Linear Modeling to Assess the Influence of Diet on the Colon Metabolomic Profile in Association with Aberrant Crypt Foci in C57BL/6N Mice

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INTRODUCTION

- Though significant differences in aberrant crypt foci (ACF) burden, early markers of colorectal cancer (CRC) development, have been associated with early lifetime diets in female C57BL/6N mice, diet-related metabolomic variations producing these changes in ACF composition (and thus CRC risk) have not yet been evaluated.
- We analyzed colon metabolomic profiles in these mice to evaluate the interplay between metabolites, diet, and ACF formation identifying metabolites associated with ACF independent of diet, those associated with diet independent of ACF, and those associated with an interaction between diet and ACF.
- Our aim is to devise appropriate pre-processing operations for the colon metabolomics data and construct linear models accurately extracting potential biomarkers for the characterization of the diet influence on CRC risk using a metabolomics approach.

STUDY DESIGN

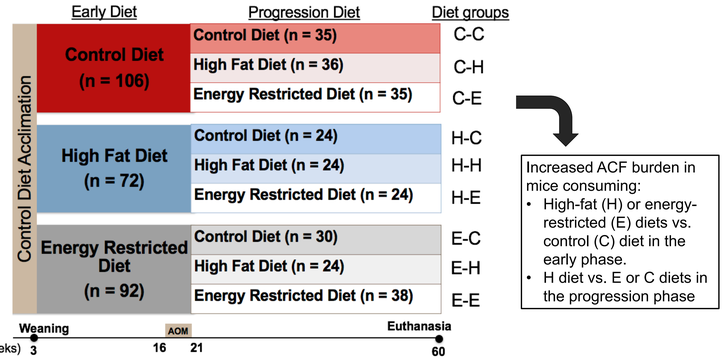
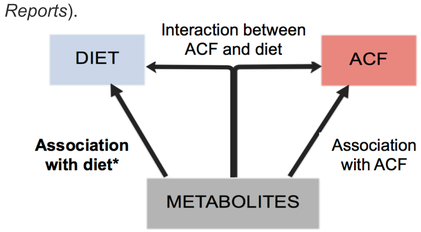


Figure 1. Schematic of the original mouse feeding study design, and relevant outcomes (adapted from Xu et al., 2016, *Sci. Reports*).



* Only metabolites independently associated with diet are analyzed in this poster.

Figure 2. Interplay between metabolites, diet, and ACF composition and our tentative approach at analyzing them.

METHODS

☑ PART 1: Sample Collection and Metabolite Extraction

- Mouse colon separation into proximal, medial, and distal sections (n = 90 each) and tissue excision from these sections.
- Monophasic extraction (2:5:2 $CDCl_3$:CD_3OD:H_2O)
- Metabolomic analysis using ultra-high-performance liquid chromatography-high resolution mass spectrometry (HILIC-QTof), feature extraction, and removal of peaks present in blanks/non-peaks

491 metabolite groups from HILIC positive mode (POS)

415 metabolite groups from HILIC negative mode (NEG)

☑ PART 2: Metabolite Data Pre-Processing

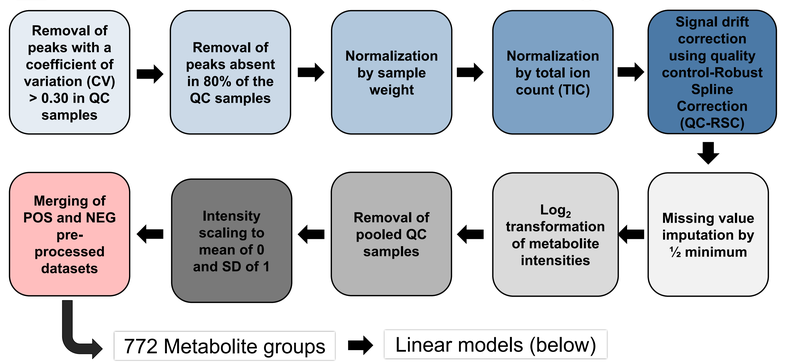


Figure 3. Schematic detailing metabolite data pre-processing operations before input into linear models

☑ PART 3: Linear models

- To detect metabolites associated with all possible pairwise comparisons of diet groups, 36 comparisons were performed.
- In each of the pairwise sub-datasets, intensities with a standard deviation in the lowest 5th percentile were removed, reducing the linear model input to 733 metabolites.
- Linear model formula:

$$Metabolite \sim \boldsymbol{Diet\ group}^{a} + ACF^{b} + weight + random\ effect^{c}$$

[a] Term of interest, used to assess statistical significance.
[b] ACF data were analyzed as binary (Presence/Absence of ACF).
[c] Random effect was added in each linear model to account for individual variability since each 3 samples were harvested from the same mouse (1|subject ID).

RESULTS

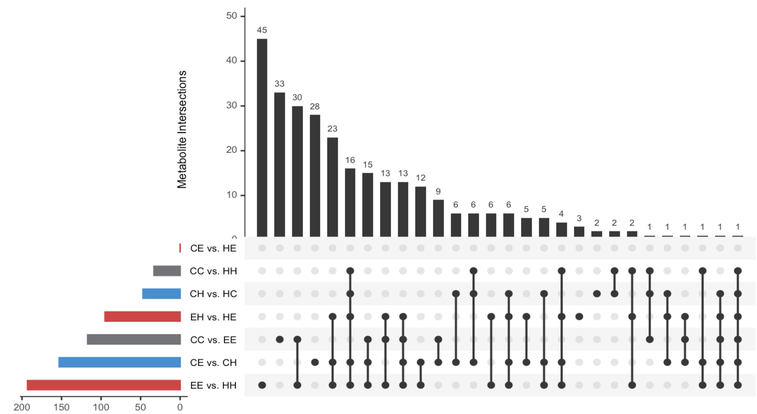


Figure 4. Summary of significant metabolites in a subset of pairwise diet comparisons and their intersections to each other.

- Significant metabolites were reported using the following cutoffs: **FDR- adjusted p-value ≤ 0.05 and -0.25 ≤ $\log_2$ fold change ≤ 0.25**.
- Significant metabolites were detected in all pairwise models except in models comparing samples with the same progression diet (e.g., CE vs. HE).
- Identification and further discussion of these detected metabolites are covered by Haley Chatelaine (second author) in her poster "*Direct comparison of early high fat, energy-restricted and control diets on colon metabolomic profiles associated with aberrant crypt foci formation in a C57BL/6N mouse model*" (abstract # 130).

FUTURE DIRECTIONS

- Linear models to detect metabolites independently associated with ACF and associated with an interaction between ACF and diet.
- Extrapolate our results to humans by evaluating how identified potential CRC biomarkers could be indicative of early CRC development in humans.

ACKNOWLEDGEMENT

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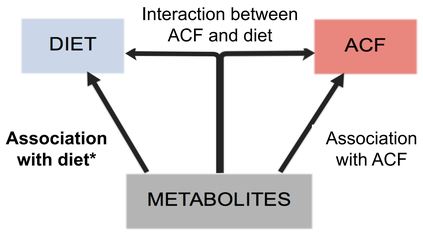
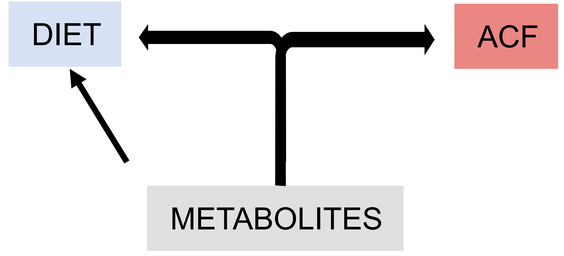

DIET
Interaction between ACF and diet
ACF
Association with diet*
Association with ACF
METABOLITES


DIET
ACF
METABOLITES