Changes in Gut microbiota Abundance with Dietary Intervention

Fall 2020 Advanced Biostats Term Project Report

By Daniel Broderick

Introduction

The study of the microbiome is a novel, rapidly growing field. While the human genome project began in the 90s, the human microbiome project was started in 2008. The mission, to capture the tremendous complexity and diversity of the human microbiome, is considered by some a challenge even *greater* than sequencing the human genome. Our guts have an estimated 20,000 orthologous groups of bacteria, less than 5,000 of which are known, few are fully sequenced, and even fewer have their functions characterized in the literature[1]. With that caveat, microbes are responsible for the digestion and production of certain nutrients (or even neurotransmitters[2]) and may be implicated in several diseases[3]. Likewise, factors like birth mode (caesarean or vaginal), antibiotic use during infancy, diet, and other lifestyle factors[4] affect the microbiome. What diet will cultivate a desirable composition of microbiota and improve human health?

A couple things we can't digest are gluten and FODMAPs; the microbes in our gut pick up the slack. Short-term effects might be feeling bloated after eating that double-stuffed dominos pizza. Even in non-celiac hosts, chronic exposure to gluten, or rather the gliadin protein, widens tight junctions in the epithelial lining of the gut making it more permeable to macromolecules. Simply, gluten causes "leaky gut", which has downstream effects like inflammation.[5] Microbes that produce gluten-degrading enzymes may mitigate these effects and are of interest to celiac and non-celiac patients alike.[6]

FODMAP stands for low–fermentable oligo-, di-, and monosaccharides and polyols, a group of particularly offensive indigestible short-chain carbohydrates. Their breakdown by gut bacteria also produces gas, bloating, abdominal pain, etc. They have long been implicated in in food intolerance, "beans, beans, the musical fruit..." (beans contain galacto-oligosaccharides).

The standard treatment is avoidance via a low FODMAP diet[7]. By observing how the gut microbiome responds to specific diets, we may be able to develop new dietary protocols to improve health, or even cure disease.

The study of interest[8] measures the microbiota composition of 78 obese individuals before and after dietary intervention using the HITChip microarray on fecal samples.[9] It is a compilation of 4 smaller studies, which mainly differed by the diet introduced: study A (n=28) eats whole grains; study B (n=24) eats low-fiber, refined wheat bread; study C eats a daily dose of 8g inulin and 8g oligofructose; study D (n=13) eats a one-week run-in diet followed by three weeks each of a resistant-starch-enriched, non-starch-polysaccharide-enriched, and high protein diets.

Hypotheses

This is a pretty standard human gut microbiome study. The basic design is to quantify the microbiome, apply some treatment and quantify the microbiome again. Then we must ask: how and why did the microbiome change? The following hypotheses are derivations of that essential question.

Does increased gluten intake affect the relative number of glutenase-producing microbiota?

Participants in studies AB (combined, see methods) are both consuming additional gluten in the form of rye and wheat bread. Certain microbes can hydrolyze gluten peptides: *Lactobacilli*, *Aspergillus niger*, *Flavobacterium meningosepticum*, *Myxococcus xanthus*, and *Sphingomonas capsulate*.[10] With more of their "food" available, we would expect see an increase in the abundance of these microbes. Such findings could inform future probiotic treatments for gluten sensitivity.

Does oligofructose/inulin consumption affect gut microbiota composition similarly to wheat or rye bread consumption?

Oligofructose and inulin are both fructans, a group of specific carbohydrates found in wheat and rye. Fructans, like any indigestible carbohydrate, cause some people digestive issues. Study C's 8g inulin and 8g oligofructose treatment is gluten-free; how it affects the microbiome as compared to study AB's wheat/rye treatment will inform our conclusions. If the change in microbiota composition in study C is similar to study AB, then fructans may have a more significant effect on the microbiome than gluten. If study C shows little change in microbiota composition compared to study AB, then gluten has a more significant effect on the microbiome. I think the latter is most likely. Fructans not broken down can be excreted while the body treats gliadin as a foreign invader[11]. An immune response implies gluten peptides pose a greater risk to human health than fructans, and more likely to affect the microbiome. The literature on exclusive oligofructose or inulin consumption is scarce, but it has generally been demonstrated to increase Bifidobacterium counts[12].

Methods

The original data[13] represents microarray signal intensities from the HITChip, a novel but highly specific and reproduceable technology. It can reliably distinguish the microbiota profiles of different individuals using the 16S rRNA gene to categorize microbiota. The data is in an excel file with 1169 columns. Two columns are used to indicate the participant ID, treatment, and when the measurement was taken; semantically inconsistent and don't differentiate studies A and B. The remaining columns are continuous, log-transformed, min/max normalized signal intensities for the 1037 phylotypes and 130 genus-like groups.

The phylotype data was not analyzed for simplicity. Approximately 23.1% of probes on the microarray do not have unique sequences; the abundances for phylotypes, especially those in diverse genera with considerable genetic overlap, can be considered unreliable. Finally, the function of most phylotypes is unknown. Let's proceed with the analysis of the genotype-like groups.

To extract the relative abundances from this data, the log-transformation and min/max normalization must be reversed, then each genotype value divided by the sum of the row. This would be useful to determine the proportion of the entire microbiome a particular genus comprises. Unfortunately, the authors did not specify the log base or min/max normalization formula used. I attempted to produce relative abundances assuming base 10 and the normalization formula: y= (x-min)/(max-min). This failed; proportions of the most abundant microbe genera like Bacteroides or Faecalibacterium were nowhere near the normal range[14]. I decide to move forward with the original data, which we can say represents absolute abundances.

When we don't fully comprehend the data we're dealing with (e.g. missing information), non-parametric, rank-based approaches are useful. We use non-metric multidimensional scaling with a Bray-Curtis distance matrix as input. Then we can extract and plot the scores, each score represents the microbiota composition of an individual; closer points are more similar in their microbiota composition.

Visualizing microbiome differences is only so useful. Next, we will quantify if the microbiome changes are actually significant. We implement an ANOSIM test with a BC dissimilarity matrix and 9999 permutations. This measures how dissimilar the microbiota composition is for specific groups. Of particular interest is comparing pre-intervention study AB

to pre-intervention study C, then also comparing post-intervention study AB to post-intervention study C.

To identify *which* microbial species are found more often in one group compared to another, we use an indicator species analysis. Why don't we test our hypotheses directly and compare abundances for a particular genus? Technically, a repeated measures NPMANOVA (or a paired t-test if we ran each individually) could be used to compare *Lactobacillus, Aspergillus niger, Flavobacterium meningosepticum, Myxococcus xanthus,* and *Sphingomonas capsulate* abundances between study AB and study C. With such a large dataset of species abundances (which tend to fluctuate even without intervention) this would be data dredging. Maybe looking at the abundances for particular genera would be appropriate for some studies. In this case however, a highly specific treatment is being applied, so the indicator species analysis should reveal significant changes. This results listed are just for the overall pre- and post-intervention group, but the analysis was also carried out to compare the pre- and post-intervention groups of study AB and study C, of just study AB, and of just study C. The results were similar regardless, except the fewer studies (e.g. smaller sample size) considered, the more significant changes in species abundance were found.

Results

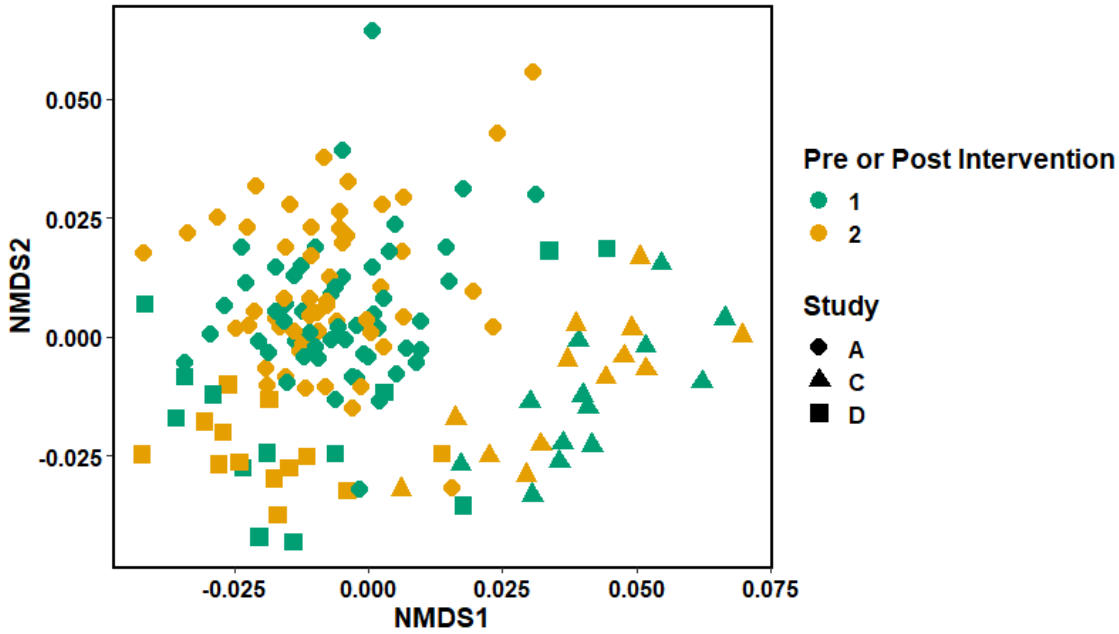

Figure 1: Pre-intervention points do not cluster together; subjects from different studies *start* with different microbiomes. We only see strong clustering by study, pre- and post- intervention.

	ANOSIM statistic R	P - value
Pre/post	0.0023	0.3027
Pre - AB/C/D	0.6714	<.0001
Post – AB/C/D	0.6351	<.0001
Pre – AB/C	0.7996	<.0001
Post – AB/C	0.7566	<.0001

Table 1: The microbiota composition between studies AB, C, and D, or just between studies AB and C, is less dissimilar in the post-intervention measurement.

Genera Pre-intervention	Stat value	P-value
Roseburia intestinalis	0.222	0.0068
Aeromonas	0.217	0.0057
Micrococcaceae	0.198	0.0149
Novosphingobium	0.177	0.0314
Mitsuokella multiacida	0.177	0.0275
Clostridium leptum	0.163	0.0422
Akkermansia	0.160	0.0474
Haemophilus	0.157	0.0491
Genera Post-intervention		
Eubacterium cylindroides	0.161	0.0436

Table 2: Only *Eubacterium cylindroides* is significantly more abundant in the post-intervention measurement.

Discussion

This study has shown that diet can rapidly alter the microbiome. Interesting, table 1 shows the subjects' microbiomes seems to have become more similar after the dietary intervention. Table 2 would also imply that microbiome diversity decreased for every intervention, because there is only one species significantly more abundant in the post-measurement, compared to eight in the pre-measurement. The species that did proliferate, *Eubacterium cylindroides* is associated with opportunistic infections[15]. The study diets were probably more restrictive than whatever the participants were eating before, so normal gut flora died and were displaced by *Eubacterium*.

I'm tentative to draw further conclusions about the effect of gluten or fructans on microbiota composition. See figure 1. Pre-intervention groups do not cluster together across studies, probably because the participants were from different countries; study AB was conducted in Finland, study C in Belgium, and study D in Britain. Ideally, the participants would come from the same sampling pool, so that their starting microbiomes are relatively similar, and the effect of different interventions could be clearly shown. An analysis to see if there was a trend in the distance/direction between the pre- and post-measurements in figure 1 or a SIMPER procedure, would be a good next step; such techniques are too daunting for me to attempt.

The lack of supplemental materials that should come with the data slightly limited my analysis. The obese and non-obese controls were left out, the dataset did not distinguish male vs female subjects, and certain biomarkers were measured but not included. This is a testament to the importance of being thorough, should peers attempt to reproduce your work. The overall decrease in microbial diversity is immensely interesting, and an important consideration for future studies that implement restrictive diets.

Literature Cited

[1] Qin, J. et al. A human gut microbial gene catalog established by metagenomic sequencing. Nature 464, 59 (2010).
[2] Reigstad, C. S. et al. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. FASEB J 29, 1395–1403 (2015).
[3] Morgan, X. C. et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biology 13, R79 (2012).
[4] Jandhyala, S. M. et al. Role of the normal gut microbiota. World J Gastroenterol 21, 8787–8803 (2015).
[5] Drago, S. et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scandinavian Journal of Gastroenterology 41, 408–419 (2006).
[6] Amador, M. de L. M., Arévalo-Rodríguez, M., Durán, E. M., Reyes, J. C. M. & Martín, C. S. A new microbial gluten-degrading prolyl endopeptidase: Potential application in celiac disease to reduce gluten immunogenic peptides. PLOS ONE 14, e0218346 (2019).
[7] Hill, P., Muir, J. G. & Gibson, P. R. Controversies and Recent Developments of the Low-FODMAP Diet. Gastroenterol Hepatol (N Y) 13, 36–45 (2017).
[8] Korpela, K. et al. Gut Microbiota Signatures Predict Host and Microbiota Responses to Dietary Interventions in Obese Individuals. PLOS ONE 9, e90702 (2014).
[9] Rajilić-Stojanović, M. et al. Development and application of the human intestinal tract chip, a phylogenetic microarray: analysis of universally conserved phylotypes in the abundant microbiota of young and elderly adults. Environ Microbiol 11, 1736–1751 (2009).
[10] Kaukinen, K. & Lindfors, K. Novel Treatments for Celiac Disease: Glutenases and Beyond. Digestive diseases (Basel, Switzerland) 33, 277–81 (2015).
[11] Caio, G., Volta, U., Tovoli, F. & De Giorgio, R. Effect of gluten free diet on immune response to gliadin in patients with non-celiac gluten sensitivity. BMC Gastroenterol 14, 26 (2014).
[12] Gibson, G. R. Dietary Modulation of the Human Gut Microflora Using the Prebiotics Oligofructose and Inulin. J Nutr 129, 1438S-1441S (1999).
[13] Korpela, K. et al. Data from: Gut microbiota signatures predict host and microbiota responses to dietary interventions in obese individuals. 3112544 bytes (2015) doi:10.5061/DRYAD.BV4K7.
[14] Arumugam, M. et al. Enterotypes of the human gut microbiome. Nature 473, 174 (2011).
[15] Jeffrey K. Actor, 12 - Clinical Bacteriology, Elsevier's Integrated Review Immunology and Microbiology (Second Edition), W.B. Saunders, 2012. Pages 105-120. https://doi.org/10.1016/B978-0-323-07447-6.00012-0