inducing mitochondrial stress. We continue our studies of oxidative NAD pools and their cell-specific metabolic signatures using the instrument purchased under W911NF-20-1-0155. The 96-well format has been essential for investigating changes in ATP, glycolysis, and oxidative phosphorylation.
Additionally, as part of Project 2B, we are examining the impact of NAD over-abundance on Glucose-6-phosphate dehydrogenase (G6PD) deficiency. ~400 million people are affected by G6PD deficiency, many unaware of their status. We observed that G6PD is inhibited by pyridone derived NADPs, potentially accentuating the response to oxidative stress in G6PD deficient individuals. We are synthesizing biosynthetic precursors, intermediates and masked NAD(P) catabolites to supplement cultured human hepatic and nephrotic cells expressing G6PD variants. Using the requested XeF96 instrument, we will soon establish if the bioavailability of NAD(P) precursors and catabolites impact glycolysis and oxidative respiration status differently in G6PD deficient cells. This project is particularly relevant to the long-term health of G6PD-deficient US service-men and women for whom B-vitamin complexes are used to complement their nutritional RDA.

Project 3: The role of vitamin B3 in ovarian cancer recurrence
We are investigating whether a shift in NAD abundance promotes OC cell survival while acting as a switch between glycolysis and oxidative phosphorylation. Further, with an increased reliance on PARP inhibition (PARPi) in the treatment of OC, cellular energetics induced by this blockade have been evaluated in the context of development of PARPi resistance (PMID: 34731617). Since NAD is generated from supplementation or dietary sources, understanding how NAD bioavailability (PMID: 34806016) can be manipulated to increase drug sensitivity and minimize the emergence of aggressive phenotype is critical to develop nutritional interventions in OC patients.
In cisplatin exposed mice, we observed that the cisplatin promoted kidney tissues degeneration and loss of renal NAD and NADH. Both could be remediated by oral supplementation with NRH, indicative of the effective biodistribution of this NAD precursor (PMID: 31767171). Since NAD loss has been shown to subvert nuclear DNA damage repair mechanisms, changes in the cellular capacity for oxidative phosphorylation and promote glycolysis in a number of cell based-assay, in cisplatin-exposed OC cells, NAD depletion was proposed to globally alter

metabolic function, DNA repair, and gene expression and regulation promoting cell survival. We first tested 17 OC cell lines for NAD biosynthetic enzymes expression, including NNMT, and observed that NNMT and NAMPT were more expressed in cancer cell lines than in normal cell lines. Importantly, NADSYN and NAPRT levels were not significantly different. This was in stark contrast with a 2019 report in Nature by Chowdhry (doi: 10.1038/s41586-019-1150-2), that showed chemoresistance associated with the NAPRT/NADSYN pathway selection. We then realized that our cells had been cultured under aseptic conditions in NAM-only-containing media (PMID: 32130883). The absence of NA in the media selected for sub-clone cells that could survive on NAM as NAD precursor rather than NA and therefore were selected for their ability to use the NAMPT/NNMT pathway. With this knowledge, we now plan to repeat these experiments and determine whether cisplatin sensitivity can be re-introduced in NAPRT-dependent cisplatin-resistant OC cell line through manipulation of the NAD levels with NA, and NAR (the acid form of NR) rather than NAM. To examine this, the effects of supplementing with NA and NAR will be measured in cell survival assays and in XeF96 seahorse measurements of ECAR and OCR, upon exposure to cisplatin. With this work, we have so far validated that the NAD biosynthetic pathways acts as drivers of chemo-resistance phenotypes, selected for by the abundance of and nature of the NAD precursors. This knowledge could offer novel strategies to enhance the efficacy of cisplatin through OC patient-centered nutritional interventions.

Training Opportunities: Nothing to Report

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Natalie Rose Gassman
Person Months Worked: 1.00
Project Contribution:
National Academy Member: N

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Date Submitted: 1/24/23 12:00AM Date Published: 1/1/21 6:00AM
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Article Title: The Biochemical Pathways of Nicotinamide-Derived Pyridones
Authors: Faisal Hayat, Manoj Sonavane, Mikhail V. Makarov, Samuel A. J. Trammell, Pamela McPherson, Natali
Keywords: NAD; nicotinamide; pyridones; redox cofactor
Abstract: As catabolites of nicotinamide possess physiological relevance, pyridones are often included in metabolomics measurements and associated with pathological outcomes in acute kidney injury (AKI). Pyridones are oxidation products of nicotinamide, its methylated form, and its ribosylated form. While they are viewed as markers of over-oxidation, they are often wrongly reported or mislabeled. To address this, we provide a comprehensive characterization of these catabolites of vitamin B3, justify their nomenclature, and differentiate between the biochemical pathways that lead to their generation. Furthermore, we identify an enzymatic and a chemical process that accounts for the formation of the ribosylated form of these pyridones, known to be cytotoxic. Finally, we demonstrate that the ribosylated form of one of the pyridones, the 4-pyridone-3-carboxamide riboside (4PYR), causes HepG3 cells to die by autophagy; a process that occurs at concentrations that are comparable to physiological conce
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Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
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Volume: 17 Issue: 12 First Page #:
Date Submitted: 1/24/23 12:00AM Date Published: 12/1/22 6:00AM
Publication Location:
Article Title: Dihydroxyacetone suppresses mTOR nutrient signaling and induces mitochondrial stress in liver cells
Authors: Arlet Hernandez, Manoj Sonavane, Kelly R. Smith, Jensyn Seiger, Marie E. Migaud, Natalie R. Gassma
Keywords: dihydroxyacetone; mitochondria; liver; metabolism; electronic cigarette; autophagy, lysosomal stress; glycolysis; oxidative phosphorylation; reactive oxygen species
Abstract: Dihydroxyacetone (DHA) is the active ingredient in sunless tanning products and a combustion product from e-juices in electronic cigarettes (e-cigarettes). DHA is rapidly absorbed in cells and tissues and incorporated into several metabolic pathways through its conversion to dihydroxyacetone phosphate (DHAP). Previous studies have shown DHA induces cell cycle arrest, reactive oxygen species, and mitochondrial dysfunction, though the extent of these effects is highly cell-type specific. Here, we investigate DHA exposure effects in the metabolically active, HepG3 (C3A) cell line. Metabolic and mitochondrial changes were evaluated by characterizing the effects of DHA in metabolic pathways and nutrient-sensing mechanisms through mTOR-specific signaling. We also examined cytotoxicity and investigated the cell death mechanism induced by DHA exposure in HepG3 cells. Millimolar doses of DHA were cytotoxic and suppressed glycolysis and oxidative phosphorylation pathways. Nutrient sensing throug
Distribution Statement: 1-Approved for public release; distribution is unlimited.
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
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Volume: Issue: First Page #:
Date Submitted: 1/24/23 12:00AM Date Published: 1/1/23 12:00PM
Publication Location:
Article Title: MYB sustains hypoxic survival of pancreatic cancer cells by facilitating metabolic reprogramming
Authors: Shashi Anand, Mohammad Aslam Khan, Haseeb Zubair, Sarabjeet Kour Sudan, Kunwar Somesh Vikra
Keywords: HIF1?; MYB; hypoxia; metabolic reprogramming; pancreatic cancer
Abstract: Extensive desmoplasia and poor vasculature renders pancreatic tumors severely hypoxic, contributing to their aggressiveness and therapy resistance. Here, we identify the HuR/MYB/HIF1? axis as a critical regulator of the metabolic plasticity and hypoxic survival of pancreatic cancer cells. HuR undergoes nuclear-to-cytoplasmic translocation under hypoxia and stabilizes MYB transcripts, while MYB transcriptionally upregulates HIF1?. Upon MYB silencing, pancreatic cancer cells fail to survive and adapt metabolically under hypoxia, despite forced overexpression of HIF1?. MYB induces the transcription of several HIF1?-regulated glycolytic genes by directly binding to their promoters, thus enhancing the recruitment of HIF1? to hypoxia-responsive elements through its interaction with p300-dependent histone acetylation. MYB-depleted pancreatic cancer cells exhibit a dramatic reduction in tumorigenic ability, glucose-uptake and metabolism in orthotopic mouse model, even after HIF1? restoration.
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
Acknowledged Federal Support: **Y**

Partners

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I certify that the information in the report is complete and accurate:
Signature: Natalie R. Gassman, PhD
Signature Date: 1/25/23 9:51AM

RPRR Final Report
As of January 23, 2023

Major Goals: Purchase a **Seahorse XFe96 Analyzer,** a high throughput extracellular flux analyzer for faculty in the College of Medicine (COM) and the Mitchell Cancer Institute (MCI) at the University of South Alabama.

Accomplishments: Project 1: Metabolic reprogramming induced by environmental exposures- Natalie R. Gassman, PhD; University of South Alabama College of Medicine (COM) and Mitchell Cancer Institute (MCI)- relocated to University of Alabama at Birmingham in August of 2021.

Environmental exposures trigger various cellular responses, from changes in cell signaling cascades to the induction of epigenetic changes or DNA damage. Environmental exposure impact genomic integrity and contribute to aging and disease development. An individual's occupation and lifestyle choices play a significant role in the chemicals and stressors they are exposed to throughout their lifetime and the subsequent risk of developing. Military personnel can experience occupational exposures from their deployments and from prolonged physical and emotional stress that can induce cellular changes impacting both their immediate and long-term health. Understanding how environmental exposures modulate cell function and induce DNA damage or chromatin changes is critical to understanding the consequences of exposure in a population.

The equipment purchased under W911NF-20-1-0155 allowed us to monitor the metabolic changes induced by environmental toxicants and oxidized metabolites. The 96-well format of the instrument allows highly accurate technical replicates and numerous cell treatment conditions to be tested. We have leveraged the power of this instrument to examine metabolic changes in two projects. The first examined metabolic changes induced by the ubiquitous environmental toxicant bisphenol A (BPA) and a chemical found in electronic cigarettes, dihydroxyacetone (DHA, Project 1A). Using our knowledge of the mechanism of action of bisphenol A, we have been confirming the oxidative stress induced by BPA induces mitochondrial stress and dysfunction. We recently published a review of the importance of BPA's effects on the mitochondria (https://doi.org/10.1007/s44169-022-00011-z), and the results generated with the instrument from W911NF-20-1-0155 will increase our understanding of BPA exposure and co-exposure effects on the mitochondria. We have also examined the mitochondrial dysfunction and nutrient-sensing changes DHA induces in liver cells. Our work on the changes induced by DHA was recently published in PLoS ONE (PMID: 36472985).

More recently, we have been screening a library of compounds from the National Toxicology Program to determine if environmental toxicants are also mitotoxicants, altering metabolism as well (Project 1B). This work is still developing, but several persistent environmental chemicals show specific mitochondrial dysfunction that reduces oxidative phosphorylation and energetics in cells. We are continuing this work and beginning to utilize advanced mitochondrial-specific fuel flex analysis to understand metabolic switches induced by environmental exposures, which likely contribute to cell senescence, reduced cell function, and contribute to various diseases, including neurotoxicity, obesity, and cardiovascular disease.

Project 2: Metabolic reprogramming induced by imbalances in energy-related B-vitamins (Marie E. Migaud, PhD; COM-MCI).

Military personnel often experience occupational micronutrient dietary deficiencies from their deployments. Such deficiencies can induce cellular changes impacting both their immediate and long-term health. Understanding how micronutrients modulate cell function, signaling and metabolism is critical to developing functional foods and optimizing nutrition in service men and women.

The equipment purchased under W911NF-20-1-0155 allowed us to monitor the metabolic changes induced by supplementation strategies that seek to increase intracellular NAD (Project 2A). NAD intracellular abundance varies greatly due to age, disease, and diet. Using equipment purchased under W911NF-20-1-0155, we also observed that basal bioenergetics were primarily under the control of the riboflavome in hepatocytes through mitochondrial respiration, and nicotinamide and thiamine abundance for glycolysis in epithelial kidney cells. We have developed a novel biosynthetic pathway to NAD, which uses the reduced form of NR, NRH, and by-passes the conventional NAD biosynthetic pathways. Creating NAD through these pathways has highlighted the need for balanced supplementation that doesn't create imbalances in NAD pools leading to increased reductive

and mitochondrial stress. We have published a detailed analysis of the effects of NRH on liver and kidney models (PMID: 33166357).

Further, we identified cell-specific metabolic consequences of nicotinamide-derived pyridines, which are consequences of oxidative NAD pools in cells (PMID: 33498933). Our novel chemical strategies have identified the existence of oxidized NAD pools that act as pathogenic molecules within cells, altering enzymatic reactions and inducing mitochondrial stress. We continue our studies of oxidative NAD pools and their cell-specific metabolic signatures using the instrument purchased under W911NF-20-1-0155. The 96-well format has been essential for investigating changes in ATP, glycolysis, and oxidative phosphorylation.

Additionally, as part of Project 2B, we are examining the impact of NAD over-abundance on Glucose-6-phosphate dehydrogenase (G6PD) deficiency. ~400 million people are affected by G6PD deficiency, many unaware of their status. We observed that G6PD is inhibited by pyridone derived NADPs, potentially accentuating the response to oxidative stress in G6PD deficient individuals. We are synthesizing biosynthetic precursors, intermediates and masked NAD(P) catabolites to supplement cultured human hepatic and nephrotic cells expressing G6PD variants. Using the requested XeF96 instrument, we will soon establish if the bioavailability of NAD(P) precursors and catabolites impact glycolysis and oxidative respiration status differently in G6PD deficient cells. This project is particularly relevant to the long-term health of G6PD-deficient US service-men and women for whom B-vitamin complexes are used to complement their nutritional RDA.

Project 3: The role of vitamin B3 in ovarian cancer recurrence (Jennifer Scalici, MD COM-MCI; Marie Migaud, PhD, COM-MCI).

NAD is substrate in protein PARylation and deacetylation; events critical to the emergence of aggressive phenotypes of ovarian cancer. Ovarian cancer (OC) patients who initially respond well to platinum-based chemotherapies, most often relapse with the eventual development of chemo-resistant disease, associated with loss of DNA damage repair capacity and dysregulated chromatin acetylome. Initial cisplatin-induced DNA damage stimulates NAD-dependent DNA repair pathways resulting in excessive consumption of NAD through PARP and SIRT activation. To maintain NAD levels and prevent feedback inhibition of PARPs and SIRTs, the effective recycling of the by-product Nam back to NAD is necessary. However, when produced in excess, Nam accumulates and unrecycled Nam is removed by a methylase (NNMT) to generate Me-Nam, without generating NAD. This leads to chromatin hypomethylation, a process which is also associated with aggressive phenotype occurrence. We are investigating whether a shift in NAD abundance promotes OC cell survival while acting as a switch between glycolysis and oxidative phosphorylation. Further, with an increased reliance on PARP inhibition (PARPi) in the treatment of OC, cellular energetics induced by this blockade have been evaluated in the context of development of PARPi resistance (PMID: 34731617). Since NAD is generated from supplementation or dietary sources, understanding how NAD bioavailability (PMID: 34806016) can be manipulated to increase drug sensitivity and minimize the emergence of aggressive phenotype is critical to develop nutritional interventions in OC patients.

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the effects of supplementing with NA and NAR will be measured in cell survival assays and in XeF96 seahorse measurements of ECAR and OCR, upon exposure to cisplatin. With this work, we have so far validated that the NAD biosynthetic pathways acts as drivers of chemo-resistance phenotypes, selected for by the abundance of and nature of the NAD precursors. This knowledge could offer novel strategies to enhance the efficacy of cisplatin through OC patient-centered nutritional interventions.

Publications using DURIP-purchased equipment

At the time of this final report, the **Seahorse XFe96 Analyzer** has been used in two published manuscripts, with two others in preparation.

1) Hayat F, Sonavane M, Makarov MV, Trammell S, McPherson P, **Gassman NR**, Migaud ME. The (bio)chemical diagnosis of nicotinamide-derived pyridones. Int J Mol Sci. 2021 Jan 24;22(3):1145. doi: 10.3390/ijms22031145. PMID: 33498933

2) Hernandez, A; Sonavane M; Smith KR; Seiger, J; Migaud, ME; **Gassman, NR**. Dihydroxyacetone suppresses mTOR nutrient signaling and induced mitochondrial stress in liver cells. PLoS One. 2022 Dec 6;17(12):e0278516. doi: 10.1371/journal.pone.0278516. eCollection 2022. PMID: 36472985

Other users of the Seahorse XFe96 Analyzer have published one manuscript and have two more in preparation. Furthre, a PhD candidate has used this instrument for her thesis investigations.

3) MYB sustains hypoxic survival of pancreatic cancer cells by facilitating metabolic reprogramming. Anand S, Khan MA, Zubair H, Sudan SK, Vikramdeo KS, Deshmukh SK, Azim S, Srivastava SK, Singh S, Singh AP. EMBO Rep. 2023 Jan 2:e55643. doi: 10.15252/embr.202255643. Online ahead of print. PMID: 36592158.

Role of DURIP purchased equipment in other DoD-funded research:

N/A

Role of DURIP purchased equipment in other non-DoD-funded research

1. R01ES032450 (Gassman, PI) 11/19/21-10/15/2025 NIEHS

total award amount $1,231,018

Dihydroxyacetone exposure induces metabolic reprogramming and mitochondrial dysfunction

This work examines the exposure effects of dihydroxyacetone.

Aim 1: DHA incorporation into metabolic pathways alters glycolysis and induces glycosylation protein damage.
Aim 2: DHA exposure alters NAD(P)H pools to induce oxidative stress.
Aim 3: DHA exposure alters cytosolic Ca^{2+} levels and disrupts mitochondrial function.

2. BRASH (Migaud, PI) TRISH/NASA 4/1/2023-3/31/2025

Total award amount $918,940

Controlling NAD(P) Hyper-oxidation to Regulate Repair and Maintenance Processes in Humans in Space

This work examines the biological role of ox-NAD(P) in cells and blood exposed to external stressors.

AIM1: ox-NAD(P) species are produced upon exposure to exogenous stressors and ROS-promoting agents.
AIM2: ox-NAD(P) species alter bioenergetics, transcription, and translation.
AIM3: Reduction of ox-NAD(P) levels through dietary supplementation with NAD precursors.

Training Opportunities: Upon receipt of the instrument, we immediately trained 21 individuals from five laboratories in the use and capabilities of the instruments. Two undergraduates, five graduate students (two masters candidates and two doctoral candidates), seven postdoctoral fellows, two medical students, three technicians, and two principal investigators participated in this training. During the reporting period, two additional principal investigators and the two postdoctoral fellows received training and began using the instrument.