Salivary biomarkers for stress and immune dysfunction

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SPACE LIFE SCIENCES
SUMMER INSTITUTE
Introduction

• A little bit about me.....
Objectives of Internship

• Learn and have fun!
• Work to complete a project with the microbiology lab and assist in other areas as needed
Project Objective

To measure an array of salivary hormones and cytokines in healthy human adults to then correlate the levels of viral shedding and any changes in the microbiota of saliva which may have occurred due to stress.
Background

• Stress has been shown to lead to immune dysfunction
• Stress can be measured in multiple ways
  Blood
  Urine
  Saliva
• Spaceflight is a stressful environment!
Background

• Immune dysfunction can lead to latent virus shedding and reactivation
  Varicella zoster virus (can cause shingles)
  Epstein-Barr virus
  etc.
• Potentially lead to major medical situations while on station
Various stressors associated with spaceflight acting through the hypothalamus-pituitary-adrenal (HPA)-axis is the basic mechanism involved. The HPA (brain and adrenal glands)-axis responding to stress increases the amount of stress hormones such as cortisol from the adrenal glands, and the resulting reduction in cellular immunity allows the viruses to emerge from their latent state into an active infectious agent.
Project Design

• Subjects- 13 total

<table>
<thead>
<tr>
<th>Age groups:</th>
<th>20-35yr old</th>
<th>36-50yr old</th>
<th>51-70yr old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total:</td>
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<td>2</td>
<td>7</td>
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</table>

• Time Points- 12 for each subject

<table>
<thead>
<tr>
<th></th>
<th>6am</th>
<th>Noon</th>
<th>4pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
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</tr>
<tr>
<td>Day 3</td>
<td>12</td>
<td>11</td>
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</tr>
<tr>
<td>Day 4</td>
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<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Total:</td>
<td>48</td>
<td>47</td>
<td>48</td>
</tr>
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</table>
Methods

• Saliva was collected by passive drool method, aliquoted, and frozen at -80°C
• Multiple stress hormones were measured using Salimetrics assays
• Viral shedding was measured by real-time PCR in DNA extracted samples
• Cytokines were measured in collaboration with Dr. Brian Crucian's lab
• Microbiota analysis was performed using illumina's MiSeq in collaboration with Dr. Doug Botkin
Results: Cortisol

Legend: Subjects (n=13) cortisol levels throughout four days of sample collection. Slight variation is seen between subjects but the overall trend is for cortisol levels to decrease throughout the day.
Results: Cortisol

**Legend:** Subjects (n=13) cortisol levels averaged for each of the four days of sample collection. And the overall average (n=48) cortisol level throughout the day.
Results: Cortisol

**Legend:** The overall average cortisol level throughout the day for females (n=24) and males (n=24). And for three different age groups, 20-35yr (n=16), 36-50yr (n=5), and 51-70yr (n=24). No difference was seen in salivary cortisol levels due to age or sex.
Results: DHEA

Legend: Subjects (n=13) DHEA levels averaged for each of the four days of sample collection. And the overall average (n=48) DHEA level throughout the day.
Results: DHEA

**Legend:** The overall average DHEA level throughout the day for females (n=24) and males (n=24). And for three different age groups, 20-35yr (n=16), 36-50yr (n=5), and 51-70yr (n=24). No difference is seen in DHEA due to sex; however, DHEA levels peak in the 36-50yr old group before drastically dropping after the age of 50.
**Results: Cortisol:DHEA ratio**

Legend: Subjects (n=13) cortisol:DHEA ratio averaged for each of the four days of sample collection. And the overall average (n=48) of cortisol:DHEA ratio throughout the day.
Results: Cortisol:DHEA ratio

Legend: The overall average cortisol:DHEA ratio throughout the day for females (n=24) and males (n=24). And for three different age groups, 20-35yr (n=16), 36-50yr (n=5), and 51-70yr (n=24). No difference is seen in cortisol:DHEA ratio due to sex; however, the ratio appears to be higher in subjects over the age of 50.
Results: $\alpha$-amylase

Legend: Subjects ($n=13$) $\alpha$-amylase concentrations averaged for each of the four days of sample collection. And the overall average ($n=48$) of $\alpha$-amylase concentration throughout the day.
Conclusion

• Salivary cortisol levels begin high in the morning and decrease throughout the day; appear to be unaffected by age or gender.

• Salivary DHEA levels have a similar diurnal pattern as cortisol; levels peak in middle aged subjects and then significantly decline after the age of 50.

• The Cortisol:DHEA ratio has a more gradual decline throughout the day; ratio increases in subjects that are older than 50.

• Salivary α-amylase levels gradually increase through the day; levels become more consistent throughout the day in subjects that are over 50 years old.
Conclusion

- Data has been collected but not analyzed for salivary cytokines
- EBV expression has been measured but not analyzed; VZV and CMV will also be measured
- Data on the microbiota of each saliva sample is currently underway
- Further samples and data collection on healthy adults are required to obtain enough information to validate the preliminary findings
- This information will serve as a baseline of data for normal values that can be used to compare against samples obtained from astronauts
Acknowledgments

- Lauren Merkle and Judy Hayes
- Missy Matthias and Diego Rodriguez
- The microbiology and immunology labs, Brian Crucian, Doug Botkin, and Cherie Oubre
- Duane Pierson, Satish Mehta, and Ashlie Renner