OSU Interdisciplinary Nutrition PhD Program (OSUN)

Untargeted Metabolomic Profiling of Early Diet Energy Intake in C57BL/6N Mice Indicates Tentative Differences Correlated with Diet and Colon Region

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INTRODUCTION

High-fat (H) and calorie-restricted (E) diets are associated with increased and reduced risk for colorectal cancer (CRC), respectively. Metabolomes 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,[34] and E in increased vitamin E metabolites and decreased amino acids of the colon relative to controls.[35] 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.

Background

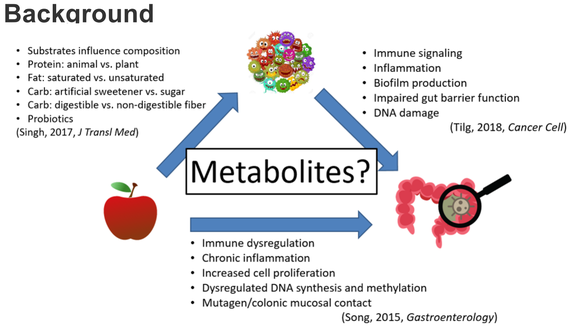

Figure 1. General overview of mechanisms by which diet and the gut microbiome mediate CRC risk.

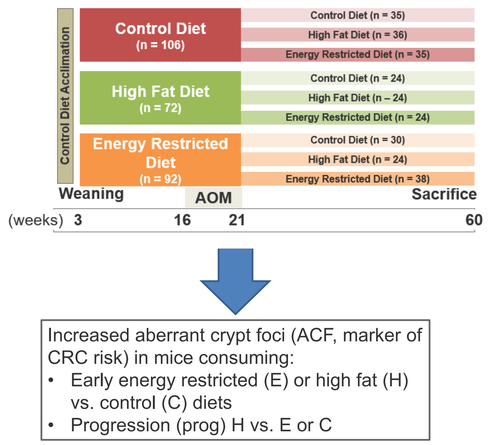

Figure 2. Schematic of the original mouse feeding study design, and relevant outcomes (adapted from Xu, 2016, *Sci. Reports*).

METHODS

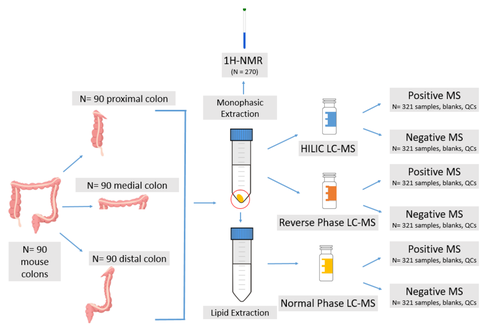

Figure 3. Colon extract preparation and metabolomic analysis using liquid chromatography-high resolution mass spectrometry (LC-HRMS) and nuclear magnetic resonance (NMR) platforms.

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

LINEAR MODELS

ACF and Diet Association[a]

Metabolite ~ ACF presence + early diet + progression diet + **ACF:early diet[b]** + **ACF:progression diet** + cohort + sample weight

*Cutoffs: FDR-adjusted *P*-value < 0.05, absolute value of estimate coefficient ≥ 0.25

Microbe Association

Metabolite ~ microbe + diet[c] + **microbe:diet** + cohort + batch + colon region

*Cutoffs: FDR-adjusted *P*-value < 0.2

[a]separate analyses for PC, MC, and DC
[b]terms of interest for each analysis are bolded
[c]separate analyses for early E vs. early C, early H vs. early C, prog E vs. prog C, prog H vs. prog C

RESULTS

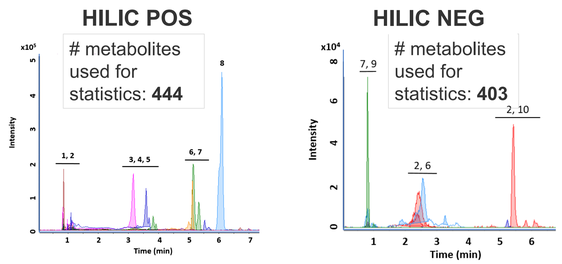

Figure 5. Representative chromatograms of mouse colon extract analyzed using HILIC LC positive (left) and negative (right) ionization MS indicating presence of anticipated classes of metabolites. 1: dipeptides, 2: bile acids, 3: acyl carnitines, 4: phosphatidylethanolamines, 5: lysophosphatidylcholines,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

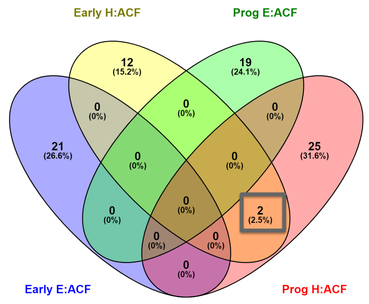

Figure 6. Metabolites found to have a significant association with early/progression diet and ACF interaction are mainly distinct to the early/progression diet group, except for 2 that overlap between early/progression H diet. (Made using Venny 2.1)

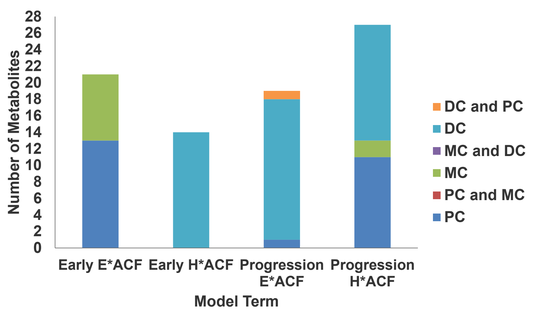

Figure 7. Colon region-specific differences in numbers of metabolites determined significant for a diet*ACF interaction in mixed-effect modeling of HILIC-MS data.

Diet Comparison	Number of significant microbe/metabolite pairs
Early E v Early C	1
Early H v Early C	2
Prog E v Prog C	0
Prog H v Prog C	35

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)
  - → warrants further study of metabolic pathways associated with H/E diets and proximal vs distal CRC risk
- Pathway analyses of these metabolites may lead to new hypotheses about diet- and microbiome-mediated CRC risk unique to each region of the colon

FUTURE WORK

- Identification of metabolites of interest using:
  a. MS/MS fragmentation
  b. metabolite databases
  c. Integration of 2D NMR data with MS data to aid in identifying novel metabolites (Bingol, 2015, *Anal. Chem.*)
  d. Authentic standards
- Pathway analyses of metabolites of interest, and integration with known metabolic processes of microbes of interest
- Testing of human colon tissue to determine if the identified metabolites may serve as early biomarkers CRC risk

ACKNOWLEDGEMENTS

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