How to manage anxiety during acutely stressful situations

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Abstract

Managing stress in our daily lives can be challenging, leading many individuals to seek substances that can help alleviate anxiety. However, some of these substances, such as alcohol or marijuana, may impair a person's functioning. Our research aimed to explore the potential of safe, non-addictive substances that could effectively and rapidly reduce anxiety before facing a stressful situation. When we encounter acute stress, the hormone cortisol is released in our bodies, which is associated with the symptoms of anxiety and triggers the stress response. To assess the stress levels of our participants, we measured their salivary cortisol levels in either micrograms per deciliter (μg/dL) or nanomoles per liter. In our study, we carefully selected 108 participants, evenly distributed between 54 males and 54 females, and randomly assigned them to one of six treatment groups. We first measured their baseline salivary cortisol levels and then administered their respective treatments, which included a 250 mL Vitamin C drink, a 250 mL coffee, 250 mL of water, a 500 mg fish oil tablet, a 500 mg aspirin, or a placebo. Following a 10-minute waiting period for the substances to take effect, we exposed the participants to a mildly anxiety-inducing situation by having them sit with a pet crocodile. Subsequently, we measured their final cortisol levels and calculated the difference between the final and initial levels as the response variable. Upon analyzing the results, we found no statistically significant reduction in the cortisol difference for any of the treatments or based on the participants' gender. Although substances used in our treatments have previously been associated with cortisol reduction, our study suggests that they may require more time or consistent long-term usage to exhibit significant effects on cortisol levels.

1. Introduction

People generally take impairing substances to stay calm before acutely stress-inducing situations, like alcohol or tobacco. Our question of interest is are there safe, non-addictive, non-impairing supplements that can measurably reduce stress?

The presence of the hormone Cortisol is closely associated with stress and anxiety. Research on the effects of many household and over the counter measures to change cortisol levels has been extensive. For example, Vitamin C consumption over the course of a week has been linked to lower cortisol levels in ultramarathon runners (Peters, et al., 2001). Fish oil supplementation,

over the course of 3 weeks, is associated with lower cortisol levels as well (Barbadoro, et al., 2013). Aspirin consumption, over the course of a month, was associated with a lower level of cortisol in rats (Bhatt, et al., 2016). Dehydration is associated with higher levels of cortisol while exercising (Maresh, et al., 2006), and coffee consumption has been linked with lower levels of cortisol as well (Yusni Y, Yusuf H, 2022).

These studies certainly suggest that all of these substances have the potential to lower cortisol, however, most of these studies tend to measure the substance's effect over the course of days, weeks, or months. All of these substances are easily accessible and safe for most people. We are interested in investigating if any of them have an immediate short term effect on cortisol levels, particularly during an acutely stressful situation.

From our preliminary research we found that a person's gender can have a large impact on their cortisol levels. Many studies relating to cortisol sample exclusively males or females. Furthermore, men tend to have more elevated cortisol levels, and possibly respond differently to stressful and traumatic events than women (Dekel, et al., 2013). Furthermore, another factor that could impact cortisol level is marital status. Being married, or living in a marital-like situation is associated with lower cortisol levels (Chin B, et al., 2017, p.71).

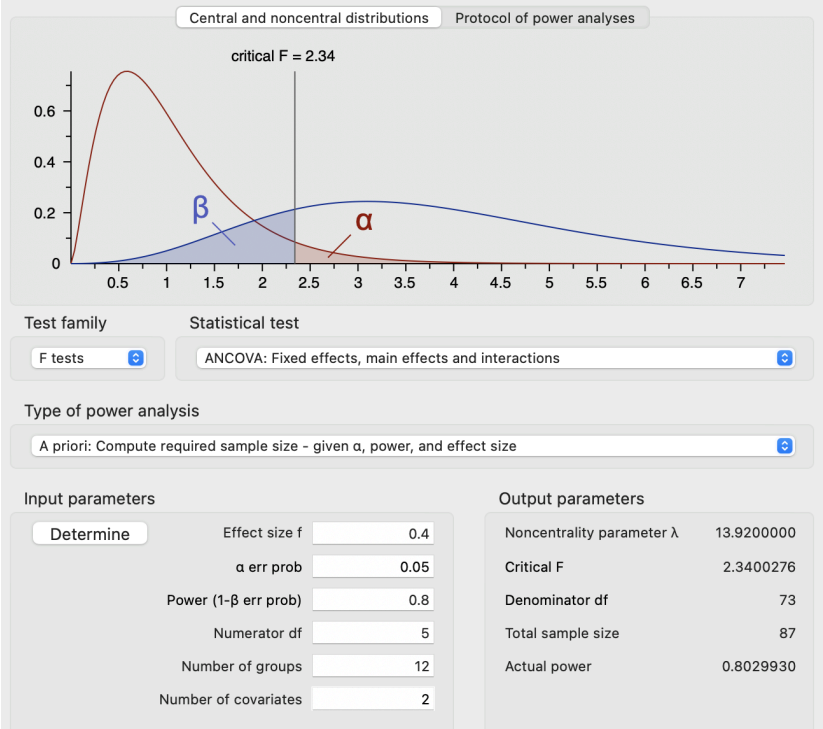
2. Methods and Procedures

2.1 Participants

All of our participants were sampled from the virtual program *The Island.* From our preliminary research, we determined that age has a significant impact on cortisol levels, decreasing through a person's twenties and thirties and increasing after their fifties (Moffat S, et al., 2020, p. 396). For this reason, we focused our study on participants aged 20-29. Considering that marital status can have an impact on cortisol levels, we focused our research on people who are currently married. Therefore, we honed our sample of participants to be married people between the ages of 20 and 30.

For the purposes of determining our sample size, we decided on a power of 0.8, giving us a 80% chance of rejecting the null-hypothesis given that it is false with an effect size of 0.4. We chose a significance level of 0.05, giving us a 5% chance of rejecting the null hypothesis given that it is true. We are using a one-way factorial design with one blocking factor. Because there are 6 levels in our treatment and 2 levels in our blocking factor, our numerator degrees of freedom is 5, our

number of groups is 2, and our number of covariates is 12. Using G*Power, we determined that we needed a total sample size of at least 87. In order to achieve a balanced design, we had a total sample size of 108 (54 males, 54 females).



Many studies relating to cortisol levels focused on either male or female participants. While many studies concerning stress level focused primarily on either males or females, it has been observed that women are associated with higher stress levels than men (Costa C, et al., 2021, p. 3). We have 5 anxiety treatments that we want to test against a placebo, therefore, we also thought it was important to test if the effectiveness of these treatments vary by gender, so we sampled an even number of males and females. We randomly assigned treatment using the R sample function.

2.2 Design

We conducted the experiment as a basic factorial design. Our response variable is change in cortisol level after treatment. While we acknowledge that many factors could affect stress level, we are only interested in two of them: type of treatment and sex. Other potential factors are held constant, including age (20-29) and marital status (married). The levels of the two design factors and the factor diagram are listed below.

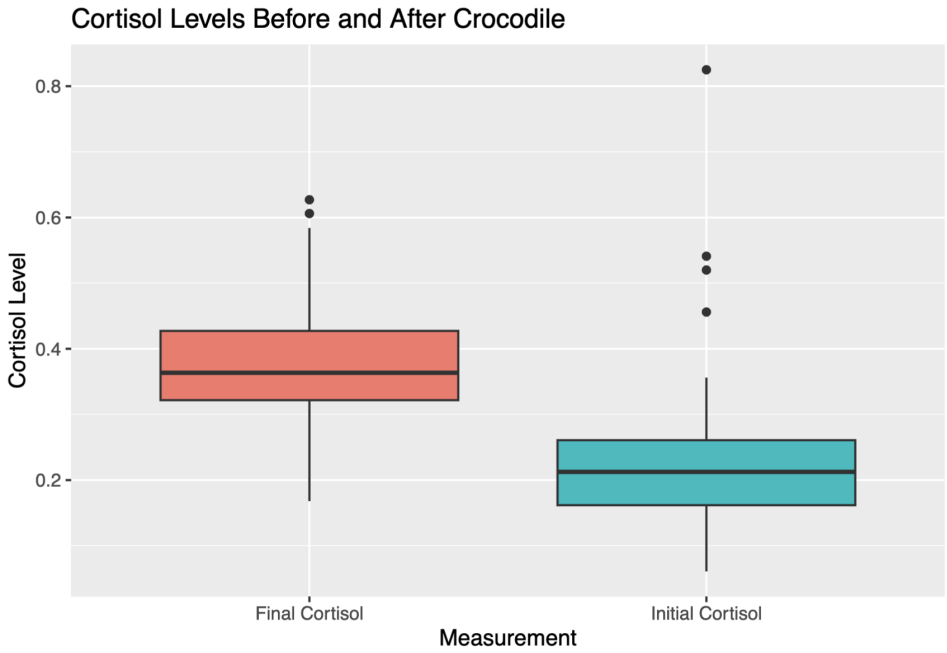
Type of treatment	Vitamin C Drink 250 mL, Coffee 250 mL, Water 250 mL, Fish Oil Tablet 500 mg, Aspirin 500 mg, Placebo
Sex	Male, Female

2.3 Materials and Procedure

Step 1: Each participant had their initial cortisol level taken via a salivary sample.
Step 2: Then, the participants were administered the treatment.

- Vitamin C Drink 250 mL
- Coffee 250 mL
- Water 250 mL
- Fish Oil Tablet 500 mg
- Aspirin 500 mg
- Placebo (No action taken)

Step 3: To induce stress, all islanders participating in the study were asked to sit with a pet crocodile for 10 minutes. In a one-sided t-test of the total difference in salivary cortisol we obtained a p-value = 2.2e-16 < 0.05. This ensures that sitting with a crocodile for 10 minutes effectively raises the cortisol level of participants.



Step 4: We measured the participants' salivary cortisol levels once again to compute the difference.

2.4 Instruments

We selected our participants and conducted our study in the online simulator, *The Islands* (https://islands.smp.uq.edu.au/index.php). We used specific tasks in this program, namely, taking salivary cortisol levels, requesting the participants to sit with a pet crocodile, and the 5 available treatment options ("Vitamin C Drink 250 mL", "Coffee 250 mL", "Water 250 mL", "Fish Oil Tablet 500 mg", "Aspirin 500 mg"). We recorded our data in an Google Sheets spreadsheet (https://docs.google.com/spreadsheets/u/0/). To analyze our data, create our model, and create the useful graphics, we used base R and RStudio (https://posit.co/download/rstudio-desktop/), as well as the "tidyverse" and "ggplot2" packages.

3. Results

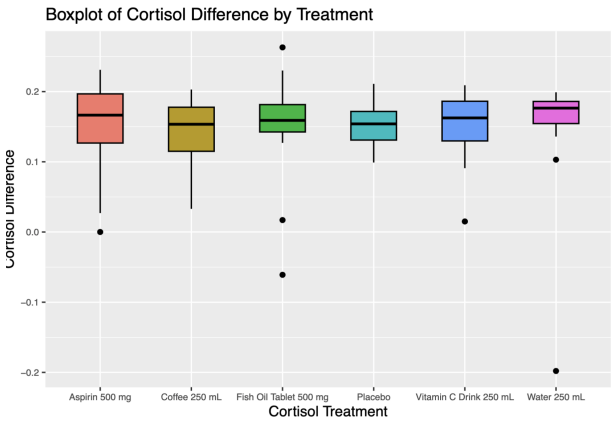
3.1 Types of Statistical Analysis

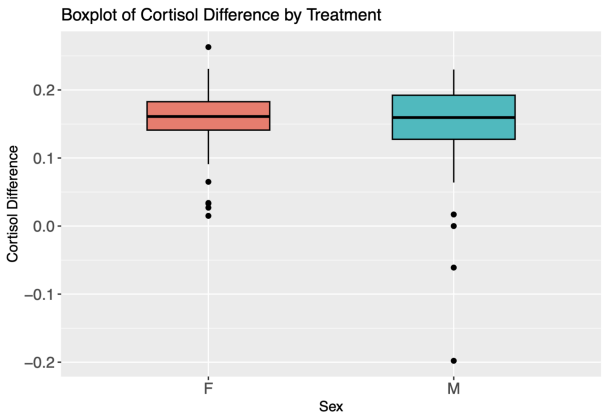
To analyze the data, we first created two boxplots for the both factors included in the study, in order to roughly understand our data. Then we generated the ANOVA table for the data to further examine if any of the factors would have an effect on the response variable. We want to conduct an F-Test to determine if there is a significant difference between cortisol difference (and by

extension, anxiety levels) for anxiety treatment. We have also set our “Placebo” group as our baseline statistic to compare our other treatment groups as well.

3.2 Data Visualization

Below are the boxplots of difference in cortisol levels against treatment and against gender. For the Cortisol Levels versus Treatment plot, we can see that despite the range for different treatment seems to vary, however, the median Cortisol levels for all 5 treatments seem to fluctuate around the same level, which is also very close to the median of no treatment (placebo/control group). For the Cortisol Levels versus Gender plot, we found that although the range for male seems to be larger than that for females, the center, or median cortisol levels for both genders are still around the same level. Based on these preliminary analysis of cortisol levels, we found that it is possible that the factors in our study have relatively minor effects on the response variable measured, but future analysis is required.





3.3 Anova Results

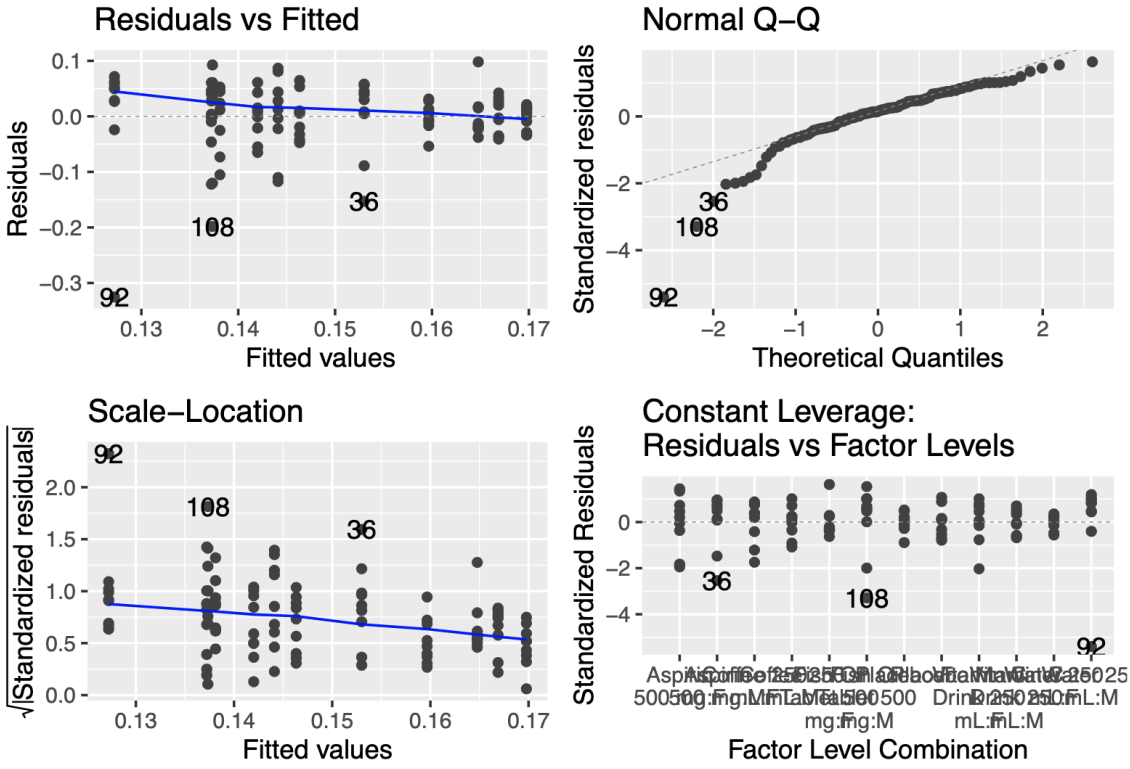
Table 1: ANOVA Table

Source	Df	SS	MS	F	Pr(>F)
Anxiety Treatment	5	0.002	0.0004	0.097	0.992
Sex	1	0.0013	0.00125	0.307	0.581
Axiety Treaatment:Sex	5	0.0155	0.0155	0.757	0.583
Residual	96	0.3922	0.00409		

We then perform ANOVA, and from the table above, we observe that the p-value for anxiety treatment, gender, and also the interaction of treatment and gender are very large, much larger than 0.05. Therefore, we conclude that neither anxiety treatment, sex, or their interaction term have a significant effect on difference in cortisol level.

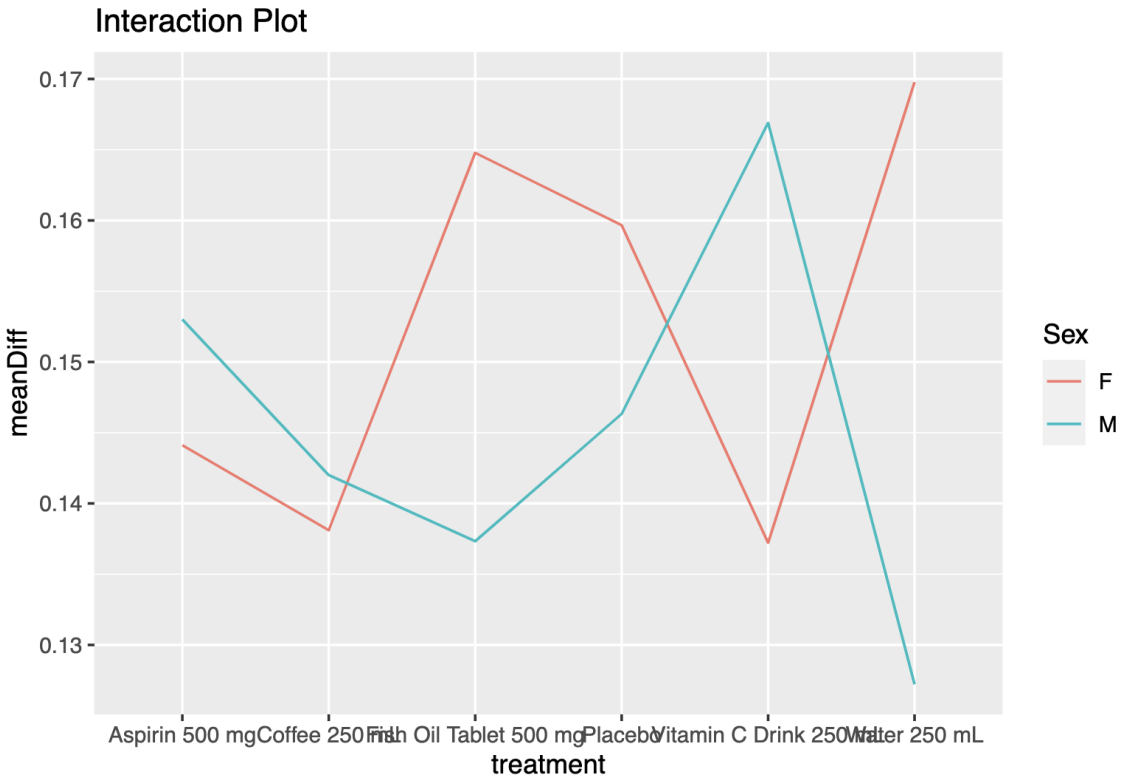
3.4 Diagnostic Plot

From the residuals vs fitted plot, we find that the residuals do not display any clear pattern and could be considered of constant variances. This conclusion is further supported by the scale-location plot, where we still do not observe any obvious pattern among the residuals. In the normal Q-Q plot, we observe that the low tail of the data points are slightly deviated from the straight Q-Q line; however, since the majority of the data points perfectly follow the line, we can still conclude that the normality assumption for the study is satisfied. Therefore, the assumptions of normal distribution and constant variance are both satisfied for our study.



3.5 Interaction Plot

To further understand the effects of the factors, we decide to create an interaction plot for cortisol levels and gender. From the graph below, although it appears that there is apparent interaction between the two factors, as the lines for female and male show very different trends, the y-scale has exaggerated the difference and make it appear to be larger than actuality. The y-scale is relatively small, which is why we observe a difference in gender that is actually quite minor. From our anova model, the interaction is not significant, which is consistent with our findings from the plot.



4. Discussion

Although we do not observe any effects of the factors on cortisol level difference, our research model has several advantages, ensuring the validity of our study. First, we obtained an adequate sample size. The minimum sample size required for our study is 87, and we have a total sample size of 108. Second, the variety of treatments is wide. For our study, we decided to incorporate 5 commonly available substances known to affect stress levels. However, there are certain parts of our study that could still be improved. First, our model could be improved in its generalization capabilities. We only included young people in our study to eliminate the impact of age on people's stress level, but we are unable to generalize to the entire age-group. Second, there exists

extraneous factors that are not considered, including socio-economic status of participants and education level, which could all have different effects on people's stress levels.

5. Conclusion

Based on our study and subsequent data analysis, we found that there is no significant relationship between either treatment, sex, or their interaction and difference in cortisol level.

Our proposed method does not reduce stress level in the setting of our study; therefore, we believe that further investigations are required. While all treatments have been shown to reduce cortisol level in other studies, some of them may require longer than 10 minutes, which is the amount of reaction time we choose for our study, to take effect. Meanwhile, some of the treatments may require repeated usage. In this case, instead of measuring the effect of these treatments after one intake, we might need to measure the effects of multiple intake or long term effect of using these treatments. Additionally, we might need to compare the cortisol levels under stressful conditions with and without the help of different treatments to have a better understanding of treatment effects.
Some of the future directions and research questions that could be answered are as follows:

- Does long term use of treatment reduce stress level?
- Are these treatments effective for longer term stress (a few hours instead of 10 minutes)?
- Are these treatments effective for a response variable other than cortisol? (Glucose level or self-reported stress level).

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