Socioeconomic State & Intestinal Microbiota

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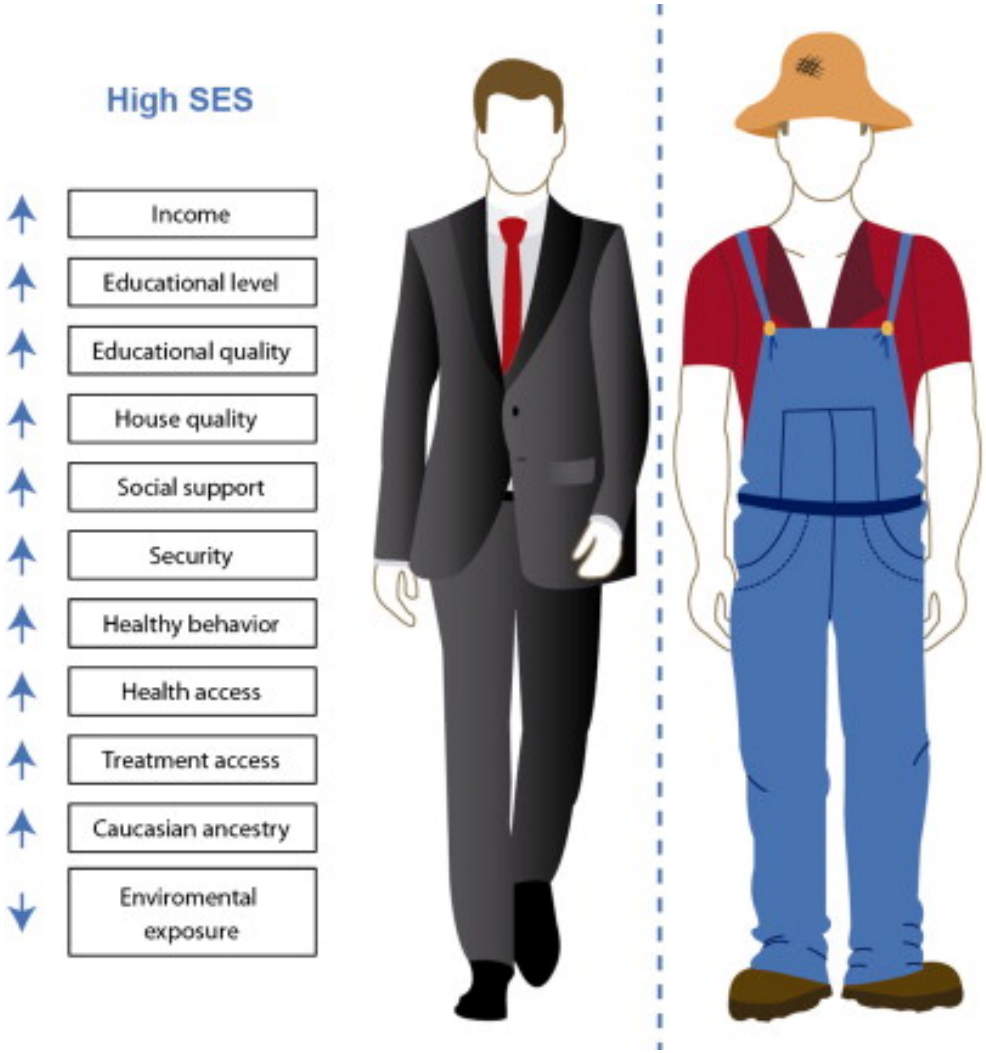
Socioeconomic Status (SES)



SES is a hierarchical social classification associated with different outcomes in health and disease

Calixto & Anaya, Autoimmunity Reviews (2014), 13(6): 641-654

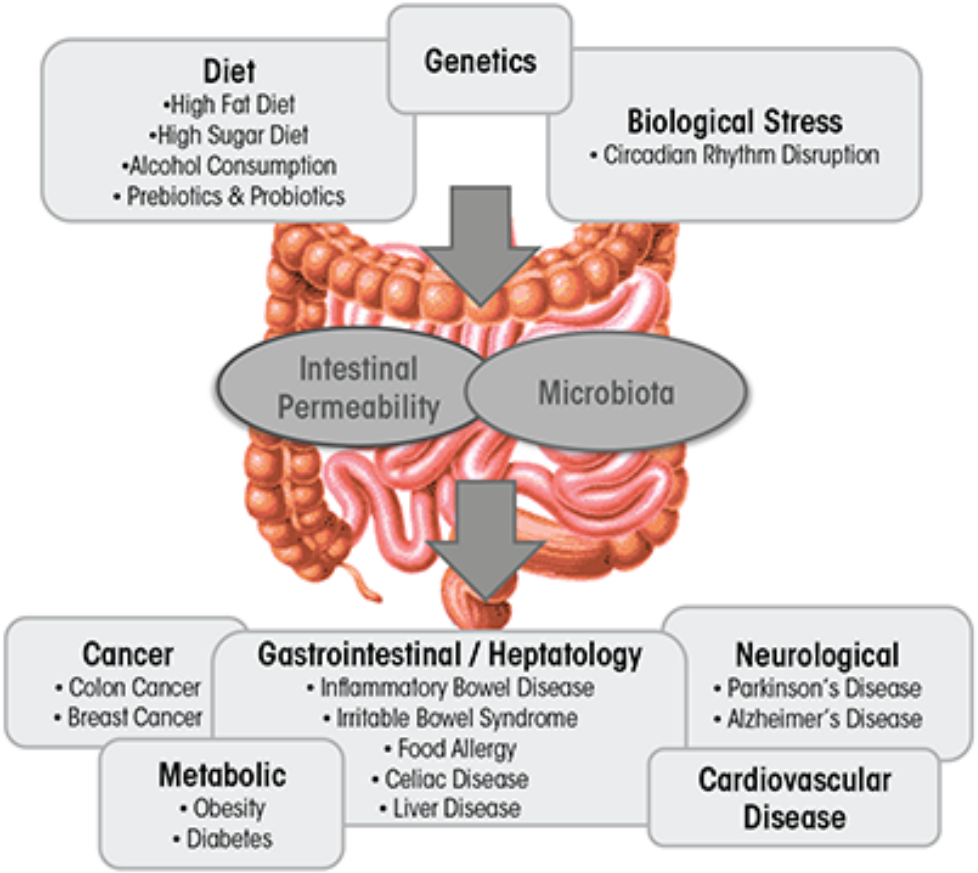
Socioeconomic (SES) and Disease

- SES disparities in morbidity/mortality from chronic disease is well-established
- Residents of low SES neighborhoods have higher rates of disease than individuals from more affluent neighborhoods including:
  - Asthma
  - Diabetes
  - Myocardial infarction
  - Stroke
  - Overall mortality

Common feature of many of these diseases…
low-grade inflammation

Inflammatory Disease and the Microbiota

- *Many inflammatory conditions are characterized by alterations in the composition of the intestinal microbiota*, with a decrease in microbiota diversity including:
  - Obesity
  - Diabetes
  - IBD
  - Asthma
  - Heart disease
  - Cancer



Engen et al., ARCR (2015), 37(2)

Intestinal Microbiota

- Genetics, life history (vaginal/cesarean, bottle/breastfed), and diet impact the intestinal microbiota and although most humans have similar microbiota no two people are exactly the same
- Cohabitating individuals tend to have similar microbiota suggesting that diet and shared environment shape the intestinal microbiota
- Lifestyle factors also influence the microbiota
  - Consumption of processed foods
  - Physical inactivity
  - Visceral adiposity
  - Psychosocial stress
  - Antibiotic use
  - Exposure to pollutants or toxicants

Socioeconomic Status (SES) & Microbiota

Could lifestyle factors associated with low SES communities influence the intestinal microbiota and predispose low SES communities to higher inflammation-mediated disease?

RESEARCH ARTICLE

Lower Neighborhood Socioeconomic Status Associated with Reduced Diversity of the Colonic Microbiota in Healthy Adults

Gregory E. Miller[1]*, Phillip A. Engen[2], Patrick M. Gillevet[3], Maliha Shaikh[2], Masoumeh Sikaroodi[3], Christopher B. Forsyth[2], Ece Mutlu[2], Ali Keshavarzian[2]

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Miller et al., PLoS One (2016), 11(2):e0148952

Methods

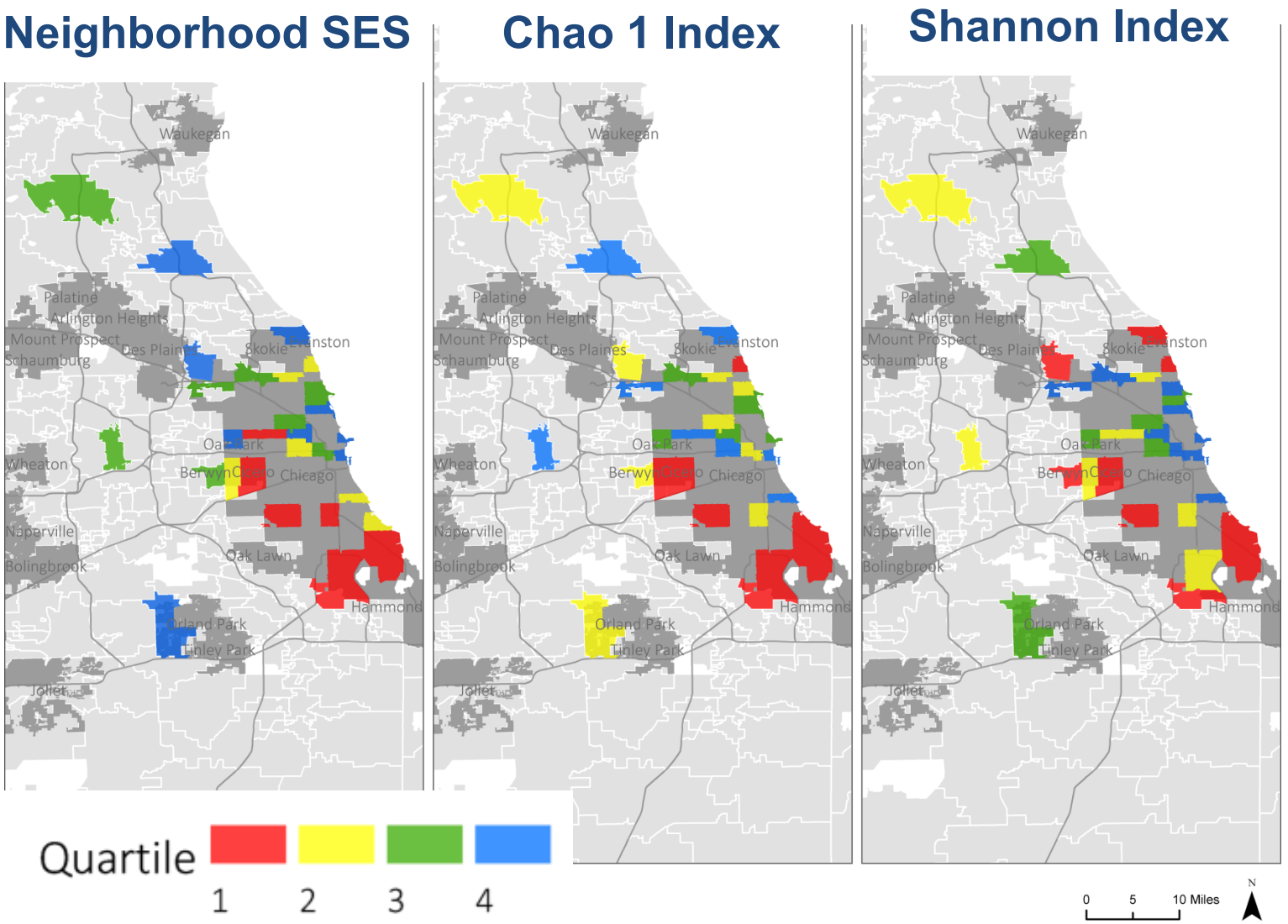
- *Neighborhood SES*
  - 3y estimates (2010-2012) of median household income, education and employment characteristics, and median home value were obtained for each neighborhood. Indicators were standardized and averaged to form a neighborhood SES composite score
  - Higher scores represent more affluent and educated neighborhoods
- *Specimen collection*
  - Mucosal biopsies (n=41) and fecal samples (n=26) were collected via a limited, un-prepped sigmoidoscopy (20–25cm from anal verge). Suction was not used during advancement of the scope and the biopsy forceps was not taken out of the channel of the scope until sample collection. Biopsies were taken from pink mucosa without visible feces at the sigmoid colon ~20cm from the anal verge

Subject Cohort

Healthy Control Subjects (n = 44)	Mean (SD) or Percent	Range
Age (years)	39.1 (14.2)	20–72
Gender (Female)	61.4%	-
Caucasian	50.0%	-
African-American	34.1%	-
Body Mass Index (kg/m^2)	27.9 (6.7)	19.6–45.4
Current Smoker	14.3%	-
Alcohol Use (years)	12.1 (9.9)	0–43
Median Household Income (2012 dollars)	58,042 (22,400)	20,100–129,570
Median Home Value (2012 dollars)	297,495 (123,762)	121,259–565,975
Percent Employed	89.1 (5.4)	65.3–95.6
Percent High School Graduates	86.4 (9.3)	61.8–99.5
Neighborhood SES Composite	0.05 (0.89)	-2.0 –+1.9
Sigmoid Mucosa Alpha-Diversity (Shannon)	6.3 (0.8)	4.3–7.7
Sigmoid Mucosa Alpha-Diversity (Chao1)	1009 (613)	124–2426
Feces Alpha-Diversity (Shannon)	7.8 (0.7)	5.7–8.8
Feces Alpha-Diversity (Chao1)	2841 (1175)	459–6182

Miller et al., PLoS One (2016), 11(2):e0148952

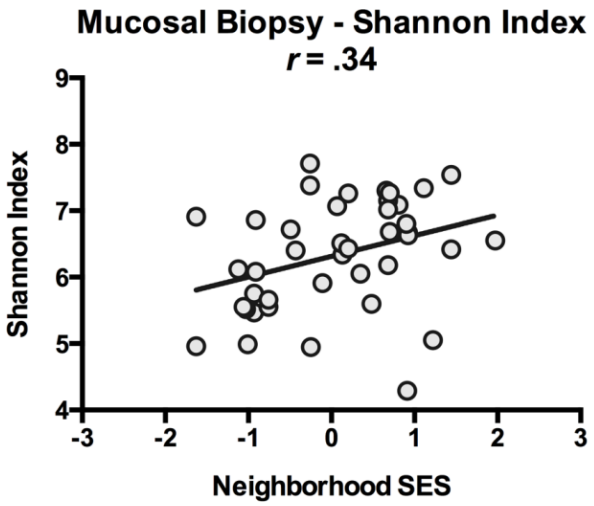
Mucosal Associated-Microbiota SES and α-Diversity

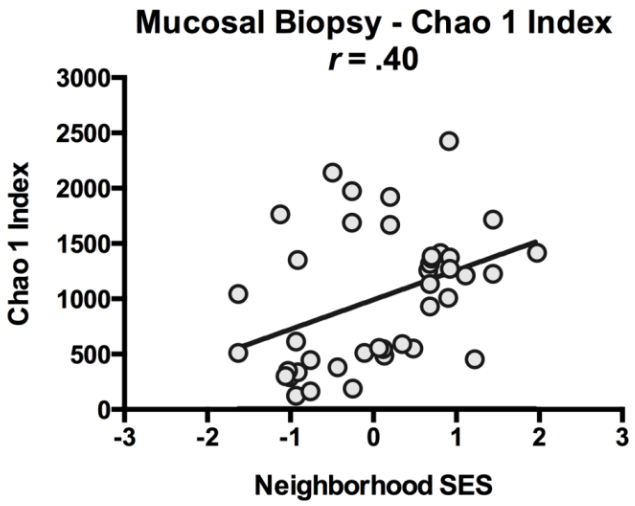


Miller et al., PLoS One (2016), 11(2):e0148952

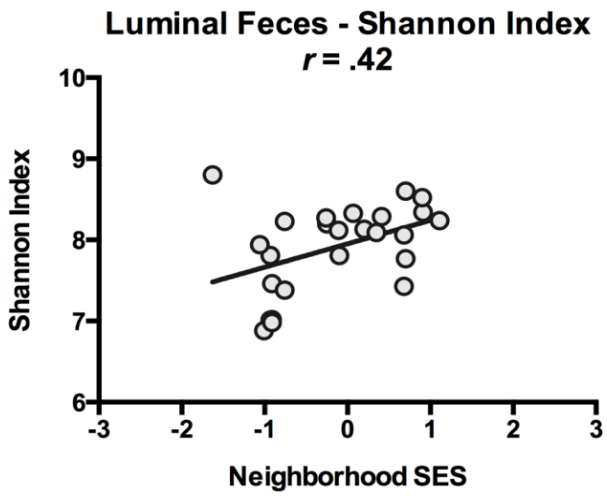
α-Diversity Positively Correlates with SES

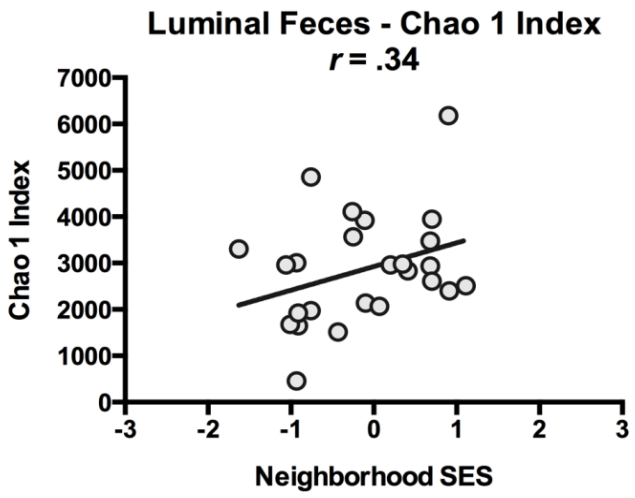
Mucosa





Lumen





Miller et al., PLoS One (2016), 11(2):e0148952

α-Diversity Positively Correlates with SES

	Mucosa		Feces	
	Sigmoid Mucosa Shannon Index *n* = 41	**Sigmoid Mucosa Chao1 Index *n* = 41**	**Feces Shannon Index *n* = 26**	**Feces Chao1 Index *n* = 25**
Unstandardized Coefficient (B)	0.31	0.27	0.29	0.51
Standard Error B	0.14	0.10	0.13	0.29
95% Confidence Interval	.03, .59	.07, .47	.02, .56	-.09, 1.12
Standardized Coefficient (β)	0.34	0.40	0.42	0.34
P-Value	**0.03***	**0.01***	**0.04***	0.09
Variance Explained (R^2)	0.12	0.16	0.18	0.12

**P*-Value < 0.05

doi:10.1371/journal.pone.0148952.t002

Miller et al., PLoS One (2016), 11(2):e0148952

Neighborhood SES Predicts α-Diversity After Adjusting for Covariates

	Mucosa		Feces	
	Sigmoid Mucosa Shannon Index *n* = 41	Sigmoid Mucosa Chao1 Index *n* = 41	Feces Shannon Index *n* = 26	Feces Chao1 Index *n* = 25
Adjusted for Demographic Covariates	**Demographic Covariates**			
Unstandardized Coefficient (B)	0.35	0.29	0.35	0.66
Standard Error B	0.17	0.12	0.16	0.36
Standardized Coefficient (β)	0.39	0.42	0.52	0.43
P-Value	0.09	**0.02***	**0.04***	0.08
Incremental Variance (ΔR^2)	0.11	0.13	0.18	0.13
Adjusted for Lifestyle Covariates	**Lifestyle Covariates**			
Unstandardized Coefficient (B)	0.45	0.30	0.28	0.77
Standard Error B	0.18	0.13	0.16	0.30
Standardized Coefficient (β)	0.45	0.41	0.42	0.54
P-Value	**0.02***	**0.03***	0.09	**0.02***
Incremental Variance (ΔR^2)	0.14	0.12	0.13	0.22

**P*-Value < 0.05

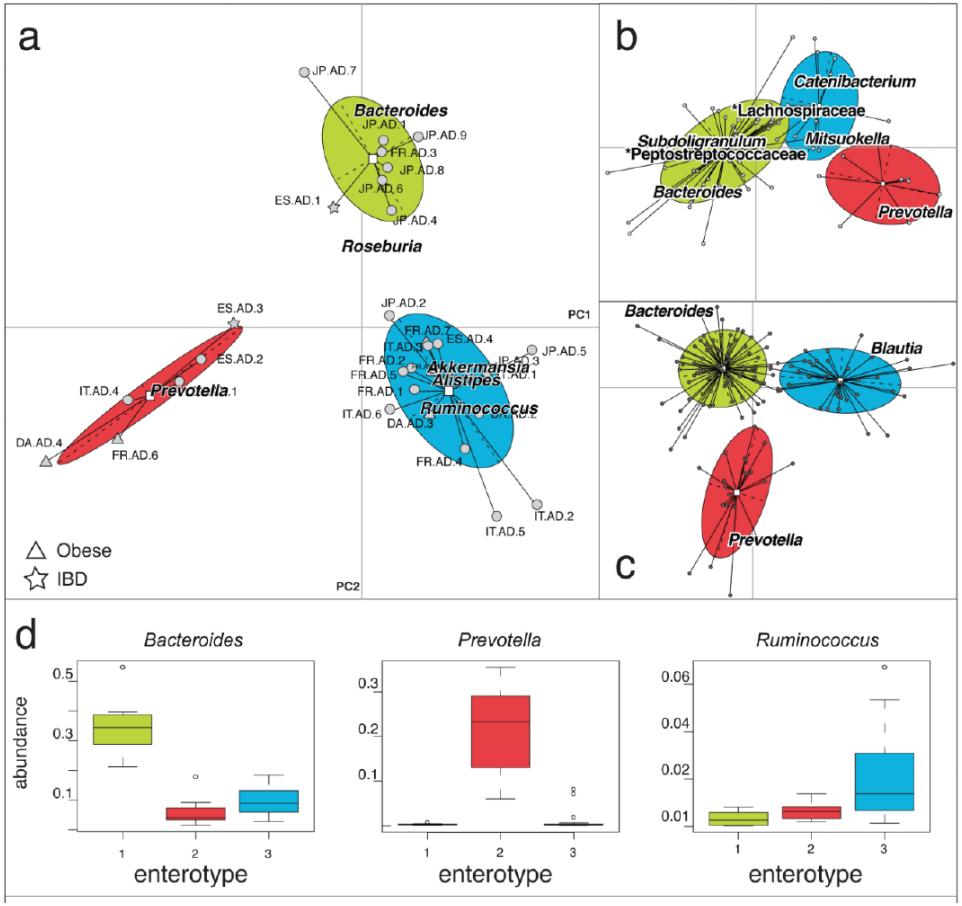
Values reflect association of neighborhood SES with alpha-diversity indicators. Demographic covariates include age, gender, and dummy codes for Caucasian and African-American. Lifestyle covariates include body mass index, smoking status, and alcohol use.

doi:10.1371/journal.pone.0148952.t003

Miller et al., PLoS One (2016), 11(2):e0148952

Microbiota Communities Can be Classified into Enterotypes

- Enterotype is a classification of the intestinal microbiota that vary in species and functional composition:
  - *Prevotella*
  - *Bacteroides*
  - *Ruminococcus*



Arumugam et al., Nature (2011), 473(7346): 174-180

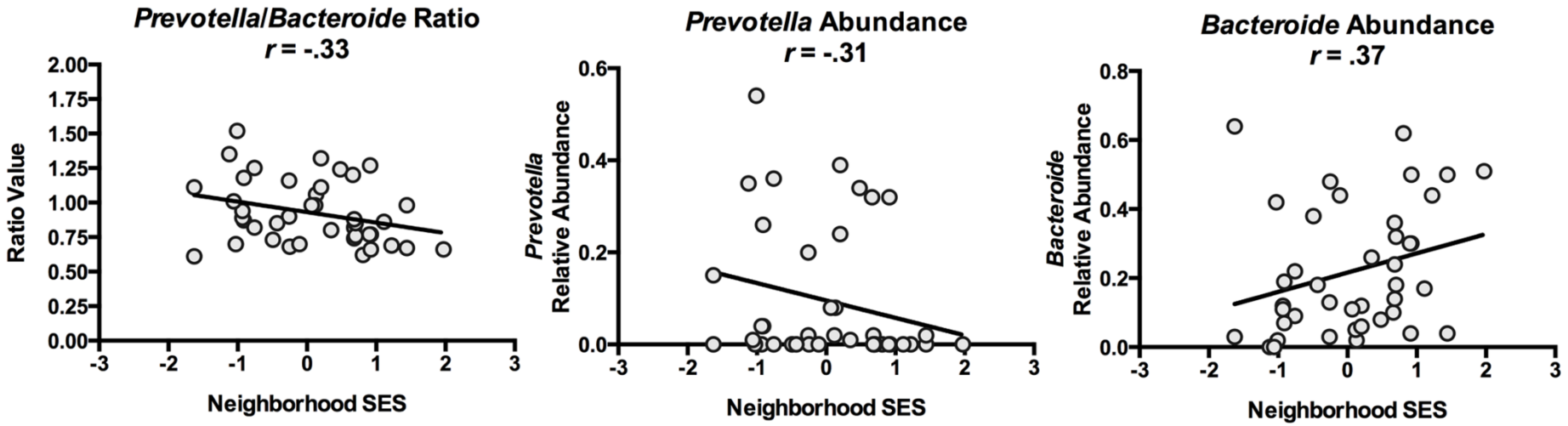
Enterotype is Associated with SES

	Prevotella n = 41	*Bacteroides n* = 41	*P/B* Ratio *n* = 41	*Ruminococcus n* = 41
Rank-order correlation	-0.31	0.37	-0.33	-0.05
P-Value	**0.05***	**0.02***	**0.04***	0.77
Variance Explained (R^2)	0.10	0.14	0.11	0.03

**P*-Value < 0.05

P/B = *Prevotella* to *Bacteroides* Ratio

doi:10.1371/journal.pone.0148952.t004



Miller et al., PLoS One (2016), 11(2):e0148952

Summary

- *Significant relationships were observed between SES and microbiota diversity* in both feces and mucosal-associated microbiota communities

- *As SES increased so did the level of diversity*
  - 10-20% of variations in diversity were accounted for by SES
  - Does not appear to be related to adiposity, smoking, or alcohol consumption and not completely explained by diet

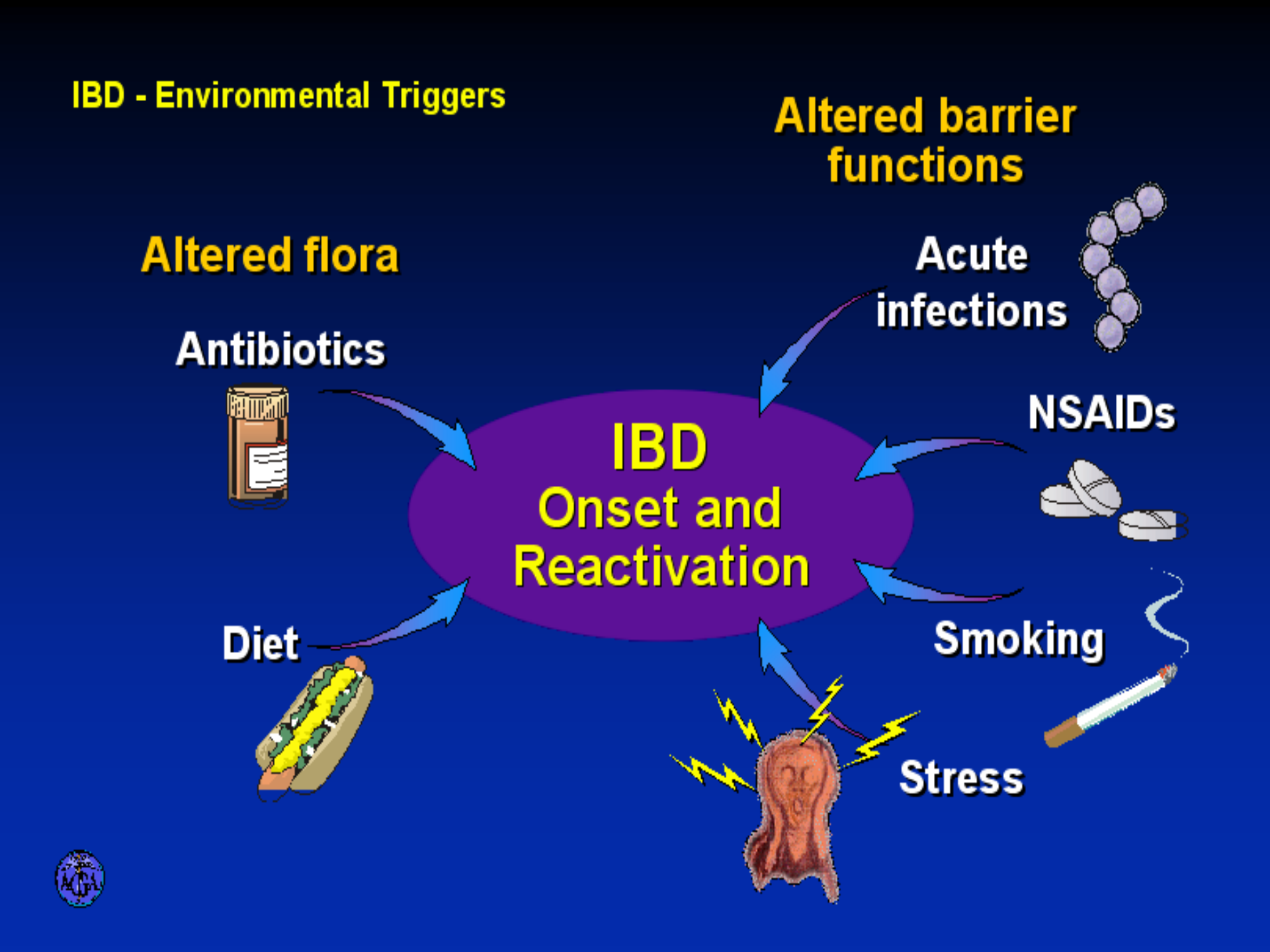
- In the greater Chicago area, *individuals from more affluent neighborhoods might have diets that are enriched in animal products* relative to carbohydrates (based on abundance of Bacteroides and Prevotella)

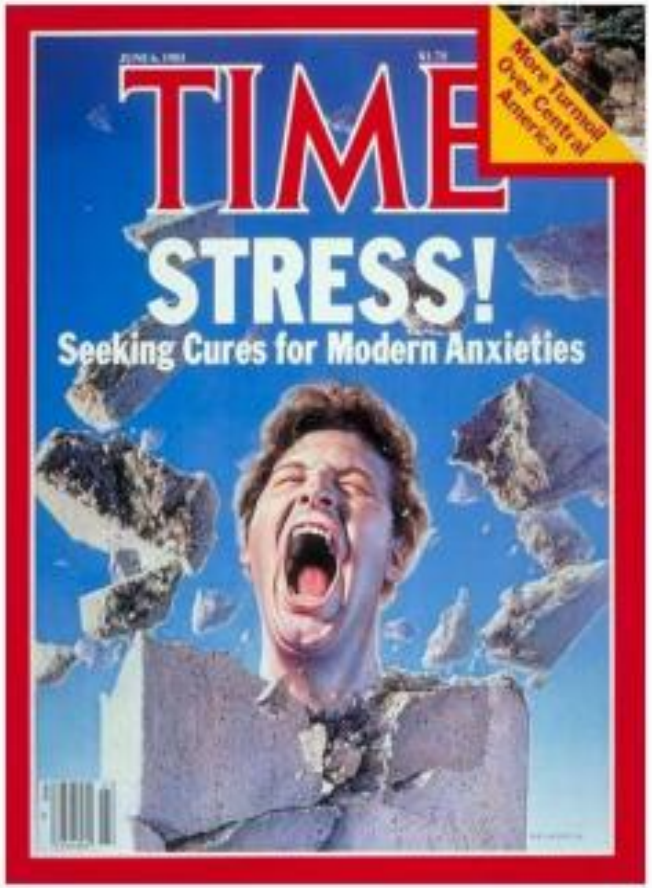
- There was no correlation between SES and serum cytokines , markers of endotoxemia and intestinal permeability

Why should SES of the host impact microbiota composition?

- Diet
- Alcohol consumption
- Physical activity/inactivity
- Antibiotic use
- Exposure to pollutants or toxicants
- Stress- social support, security
- Early life events
- “Generational” factors
- Sleep/Circadian Misalignment

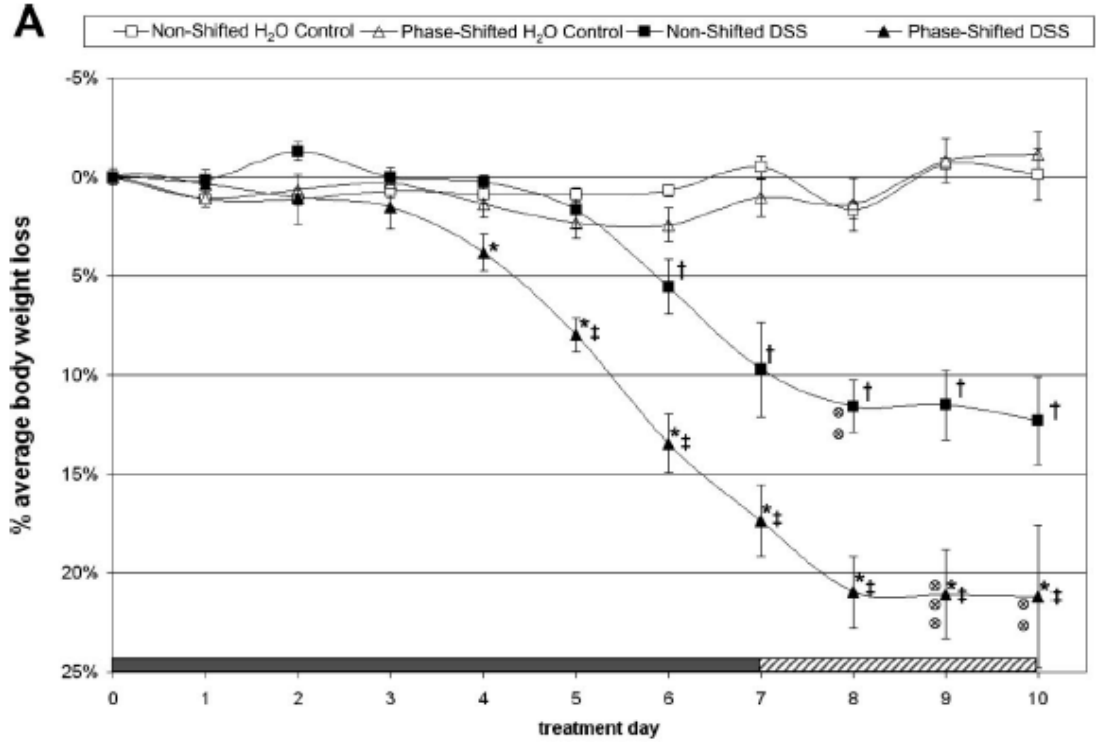
IBD - Environmental Triggers

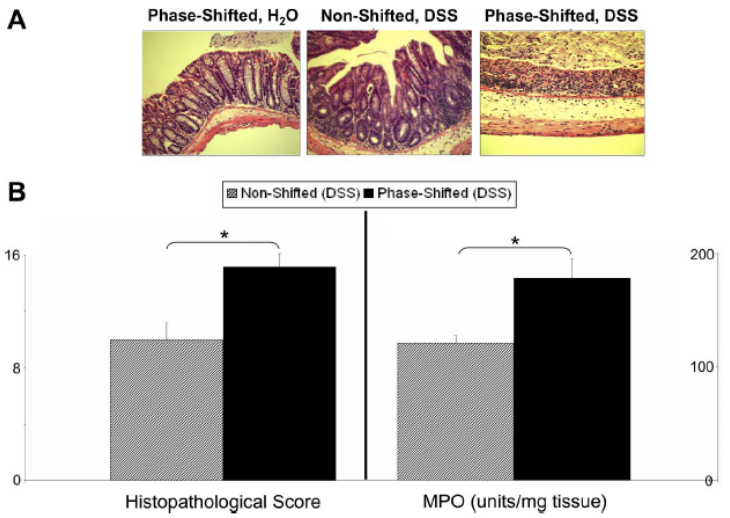



TIME
More Turmoil Over Central America
STRESS!
Seeking Cures for Modern Anxieties

Stressed & Sleep deprived

Circadian disruption augments vulnerability to chemically induced intestinal injury (DSS)





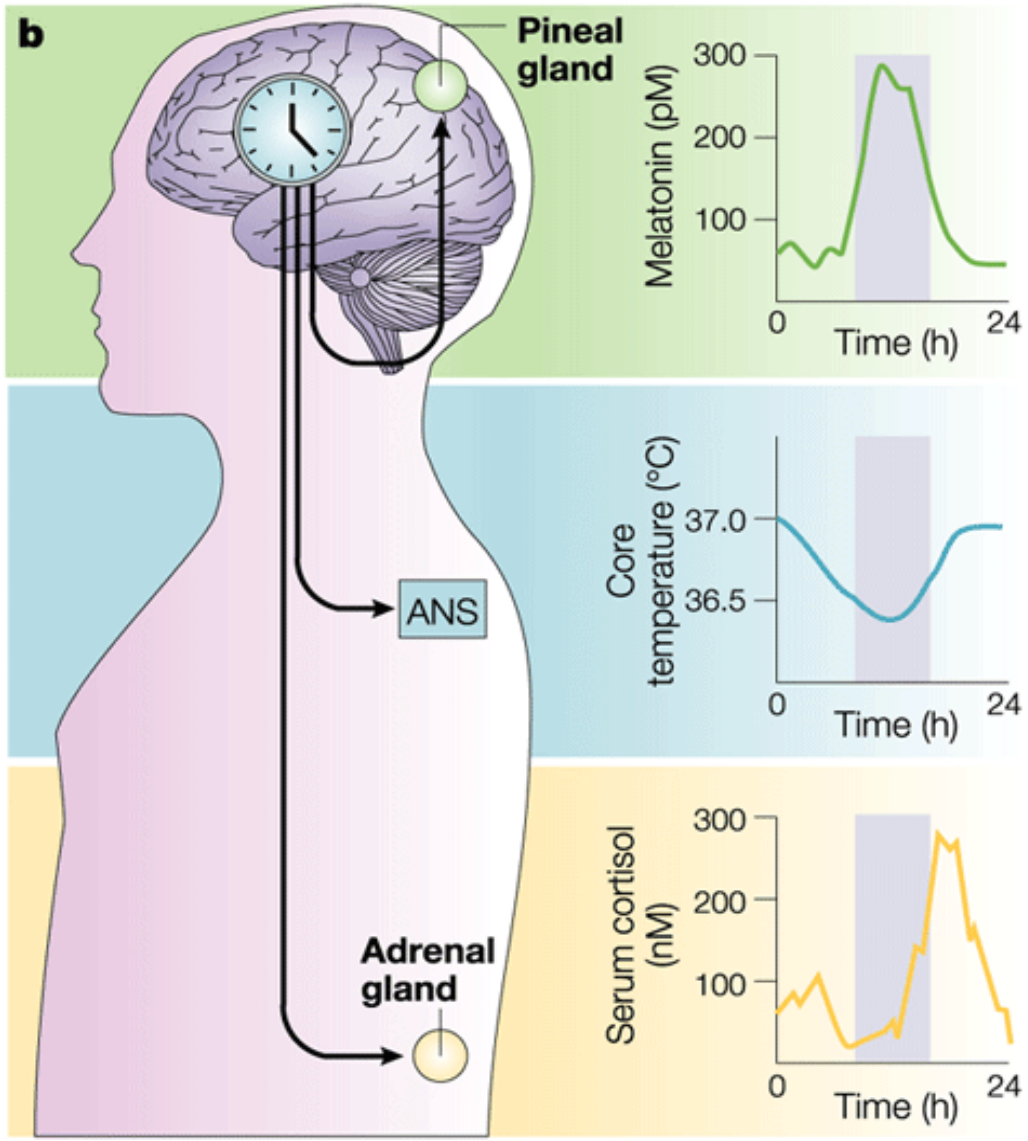
Preuss et al. *Am. J. Physiol.* 2008. **295**: R2034-R2040.

What are Circadian Rhythms?

- A circadian rhythm is any biological process that displays an endogenous, entrainable, oscillation of ~24h
  - *Endogenous:* self-sustained
  - *Entrainable:* adjust to cues in the environment
  - *Circadian* comes from the Latin words *circa (*"around") and *diēs (*"day")

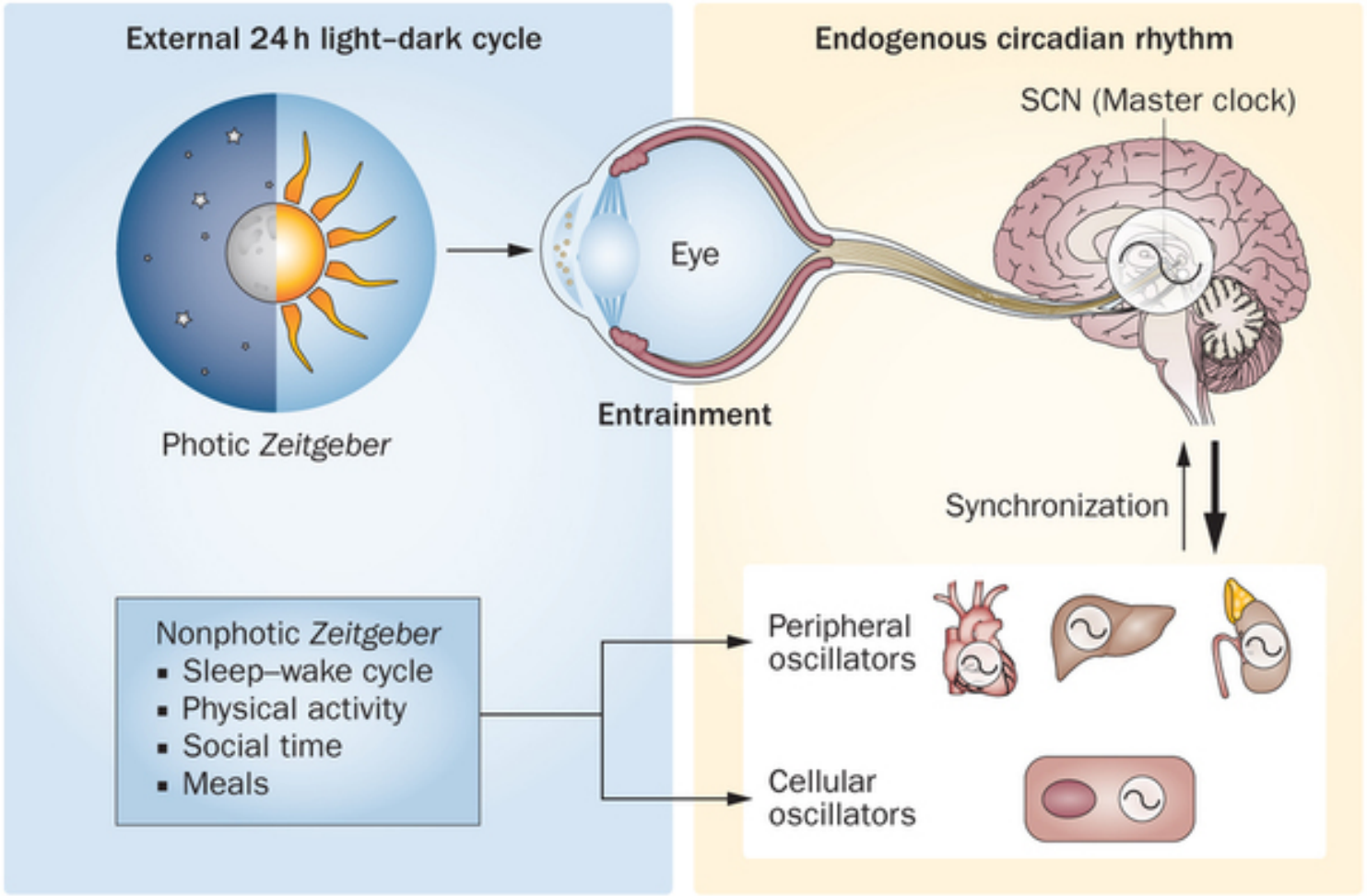
What are Circadian Rhythms?

- Allow an organism to prepare for predictable changes in the environment
  - Light:dark cycles
  - Food availability
  - Physical demands
- Regulate nearly everything!



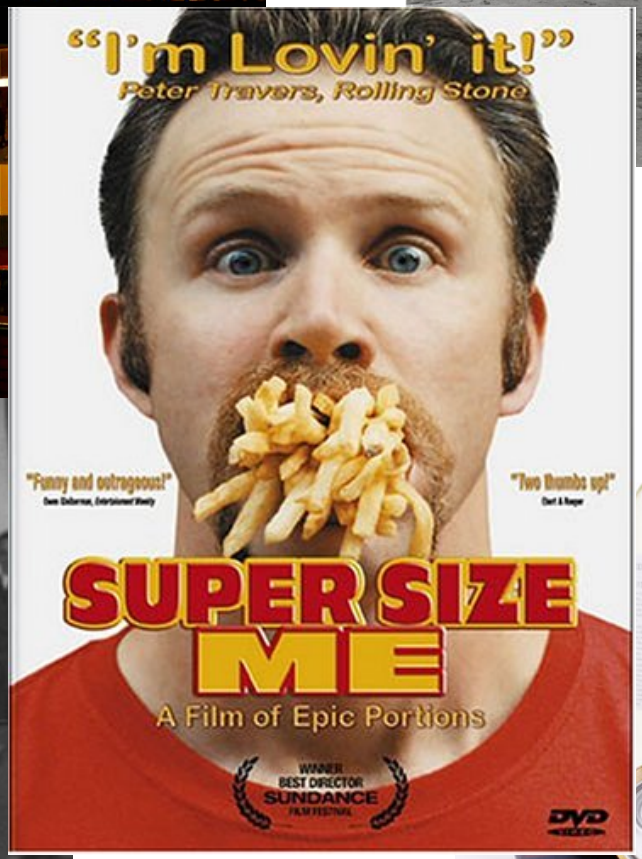
Hastings et al, Nature Reviews Neuroscience 4: 649-661, 2003

What Factors Disrupt the Circadian Clock?



Buttgereit et al, Nature Reviews Rheumatology 11: 349-356, 2015

Threats to Good Sleep & Circadian Homeostasis

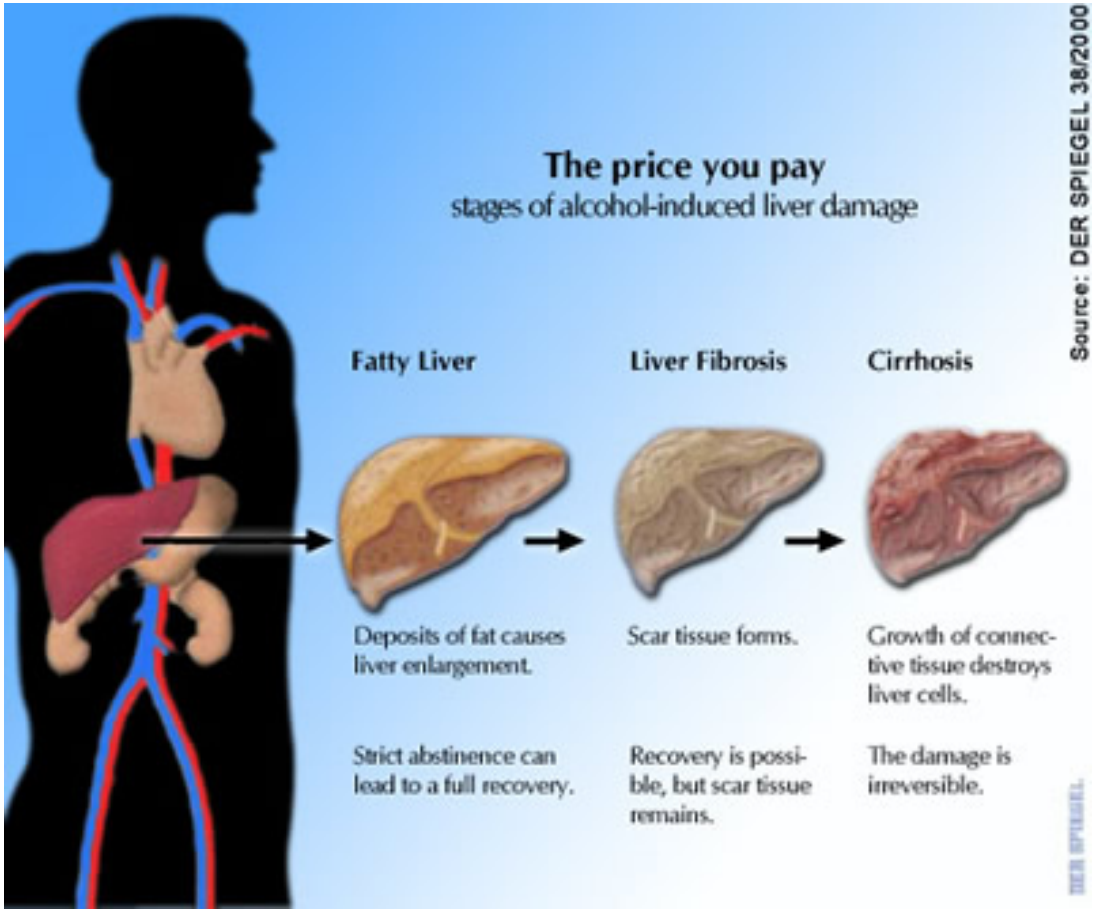


Diseases Associated with Shift Work

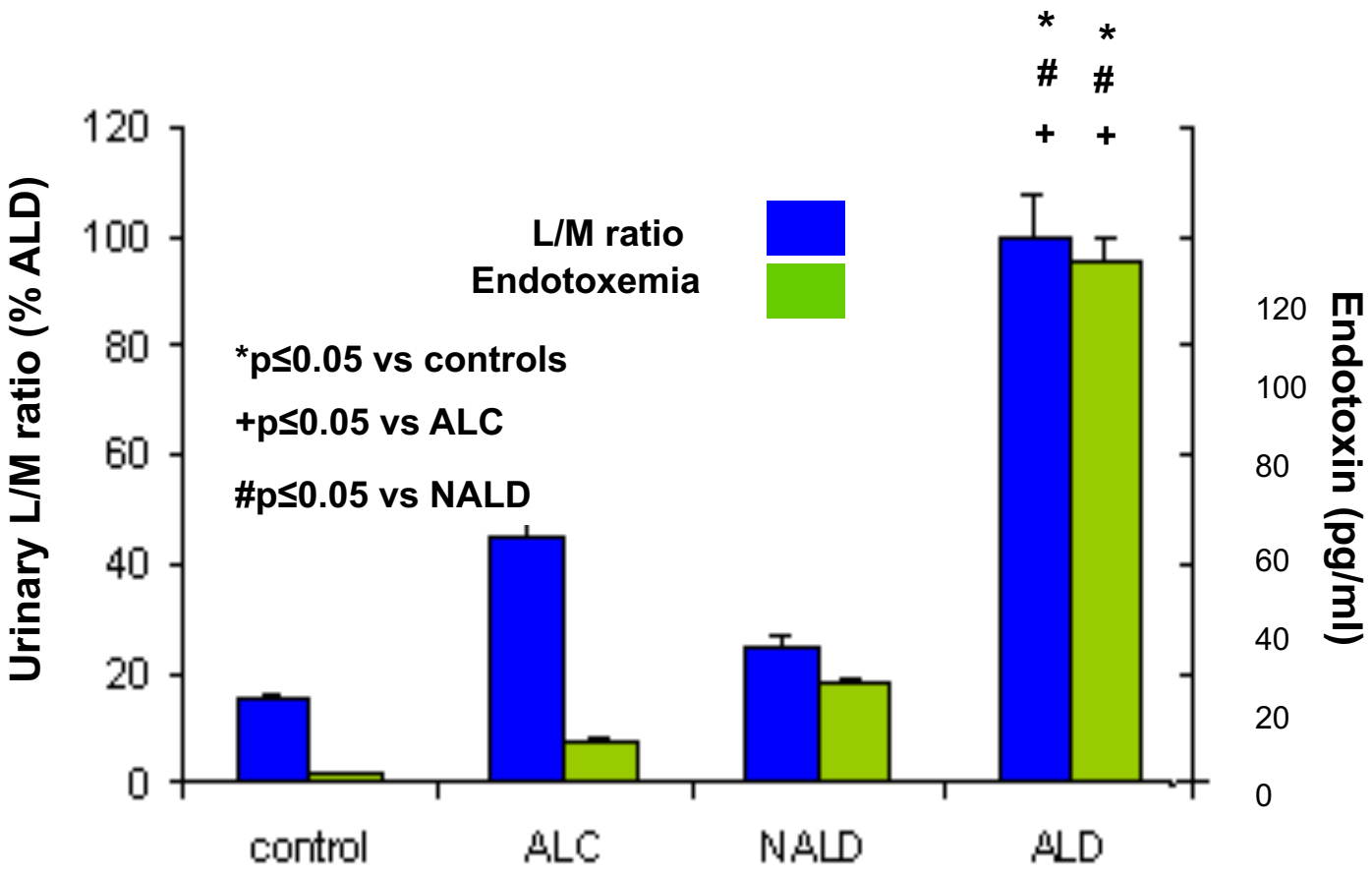
- Obesity, **NASH**
- Metabolic Syndrome, Diabetes
- Cancer (breast, prostate, colon)
- Cardiovascular Disease
- Inflammatory Bowel Disease (IBD)

Inflammation

Alcoholic Liver Disease

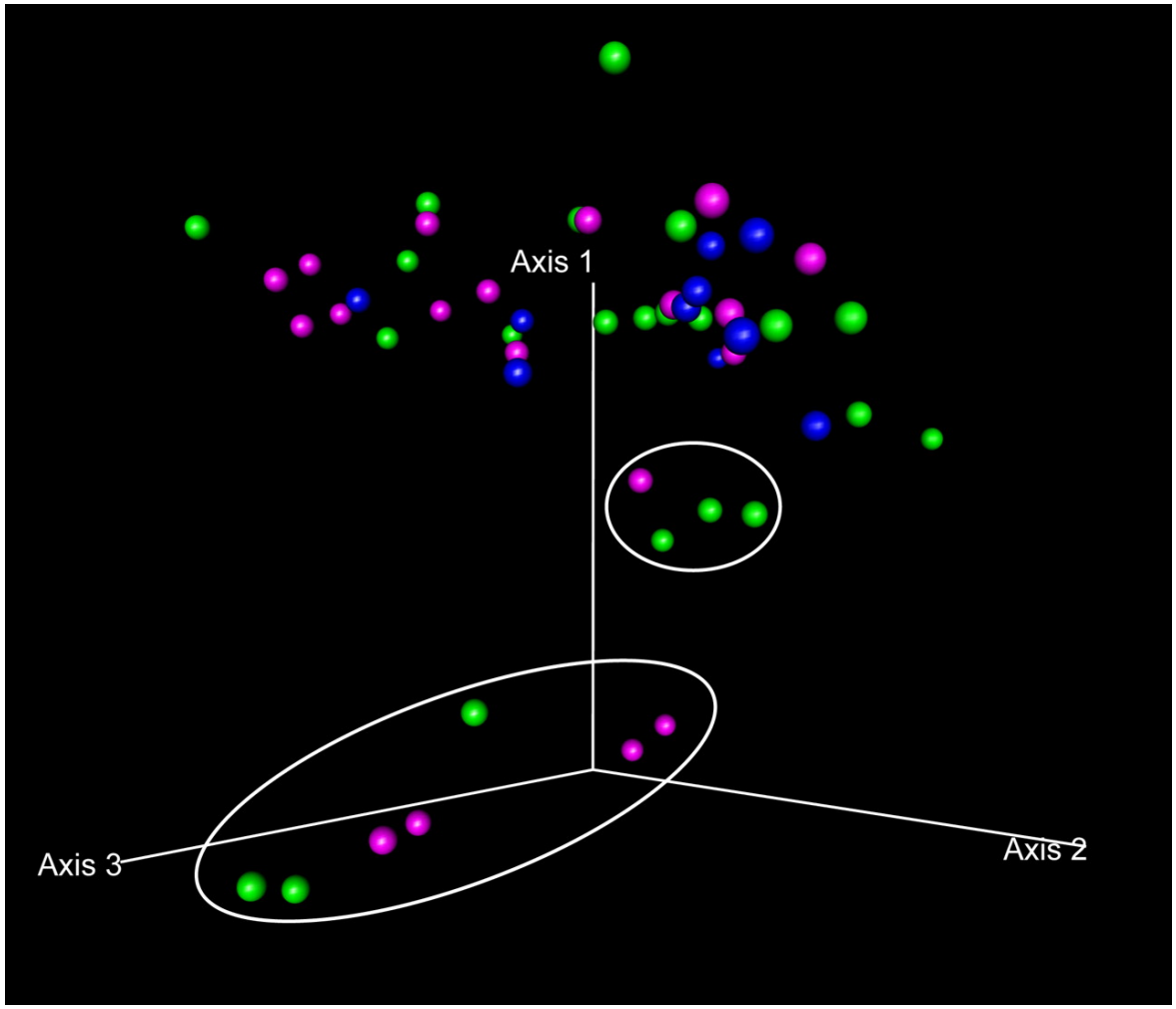


Alcoholics with ALD had endotoxemia and gut leakiness.



Keshavarzian A. et al., *Am J Gastroenterol*. 1999;94:200-207

25% of Alcoholics Differ from the Main Cluster



Healthy Controls

ALC

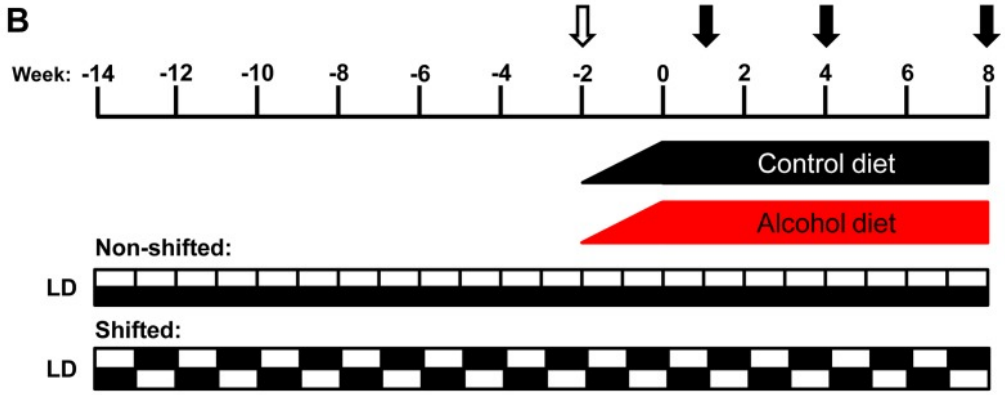
ALD

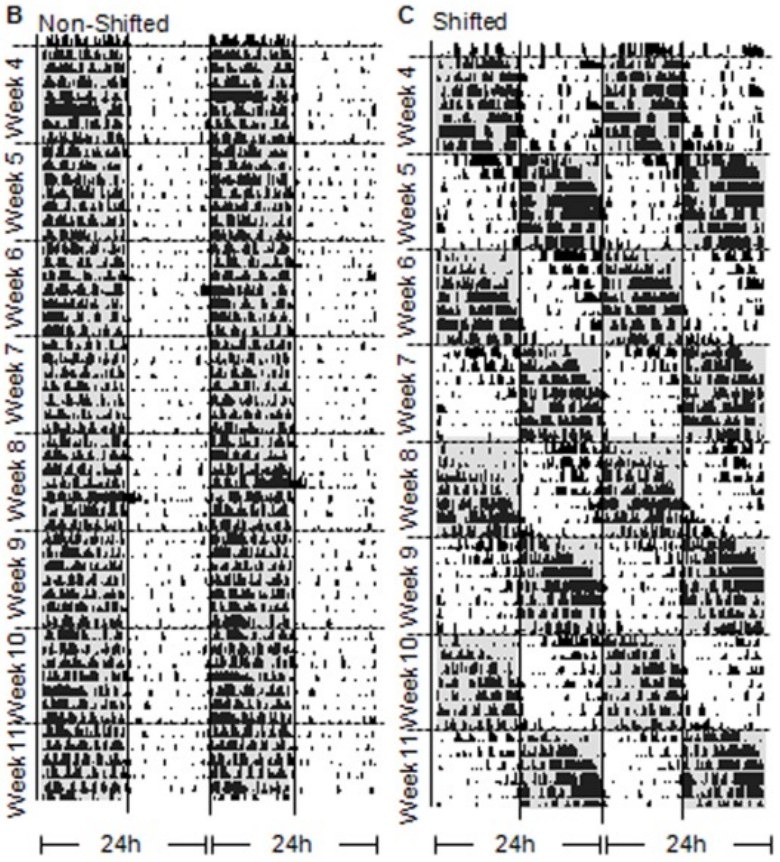
Mutlu, et al. Am J Physiol Gastrointest Liver Physiol 2012;302:G966-G978

Can disrupted circadian homeostasis promote gut leakiness and dysbiosis in alcohol fed mice?

Models of Circadian Rhythm Disruption

Environmental Circadian Rhythm Disruption





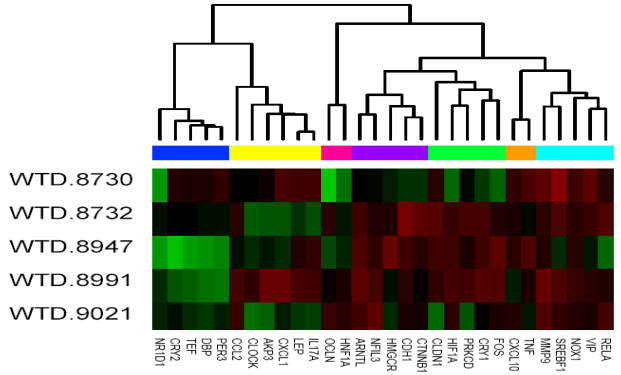
Summa, Voigt, et al PLoS One 2013 8(6): e67102 & Voigt, et al PLoS One 2014 9(5): e97500

Experimental Outcomes

Intestinal Barrier Function: Intestinal Permeability

Administer: Sucralose, Sucrose, Lactulose, Mannitol
Measure: Urinary sugar content

Gene Expression



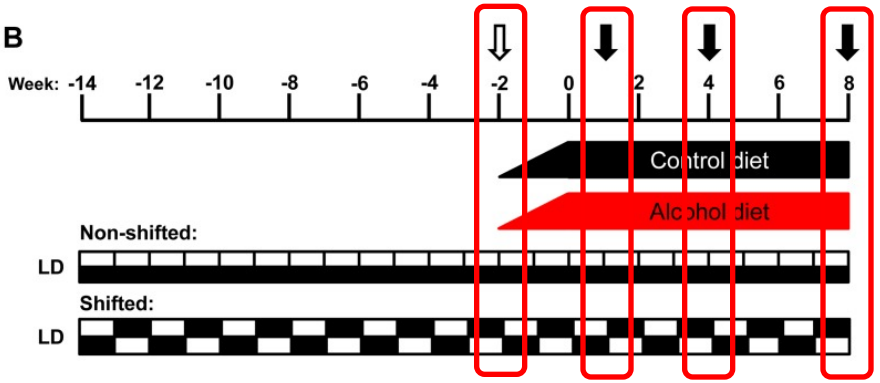
Microbiome: Stool Microbiota

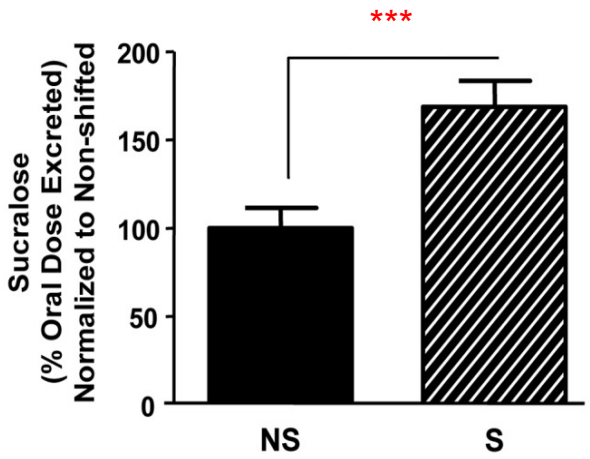
End Organ Damage: Liver Pathology

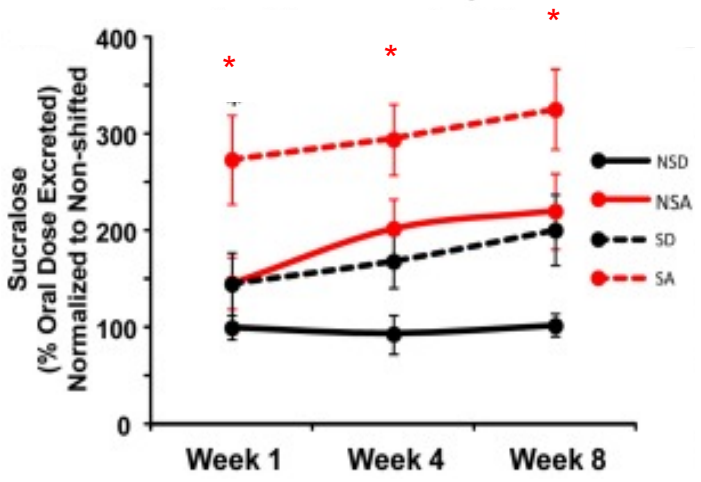
Steatosis & Lobular Inflammation

Disrupted Circadian Rhythm Promotes alcohol-induced Intestinal Permeability

Environmental Circadian Rhythm Disruption

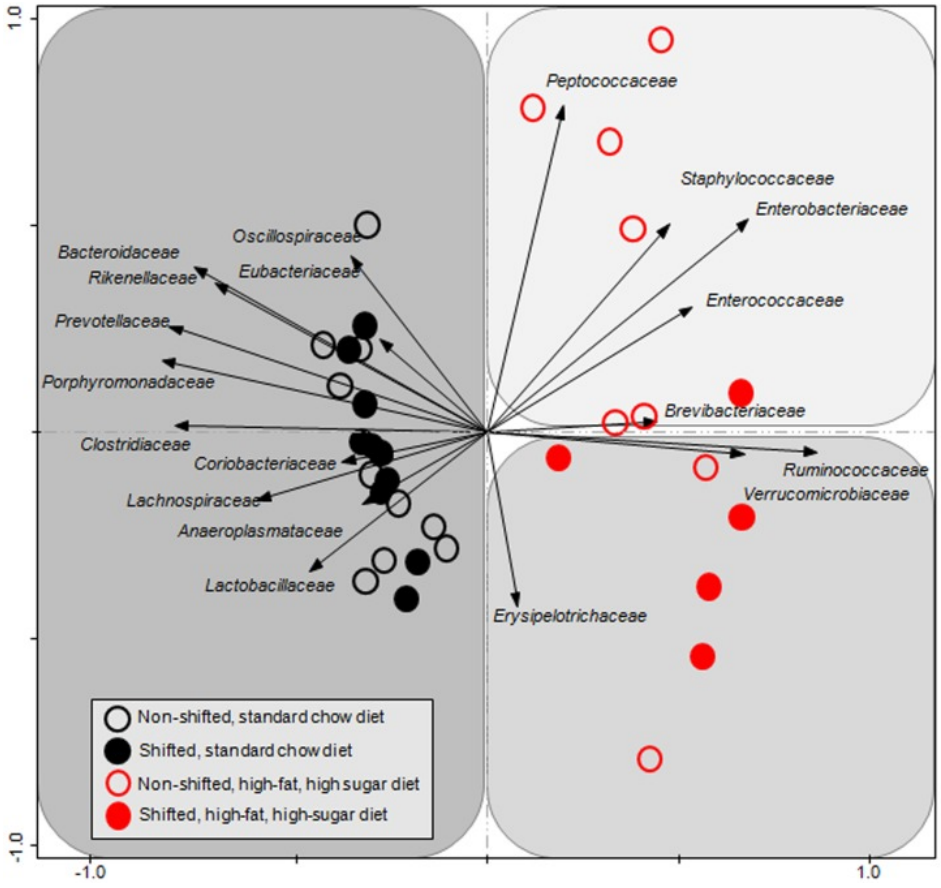




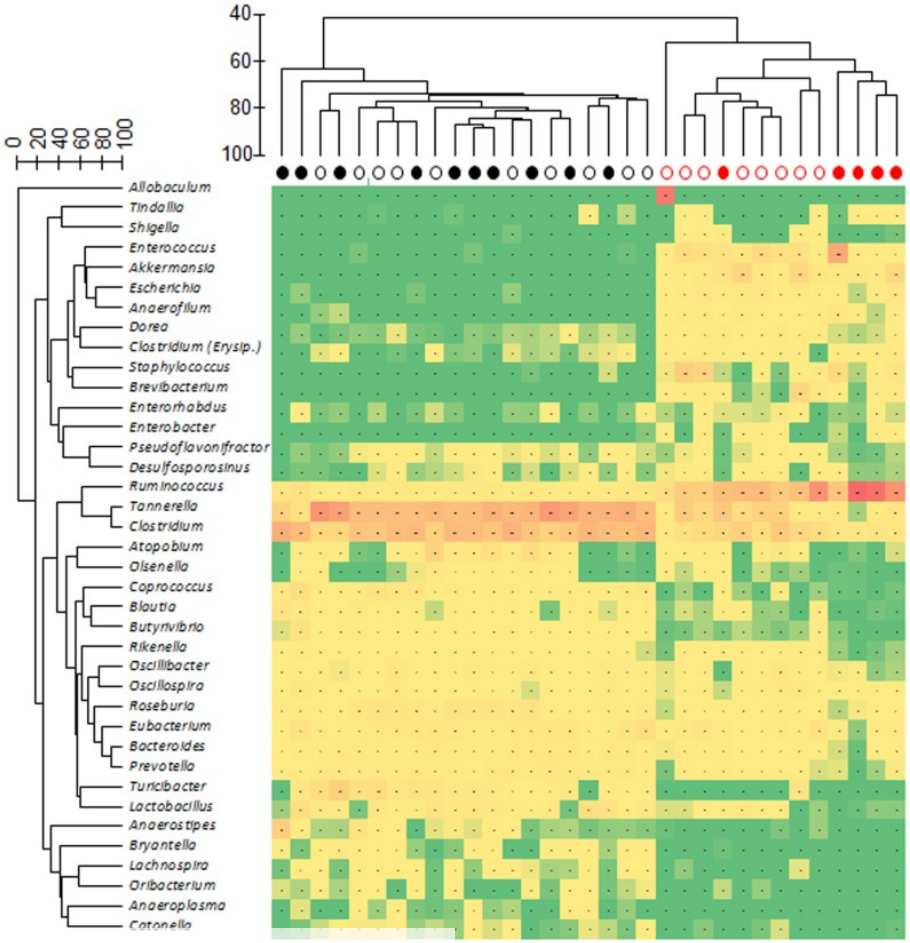


Summa, Voigt, et al PLoS One 2013 8(6): e67102

Environmental Disruption Alters the Microbiota Under "Challenging" Conditions



Family level

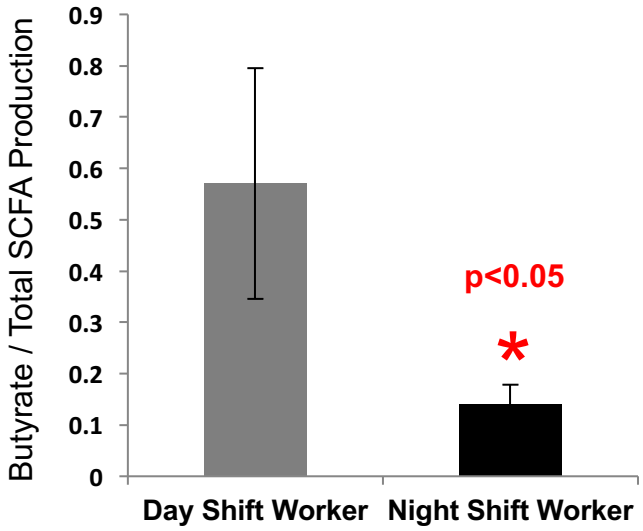


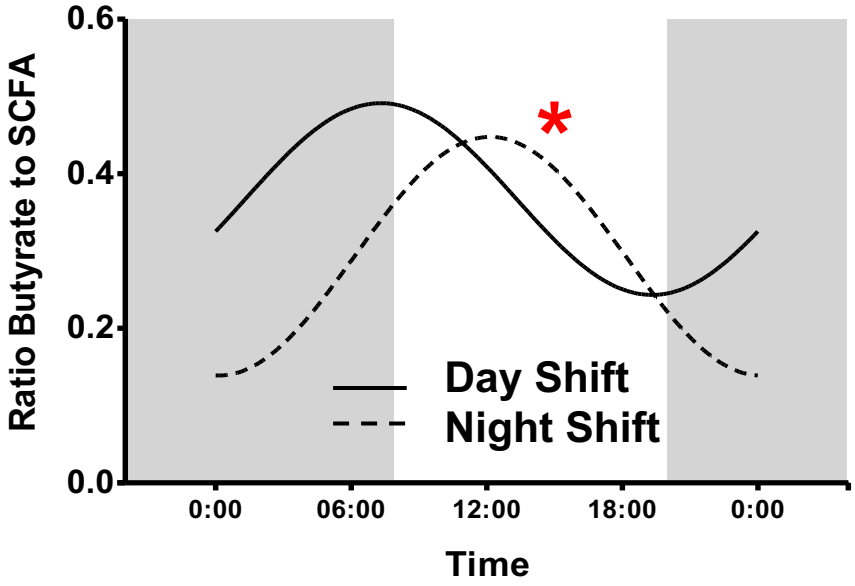
Genus level

Voigt et al, PLoS One, 2013

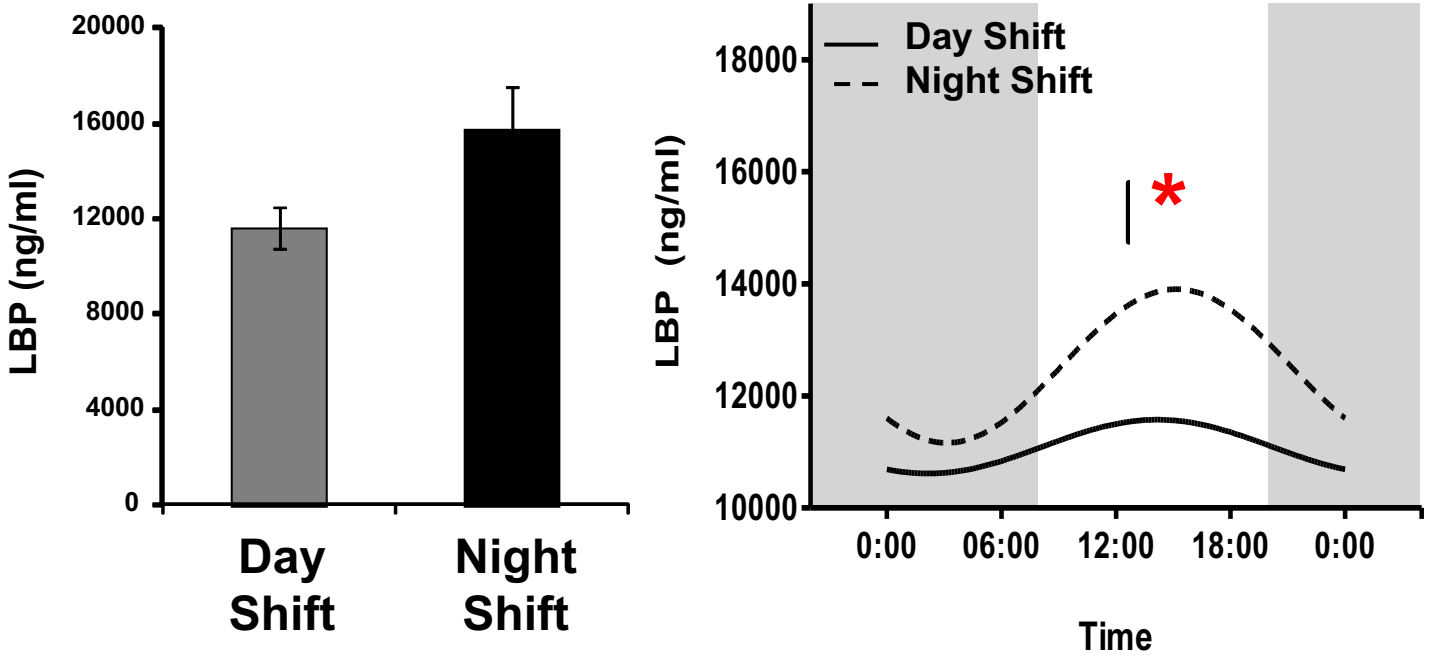
Can disrupted circadian homeostasis promote gut leakiness in humans?

Butyrate Production as a Ratio of Total SCFA Production

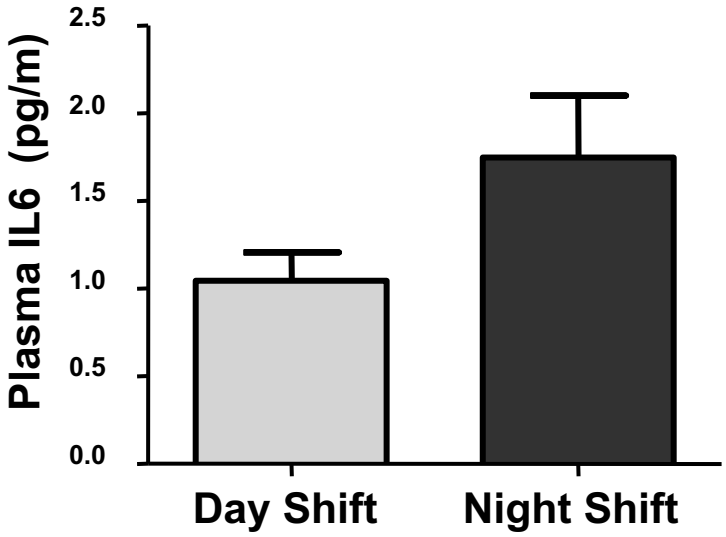


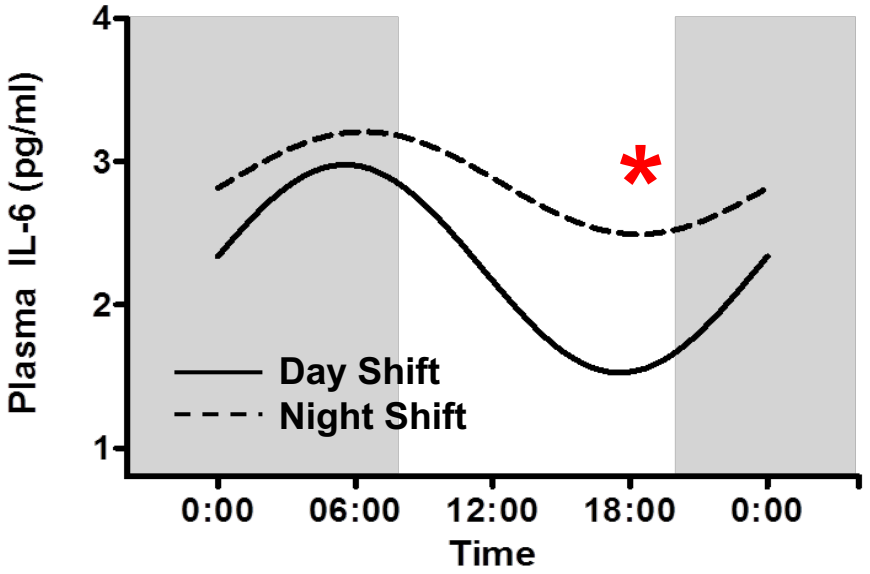


Serum LBP in Day Shift & Night Shift Workers

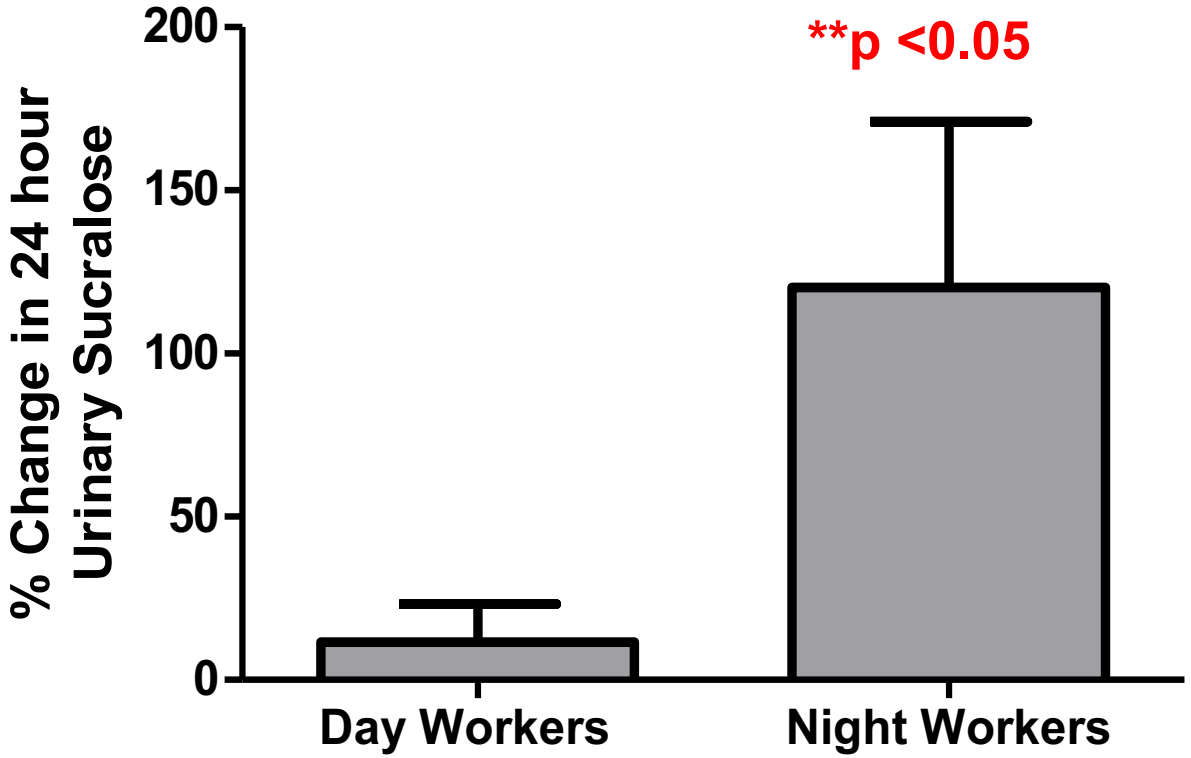


Plasma IL6 in Day Shift and Night Shift Workers

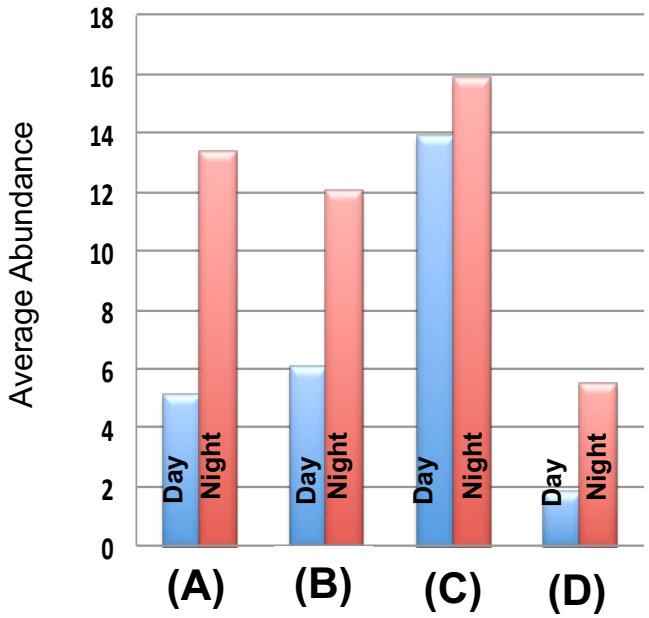




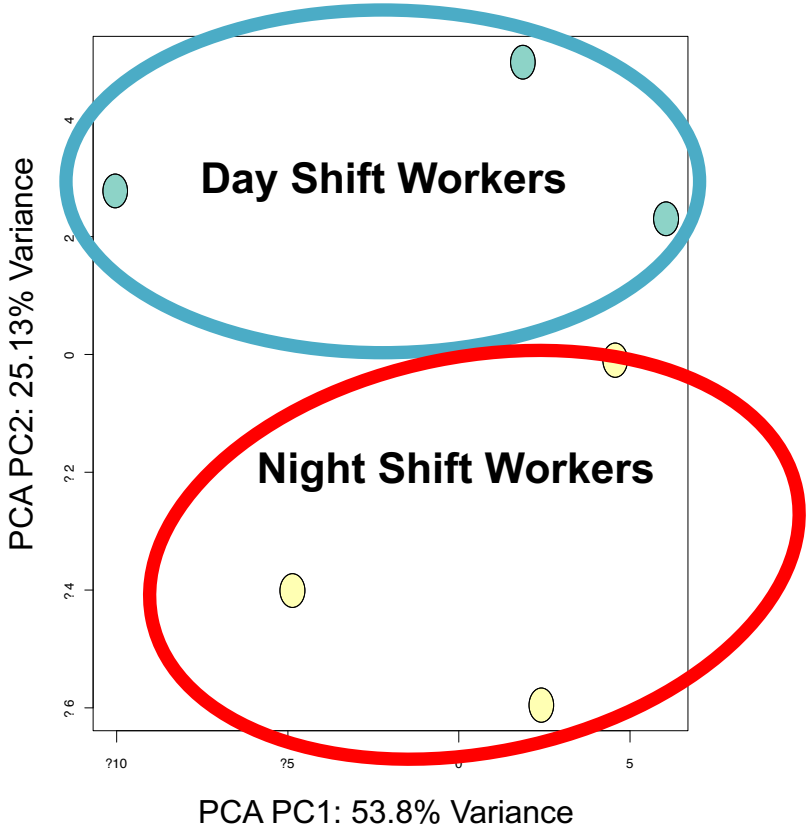
Change in Intestinal Permeability After Moderate Alcohol Consumption



Fecal Microbiota Analysis in Day Shift and Night Shift Workers



(A) Peptostreptococcaceae
(B) Turicibacteraceae
(C) Clostridiaceae
(D) Pseudomonadaceae



Conclusion

- Health disparities due to SES may be related to sub-optimal intestinal microbiota communities associated with different lifestyle, genetic, or environmental factors associated with different neighborhoods

- Targeting the intestinal microbiota with strategies to alter the intestinal microbiota (high fiber diet or dietary supplementation) may be a viable strategy to reduce SES related health disparities

Impact of SES on microbiota was reported in A Subsequent Study

ORIGINAL ARTICLE: GASTROENTEROLOGY

Gut Microbiota Differences in Children From Distinct Socioeconomic Levels Living in the Same Urban Area in Brazil

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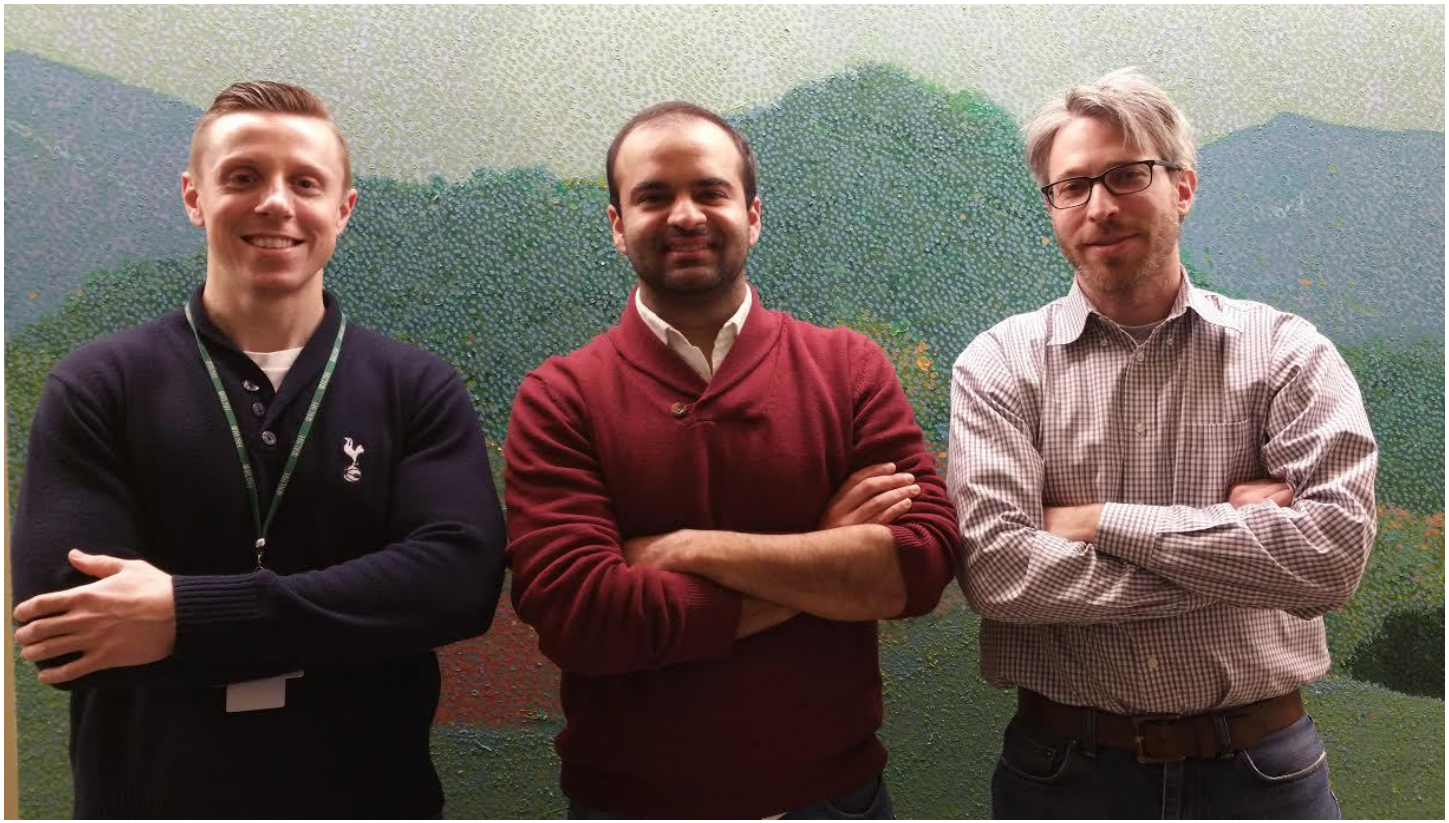
TABLE 3. Bacterial genera and species (colony-forming units: CFU/g feces) in stool samples representing the colonic microbiota of the slum children and children from the private school

		Slum (n = 100)	Private school (n = 30)	P^*
Total eubacteria	CFU/g ($\times 10^{14}$)	2.32 (0.40–10.88)	0.02 (0.0–0.05)	<0.001
Bacteroidetes phyla	CFU/g ($\times 10^{9}$)	1.61 (0.51–2.53)	0.12 (0.02–0.46)	<0.001
Bacteroides fragilis	CFU/g ($\times 10^{10}$)	1.22 (0.21–6.12)	0.43 (0.05–4.22)	0.219
Firmicutes phyla	CFU/g ($\times 10^{8}$)	0.96 (0.35–3.07)	0.13 (0.04–0.43)	<0.001
Lactobacillus spp.	CFU/g ($\times 10^{7}$)	6.45 (1.71–31.15)	2.56 (0.23–12.50)	0.016
Clostridium difficile	CFU/g ($\times 10^{3}$)	1.69 (0.78–7.26)	8.85 (2.1–17.78)	0.002
Clostridium perfringens	CFU/g ($\times 10^{5}$)	0.71 (0.17–6.08)	10.91 (1.52–39.76)	<0.001
Staphylococcus aureus	CFU/g ($\times 10^{5}$)	5.20 (1.81–24.50)	5.23 (0.19–17.56)	0.483
Bifidobacterium spp.	CFU/g ($\times 10^{5}$)	4.30 (0.97–21.18)	4.31 (2.13–14.58)	0.719
Salmonella spp.	CFU/g ($\times 10^{2}$)	2.13 (0.84–7.29)	8.86 (3.02–22.35)	0.046
Escherichia coli	CFU/g ($\times 10^{9}$)	1.38 (0.30–11.84)	0.37 (0.07–2.75)	0.037
Methanobrevibacter smithii	CFU/g ($\times 10^{7}$)	3.34 (1.04–8.71)	0.02 (0.0–0.55)	<0.001

*Mann-Whitney test (median and 25th and 75th percentiles).

Microbiota and Bioinformatics Collaboration Team



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Neighborhood SES Predicts α-Diversity After Adjusting for Covariates

S4 Table. Correlations between neighborhoods SES, alpha-diversity indices, and covariates.

	Neighborhood SES Composite *n*=44	Sigmoid Mucosa Shannon Index *n*=41	Sigmoid Mucosa Chao1 Index *n*=41	Feces Shannon Index *n*=26	Feces Chao1 Index *n*=25
Age	0.15	-0.06	-0.07	-0.08	0.10
Gender	0.03	-0.05	-0.05	-0.14	-0.31
Caucasian	0.47 ^	0.24	0.24	0.14	0.12
African-American	-0.51 ^	-0.21	-0.19	-0.01	0.04
Body Mass Index	-0.39*	-0.27	-0.26	-0.24	-0.12
Current Smoker	0.04	0.16	0.17	-0.23	-0.26
Alcohol Use	-0.04	0.07	0.06	0.04	0.34

Values are Pearson (for age, body mass index, alcohol) or Point-Biserial correlations (for Gender, Caucasian, African-American, and Smoking). Gender is coded as 0 = Male, 1 = Female. Caucasian, African-American, and Smoker are coded as 0 = No, 1 = Yes. For sigmoid mucosa, where $n = 41$, the critical value of r at $\alpha = 0.05$ is 0.30. For Feces, where $n = 26$, the critical value of r at $\alpha = 0.05$ is 0.37. * $p < 0.01$; ^ $p < 0.001$.

S5 Table. Percent variance in alpha diversity explained by covariates.

	Sigmoid Mucosa Shannon Index *n*=41	Sigmoid Mucosa Chao1 Index *n*=41	Feces Shannon Index *n*=26	Feces Chao1 Index *n*=25
Age	0.004	0.005	0.006	0.010
Gender	0.003	0.003	0.020	0.096
Caucasian	0.058	0.058	0.020	0.014
African-American	0.044	0.036	0.000	0.002
Body Mass Index	0.073	0.068	0.058	0.014
Current Smoker	0.026	0.029	0.053	0.068
Alcohol Use	0.005	0.004	0.002	0.116

Table shows R^2 values, reflecting percentage of variance in alpha diversity indices explained by each covariate (for age, body mass index, alcohol). Gender is coded as 0 = Male, 1 = Female. Caucasian, African-American, and Smoker are coded as 0 = No, 1 = Yes. For sigmoid mucosa, where $n = 41$, the critical value of r at $\alpha = 0.05$ is 0.30. For Feces, where $n = 26$, the critical value of r at $\alpha = 0.05$ is 0.37. * $p < 0.01$; ^ $p < 0.001$.