THE EFFECT OF EXERCISE ON THE MICROBIOME

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INTRODUCTION

• 78.6 million adults in the United States are classified as obese.

• Sedentary lifestyle is associated with greater incidence of:
  – Cardiovascular disease
  – Type 2 diabetes
  – Cancer
  – Metabolic syndrome

LIFESTYLE + MICROBES = DISEASE INTERACTIONS

“Sitting has become the smoking of our generation.”

- NILOFER MERCHANT

POTENTIAL EXERCISE MECHANISMS

- Hypothalamic-Pituitary-Adrenal Axis releases hormones
- Short-chain fatty acid influence lipid and glucose metabolism
- Gut transit time alters microbial composition
- Microbial metabolites interact with muscle receptors

EXERCISE, DIET AND GUT MICROBIOTA

Do diet and exercise of elite rugby players have differential effects on the gut microbiome compared to healthy and obese controls?

<table>
<thead>
<tr>
<th></th>
<th>Elite athletes (n=40)</th>
<th>Low BMI controls (n=23)</th>
<th>High BMI controls (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.8±3.8</td>
<td>28.1±5.1</td>
<td>30.8±5.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.1±3.0*</td>
<td>22.7±1.8**</td>
<td>31.2±3.0¥</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>101.3±13.8</td>
<td>74.3±6.3</td>
<td>103.1±13.8</td>
</tr>
<tr>
<td>Body fat % (kg)</td>
<td>16.9±6.1++</td>
<td>15±4.6*</td>
<td>33.9±8.8¥</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>80±8.9++</td>
<td>55.4±5.6**</td>
<td>65±8¥</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.8±0.04++</td>
<td>0.8±0.05</td>
<td>0.9±0.07¥</td>
</tr>
</tbody>
</table>

Data shown as mean±SD, *p<0.01 or **p<0.0005 athletes versus low BMI controls
+p<0.01 or ++p<0.0005 athletes versus high BMI controls ¥p<0.0005 high BMI versus low BMI controls. Note only data for 39 athletes was available for waist/hip ratio while only data for 22 controls was assessed for fat (kg) and lean mass (kg).

MICROBIAL COMMUNITIES DIFFER AMONG ELITE RUGBY ATHLETES AND NON-ATHLETES

- Similarity of sample pairs/types
  - Elite rugby players were different from both control groups
- N=86 participants
  - Black = Rugby players
  - Green = High BMI
  - Red = Low BMI

RUGBY ATHLETES HAD GREATER GUT MICROBIOME RICHNESS/DIVERSITY

- Richness of the microbiomes differed in rugby players compared to high and low BMI controls

PROTEIN INTAKE POSITIVELY CORRELATED WITH ALPHA DIVERSITY

ANIMAL MODELS OF EXERCISE

- Majority of evidence is in animal models
- Exercise vs. sedentary mice - greater abundance of Lactobacillales and Enterococcus faecium and lower abundance of Tenericutes genera\(^1\)
- High-fat diet vs. control diet in rats (both with and without exercise)\(^2\):
  - Exercise counteracted microbiota changes induced by high-fat diet
- Exercise effect depends on metabolic state\(^3\)
  - Exercise increased Bacteroides/Prevotella
  - Increased Bifidobacteria species in healthy compared to diabetic rats

AGE AT INITIATION OF EXERCISE MAY IMPACT THE GUT MICROBIOME

- Bacterial diversity increases with age\textsuperscript{1}
- Children have greater interpersonal variation compared to adults\textsuperscript{2}
- Gut microbial communities may be more plastic or malleable earlier in life\textsuperscript{1,2}

EARLY LIFE EXERCISE ALTERED ALPHA DIVERSITY

EARLY LIFE EXERCISE ALTERED BETA DIVERSITY

- PCoA using unweighted UniFrac distances
- Microbial diversity clustered by time
- Early life exercise influenced clustering which persisted after exercise cessation

EARLY LIFE EXERCISE RESULTED IN SIGNIFICANT PHYLTA DIFFERENCES

AGE AT ONSET OF EXERCISE MAY INFLUENCE WEIGHT MAINTENANCE

EARLY LIFE CHOICES AND POTENTIAL FOR THE FUTURE

• Microbial diversity and functional stability decline with age

• Pre-adolescent and adolescent children have unique intestinal microbial communities compared to adults
  – Greater Bifidobacteria and Clostridium among adolescents aged 11-18

• Exercise (wheel running) among adolescent-aged rats
  – Improved gut microbial diversity
  – Increased Streptococcaceae relative to sedentary and weight-matched high-fat diet controls

EARLY LIFE CHOICES AND POTENTIAL FOR THE FUTURE

• Bifidobacterium and Lactobacillus increased with wheel running among rats\textsuperscript{1}

• Bifidobacteria strains when given early in life normalize exaggerated HPA-responses in germ-free mice\textsuperscript{2}

• Clostridium species have been associated with improved anti-inflammatory responses\textsuperscript{3}

THE WEIGHT OF COLLEGE LIFE

• Emerging adulthood is a vulnerable period for weight gain and obesity risk

• Average college freshman weight gain: 1.5 – 6.8 pounds in the first semester

• Intestinal microbiota change with obesity and may contribute to weight gain

STUDY PURPOSE

• To assess whether associations exist between physical activity and fecal microbial abundance during the first year of college

  – Secondary analysis of cross-sectional data

  – Self-reported physical activity and screen time
METHODS

- Data collected during the 2014-2015 school year at Arizona State University
- Anthropometric data: height and weight
- Physical activity assessment: Godin-Shepard Leisure-Time Physical Activity Questionnaire
  - Moderate-to-vigorous physical activity (MVPA) was reported in min/day
  - Screen time was reported in hours/day
**MICROBIOME METHODS**

- Illumina MiSeq of PCR amplified 16S rRNA gene (V4 region)
- Bacteroidetes:Firmicutes ratio to assess phyla-level differences in microbial abundance by BMI and associations with self-reported activity
- Diversity
  - Alpha-diversity
    - Phylogenetic diversity for within-sample differences
    - Relates to richness of sample taxa
    - Faith’s index based on phylogenetic branch lengths
  - Beta-diversity
    - Principal Coordinates Analysis (PCoA) for between-sample differences
    - Group similarities among phylogeny
    - Weighted (presence + frequency) and unweighted (presence/absence) UniFrac
# PARTICIPANT CHARACTERISTICS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Male, % (n)</td>
<td>44.9 (40)</td>
</tr>
<tr>
<td>Age, y</td>
<td>18.6 ± 0.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4 ± 5.3</td>
</tr>
<tr>
<td>Underweight, % (n)</td>
<td>5.6 (5)</td>
</tr>
<tr>
<td>Normal weight, % (n)</td>
<td>57.3 (51)</td>
</tr>
<tr>
<td>Overweight, % (n)</td>
<td>23.6 (21)</td>
</tr>
<tr>
<td>Obese, % (n)</td>
<td>13.5 (12)</td>
</tr>
<tr>
<td>MVPA, min/d</td>
<td>53.4 ± 31.1</td>
</tr>
</tbody>
</table>

Underweight, normal, overweight and obese categories were classified as <18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m², respectively.

MVPA, moderate to vigorous physical activity

- N=89 participants with activity and microbiome data
- Race and ethnic distribution:
  - 30.3% Hispanic
  - 41.6% Non-Hispanic White
  - 7.9% Non-Hispanic African American
  - 1.1% American Indian/Alaska Native
  - 10.1% Asian
  - 9.0% Mixed
BACTERIOIDETES: FIRMICUTES RATIO AND MVPA DID NOT DIFFER BY BMI GROUP

Wilcoxon-rank; p=0.394

Wilcoxon-rank; p=0.129
BACTEROIDETE:FIRMICUTE RATIO AND MVPA WAS NOT CORRELATED

Most animal studies have observed mixed results:

- **Decreased Firmicutes**

- **Increased Firmicutes**

- **No difference in ratio**

\[ R^2 = 0.004, \ p = 0.551 \]
Alpha diversity did not differ by self-reported activity.

**MVPA**

**SCREEN TIME**

<table>
<thead>
<tr>
<th>Phylogenetic Diversity</th>
<th>MVPA Quartiles (min/d)</th>
<th>Phylogenetic Diversity</th>
<th>Screen time (hrs/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;28.8</td>
<td>30&lt;8.8</td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td>28.8-55.7</td>
<td>50&lt;7.2</td>
<td>2-4</td>
<td></td>
</tr>
<tr>
<td>55.8-79.2</td>
<td>70&lt;10.2</td>
<td>4-6</td>
<td></td>
</tr>
<tr>
<td>&gt;79.3</td>
<td>90&lt;10.9</td>
<td>&gt;6</td>
<td></td>
</tr>
</tbody>
</table>
PCOA PLOTS OF GUT MICROBIAL COMMUNITIES BY MVPA QUARTILES

**UNWEIGHTED UNIFRAC**

PC2 (3.9%)
PC1 (6.28%)
PC3 (3.31%)

**WEIGHTED UNIFRAC**

PC2 (22.38%)
PC1 (26.38%)
PC3 (9.79%)

< 28.8 min
28.8-55.7 min
55.8-79.2 min
79.3-102.9 min
PCOA PLOTS OF GUT COMMUNITIES BY SCREEN TIME QUARTILES

UNWEIGHTED UNIFRAC

WEIGHTED UNIFRAC

PC2 (3.9%)  
PC1 (6.28%)  
PC3 (3.31%)

PC2 (22.38%)  
PC1 (26.38%)  
PC3 (9.79%)

< 2 hrs  
2-4 hrs  
4-6 hrs  
> 6 hrs
BETA DIVERSITY DIFFERED BY SELF-REPORTED ACTIVITY

MVPA

SCREEN TIME

***p<0.0001; **p<0.01
BETA DIVERSITY DIFFERED IN OBESITY-PRONE ADOLESCENT RATS

- Beta diversity of gut microbial communities differed by treatment group (p=0.028)
  - Exercise vs. Sedentary
  - Weight-maintenance vs. Sedentary

- Exercise was metabolically advantageous
  - Improved insulin sensitivity
  - Greater LDL reduction
  - Increased fat oxidation

BACTEROIDES AND PREVOTELLA IMPACTED OVERALL BETA DIVERSITY

Ten Most Influential Genera
- Bacteroides
- Prevotella
- Coprococcus
- Parabacteroides
- Ruminococcus
- Faecalibacterium
- Bifidobacterium
- Blautia
- Sutterella
- Dialister

MVPA?? (p=0.010)
Screen time?? (p=0.040)
CONCLUSIONS

• Beta diversity distance metrics suggest that extreme levels of MVPA but not screen time may have larger effects on gut microbial diversity

• Combined with findings from animal studies, these data suggest the importance of adjusting or controlling for numerous lifestyle and other factors
  – Animal strain
  – Dietary intake

• More work is needed to clearly elucidate the role of physical activity in modulating intestinal microbial composition
  – Age
  – Type of exercise
  – Exercise dose and duration
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Team

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