Abstract:
Background & Aims: Immune-mediated pathologies such as Crohn’s disease and rheumatoid arthritis exhibit an increase in inflammatory cytokines, leukocytes, tissue thickening and damage. Glycolysis, a critical metabolic pathway, is increased in inflammatory conditions such as Crohn’s disease and rheumatoid arthritis. Glycolysis is associated with an enhanced glucose uptake which provides a target that can be exploited for diagnosis and/or treatment. We hypothesize that components of the glycolysis pathway targeted in models of disease will reduce inflammation. To test this, TNFΔARE mice lack the AU rich element (ARE) in the Tnfα gene which delays the decay of TNF mRNA. The excess TNF leads to ileitis and arthritis and provides a model of immune-mediated inflammatory disease. The K/BxN serum transfer model was used as another model of arthritis in HK2ΔARE mice, which ubiquitously express tamoxifen inducible Cre in order to systemically delete hexokinase 2 (HK2). Position emission tomography (PET) scans with [18F]2-deoxyglucose were used to detect glucose uptake in inflamed tissues. TNFΔARE were treated with either PBS or 3-bromopyruvate, an inhibitor of HK2 that reduces glycolysis. K/BxN arthritis was induced injecting intra-peritoneally 150 μL of serum 3 weeks after tamoxifen treatment in the 8-week-old K/BxNΔARE mice and clinical scores were assessed daily. Tissues were collected for histology and scored for inflammation using a system developed to evaluate multi-organ inflammation. Immunohistochemistry and RT-PCR were done to validate biomarkers of glycolysis and inflammation. Results: Preliminary data show that inflamed tissues take up glucose and express molecules associated with glycolysis and inflammation. Inhibiting glycolysis decreases inflammation scores in the gut and joints. Conclusions: Glycolysis is active in inflammation and may provide a target to treat immunemediated disease.

Background:
The Warburg effect has long demonstrated that tumor cells increase glycolytic metabolism that enables proliferation. This shift in metabolism has more recently been recognized in immune-mediated inflammation. While the role of RA and Inflammatory Bowel Disease differ, both show marked inflammation in target tissues with a metabolic shift from oxidative phosphorylation to glycolysis. This shift to glycolysis causes dephosphorylation of AMPK leading to activation of mTOR and transcription factors such as HIFα and a downstream increase in inflammation. HK2 further enhances the transcription of several genes required for glycolysis (Figure 1). In RA, these inflammatory conditions activate and impair Fas/FLIP-like TNFα-synovocytes leading to synovial inflammation, thickening of the synovial lining, and cartilage erosion. In IBD, this shift leads to mucosal lesions, vilus blunting, loss of goblet cells, and cellular infiltrates that cause tissue thickening, all of which contribute to tissue damage and reduced nutrient absorption. By targeting factors of glycolysis for inhibition, inflammatory cells are starved of glucose and become apoptotic, reducing overall tissue inflammation.

Methods:
- Treatment of TNFΔARE mice with 5 mg/kg of 3-bromopyruvate in 0.1 mL PBS vs 0.1 mL PBS injection
- Treatment of 8-week-old K/BxNΔARE mice with tamoxifen and 3 weeks later, given an IP injection with 150 μL of K/BxN serum to induce transfer model
- PET scans of affected mice
- Collection of ileum and ankle joints for histology
- Preparation of tissue – Blending, cutting in half and preparing Swiss roll before fixing tissue in formalin
- Immunohistochemistry and RT-PCR for genes associated with inflammation and/or glucose metabolism

Summary and Conclusions:
- PET scans showed that inflamed tissues in the JARE mice (joints) and K/BxN transfer model (joints) have increased glucose uptake compared to control BL/6 mice. While we did not find evidence of glucose uptake in the ileum on PET, this may be due to the low amount of ileal tissue present in the JARE. PET had previously been successful in the SAMP1/WyImo model of ileitis.
- A significant difference was noted in the inflammatory score of BL/6 vs JARE mouse models. The thickness of the full tissue and mucosal tissue were also noted and can be used as quicker means to compare tissues in some models.
- Inflammatory scores of JARE mice treated with 3-bromopyruvate showed a significant decrease in comparison to those treated with PBS. RT-PCR supported the reduced inflammation seen in histology.
- The JARE/HK2ΔARE mice presented with lower clinical and histological arthritis scores than wild type mice.
- Both murine models presented with similar cellularity and inflammatory changes when compared to the human diseased tissues they represent.
- Glycolysis is increased in inflammatory disorders such as Crohn disease and rheumatoid arthritis. Glycolytic inhibition, such as inhibition of hexokinase 2 could be an effective treatment strategy.