A microbiome-focused multi-omic assessment of the impact of intermittent fasting on gut bacteria

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Abstract

Most humans will go through one or more dietary modifications or interventions during their lifetime (fasting, supplementation or other dietary changes). Several studies have shown that dietary modifications can have beneficial impacts on the host, some of which may be mediated through changes in the gut microbiome and/or its activity. In this research, we employed a longitudinal, multi-omic approach (shotgun metagenomics, metatranscriptomics and metabolomics) to study the impact of intermittent fasting (IF) on the gut microbiome of healthy individuals. In general, metatranscriptomics (MTS) sequencing profiles were much more variable and dynamic than metagenomic (MGS) sequencing profiles, with select individuals showing a strong correlation between diet and MTS functional profiles. Additional metabolic results and analyses indicated pathway enrichment related to fasting. In conclusion, our results indicate that metatranscriptomics can reveal discrete changes in microbial community functional profiles that are not detected by DNA and may be better suited to understand biological responses to dietary modifications such as intermittent fasting.

Methodology

Seven healthy volunteers were recruited for an intermittent fasting regimen and at-home stool collection for 2 weeks.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	60
Diets	Normal				Fasting		Normal				Fasting			Normal					
Stool collection	✓	✓			✓	✓	✓		✓			✓	✓	✓				✓	✓

Week 1 consisted of 2 consecutive fasting days (600-800 Cal/day) and 5 days of normal diet (1,500-2,000 Cal/day). Week 2 consisted of 3 consecutive fasting and 4 normal diet days.

The fasting diet was vegetarian, consisting of fruits and vegetables, while the normal diet was balanced meals providing all macronutrients (carbohydrates, fats, fiber and proteins).

To capture the effect of the fasting and normal diets on gut through microbiome DNA, RNA and metabolites, participants self-collected fecal samples in OMNIgene®•GUT DNA and RNA and OMNImet®•GUT devices. A total of 154 samples were collected, with each participant sampling daily with both devices over 11 days. The collected samples were held at room temperature for 5-7 days before extraction, to mimic the transport time for at-home collected samples to reach a processing laboratory.

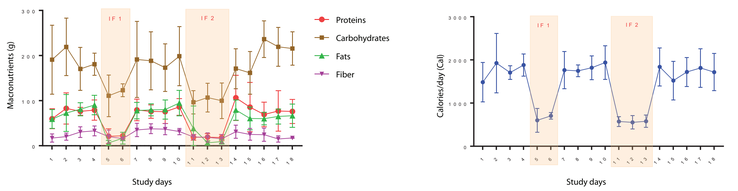

Figure 1. Average macronutrient and calorie intake by the participants shows high compliance to the intermittent fasting (IF) diet regimen. The graph shows a drastic reduction of protein, fat and caloric intake during fasting (IF 1 or IF 2) days, compared to normal diet days.

DNA and RNA extractions, sequencing and data analysis

Extractions: DNA and RNA were extracted from OMNIgene®•GUT DNA and RNA using RNeasy PowerMicrobiome kit (QIAGEN).
Library preparation: DNA Prep Kit (Illumina) and Stranded Total RNA Prep with Ribo-Zero Plus Microbiome Kit (Illumina)
Metagenomics (DNA) and metatranscriptomics (RNA) sequencing and sequence analysis were completed at Diversigen. Sequence data were processed through the Diversigen automatic QC and annotation pipelines to generate taxonomic and functional feature tables.

Metabolite sample processing and analysis at Metabolon

The metabolites were analyzed by reversed-phase ultra-performance liquid chromatography-mass spectrometry (RP-UPLC-MS) with positive and negative ion mode electrospray ionization (ESI) and hydrophilic interaction liquid chromatography (HILIC) with negative ion mode ESI methods in the global metabolomics platform (Metabolon) using their proprietary analysis software.
The bioinformatics team at Diversigen used gene set enrichment analysis (GSEA) to detect whether sets of genes in metabolic sub-pathways responded in a similar way to dietary conditions.

Methodology (continued)

End-to-end multi-omic analysis of stool samples with OMNIgene®•GUT DNA and RNA and Diversigen MGS and MTS pipelines

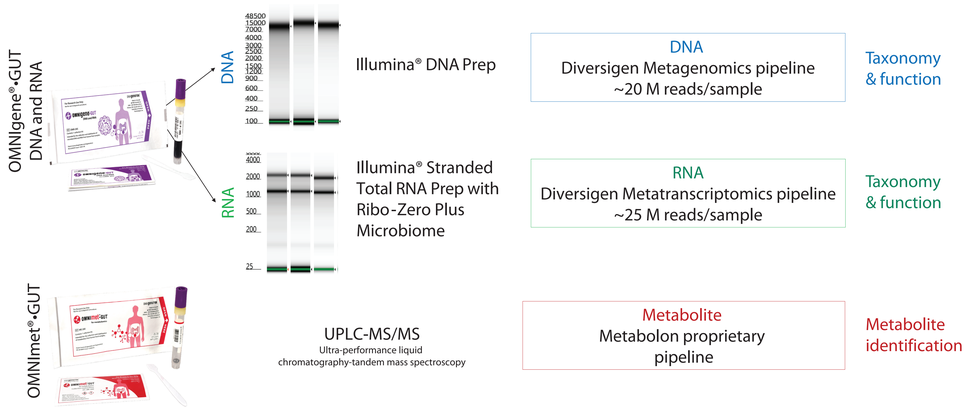

Figure 2. OMNIgene®•GUT DNA and RNA kits stabilize high-quality DNA and RNA from stool, to generate robust libraries for MGS and MTS analysis (Diversigen). OMNImet®•GUT kits stabilize gut metabolites to enable metabolomics analysis.

Results

DNA and RNA at species level and metabolite profile show strong clustering of the subjects irrespective of the analyte, highlighting the unique microbiome of each subject

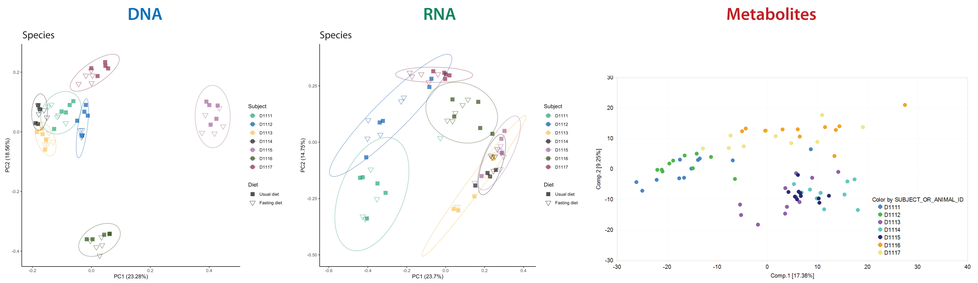

Figure 3. Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity index of species shows very strong grouping of MGS and MTS samples by subject, indicative of the unique microbial composition of every individual. Analysis of all subjects showed no clear association of microbial community to diet even though all participants followed strict diet routines. PCA plots based on metabolites also show clustering within subject implying that gut metabolite composition is also unique to an individual.

Individualized response to diet: 'Responder' vs. 'Non-responder'

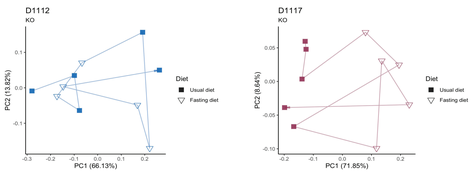

Figure 4. PCoA based on MTS abundances of KEGG orthologs (KOs) in D1117 shows clear and consistent differences between fasting and normal diet days (highlighted by ellipses), while D1112 also shows variability without a clear pattern based on diet.

Differentially abundant KOs in normal vs. fasting days in MTS and MGS

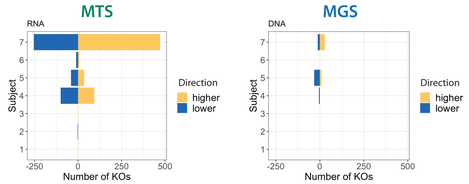

Figure 5. Number of KOs that are differentially abundant in normal vs. fasting days in MTS and MGS. Donors D1117 and D1114 display a higher number of differentially-abundant KOs associated with diet in MTS than MGS, suggesting broader functional changes at the transcriptional level in response to diet than composition of microbiome.

Results (continued)

Heatmap of the top 100 differentially-abundant functions shows D1117 strong response to diet by MTS

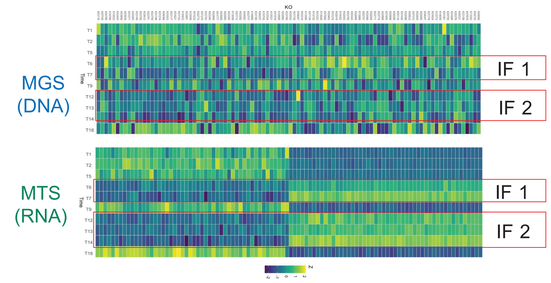

Figure 6. Heat plots showing the most differentially-abundant KOs in MTS vs. MGS for D1117. MTS shows consistent functional shifts in response to fasting, while DNA profiles are variable but do not correlate with diet. The KOs on the right show higher abundance in fasting days, while the KOs on the left show lower abundance on fasting days.

Understanding the biological impact of intermittent fasting on gut microbiome

A. Metatranscriptomics: Differentially abundant functions in 'responder' D1117

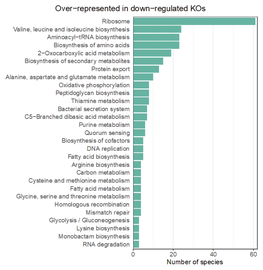

Figure 7A. Differential expression analysis shows fasting-correlated down-regulation of KOs involved in ribosome, amino acid, lipid metabolism and other pathways by species of the gut microbial community of donor D1117. Some of the same pathways were found to be up-regulated in other species (data not shown) highlighting the complexity of gut microbiome and its response to diet intervention.

B. Metabolomics: Differentially expressed sub-pathways across subjects

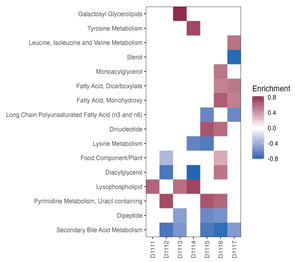

Figure 7B. The Secondary Bile Acid Metabolism sub-pathway was decreased in the majority of donors (q-value < 0.1) during fasting, potentially due to decreased bile acid secretion as a result of reduced fat consumption. Fatty Acid Metabolism and amino acid metabolism were enriched in some donors.

Conclusions

Intermittent fasting induces highly personalized responses, with MTS more suited to capture the dynamic changes in functional profiles than MGS.

Differential pathway analysis showed higher or lower expression of certain KOs in donors that responded to intermittent fasting, which were related to biosynthesis of amino acids, protein metabolism, glycolysis and cell signaling. Some of these pathways show overlap with the results of metabolomics analysis.

A subset of participants showed a measurable microbial response to fasting. Confounding factors such as transit time and microbial auxotrophy could have impacted our ability to detect changes.

DNA Genotek collection devices paired with Diversigen metagenomics and metatranscriptomics sequencing and analysis pipelines enable end-to-end multi-omic assessment of human stool samples that help us understand the role of the fecal and gastrointestinal microbiome in health and disease.

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2 Mesnage R, Grundler F, Schwiertz A, et al. (2019). *J NutrSci*. 8:e36. doi: 10.1017/jns.2019.33
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