### INTRODUCTION

High-fat (H) and calorie-restricted (E) diets are associated with increased and reduced risk for colorectal cancer (CRC), respectively. Metabolites associated with H- vs. E-associated CRC risk have never been directly compared. How these diets influence proximal (PC), medial (MC), and distal (DC) colon microbiomes and metabolomes has also not been studied. Metabolites that differentiate these groups may aid in developing region-specific biomarkers for diet-associated CRC risk, as well as guide future experiments regarding microbiome-mediated risk for CRC.

### AIM

To characterize the metabolomic profiles resulting from interactions between diet, host, and microbiome that are indicative of region-specific CRC risk.

### Hypothesis:
We hypothesize that H will result in decreased bile acids and increased unsaturated fatty acids of the colon relative to controls, and E in increased vitamin E metabolites and decreased amino acids of the colon relative to controls. We further hypothesize that metabolites associated with differences in the diet will be associated with profile changes in the microbiome and region-specific CRC risk.

### METHODS

**Figure 3.** Colon extract preparation and metabolomic analysis using liquid chromatography-high resolution mass spectrometry (LC-HRMS) and nuclear magnetic resonance (NMR) platforms.

**Figure 4.** Data preparation workflow for peaks detected using HILIC LC positive and negative ionization mass spectrometry. (*Kirwan, 2013, Anal Bioanal Chem*).

**Figure 5.** Representative chromatograms of mouse colon extract analyzed using HILIC LC (positive and negative) ionization MS indicating presence of anticipated classes of metabolites. 1: dipeptides, 2: bile acids, 3: acyl carnitines, 4: fatty acids, 5: lysophosphatidylcholines, 6: amino acids, 7: vitamin E metabolites, 8: glycerophosphocholines, 9: vitamin D metabolites, 10: sugars.

### RESULTS

**Figure 6.** Metabolites found to have a significant association with early/progression diet and ACF interaction are mainly distinct to the early/progression diet group, except for 2 that overlap between early/progression H diet. (Made using Venny 2.1).

**Figure 7.** Colon region-specific differences in numbers of metabolites determined significant for a diet*ACF interaction in mixed-effect modeling of HILIC-MS data.

### CONCLUSIONS

- Metabolites significantly associated with diet and ACF presence are distinct between:
  - H and E diet treatment groups
  - Early and progression diet phases
  - Colon regions
- Metabolism related to the microbiome may be most altered when an H, rather than E or C, diet is consumed during CRC progression
- Distinct metabolites due to diet, ACF presence, and colon region suggest that there are region-specific changes associated with H/E diets, consistent with existing epidemiological literature of different influences of diet on proximal vs. distal CRC risk (Hu, 2007, Eur J Cancer Prev)
- Pathway analyses of these metabolites may lead to new hypotheses about diet- and microbiome-mediated CRC risk unique to each region of the colon.

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