



Bio-Analytical Redox Biology (BARB) Core



The BARB Core is a **Diabetes Research Center** Metabolic and Redox Sub Core (NIDDK P30DK079626) and **UAB Institutional Research Pilot Core**. We are also supported by the **Department of Nutrition Sciences**, the **Heersink School of Medicine**, the **Nutrition Obesity Research Center** (NIDDK P30DK056336), and the **Center for Clinical and Translational Science** (NIH UL1TR003096).



Director: Douglas R. Moellering, PhD,



Operational Director: Melissa J. Sammy, PhD;



Research Assistant: Kelley Smith-Johnston, BS



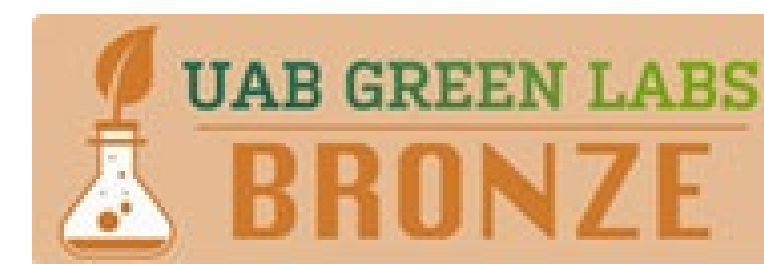
Highlights

State-of-the-Art Equipment, Materials and Facilities

- Psychological/Physiological Stress
- Mitochondrial Electron Transport Chain (ETC) Kinetics
- Oxidative Stress
- High Resolution Respirometry (HRR)
- Extracellular Flux Analysis (XFe96)
- Mitochondrial Isolation



Comprehensive Training



Consultations with Expert Staff

- Experimental Design, Troubleshooting & Data Interpretation.
- Grant Writing (strong letters of support, assay design, methods, budgeting, technical advice, collaboration).

All BARB Core Assays are of the highest quality, rigorously optimized, reproducible and standardized.

Mitochondrial Electron Transport Chain (ETC) Kinetics

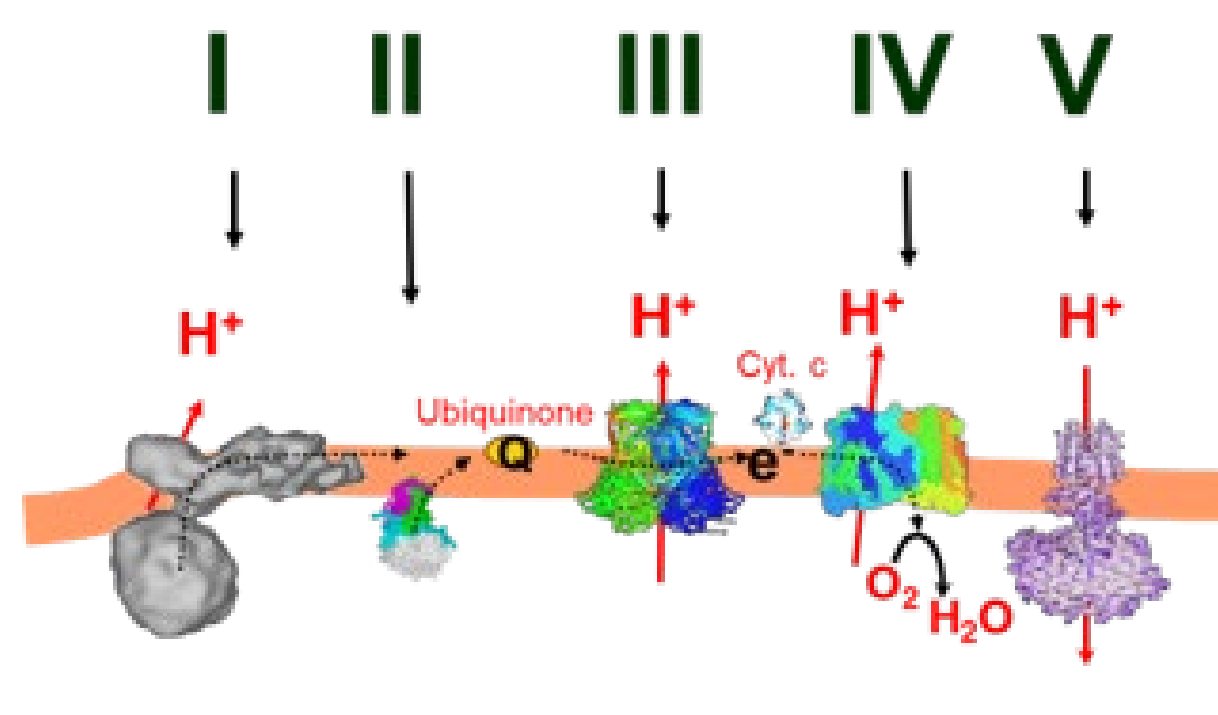
Homogenate or isolated mitochondria can be analyzed for differences in kinetics of each mitochondrial ETC complex in experimental samples compared to controls. Citrate synthase can also be measured as a surrogate for mitochondrial content.

- Complex I (NADH:Ubiquinone Oxidoreductase)
- Complex II (Succinate-Ubiquinone Oxidoreductase)
- Complex III (Decylubiquinol Cytochrome C oxidoreductase)
- Complex IV (Cytochrome c oxidase)
- Complex V (ATP hydrolysis)
- Citrate Synthase

Samples: fresh or frozen tissues or cells (human, rodent, fish, *Drosophila*)

Equipment: Beckman DU 800 Spectrophotometer, Custom-Built Mitochondrial Isolation Stations with Recirculating Chiller

Publications: References 6 - 12.



Cortisol – Chronic Stress

Cortisol is extracted from human nails (finger and/or toenail) and/or hair to measure cortisol levels as a biomarker of stress.

Samples:

- human hair (3 cm length from scalp, 0.65 cm in diameter),
- human finger/toenails (25-50 mg)

Sample Preparation: Non-invasive, collected, stored and shipped to the BARB Core at room temperature, using regular mail, without any special biohazard requirements or costs.

Equipment: Qiagen TissueLyser III, Biotek Synergy 2 Multi-mode microplate reader.

Publications: References 1 - 4.



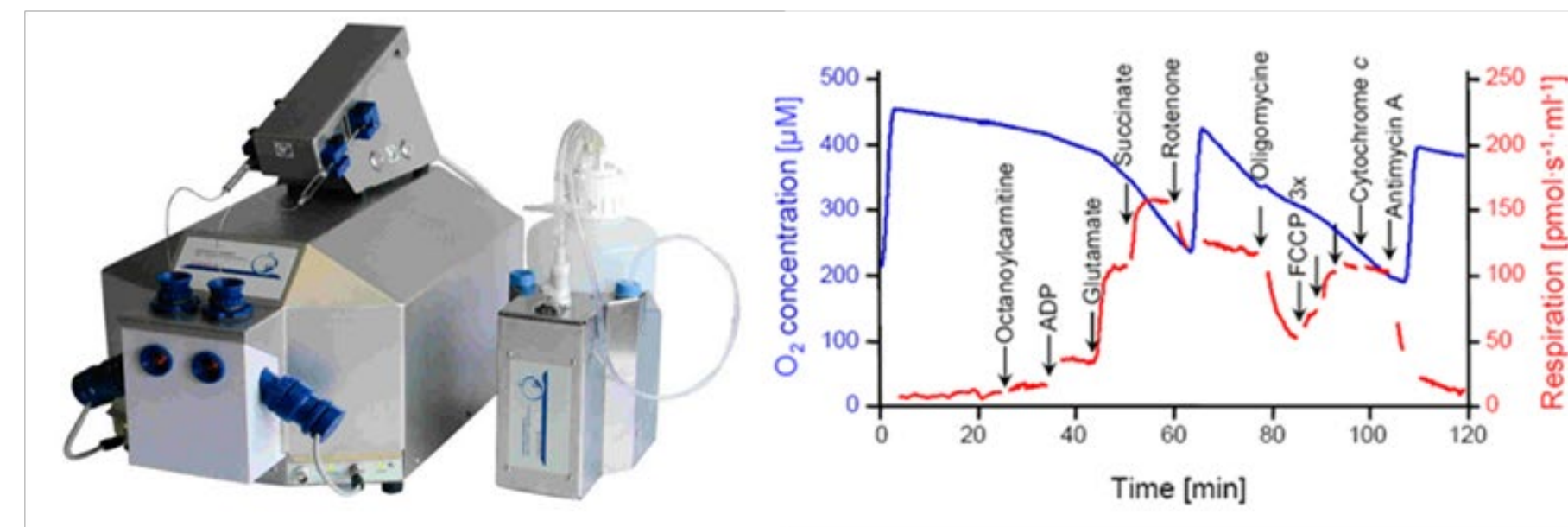
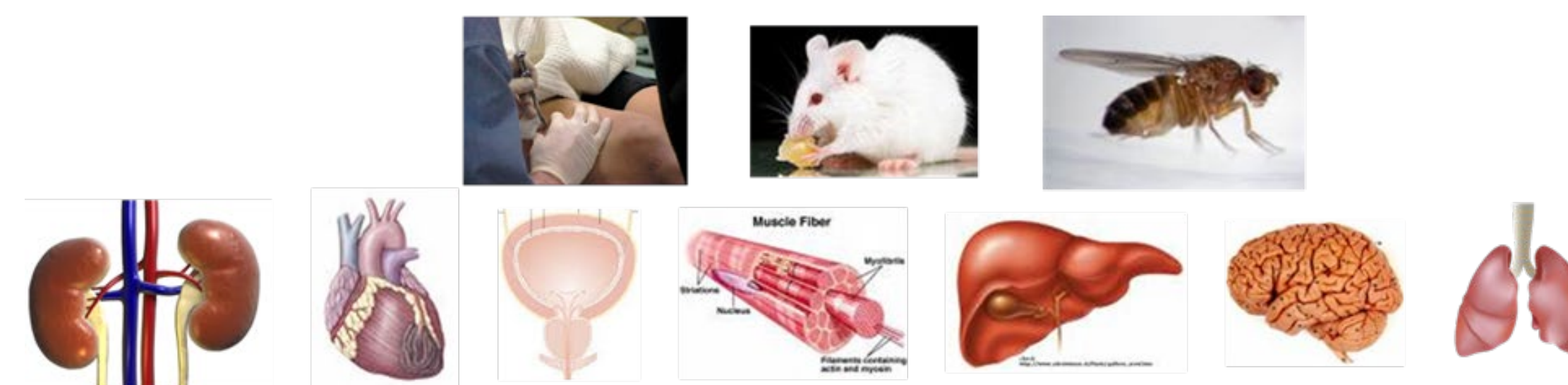
High Resolution Respirometry (HRR)

Mitochondrial function and dysfunction in freshly excised, permeabilized tissue or isolated mitochondria is measured in experimental versus control samples.

Samples: fresh, permeabilized, ex vivo tissues (human muscle, rodent renal cortex, medulla, and microvessels, embryonic and adult heart, gastroc and soleus muscle, hippocampus, bladder, liver) or isolated mitochondria (rodent, fish, *Drosophila*)

Equipment: 3 x Oroboros Oxygraph-2k Fluorometers (LED2-Module Amperometric Add-On)

Publications: References 7, 8, 10, 13 - 18.



Mitochondrial DNA (mtDNA) DAMPs

Cell-free mtDNAs are extracted from human or mouse serum/plasma and measured using qPCR. mtDNAs are damage associated molecular patterns and biomarkers of inflammation, aging and many other diseases.

Samples: frozen serum/plasma (human, mouse)

Equipment: Applied Biosystems StepOnePlus Real-Time PCR System, Applied Biosystems DynaMag Magnet, Biotek Synergy 2 Multi-Mode Microplate Reader, LabNet Plate Spinner

Publications: Reference 5.



Mitochondrial Electron Transport Chain (ETC) Kinetics

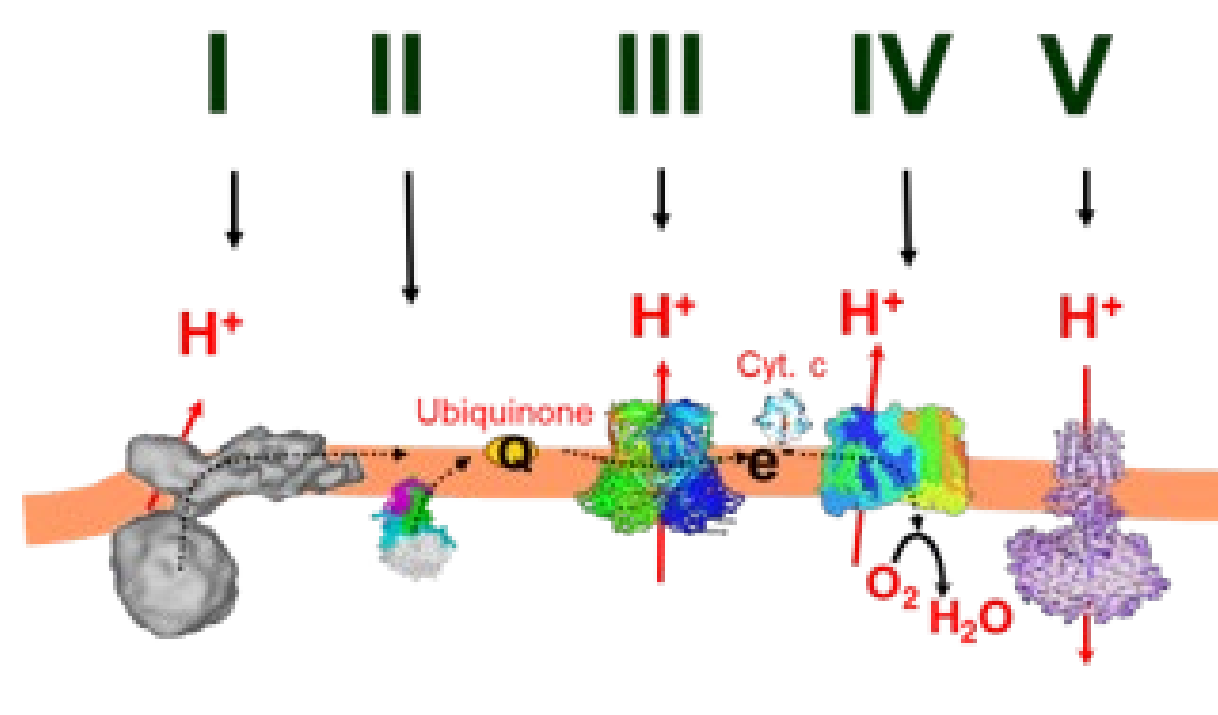
Homogenate or isolated mitochondria can be analyzed for differences in kinetics of each mitochondrial ETC complex in experimental samples compared to controls. Citrate synthase can also be measured as a surrogate for mitochondrial content.

- Complex I (NADH:Ubiquinone Oxidoreductase)
- Complex II (Succinate-Ubiquinone Oxidoreductase)
- Complex III (Decylubiquinol Cytochrome C oxidoreductase)
- Complex IV (Cytochrome c oxidase)
- Complex V (ATP hydrolysis)
- Citrate Synthase

Samples: fresh or frozen tissues or cells (human, rodent, fish, *Drosophila*)

Equipment: Beckman DU 800 Spectrophotometer, Custom-Built Mitochondrial Isolation Stations with Recirculating Chiller

Publications: References 6 - 12.



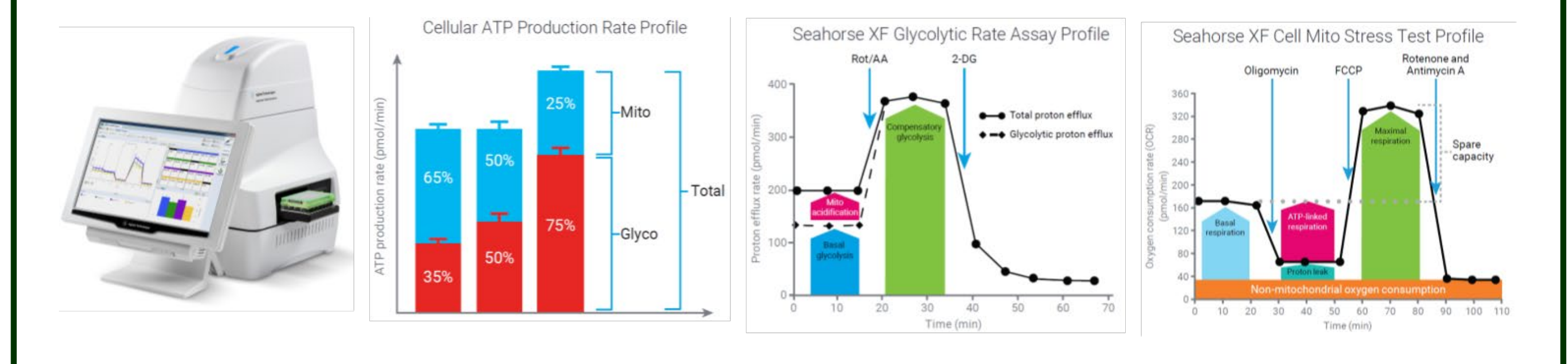
Seahorse Extracellular Flux (XFe96)

Mitochondrial respiration and glycolysis is measured simultaneously in a high throughput 96-well format in adherent cells.

Samples: cultured cells or freshly isolated mitochondria (human, rodent), mycoplasmas.

Equipment: Agilent Seahorse XFe96, high throughput, 96-well format

Publications: References 9, 13, 18 - 24.



Redox Assays

Catalase

Sample: plasma, serum, homogenate
Glutathione Tietze (GSSG and GSH)
Sample: plasma, whole blood
Glutathione Peroxidase (GPX)
Sample: plasma, homogenate

Total Antioxidant Capacity (TAC)

Sample: plasma, serum, urine, saliva
Ferric Reducing Antioxidant Power (FRAP)
Sample: plasma

References

- Knight, R., et al. Clin Nutr ESPEN. 2022.
- Cedillo, Y.E., et al. Int J Behav Med. 2020.
- Fruge, A.D., et al. Cancer Causes Control. 2018.
- Cases, M.G., et al. Contemp Clin Trials. 2016.
- Gilstrap, S.R., et al. Mol Pain. 2023.
- Kelly, N.A., et al. J Appl Physiol. 2014.
- Hunter, G.R., et al. Med Sci Sports Exerc. 2017.
- Moellering, D.R., et al. Arthritis Res Ther. 2023.
- Chocron, E.S., et al. Commun Biol. 2022.
- Ramani, M., et al. Sci Rep. 2019.
- Farias Quipildor, G.E., et al. Geroscience. 2019.
- Bell, M.B., et al. J Appl Physiol. 2019.
- Vamesu, B.M., et al. JCI Insight. 2023.
- Zhao, Q., et al. J Cell Sci. 2022.
- Graham, Z.A., et al. Physiol Rep. 2022.
- Warren, J.L., et al. Med & Sci Sports & Exer. 2020.
- Hunter, G.R., et al. J Appl Physiol. 2019.
- Saini V., et al. Nat Commun. 2020.
- Jones, R.B., et al. Glycobiology. 2023.
- Hinshaw, D.C. et al. Cancer Immunol Res. 2023.
- Hazra, S., et al. Sci Rep. 2022.
- Gupta, P., et al. Cell Rep. 2022.
- Hinshaw, D.C., et al. Cancer Res. 2021.
- Pitale, P.M., et al. Diabetes. 2021.