

# Comparison of Salivary Cortisol Levels in Various Academic Majors Using Enzyme-Linked Immunosorbent Assay

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## Abstract

The purpose of this study was to examine the impact that different majors at Embry-Riddle Aeronautical University have on salivary cortisol levels. Cortisol is a glucocorticoid synthesized in the zona fasciculata of the adrenal cortex. Secretion of cortisol is regulated by a hypothalamic hormone and a pituitary hormone. Corticotropin-releasing hormone (CRH) is secreted from the hypothalamus, and adrenocorticotropic hormone (ACTH) is secreted from the pituitary gland. Cortisol is stress hormone that is secreted in response to physical or emotional stressor. Different academic majors were anticipated to have different salivary cortisol levels when tested with competitive enzyme-linked immunosorbent assay (ELISA). Competitive ELISA uses cortisol standards and salivary samples to compete with cortisol conjugated to horseradish peroxidase to detect binding sites on a microtitre plate with coated anti-Cortisol antibodies. Bounded cortisol enzyme conjugates are measured by the reaction to the peroxidase to the substrate tetramethylbenzidine (TMB). Optical density is read on a plate reader, and the amount of cortisol in the sample is inversely proportional to the amount of enzyme conjugate detected from the plate well. The characterization of cortisol levels is classified by utilizing a standard curve line.

## Introduction

Cortisol is one of the most important hormones secreted by the cortex of the adrenal gland. Cortisol affects the metabolism of carbohydrates, fats and proteins and it is involved in the response to stress. Cortisol follows a circadian rhythm with levels peaking in the early morning and dropping to lowest values at night.

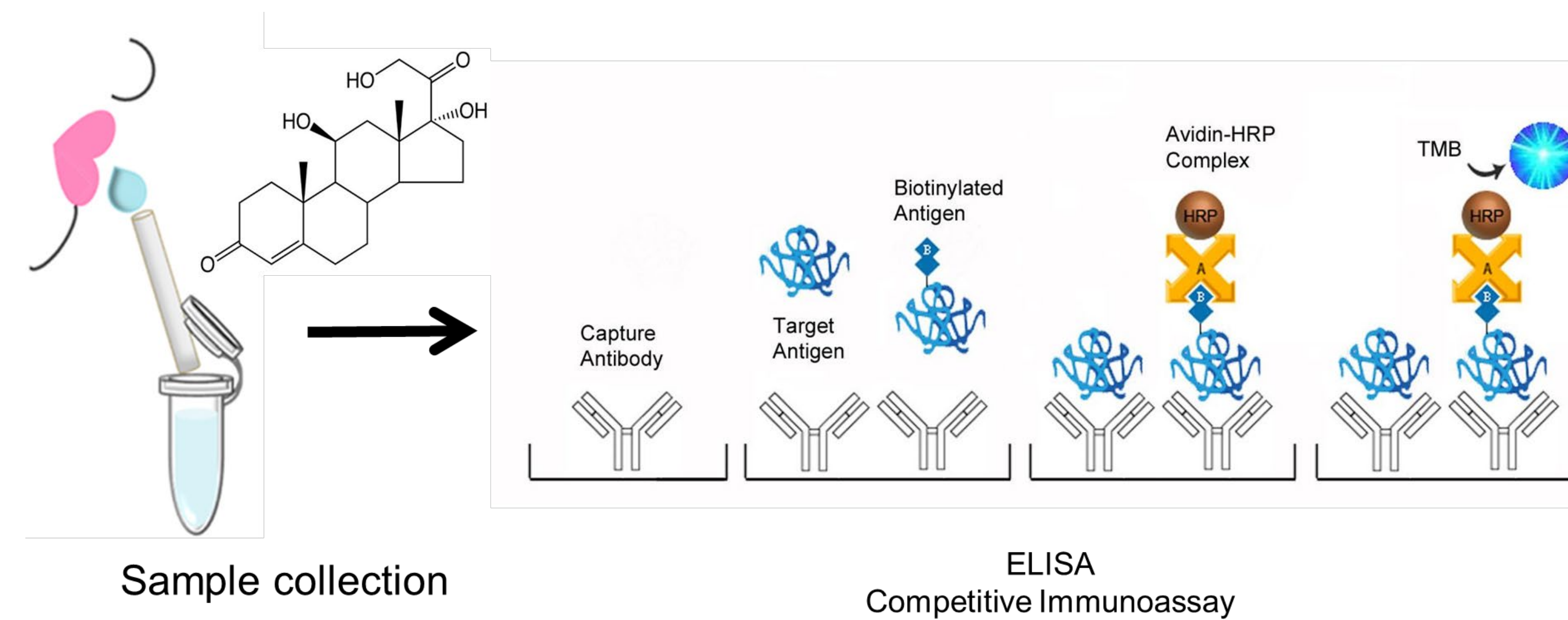
In blood, only around 10% of Cortisol is in its biologically active form, the remaining is bound to serum proteins. Unbound cortisol is also present in saliva (which is unbound to protein). Salivary cortisol levels can fluctuate and affected by salivary flow rate and are relatively resistant to degradation from enzymes. Previous studies have reported an accurate correlation between serum and saliva cortisol (meaning that salivary cortisol levels estimate with high reliability serum Cortisol levels).

Many College and University students face novel daily demands of their academic life. These daily stressors have been linked to poor physical and physiological health and amounts of stress-related hormones (including cortisol) can fluctuate according to the general capacity to cope to challenges of college life. Thus, characteristic college stressors have been linked to changes in Cortisol. Yet it is unclear the degree of these changes according to specific majors which can help to describe the general coping capacity between students.

**Objective:** In order to extend available laboratory evidence, we examined cortisol levels in saliva samples collected from different students and different majors at Embry-Riddle Aeronautical University. We expected that elevated levels of saliva cortisol would be associated with majors that are more demanding or with students that report poor academic coping efficacy.

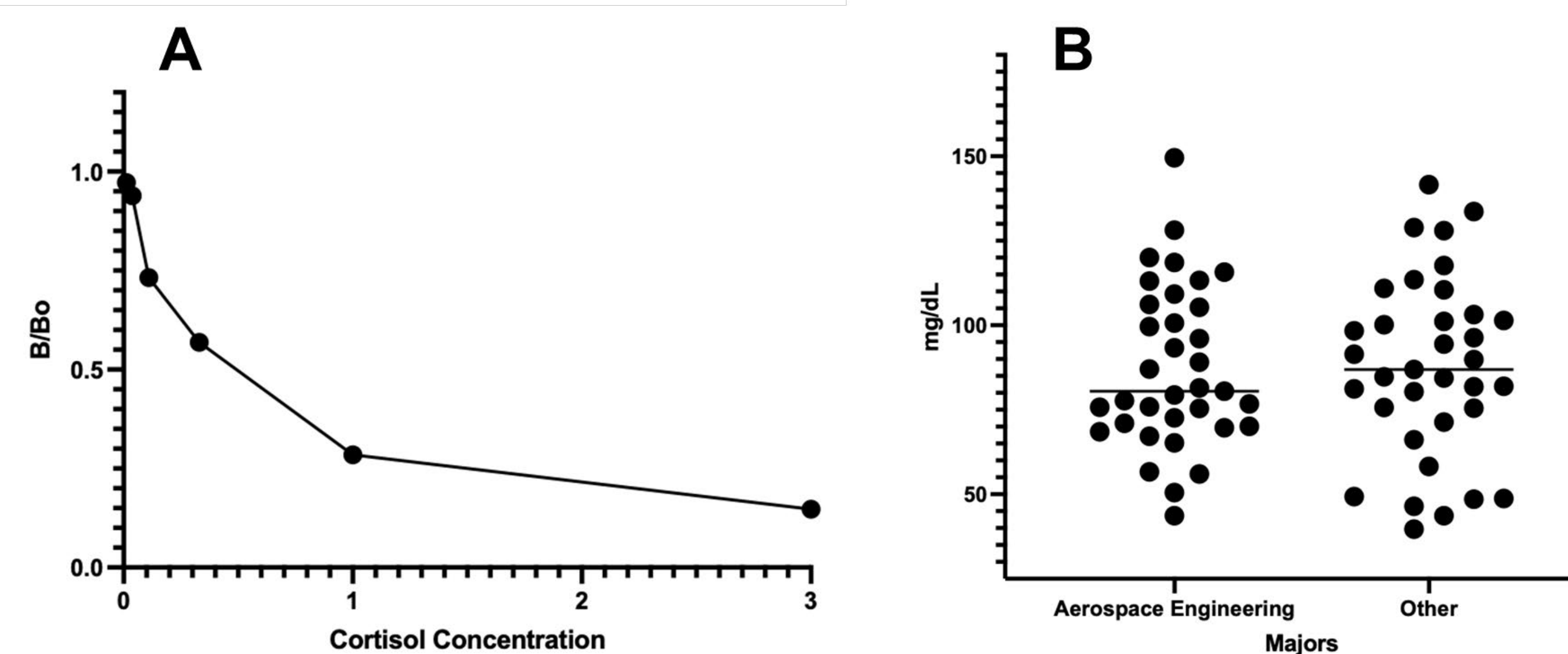
## Test Principle

This is a competitive immunoassay kit. Cortisol in standards and samples compete with cortisol conjugated to horseradish peroxidase for the antibody binding sites on a microtitre plate. After incubation, unbound components are washed away. Bound Cortisol Enzyme Conjugate is measured by the reaction of the horseradish peroxidase enzyme to the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with an acidic solution. The optical density is read on a standard plate reader at 450 nm. The amount of Cortisol Enzyme Conjugate detected is inversely proportional to the amount of Cortisol present in the sample.



**Figure 1.** Summary of the Cortisol ELISA detection method. Saliva samples were analyzed in duplicate after a disinfection procedure.

## Results



**Figure 2.** Results of the analysis. (A) Calibration curve of cortisol standards and (B) Concentration of cortisol in different saliva samples (in mg/dL). Analysis was performed using a t-test comparison between Aerospace Engineering students and the rest of the majors. There was no significant statistical differences between groups ( $p > 0.05$ ).

## Discussion

- Our results demonstrate that Cortisol concentration is not significantly different between majors
- The cortisol levels in general are slightly elevated when compared to normal parameters for young adults.
- Saliva samples were taken from different students that attended classes at different times during the day (ranging between 8:00 AM to 5:00 PM).
- Our samples size included students from different ages and activity levels.
- Limitations of our study: Our sample number was modest in size, disproportionately male with Aerospace Engineering as selective major. We did not assess perceived controllability of the stressors and did not explore how these relations may vary with the type of stressor (e.g., academic, interpersonal).

## Conclusions

We were not able to suggest whether academic degree (or academic field) had a correlation with elevated cortisol levels in saliva. Our findings serve as a backbone to continue this study with more diverse samples and greater sample population.

## Future Directions

- To continue analysis of Cortisol levels with an increased sample size
- To analyze individual differences in saliva cortisol at different times during the day and perform a more detailed high throughput analysis.

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