A Clinical Study on Islanders to Determine the Potential Therapeutic Properties of THC and Nicotine on Ghrelin Homeostasis.

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1 Abstract

Eating disorders and diabetes are a growing health concern in the United States. If a therapy could be developed to help modulate physiological signaling of homeostasis, these could be used to help treat the previously mentioned disorders. We propose that ghrelin could be a potential target to modulate. We aim to determine if the drugs THC and Nicotine are effective modulators in the levels of ghrelin. Our study is set up to focus on a representative sample of the islander's population using a Two-Way Randomized Block Design analyzed using a two-way ANOVA with blocking. These results could determine if THC and Nicotine could be potential therapies to control abnormal ghrelin levels and thus the treatment of eating disorders and diabetes.

2 Introduction

In the United States, there are over 30 million people who are diagnosed with some form of an eating disorder. In addition, there are 30.3 million people who are predicted to have some form of diabetes. The prevalence of these disorders underscores the importance of discovering novel methods which could potentially be used to treat these disorders. Previous research suggests that a potentially reasonable approach towards treating these types of disorders is through modulation of hormone levels which in turn could restore the homeostasis of individuals. In this study, we chose to focus on the modulation of levels of ghrelin.

Ghrelin is a hormone releasing peptide which is produced in mucosal endocrine cells in the stomach and intestines. Normally, low levels of food and glucose in the stomach trigger the production and release of ghrelin into the bloodstream. Ghrelin then activates AGRP neurons which are responsible for stimulating hunger. Ghrelin plays a role in the control of blood glucose levels where it is involved with the temporary inhibition of the release of insulin. However, in type two diabetics, the levels of ghrelin stay abnormally elevated, thus prolonging the inhibition of the production of insulin, exacerbating the diabetes condition. Therefore, we propose that modulating the levels of ghrelin is a therapeutic target in modulating the progression of diabetes. In addition, modulating the levels of ghrelin can be used to treat the psychological issues involved with eating disorders. One issue with direct administration of ghrelin or inhibition of the production of Ghrelin is that these treatments provide a short burst of effect rather than the body's consistent and slow release of ghrelin. Therefore, it is recommended that we use drugs which can directly modulate the cells which control the release of ghrelin.

Therefore, our study aims to determine if two drugs can serve to modulate the effects of ghrelin: THC and Nicotine. We propose that THC can modulate the levels of Ghrelin through its function as a CB1 receptor and AMPK receptor anagonist. The AMPK receptor functions to sense cellular energy status (such as the sensing of glucose levels). The activation of the receptor along with AMPK both function to down-regulate the production of an enzyme (phoenolpyruvate carbokinase) which then leads to the decrease of glucose levels. We propose that this decrease in glucose will trigger the decrease in ghrelin levels. In addition, we propose that nicotine can decrease the levels of ghrelin through its function as an antagonist of the nicotinic acetylcholine receptor. The effect of nicotine has been shown to activate the sympathetic nervous system, where its activation leads to a decrease in glucose levels. As such, we propose that the decrease in glucose levels will trigger the modulation of ghrelin levels.

3 Methods

3.1 Participants

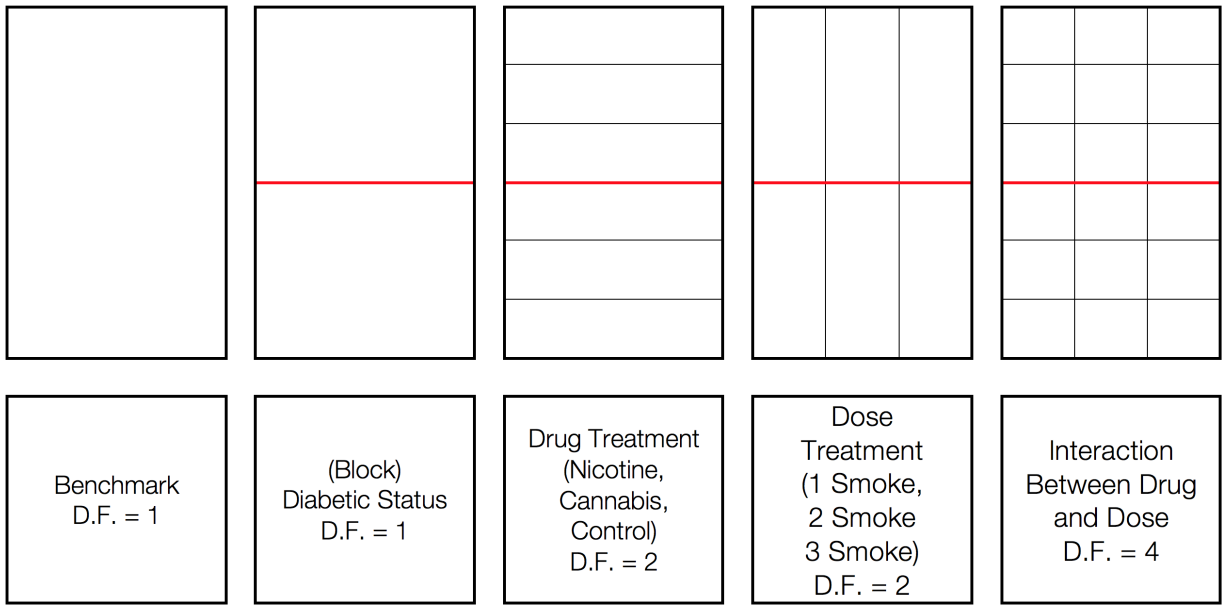
The participants will be islanders. A complete list of the whole population has been collected using python webscraping techniques. From this list, we will assign each islander a number, then we will use R sample function to randomly select participants. Treatments will be randomly assigned using R sample function. The seed will be set at 100 for both cases. We do not aim to exclude populations to have a representative sample for the whole island.

3.2 Design

The study will be set up as a Two-Way Randomized Block Design. The parameters for the design are detailed here:

Response Variable	Blood Ghrelin Levels		
Treatment 1 (Drug)	Control	Cannabis	Nicotine
Treatment 2 (Dosage)	One Inhalation	Two Inhalations	Three Inhalations
Blocking (Diabetes)	Diabetes	No Diabetes	

The factor diagram is detailed below:



We chose to focus on THC and nicotine based on their previously known properties of modulating hunger through their specific mechanism of actions. We aim to determine of one of those mechanisms of action is involved with ghrelin levels. We focus on dosage since the known mechanism of actions of THC and nicotine are dosage dependent, and we want to determine if THC and nicotine's effect on ghrelin level is

dose dependent. Finally, we will block with diabetes since it is known that levels of ghrelin are significantly different between healthy individuals and those with diabetes.

3.3 Instruments

Ghrelin levels will be measured from the islanders using blood samples. The drugs (THC and Nicotine) will be administered through inhalation. We chose an inhalation method because of the rapid delivery of the drugs into the bloodstream. Through its lipophilic properties, both drugs can cross the blood brain barrier and produce a physiological change in 10-20 minutes. Measuring ghrelin levels through blood samples is reliable due to the nature of ghrelin as a hormone, in which its primary method of distribution is through the bloodstream.

3.4 Procedure

Step 1: Find subjects from the Island willing to be a part of our experiment (finding an equal number of individuals who are diabetic and non-diabetic). After this, we use R to generate a random sample of Islanders to take part in our experiment.

Step 2: Randomly assign these groups (already divided by block) into different treatment groups for studying. The different groups are:

1) One inhalation of cigarette, two inhalations of cigarettes, three inhalations of cigarette
2) One inhalation of reefer, two inhalations of reefer, three inhalations of reefer
3) One inhalation of air, two inhalations of air, three inhalations of air

Step 3: For each unit (one islander), measure their blood ghrelin levels.

Step 4: For each unit, apply the assigned treatments to the islanders by having them smoke a cigarette (nicotine), a reefer (cannabis), or just breathing air (control) at a certain dose

Step 5: For each unit, measure their blood ghrelin levels again

Step 6: For each unit, compute the difference in blood ghrelin levels before and after treatment (this will be our response variable).

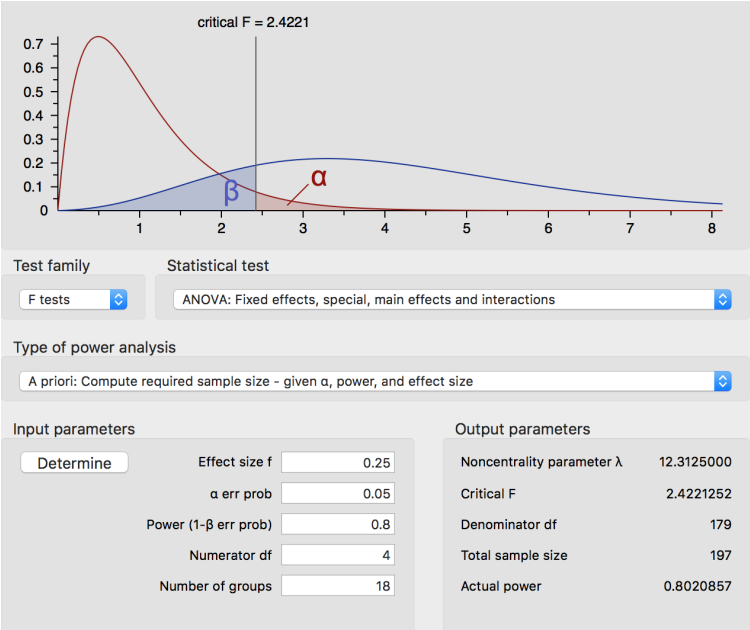
4 Data Analysis

4.1 Type of Statistical Analysis

Using R, we will conduct an ANOVA on the data. The statistical analysis that we plan to use to test if THC or nicotine influence an individual's ghrelin levels are using F-testing within treatments and blocks to determine if there is indeed a significant difference between ghrelin levels of our groups and if there is an interaction between dosage and treatment. We are going to use our control group as a baseline statistic; that way we have something to compare our treatment groups (diabetic - nicotine, diabetic - THC, non-diabetic - nicotine, non-diabetic - THC).

4.2 Sample Size Determination

We decided to use a power of .8, which means that the probability that we will correctly reject the false null hypothesis. We used an alpha level of .05, which is the probability of falsely rejecting the null hypothesis. We used a conservative effect size of .25, which is a way of quantifying the difference between groups. We will be using a two-way complete block design. Using G*Power, we determined that the sample size required is 197, based on the factor with the largest degree of freedom (4 for interaction). However, to have a balanced design, we will have a sample size of 198, so that each group will have 11 samples.



5 Results

5.1 ANOVA Analysis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
dose	2	1055.103	527.5516	0.7708073	0.4637719
drug	2	10664.627	5332.3135	7.7910597	0.0005258
Diabetic	1	5460.036	5460.0357	7.9776751	0.0051305
dose:drug	4	8764.278	2191.0694	3.2013783	0.0138193
Residuals	242	165628.286	684.4144		

Table 1: Two way with Blocking and Interactions ANOVA table. A p-value of 0.46 suggests that dose by itself does not make a statistically significant influence on change in ghrelin. However, the type of drug and and the interactions between dose and the drug, with p-values of 0.0005 and 0.0138 respectively, does make a statistically significant effect in change in ghrelin levels. Finally, with a p-value of 0.0138, the ANOVA suggests that our diabetic blocking was effective.

5.2 Tukey HSD Adjusted P-values

Comparison	Difference	Lower	Upper	P Value Adjusted
Control-Cannabis	15.54762	5.741119	25.3541195	0.0006712
Nicotine-Cannabis	4.75000	-5.056500	14.5565004	0.4891486
Nicotine-Control	-10.79762	-20.604119	-0.9911186	0.0269049

Table 2: Post-Hoc Analysis of Differences in Change of Ghrelin Levels between Different Treatment Types. Using Tukey's Honesty Significance Difference to correct against type I errors, comparisions between the average change in ghrelin levels between different treatments were done. Both the change in ghrelin levels between nicotine and control (p-value: 0.0269) and cannabis and control (p-value: 0.0006) had significant differences. However, there is not sufficent evidence (p-value: 0.4891) to suggest that there is a difference in the change in ghrelin levels between nicotine and cannabis.

5.3 Residual Diagnositics

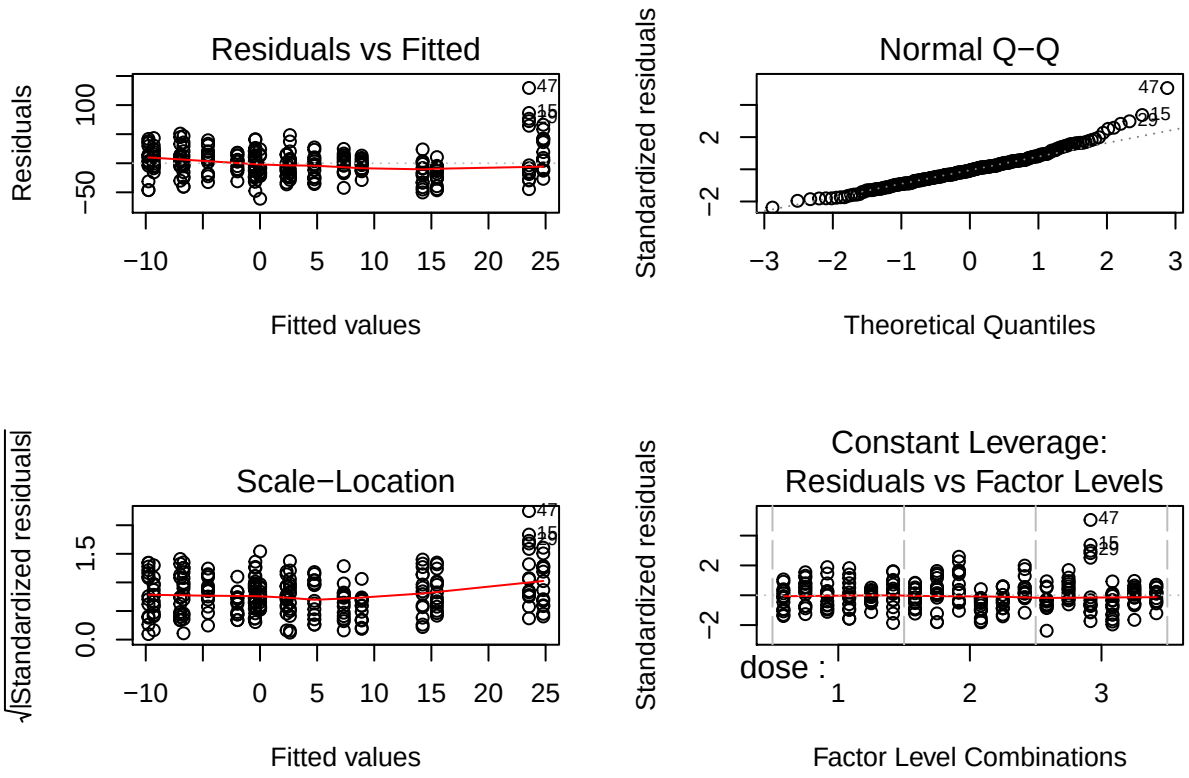


Figure 1: Summary Plots of Residuals for ANOVA Results. Each of the four plots (Residuals vs Fitted, Normal Q-Q, Scale-Location, Constant Leverage) suggests that the residuals stay constant. This suggests that the assumption of constant variance is maintained.

5.4 Interaction Plots

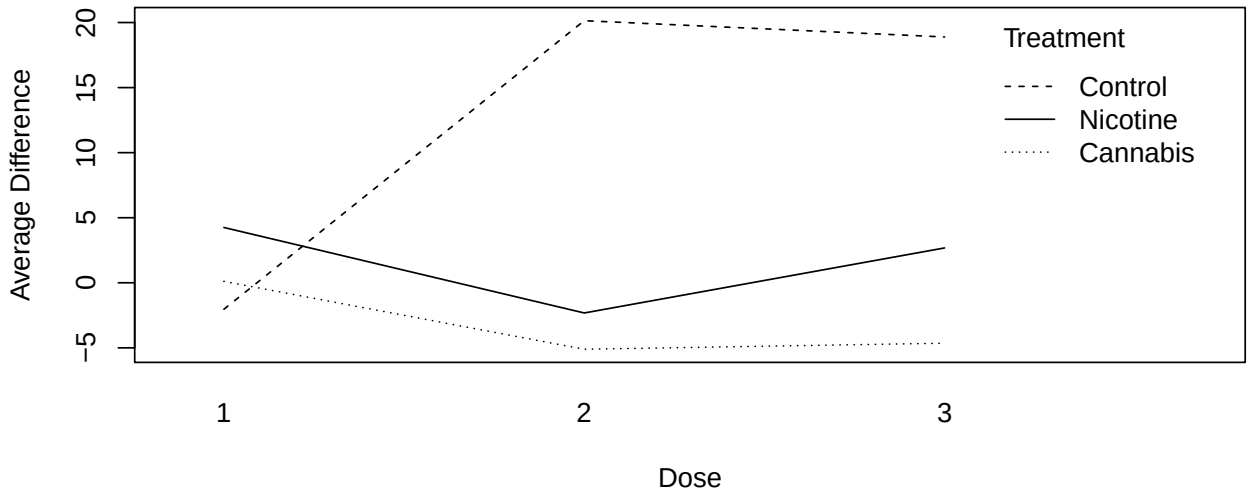


Figure 2: Interaction Plot of Treatments and Dose with Change in Ghrelin as Response. The plot suggests that there is no interaction going on between the nicotine and cannabis treatments due to change in dose. However, interaction is suggested within the control treatment due to dose.

5.5 Box Plots

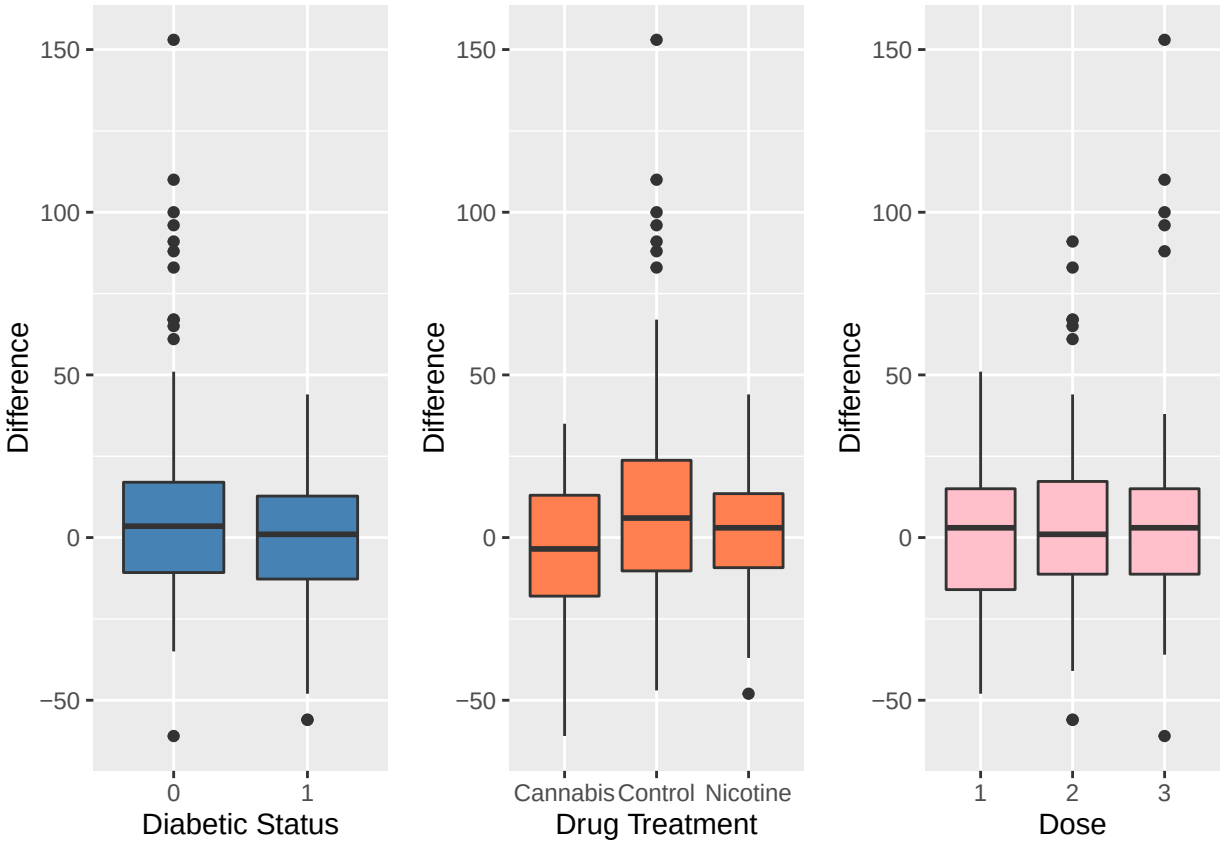


Figure 3: Box plots Comparing change in Ghrelin Levels between Diabetic Status, Drug Treatment, and Dose. The median is represented by the black bar in the middle, where the "box" depicts the 1st and 3rd quantiles. Potential outliers are displayed as black dots.

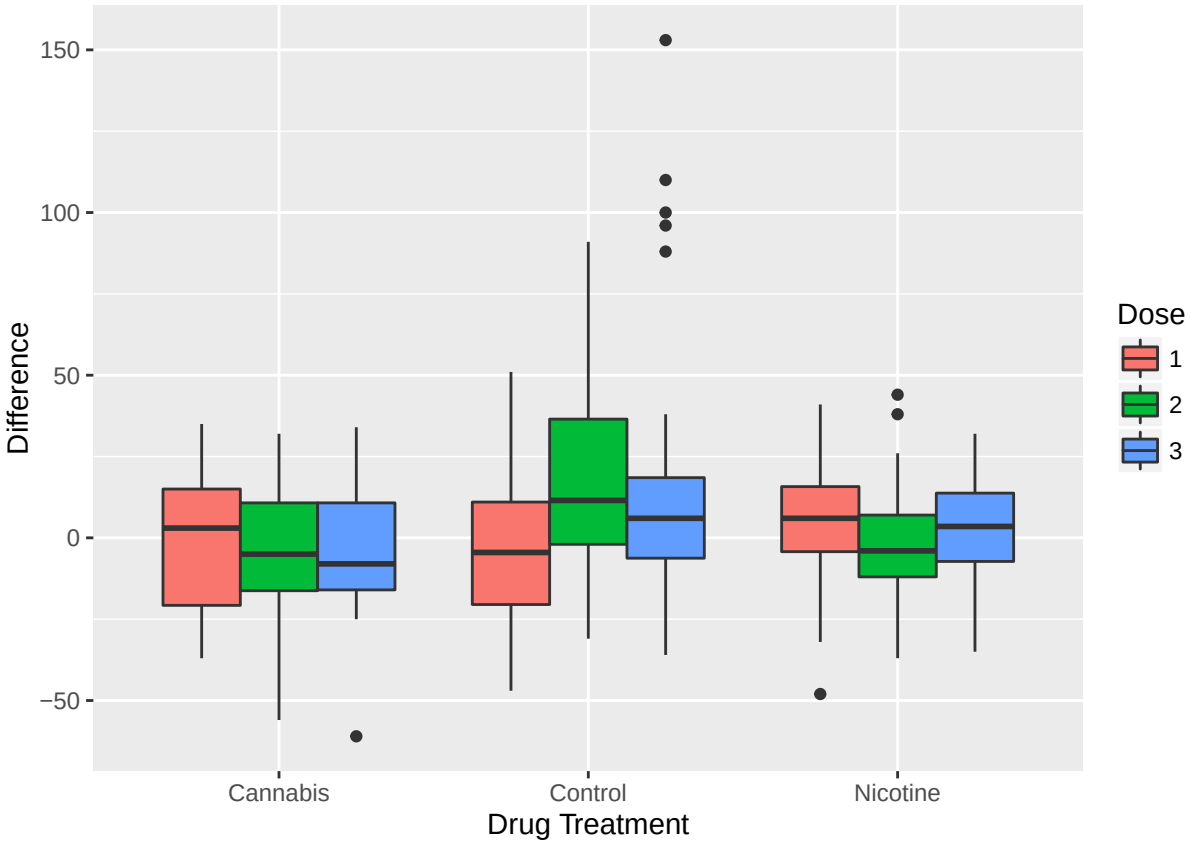


Figure 4: Side by Side Box Plot of Doses Clustered by Treatment. The median is represented by the black bar in the middle, where the "box" depicts the 1st and 3rd quantiles. Potential outliers are displayed as black dots.

6 Discussion

The aim of our study was to determine the effectiveness of cannabis and nicotine in changing ghrelin levels and the impact of different levels of dosages. Our results attempt to support or refute the idea of using THC and/or nicotine as a potential treatment for diabetes through ghrelin's interactions with insulin homeostasis.

We intended to have a sample size of 197 to achieve a power of 0.8, however, we ended up sampling 252 people which resulted in a power of 0.9. After running our experimental design on the Islanders, our ANOVA analysis demonstrated that certain factors were more impactful than others in regards to changing their blood ghrelin level. We noticed that from our two factors of interest, type of treatment and dosage level, the data revealed there was a statistically significant difference in ghrelin levels among Islanders based on the treatment given and the combination of the treatment type and dosage level. The significance of the interaction between our two factors could help explain why our results found no statistically significant

evidence that the dosage levels alone impacted the Islanders' blood ghrelin levels. Our experimental design used a block by diabetic status to take into account the impact that condition may have on blood ghrelin levels and its fluctuation. Because of this, its statistical significance as a factor is worth acknowledging for further analysis but its p-value on our ANOVA table does not denote its true impact on this study.

Going deeper into our analysis, our data revealed that nicotine and cannabis causes a significant difference in blood ghrelin levels when in comparison with our control. Our Tukey HSD confidence intervals give us a range as to the sizable differences amongst treatments and they reveal that there is a statistical difference in blood ghrelin levels for both nicotine and cannabis when compared to our control group. However, there is no conclusive evidence that suggests nicotine and cannabis changes blood ghrelin levels in different ways as seen by our confidence interval between those two treatment groups having our null hypothesis in between.

Our interaction plot reveals the relationship between our different treatments and treatment dosages, with one distinction being that the variability in differences within our control group fluctuates as dose levels increase. We acknowledge that within the limitations of the Island's treatment options, we could not give a concrete "dose" of air like we originally designed which may contribute to the level of fluctuation we recorded in our design.

The boxplots we made from our data helps reveal information about the subgroups within our study. Our control group boxplot reflects our finding that its variability is greater; however, despite this, there is still a significant difference in means between our control group and that of our nicotine and cannabis treatment groups. Not only that, but the boxplots pertaining to dosage levels also reveal that an increase in dose resulted in an increase in outliers. In addition, our boxplots also supports our initial conclusion that our blocking method by diabetic status was effective in helping us accurately detect differences in ghrelin levels amongst our treatment groups.

We acknowledge that there were some limitations to our experimental design which can be improved upon for any follow up studies. One of our primary concerns was the type of instrument which was used for this study. Changes in blood ghrelin levels were measured during this experiment. However, it is possible that THC and nicotine induce other biological changes besides the protein expression level. For example, there is a possibility that mRNA expression could change. It previously has been established the increases in mRNA expression does not necessarily lead to increases in protein levels. Therefore, there should be instruments that also measure mRNA ghrelin levels and ribosome profiling analysis to determine the rate of conversion from mRNA to protein. In addition, the time of day which the levels of ghrelin were analyzed was not well controlled. Therefore, perhaps some variation could be controlled by strictly conducting these experiments during a certain time since ghrelin levels fluctuate according to meal times since ghrelin is a hunger inducing hormone. Therefore, potential future directions could include an experimental design where we control for time of day and amount of food in the stomach of the subject to control for some of the subject to subject variation in the levels of ghrelin. In addition, it is possible that we can look at other hunger hormones to determine if perhaps other hormones could be targeted to eventually change insulin levels. Regardless of the hormone, the end goal is to eventually determine if changing the levels of these hormones can naturally change the levels of insulin in the body. If this indeed is the case, then THC and nicotine have the potential to be prescribed as in addition or a replacement of existing insulin treatments to help treat diabetics in an non-invasive manner.

7 References

1) Cannabinoids and Ghrelin Have Both Central and Peripheral Metabolic and Cardiac Effects via AMP-activated Protein Kinase. Korbonits et.al. The Journal of Biological Chemistry. 2005

2) Nicotinic Receptor-Mediated Effects on Appetite and Food Intake. Role et.al. Journal of Neurobiology. 2002

3) Nicotine enhances modulation of food-cue reactivity by leptin and ghrelin in the ventromedial prefrontal cortex. Smolka et.al. Addiction Biology. 2014

4) Plasma Ghrelin Levels in Lean and Obese Humans and the Effect of Glucose on Ghrelin Secretion. Matsukura et.al. The Journal of Clinical Endocrinology & Metabolism. 2002

5) Effects of Continuous Versus Intermittent Exercise, Obesity, and Gender on Growth Hormone Secretion. Veldhuis et.al. The Journal of Clinical Endocrinology & Metabolism. 2008

6) http://www.anad.org/get-information/about-eating-disorders/eating-disorders-statistics/. Accessed February 18, 2018

7) http://www.diabetes.org/diabetes-basics/statistics/?referrer=https://www.google.com/. Accessed February 18, 2018

8) The Pharmakinetics of THC in fat and brain: Resulting functional responses to marihuana smoking. Nahas. Human Psychopharmacology. 2001

9) Central functions of the Ghrelin Receptor. Smolders and Portelli. Humana Press. 2014