Exercise can help modulate human gut microbiota

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04/07/2014

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Abstract

Moderate exercise has been shown to be beneficial to health in many ways, including reducing stress, building a stronger immune system and increasing cardiovascular health. Recent studies in the human gut microbiome have demonstrated benefits of certain microorganisms in aiding human pathogen resistance and reduction of inflammation. The hypothesis is that exercise can help modulate the human gut microbiota. Here we show that increasing exercise frequency selects for a diverse community of microbes that contribute to a healthier environment. Individuals who exercised more often showed a significant elevation in their diversity, as well as a significant elevation of certain members of the Firmicutes phylum (including *Faecalibacterium prausnitzii*, uncharacterized species of genus *Oscillospira*, *Lachnospira*, *Coprococcus*, and uncharacterized families of Clostridiales).

Introduction

There are up to 100 trillion microbes in the human body, tenfold the numbers of human cells. The human gut holds more than seventy percent of all the microbial mass in and on the body. (Hattori M et al. 2009) Recent discoveries have shown that the microbial biota of the human body may be the first line of defense against pathogens. (Hooper LV et al. 2010) Intestinal bacteria help drive the formation of lymphoid tissues and bacteria are known to promote development of preimmune Ab repertoire which results in positive selection of B cells in GALT. (Severson et al. 2010)

The human gut contains many of the microbes crucial for digestion and processing of organic material. Without certain bacteria, such as Bacteriodetes, processing of many crucial foods may not be possible. (Bolam et al. 2011) An unhealthy gut can contain unwanted bacteria, or

become dominated by normally rare taxa, such as an overgrowth of Proteobacteria. These unhealthy bacteria can cause inflammation and have other harmful effects on the body. Good bacteria are necessary to reduce inflammation and suppress the inflammation due to Proteobacteria and other pro-inflammatory taxa. (Hattori M et al. 2009) A deficiency in the TLR5 gene, which is a component of the innate immune system and recognizes bacterial flagellin, can result in unstable gut microbiota associated with low-grade inflammation, and harboring Proteobacteria can drive and/or instigate chronic colitis (Carvalho FA et al. 2012.) The complex interaction between bacteria and human defenses can be affected greatly by antibiotic treatment, stress, and dietary choices. Moderate exercise is known to decrease levels of stress and build immunity. Lymphoid tissue associated with the gut contains high levels of immune cells found within Peyer's patches and lamina propria. Moderate exercise has been shown to modulate the immune system and exhaustive frequent exercise can result in immunosuppression. (Valdes-Ramos et al. 2010) As such it seems reasonable

to think of exercise as being crucial to the interactions within the gut microbiota (Choi J et al. 2013).

Moderate exercise is shown to produce reactive oxygen species and change the cellular/metabolic rate redox balance to that of a pro-oxidative/pro-inflammatory state. This results in anti-oxidant enzymes responding by being up regulated to decrease the inflammation, with a net effect of decreased oxidative stress. No exercise would result in a low endogenous antioxidant level response. Moderate to frequent exercise would cause a full response of endogenous antioxidants; vigorous exercise could overwhelm the endogenous antioxidant response, which could lead to a chronic pro-oxidative/pro-inflammatory state. (Herder et al. 2004) Relatively little is known about how the gut microbiota vary with exercise in humans. As our knowledge of what defines healthy guts and those with dysbiosis are limited, understanding how gut microbes are shaped in individuals with a healthy lifestyle may lead to a better definition of a healthy gut which could lead to practical applications of remodeling the gut microbiota for improved human health.

Materials and Methods

Fecal samples from participants of the American Gut Project, a crowd-sourced endeavor where individuals volunteered to sequence their own microbiomes. Participants who specifically identified themselves as exercisers or non-exercisers were analyzed, totaling 1493 participants. Each participant was categorized according his or her exercise frequency, Never, Rarely (a few times a month), Occasionally (1-2 times per week), Regularly (3-5 times per week) and Daily. DNA was extracted from the participants' feces, and the 16S rRNA gene V4 hypervariable region was sequenced on the lllumina MiSeq platform according to the manufacturer's specifications, with addition of 15% PhiX. 175 base pair were generated in each direction. The base pairs were further processed in a data curation pipeline implemented in QIIME 1.8.0 with closed reference OTU picking against the August 2013 Greengenes database. A 97% identity was used for clustering

reads into OTUs (operational taxonomic units) discarding reads that fail to match the reference sequences. Taxonomy is already associated with each of the reference sequences from Greengenes. These data were then used to generate an OTU table. Antonio Gonzales did these preprocessing steps up to generating an OTU table. Members of the Knight lab did sample preparation, extraction, and sequencing. Robert McFadzean performed the following post-processing data analyses steps with supervision by William Walters.

QIIME 1.8.0, an open source software package was utilized for analysis and comparison of microbial communities from high-throughput sequencing data. Statistical measures can be run in QIIME to compare and contrast microbial communities, and measure significant differences between communities. (Caparaso et al. 2010) Alpha and beta diversity analysis were run using even sampling at 1000 sequences per sample. Alpha diversity shows overall diversity within a community, whereas beta diversity is the differences between samples. In this case, alpha diversity was calculated by

using the unique OTUs (known as the observed species metric) in each exercise frequency category, and beta diversity was calculated using the phylogenetic-based UniFrac metric (Lozupone C et al. 2005). Taxa Summaries were generated for each exercise group, with standard error calculated in excel across each phylum. A Kruskal-Wallis test was performed to find distributions of OTUs that were significantly different among exercise groups. Bonferonni and FDR P values were calculated for all groups. Further analysis was performed on the Firmicutes to determine if there may be a dose response for any taxon with exercise and to further breakdown the taxa down to the species level.

Results

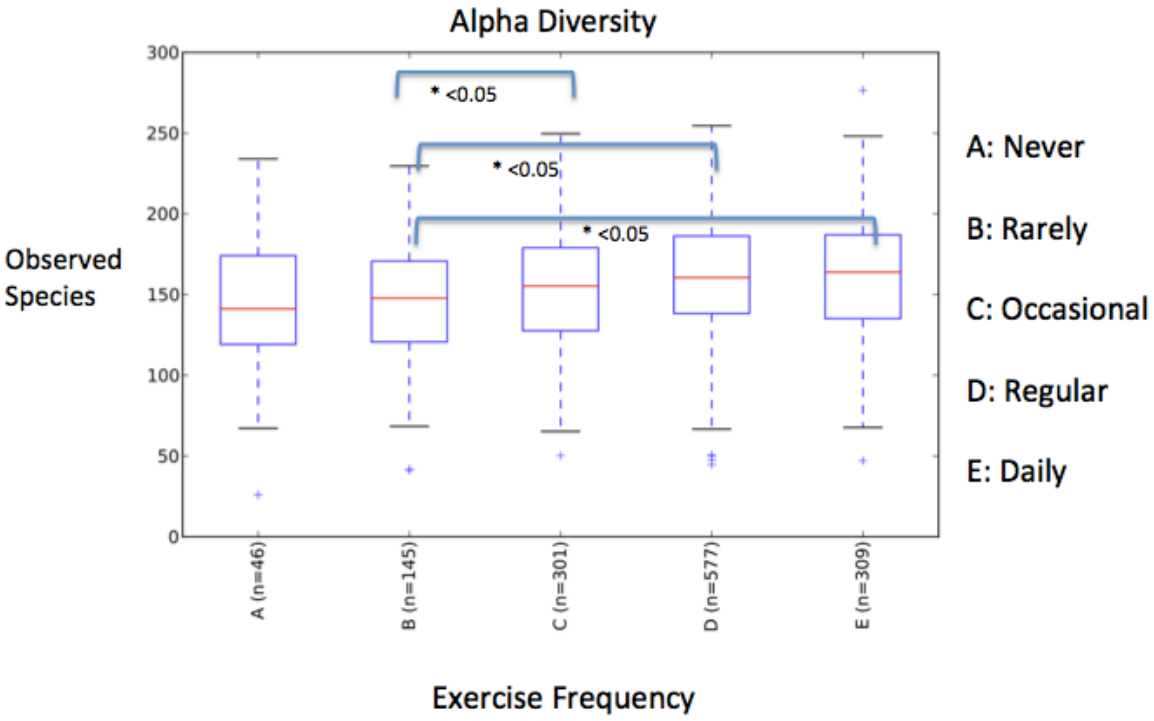


Figure 1: Alpha diversity by exercise frequency. A: Never B: Rarely C: Occasional D: Regular E: Daily exercisers. Significant P-Value differences in diversity can be seen in P * < 0.05 between groups. Significant groups are between Group B and C, B and D, B and E.

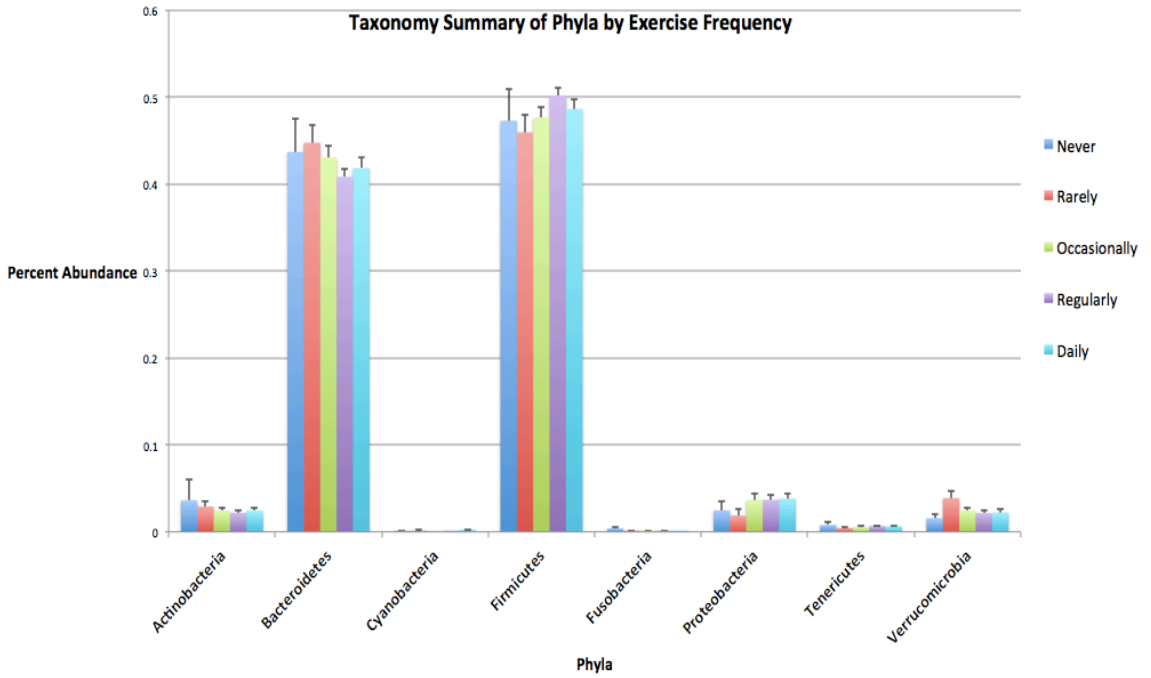


Figure 2: Taxonomy summary of phyla by exercise frequency: Bacteriodetes are more abundant in the Never exercisers and Rarely exercisers. Firmicutes have highest abundance in the Regularly, Occasionally and Daily exercisers. Standard error bars are shown to be highest on Never and Rarely exercisers.

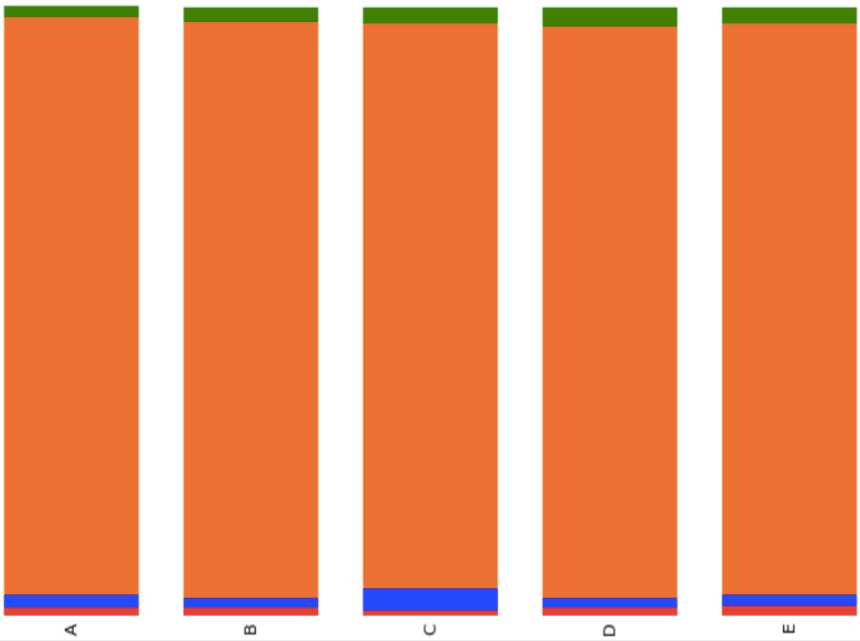
Relative Abundance

Bonferroni_P /FDR_P	Never	Rarely	Occasionally	Regularly	Daily	Taxonomy
0.000199	1.886	1.095	1.927	2.434	2.842	o__Clostridiales; Unclassified
0.0021	2.204	1.838	1.841	3.007	3.296	o__Clostridiales; f__Ruminococcaceae; Unclassified
0.0031	8.613	8.845	11.776	10.77	9.286	o__Erysipelotrichales; f__Erysipelotrichaceae; Unclassified

0.0131	2.75	1.985	2.8728	3.805	3.595	o__Clostridiales; f__Lachnospiraceae; g__Lachnospira; Unclassified
0.0195	9.931	11.19	15.343	16.72	17.94	o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii
0.0312	0.7727	0.691	1.3161	1.665	1.486	o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; Unclassified
0.0099	3.977	2.6176	2.9141	4.5992	4.7039	o__Clostridiales; f__Lachnospiraceae; unclassified
0.0099	6.5	5.1911	11.5738	14.348	18.891	o__Clostridiales; Unclassified
0.0108	4.25	2.3161	3.2509	4.6225	4.5065	o__Clostridiales; f__Lachnospiraceae; g__Lachnospira; Unclassified
0.0128	2.159	4.2573	4.2405	5.0089	4.2105	o__Clostridiales; f__; g__; s__
0.0151	1.477	1.7794	1.8247	2.2021	2.8289	o__Clostridiales; f__Lachnospiraceae; Unclassified
0.0212	17.5	19.080	23.9347	28.0429	27.529	o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii
0.0366	1.045	0.9705	1.6632	1.5813	1.4605	o__Clostridiales; f__Ruminococcaceae; g__; s__
0.0468	1.159	1.6617	1.7422	1.7316	2.75	o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__

Table 1: Significant Bonferroni and FDR P values for OTUs. Taxonomy for each OTU is in Firmicutes showing order, family, genus and species where available. All significant differences are in the phylum Firmicutes.

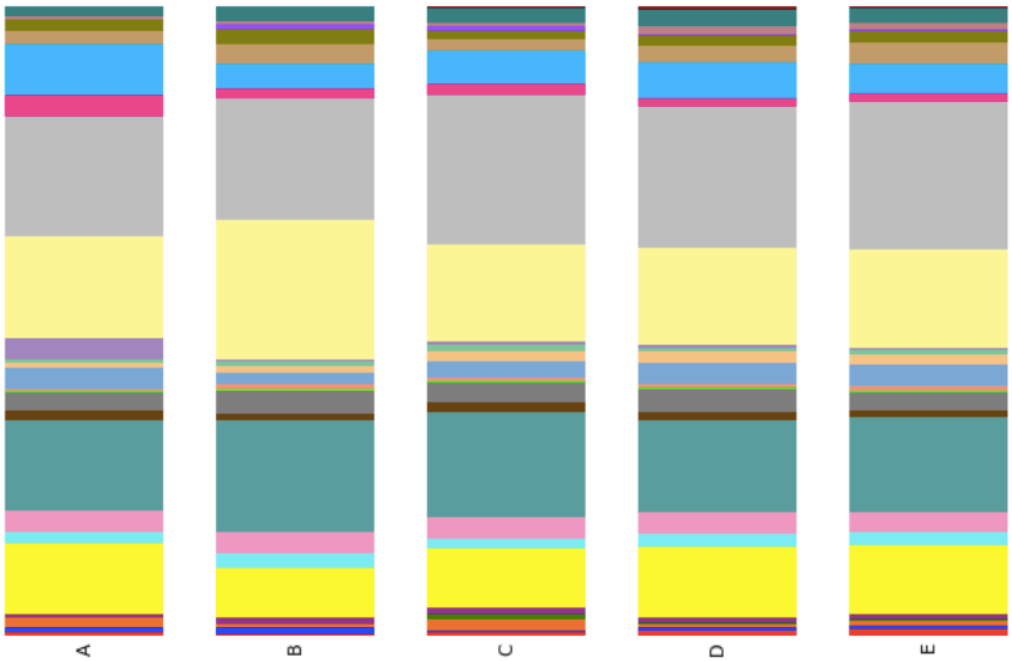
Summary of Taxonomy at Order Level



Legend	Taxonomy	Total count	Total %	A %	B %	C %	D %	E %
	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales	0	1.4%	1.3%	1.3%	1.0%	1.4%	1.7%
	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales	0	2.1%	2.1%	1.7%	3.4%	1.6%	1.8%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales	5	94.0%	94.9%	94.6%	92.9%	93.9%	93.7%
	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales	0	2.5%	1.7%	2.4%	2.6%	3.1%	2.8%

Figure 3: Firmicutes taxonomy shows an increase in the order Erysipeiolotrichales as exercise increases. (A=Never; B=Rarely, C=Occasional, D=Regularly, E=Daily)

Summary of Taxa to the Species Level



Legend	Taxonomy	Total count	Total %	A %	B %	C %	D %	E %
	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus; s__	0	0.7%	0.5%	0.4%	0.6%	0.8%	1.0%
	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Planococcaceae; g__; s__	0	0.7%	0.8%	0.9%	0.4%	0.6%	0.7%
	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Enterococcaceae; g__Enterococcus; s__	0	1.0%	1.5%	0.5%	1.7%	0.6%	0.6%
	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__zeae	0	0.3%	0.1%	0.1%	0.9%	0.1%	0.4%
	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__	0	0.8%	0.5%	1.1%	0.9%	0.8%	0.8%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	1	10.1%	11.3%	7.7%	9.4%	11.3%	10.8%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__; s__	0	2.0%	1.9%	2.4%	1.7%	1.9%	2.1%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__	0	3.3%	3.2%	3.3%	3.4%	3.4%	3.3%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	1	15.7%	14.5%	17.8%	16.7%	14.8%	15.0%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__	0	1.3%	1.5%	1.2%	1.4%	1.2%	1.1%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	0	3.1%	2.8%	3.5%	3.1%	3.6%	2.8%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Dorea; s__	0	0.3%	0.3%	0.3%	0.3%	0.4%	0.3%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnobacterium; s__	0	0.6%	0.4%	0.7%	0.5%	0.5%	0.8%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospira; s__	0	2.9%	3.3%	1.8%	2.6%	3.3%	3.3%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__faecis	0	1.4%	0.9%	1.1%	1.7%	1.8%	1.5%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__	0	0.7%	0.5%	0.7%	0.9%	0.6%	0.7%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__gnavus	0	1.0%	3.2%	0.5%	0.6%	0.4%	0.3%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	1	16.9%	16.2%	22.0%	15.3%	15.3%	15.5%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii	1	21.6%	19.1%	19.2%	23.9%	22.5%	23.5%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	0	1.8%	3.3%	1.7%	1.6%	1.2%	1.4%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	0	5.6%	8.1%	3.8%	5.4%	5.8%	4.7%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Dialister; s__	0	2.6%	1.9%	3.3%	1.8%	2.6%	3.4%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Phascolarctobacterium; s__	0	1.7%	1.8%	2.3%	1.3%	1.6%	1.6%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__Finegoldia; s__	0	0.4%	0.2%	0.6%	0.6%	0.3%	0.3%
	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__WAL_1855D; s__	0	0.8%	0.4%	0.7%	0.7%	1.3%	1.0%
	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__; s__	0	2.2%	1.7%	2.1%	2.3%	2.5%	2.3%
	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Catenibacterium; s__	0	0.3%	0.0%	0.2%	0.3%	0.6%	0.5%

Figure 4: Going down to the species level within the phylum of Firmicutes we see an increase for exercisers in the species *Faecalibacterium prausnitzii*. We also see a decrease in species *Ruminoccocus gnavus* with increasing exercise. The species *Lactobacillus zeae* showed no obvious trend with exercise. These were the only classified species at the species level; other species shown are unclassified at this level. (A=Never; B=Rarely, C=Occasional, D=Regularly, E=Daily)

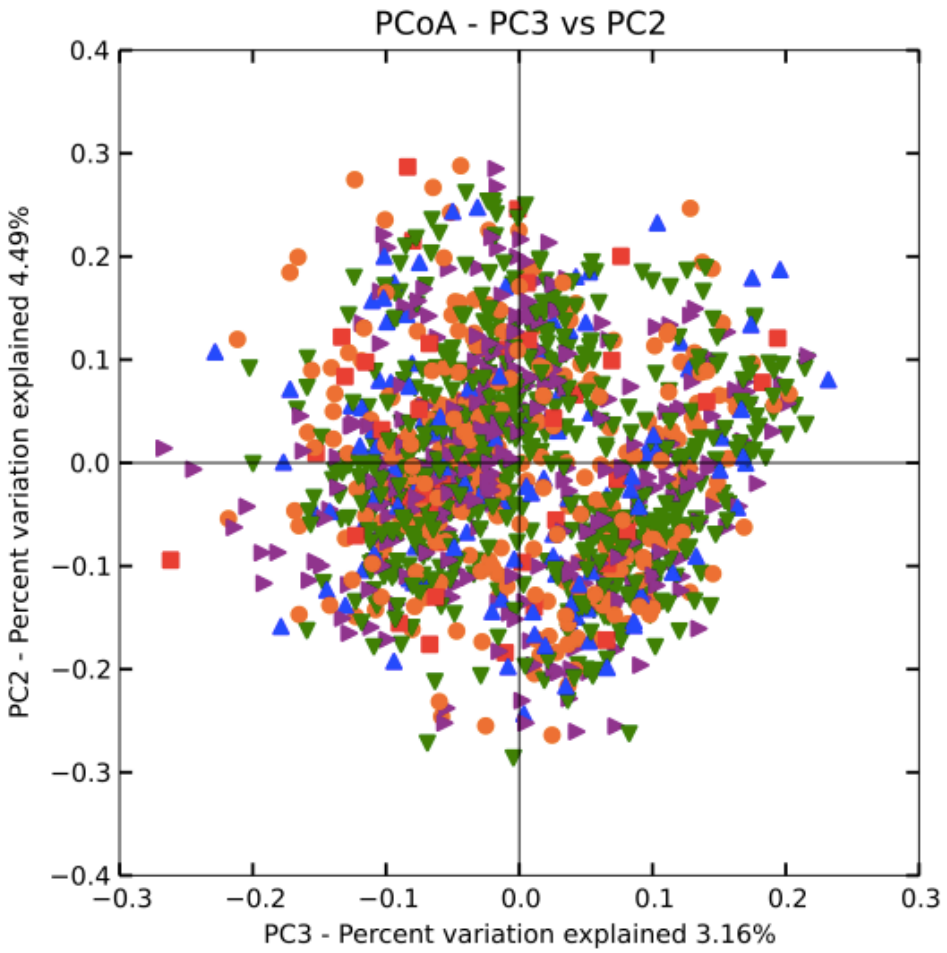


Figure 5: Unweighted Unifrac Beta analysis Showing Principal co-ordinates analysis PC3 Vs. PC2. Colorized by exercise frequency (Never: Red; Blue=Rarely; Green=Regular; Purple=Daily; Orange=Occasional). A clear split is shown, however it does not appear to be associated with exercise frequency. There are no distinctly different clusters according to Exercise.

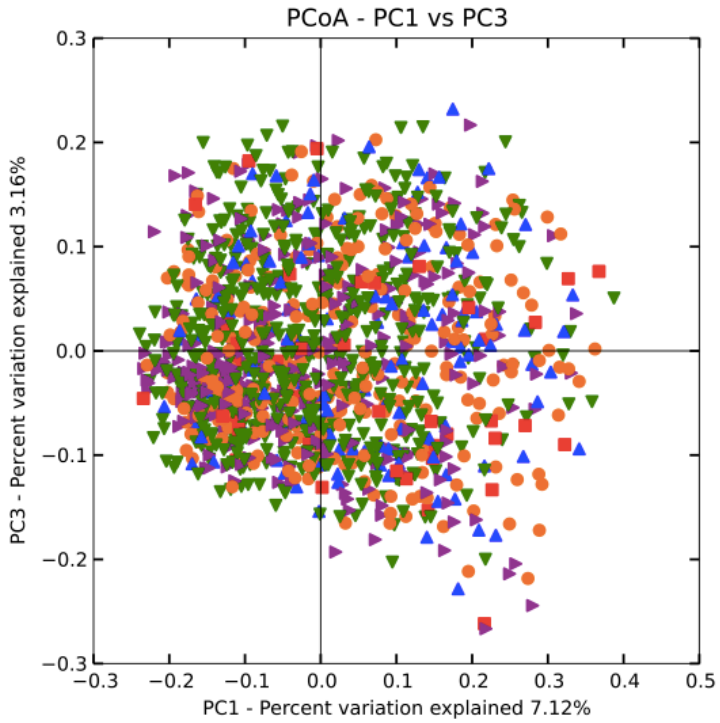


Figure 6: Unweighted Unifrac Beta analysis Showing Principal co-ordinates analysis PC1 Vs. PC3. (Never: Red; Blue=Rarely; Green=Regular; Purple=Daily; Orange=Occasional). There are no distinctly different clusters according to Exercise.

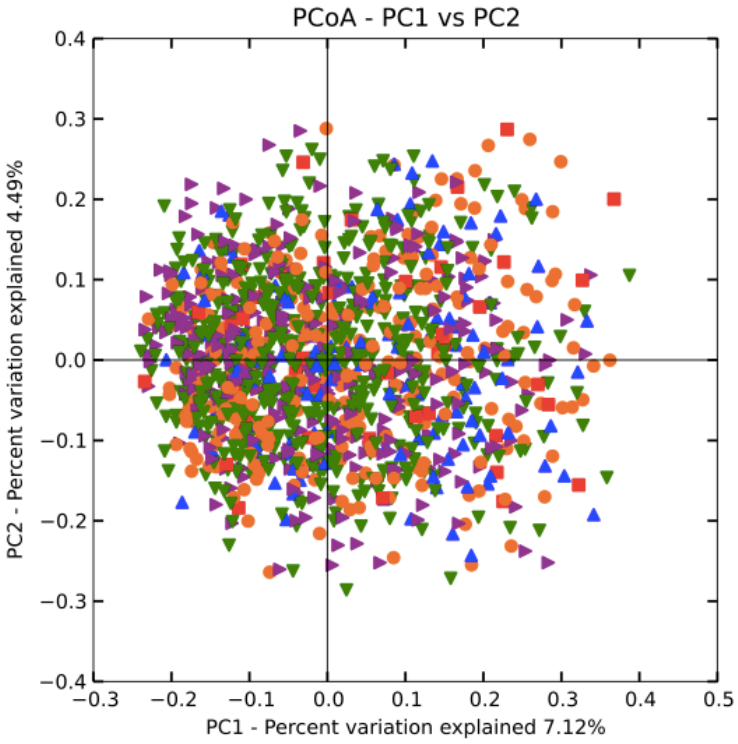


Figure 7: Unweighted Unifrac Beta analysis Showing Principal co-ordinates analysis PC1 Vs. PC2. (Never: Red; Blue=Rarely; Green=Regular; Purple=Daily; Orange=Occasional). There are no distinctly different clusters according to Exercise.

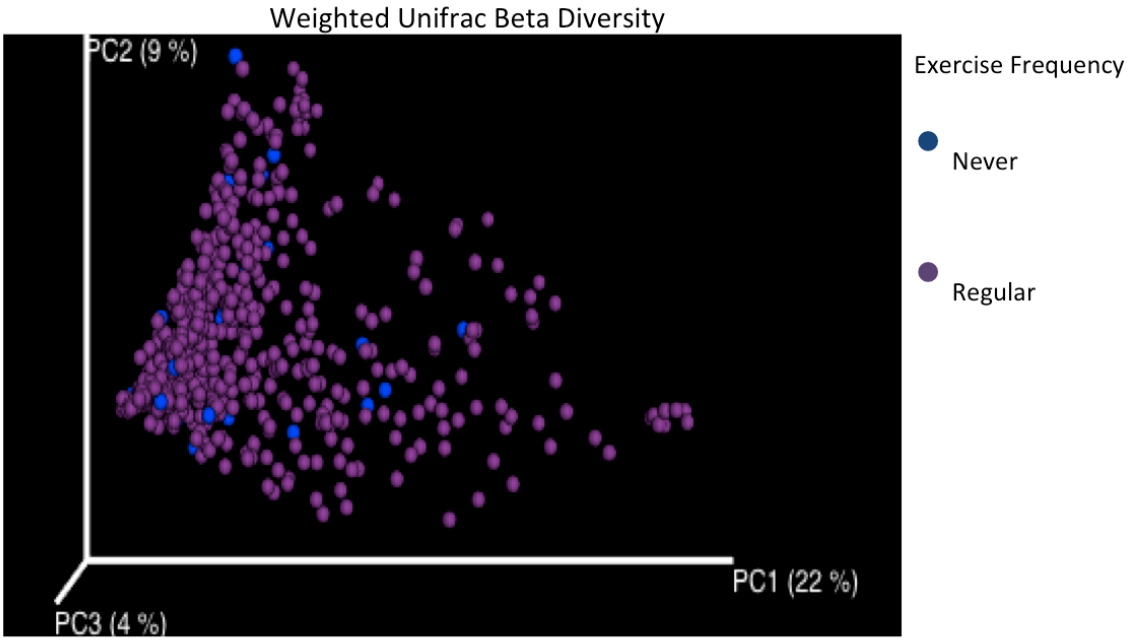


Figure 8: Weighted Unifrac Beta Diversity shows a trend. Beta Analysis of each group OTU's shows a trend in the weighted unifrac distances between the samples. Weighted UniFrac metric takes into account abundance of sequence data. Difference between Never and Regular exercisers may be due to the number of groups of each category. The weighted unifrac treats each sample equally instead of each unit of the branch equally. The distances between all samples are calculated and the results indicate a trend. It did not appear to be a gradient mapped to Exercise Frequency.

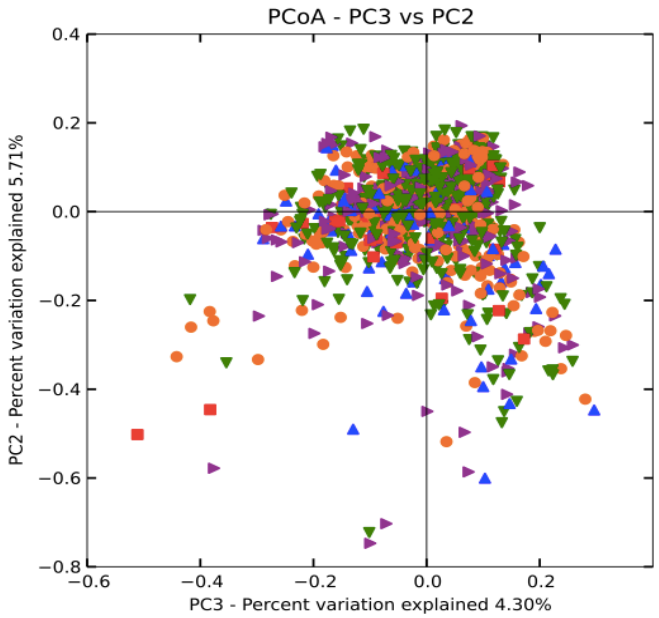


Figure 9: Weighted Unifrac beta diversity plot generated in QIIME with make_2d_plots. Showing Principal co-ordinates analysis PC3 Vs. PC2. Colorized by exercise frequency. (Never: Red; Blue=Rarely; Green=Regular; Purple=Daily; Orange=Occasional) No apparent strong clustering according to exercise is visible here.

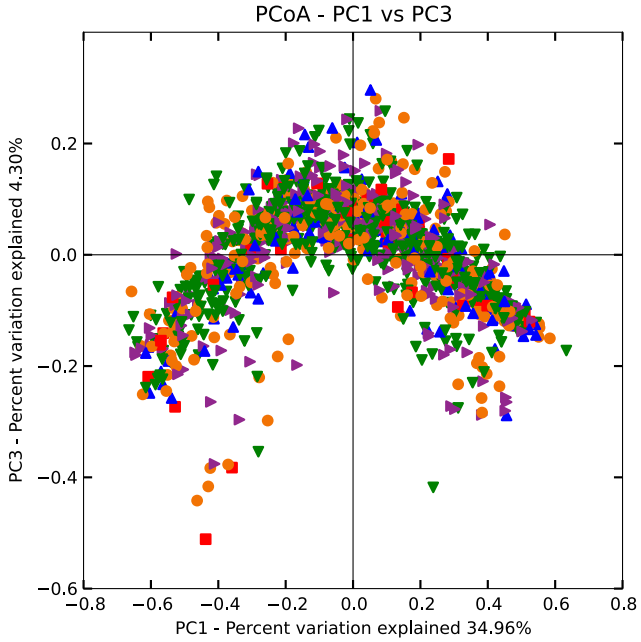


Figure 10: Weighted Unifrac beta diversity plot generated in QIIME with make_2d_plots. Showing Principal co-ordinates analysis PC1 Vs. PC3. Colorized by exercise frequency. (Never: Red; Blue=Rarely; Green=Regular; Purple=Daily; Orange=Occasional) No apparent strong clustering according to exercise is visible here.

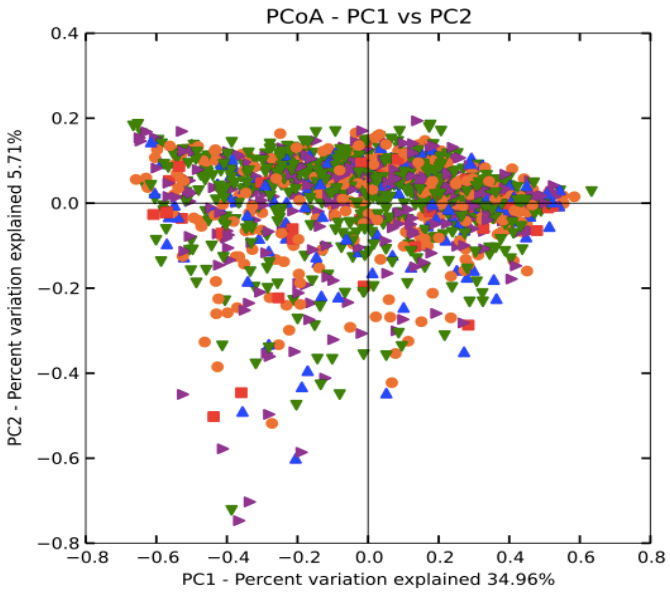


Figure 11: Weighted Unifrac beta diversity plot generated in QIIME with make_2d_plots. Showing Principal co-ordinates analysis PC1 Vs. PC2. Colorized by exercise frequency. (Never: Red; Blue=Rarely; Green=Regular; Purple=Daily; Orange=Occasional) No apparent strong clustering according to exercise is visible here.

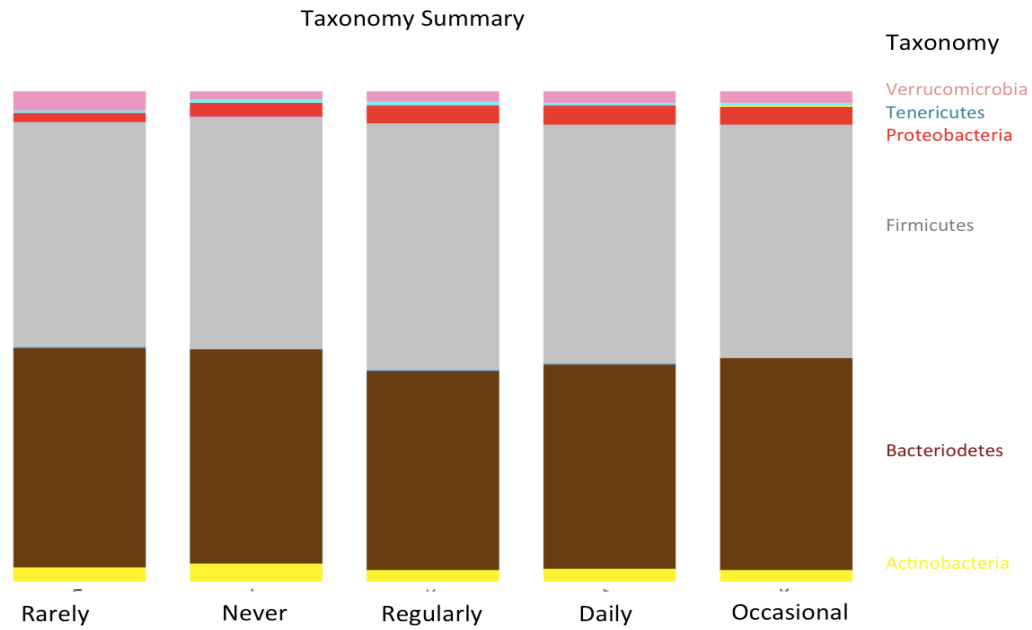


Figure 12: Summary of Taxa between groups, showing greatest change in distribution of Firmicutes and Bacteriodetes. Individuals who exercised Regularly, Daily or Occasionally showed an increase in Firmicutes and as compared to Rarely or Never exercisers.

Discussion

An increase in diversity has been associated with a healthier system due to the ability to respond to adverse selection pressures. (Chatelier et al. 2013) Healthy children from Bangladesh showed a far greater diversity in their gut microbiota than healthy children from the United States. This diversity may confer resilience adding to ecosystem stability though ensuring functional redundancy. As such healthy children in Bangladeshi contained greater diversity compared to sibling counterparts recovering from acute diarrhea. (Lin A et al. 2013) Here we demonstrate a greater diversity in groups of individuals who exercise more frequently (figure 1). The taxa distribution between exercise groups is significantly different for certain commensal bacteria in the Firmicutes phylum using a Kruskal-Wallis test, as shown in (table 1). These Firmicutes are all elevated in those individuals who exercise more frequently. Decreases in biodiversity and amount of intestinal bacteria from the dominant phyla Firmicutes occurs frequently within patients with Crohn's disease. *Faecalibacterium prausnitzii* was the only species level

taxon we observed to be significantly different among exercise categories. Here we showed an increase in *Faecalibacterium prausnitzii* as exercise increases. (figure 4) The depletion of *Faecalibacterium prausnitzii* is often related to an increased risk of recurring Crohn's disease. (Sokol H et al. 2008) There is a significant increase in alpha diversity among individuals who exercise more frequently (figure 1), and lower variability among the predominant taxa (figure 2) as can be seen by the standard error. This suggests that exercise can help maintain a healthier gut microbiota by increasing diversity, selecting for microbes that impart a healthy phenotype, and potentially stabilizing the microbial configuration. Many other factors may however also influence the human gut such as diet, antibiotic treatment and age. The prevention of colon cancer is often associated with healthy lifestyle choices such as exercising regularly. Genetic variation also has an upward shift associated with physical activity and diet, which can lead to higher prevention of developing colon cancer. (Zeng et al. 2014) Future experiments may include keeping individuals with a controlled diet and exercise regimen to determine effects of exercise as the driver diversifying

or enriching the human gut microbiota. This analysis only included individual time points-if a longitudinal human study were performed, a volatility analysis with given time stamps of samples would clarify whether exercise acts to stabilize the microbial community. Probiotics have been known to help with homeostasis of the human immune system after exercise. (West et al. 2009) It would also be beneficial to look deeper into the specific dose-response within the different levels of exercise and determine the actual magnitude of species affect on health and the ability to perform at a high level. Additional study is needed with a higher sample population of 'Never' exercisers, which was the lowest in our participant sample. This low sample size may cause significant differences not to be seen. Additionally it would be worth looking into whether human fecal transplants from regular exercisers into gnotobiotic mice can increase resistance to infection, change activity levels or decrease the propensity to gain weight, in a similar fashion to the fecal transplants from human twins who were discordant for BMI into mice (Turnbaugh et al. 2009). Further study could help identify species, which may be useful as treatment for certain known diseases such as irritable bowl

disease. (Sokol H et al. 2008) It would also be beneficial to investigate how certain bacteria may be enriched through exercise, and help with performance in athletics. Recent studies in mice have shown a correlation between exercise and shifts in gut microbiota as a result, which led to weight loss. (Evans C et al. 2014) Studying elite athletes, their gut microbiota and how it may be different than average individuals may further help with diagnosing differences in species. This may serve to identify species helpful for athletic achievement. In addition to giving us a better understanding of how a healthy lifestyle shapes the gut microbiota, these studies could lead to potential treatments or probiotics for ailments or potentially improving athletic performance.

Conclusion

Exercise leads to an increase alpha diversity amongst individual's microbiome, especially the Firmicutes and Clostridiales. Significant differences were seen in commensal bacteria within the phylum, Firmicutes. These included *Faecalibacterium prausnitzii*, uncharacterized species of genus *Oscillospira*, *Lachnospira*, *Coprococcus*, and uncharacterized families of Clostridiales. These differences in commensal bacteria can lead to healthier individuals more able to fight off certain pathogens. For example, *Faecalibacterium prausnitzii* is a butyrate producer, which has been shown to be important in maintaining intestinal epithelial health. (Blottere HM et al. 2003) Understanding how the microbial diversity and enrichment of commensurate bacteria in the gut is modulated through exercise could aid in treatment and prevention of health issues.

Acknowledgement

Dr. Rob Knight

Tony Walters

Dr. Antonio Gonzales Pena

References

1. Belkaid Y, Naik S. Compartmentalized and systemic control of tissue immunity by commensals. Nature Publishing Group USA. 2013 DOI 10.1038/ni.2604

2. Bolam, David N., and Justin L. Sonnenburg. "Mechanistic insight into polysaccharide use within the intestinal microbiota." *Gut microbes* 2.2 (2011): 86-90.

3. Blottière HM, Buecher B, Galmiche JP, Cherbut C. Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. Proc Nutr Soc. 2003;62:101–106.[PubMed]

4. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne

NM. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;5(8):1091–103. doi: 10.1136/gut.2008.165886. [PMC free article] [PubMed] [Cross Ref]

5. Carvalho FA, Koren O, Goodrich JK, Johansson ME, Nalbantoglu I, et al. Transient inability to manage proteobacteria promotes chronic gut inflammation in TLR5-deficient mice. Cell Host Microbe. 2012;12:139–152. [PubMed]

6. Emmanuelle Le Chatelier, Trine Nielsen, Junjie Qin, Edi Prifti, Falk Hildebrand, Gwen Falony, Mathieu Almeida, Manimozhiyan Arumugam, Jean-Michel Batto, Sean Kennedy, Pierre Leonard, Junhua Li, Kristoffer Burgdorf, Niels Grarup, Torben Jørgensen, Ivan Br (2013). Richness of human gut microbiome correlates with metabolic markers*Nature* DOI: 10.1038/nature12506

7. Choi JJ, et al. Exercise attenuates PCB-induced changes in the mouse gut microbiome. Environ Health Perspect 1216725–730.7302013. http://dx.doi.org/10.1289/ehp.1306534. [PMC free article] [PubMed]

8. Christian C. Evans, Kathy J. LePard et al. Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. PLoS One. 2014 Mar 26;9(3):e92193. doi: 10.1371/journal.pone.0092193. eCollection 2014

9. Hattori M, Taylor TD. The human intestinal microbiome: a new frontier of human biology. DNA Res. 2009;16:1–12. [PMC free article] [PubMed]

10. Herder R, Demmig-Adams B.The power of a balanced diet and lifestyle in preventing cardiovascular disease. Nutrition in Clinical Care **7**(2), 46-55

11. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol.

2010;10:159–169. [PubMed]

12. Lanning D, Sethupathi P, Rhee KJ, Zhai SK, Knight KL. Intestinal microflora and diversification of the rabbit antibody repertoire. J. Immunol. 2000;165:2012–2019.[PubMed]

13. Lin A., Bik E.M., Costello E.K., Dethlefsen L., Haque R., Relman D.A., Singh U. Distinct distal gut microbiome diversity and composition in healthy children from Bangladesh and the United States. PLoS ONE. 2013;8 doi: 10.1371/journal.pone.0053838. [PMC free article] [PubMed][Cross Ref]

14. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71:8228–8235. [PMC free article] [PubMed]

15. K. M. Severson, M. Mallozzi, A. Driks, K. L. Knight. B Cell Development in GALT: Role of Bacterial Superantigen-Like Molecules. *The Journal of Immunology*, 2010; 184 (12): 6782 DOI: 10.4049/jimmunol.1000155

16. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc. Natl. Acad. Sci. USA. 2008;105:16731–16736. [PMC free article]

17. Ray K. Gut microbiota: Colorectal cancer—driven by inflammation and gut bacteria? Nat. Rev. Gastroenterol. Hepatol. 2012;9:558. [PubMed]

18. Vanessa K Ridaura, Jeremiah J Faith, Federico E Rey, Jiye Cheng, Alexis E Duncan, Andrew L Kau, Nicholas W Griffin, Vincent Lombard, Bernard Henrissat, James R Bain, Michael J Muehlbauer, Olga Ilkayeva, Clay F Semenkovich, Katsuhiko Funai, David K Hayashi, Barbara J Lyle,

Margaret C Martini, Luke K Ursell, Jose C Clemente, William Van Treuren, William A Walters, Rob Knight, Christopher B Newgard, Andrew C Heath, Jeffrey I Gordon. Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice. *Science* 6 September 2013: vol. 341 no. 6150 DOI: 1126/science.1241214

19. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, et al. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest.Nature 444: 1027–1031. [PubMed]

20. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Gonzalez Pena A, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. **2010**. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5): 335-336.

21. Paolillo R, Romano Carratelli C, Sorrentino S, Mazzola N, Rizzo A. Immunomodulatory effects of Lactobacillus plantarum on human colon cancer cells. Int Immunopharmacol. 2009;9:1265–1271. [PubMed]

22. Valdés-Ramos R, Martínez-Carrillo BE, Aranda-González II, Guadarrama AL, Pardo-Morales RV, et al. Diet, exercise and gut mucosal immunity. Proc Nutr Soc. 2010;69:644–650. [PubMed]

23. Zeng H, Lazarova DL, Bordonaro M. Mechanisms linking dietery fiber, gut microbiota and colon cancer prevention. World J Gastrointest Oncol. 2014 Feb 15;6(2):41-51

24. West N.P., Pyne D.B., Peake J.M., Cripps A.W. Probiotics, immunity and exercise: A review. Exerc. Immunol. Rev. 2009;15:107–126.[PubMed]