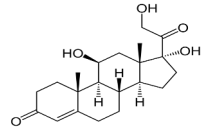


Correlating Cortisol and Academic Performance

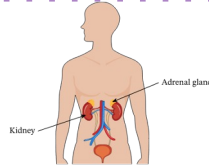
Rodolfo Garcia

Abstract:

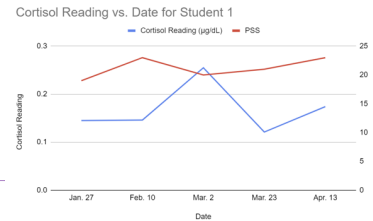
- Stress has far-reaching consequences, not just for individuals but society generally. 5-8% of total healthcare costs and 120,000+ deaths are attributed to stressful workplaces and burnout per year (Goh 2016). How do we mitigate these consequences of stress and workload?
- This study was unable to make strong conclusions about correlations between cortisol but weak correlations exist which may be more well-emphasized by a larger sample size. This study helped to generate a concrete future methodology that may be replicated in the future at greater length.



- cortisol (above), a steroid hormone
- produced by the adrenal glands
- atop the kidneys (below). Chronic exposure is correlated with failing physical health.



At left, the chain reaction that occurs, involving the brain's hypothalamic-pituitary-adrenal axis, and the adrenal gland which produces cortisol.



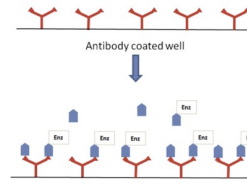
Above, an example of a student's stress profile over the course of the study.

Methodologies

Sample Collection:

- Ten students answered perceived stress questionnaires asking about their experiences in the past month. Students will then swab for saliva according to instruction for SOS (SaliOralSwab) kit.
- Students then engaged with 30 minute meditation in peaceful ambient room (The Well) to mitigate confounding acute stressors and ground everyone at a similarly relaxed state.
- Students then swabbed one more time after meditation before departing.
- Students repeated this process 6 times total, 5 during the semester (3 weeks in-between sessions).

Competitive ELISA



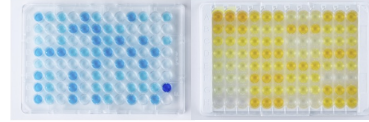
(Cox, 2019)

Above, a diagram depicting the ELISA immunoassay technique. Prepared cortisol in the form of an enzyme conjugate solution binds to antibodies in the well. Tetramethylbenzidine (TMB) will then bind to these enzyme complexes, causing them to change to a light blue color. Sample cortisol (in this case, salivary cortisol) will also bind to the antibodies in the wells of the plate, but unlike the enzyme complexes, TMB will not bind to salivary cortisol. This means that the deeper the blue of the solution, the less cortisol that was in the introduced sample. A plate reader is a machine which can then determine exactly how much color change occurred and can calculate a resulting concentration from this data.

Methodologies

Sample Analysis: Salivary Cortisol ELISA

Competitive binding assay, horseradish peroxidase will bind to cortisol in plate if there is no sample cortisol to do so and will create noticeable change in solutions. If there IS sample cortisol to bind to antibody binding sites on plate, horseradish peroxidase will not bind, won't cause a change in the plate color and will be washed away. Standard plate reader at 450 nm used to observe changes. Horseradish peroxidase reaction is blue. The amount of color change that occurs in a sample can then be compared to a set of standards, which we know the concentrations of cortisol for. Interpolating how much color change has occurred compared to known cortisol standard's color change will help us understand the concentration of cortisol in a sample.

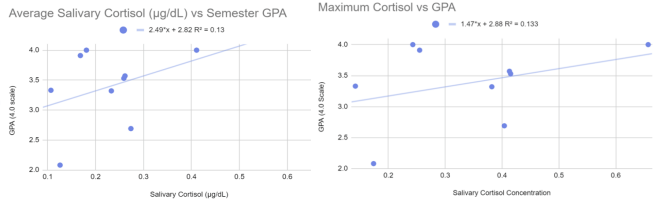


At left, visualization of the microtiter plates after administration of tetramethylbenzidine (TMB) and sulfuric acid stop solution. The saturation of the color is inversely proportional to how much cortisol in the saliva sample.

Conclusions:

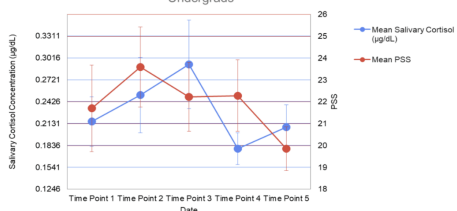
- There is not enough evidence to support the existence of a meaningful correlation between the predictor variables and dependent variables but this study has laid the foundation for potential replication studies. If this study were to be done again, one possible venue that would be of great success would be a yoga course done at UAF where a yoga instructor can assist greatly with helping students achieve a similar basis of relaxation prior to sampling. More research may also be done in the abilities of students to relax after Thanksgiving, or Spring breaks, and how that may correlate with academic success as well.

Results and Discussion



Above are linear correlations between two different measures of cortisol throughout the semester and semester GPA. With a higher sampling frequency, it is possible the difference between the two measures would be more pronounced and one may hold more predictive power than the other. In both cases, however, Pearson's Correlation coefficient is somewhat low, even for human research.

Mean Salivary Cortisol and Mean PSS Over Spring 2024 for UAF Undergrads

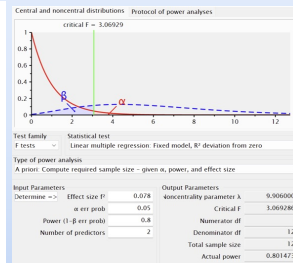


At left: perceived stress score overall remained relatively the same during the entire semester.

The standard deviation of the average PSSs at each timepoint over the entire semester is calculated to be ~1.356. Standard deviation of average salivary cortisol was ~0.0924 µg/dL. Standard error bars are defined by $\pm (\text{sd} / \sqrt{n})$, where $n = 9$. The difference between Time Point 3 and Time Point 4 is of note, as salivary cortisol experiences a harsh drop. Finding the cause of this may prove to be a worthwhile question.

```
> gam_model <- gam(GPA ~ s(CortAvegs, k = 5) +  
+ s(PSSAvegs, k = 5), data = averageddf)  
> summary(gam_model)  
  
Family: gaussian  
Link function: identity  
  
Formula:  
GPA ~ s(CortAvegs, k = 5) + s(PSSAvegs, k = 5)  
  
Parametric coefficients:  
              Estimate Std. Error t value Pr(>|t|)  
(Intercept)   3.3811      0.2056    16.45 3.22e-06 ***  
---  
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1  
  
Approximate significance of smooth terms:  
              edf Ref.df F p-value  
s(CortAvegs)    1     1 1.800  0.228  
s(PSSAvegs)     1     1 1.507  0.265  
  
R-sq.(adj)  = 0.0724 Deviance explained = 30.4%  
GCV = 0.57053 Scale est. = 0.38035 n = 9
```

A GAM like the one modeled above on RStudio can be used to fit smooth lines to data when the underlying mathematical function is non-linear and unknown. This method is more flexible than a strictly straight linear regression. This method works well for data where the underlying distribution is not well-known. In this regression, the p-value is about 0.02 lower than the regression that is a straight linear regression.



The above G*Power calculation shows that a sample size of 127 is desired to have sufficient power for such a study where a multiple linear regression's p-value will be safe to trust. A separate simulation done in RStudio suggested that for a simpler difference of means test approach, sample size would have to be 30. However, such an approach would be unable to account for other variables efficiently.

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