

The Impact of Energy Drink Factors on Mental Vigilance

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STATS 101B: Introduction to Design and Analysis of Experiment

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Introduction

This research study investigates the effect of different energy drink formulations upon human mental vigilance. The effect of various substances on human performance is often an important topic to research as humans can be interested in ways to enhance their abilities to perform certain tasks. Energy drinks are a common substance to do so. These drinks are drinks often with stimulants inside, most commonly caffeine, and are marketed as products that will enhance physical and mental performance. Because of the variety of energy drink formulations that one can purchase, it begs the question of what component of these drinks actually helps enhance performance. Therefore, this experience tests the following research question: What is the effect of sugar and caffeine on mental vigilance?

Design of Experiment

Sugar is often known to give people an initial boost of energy from the release of glucose in the bloodstream (Bellisle, 2001), but caffeine is also proven to decrease levels of tiredness in humans (Winston et. al, 2005). Therefore, we examine the main effect of each factor and possibly their interaction effect on mental performance. We expect that the presence of higher levels of sugar and caffeine to increase vigilance levels since each component individually is known to increase human mental performance.

Since we not only want to investigate the main effect of our two factors but also their interaction effect, we used a replicated 2^2 factorial design with one block. We chose to use a replicated design to increase the power of the test. Each factor has two levels: high and low, where the factor is either present or not present in the energy drink. Table 1 shows the four possible energy drink formulations below.

| | | Sugar | |
|----------|----------|---------------------------|---------------------------|
| | | Low (0) | High (1) |
| Caffeine | Low (0) | Caffeine-free, sugar-free | Caffeine-free, with sugar |
| | High (1) | Sugar-free, with caffeine | With caffeine and sugar |

Table 1. One replicate of four energy drink treatments.

Each replicate of the experiment was performed within a different age block since age is linked to mental performance (Corriea et. al, 2018), so it may be a nuisance factor for the response variable of vigilance. Four age blocks were used:

- Children: 0-14 years old
- Youth: 15-24 years old
- Adult: 25-65 years old
- Senior: 65+ years old

The age blocks are based upon common age groupings of human development. For instance, humans go through puberty around 14 and finish developing their brains around 25 (The University of Queensland, 2022). Since cognitive loss may start later in life (Ebrahimi et. al, 2022), we set the last age block to be 65+, a common retirement age. Variance in developmental speed, especially in the first age block from 0 to 14 years old, may still be prevalent.

Participants were randomly sampled from ten different cities across the island of Bonne Santé: Talu, Pauma, Eden, Colmar, Mahuti, and Riroua. Random sampling was done using birth records and schools, and were taken from only one island to limit cross-island variation. During the experiment, participants first take a baseline vigilance test, where they are timed to see how fast they can circle all the letter Z's on a page of 2000 other letters. Then, they ingest a 250ml of an energy drink that has a randomly assigned treatment combination and wait thirty minutes for the drink to take effect since the effects of caffeine take time to kick in (American Medical Association, 2022). Finally, they retake the vigilance test. The difference in time in which they carry out the vigilance test is the response variable for the study, measured in minutes.

```
> summary(model_no_block)
```

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|--------------------------------|----|--------|---------|---------|----------|
| factor(sugar) | 1 | 0.1806 | 0.18063 | 5.522 | 0.0367 * |
| factor(caffeine) | 1 | 0.0156 | 0.01563 | 0.478 | 0.5026 |
| factor(sugar):factor(caffeine) | 1 | 0.0006 | 0.00063 | 0.019 | 0.8923 |
| Residuals | 12 | 0.3925 | 0.03271 | | |

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(model_block)
```

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|--------------------------------|----|---------|---------|---------|----------|
| factor(sugar) | 1 | 0.18063 | 0.18063 | 6.359 | 0.0327 * |
| factor(caffeine) | 1 | 0.01563 | 0.01563 | 0.550 | 0.4772 |
| factor(block) | 3 | 0.13688 | 0.04563 | 1.606 | 0.2554 |
| factor(sugar):factor(caffeine) | 1 | 0.00062 | 0.00062 | 0.022 | 0.8853 |
| Residuals | 9 | 0.25563 | 0.02840 | | |

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> #no block
> d <- 0.2
> MSE <- 0.03271
> #install.packages("pwr")
> library(pwr)
> pwr.anova.test(k = 4, f = d/sqrt(MSE), power = 0.9)
```

Balanced one-way analysis of variance power calculation

k = 4
n = 4.035544
f = 1.105833
sig.level = 0.05
power = 0.9

NOTE: n is number in each group

Figure 1. Anova of Variance, With and Without Blocking for 1 Run, and Power Anova Test

To determine the number of design replicates needed for a power of 0.9, the power anova test in R was used as seen in Figure 1. After dividing the desired maximum difference of 0.2 by the square root of the mean square effect, the effect size was calculated to be 1.106. We also decided to do a preliminary test on this one run and found blocking was unnecessary; the mean square for block did not have a very big ratio to mean square of error. With four different treatment groups, a minimum of five replicates was needed. In this study, six replicates of the full design were run. Each run contained four age blocks of four treatment combinations each, equalling to sixteen observations per run. Hence, in total, the experiment was conducted on 96 subjects.

Results and Interpretation

| <pre>{r} model2 <- aov(Vigilance.Test.Improvement ~ factor(Sugar)*factor(Caffeine) + factor(Age.Block), data = data) summary(model2) ---</pre> | <pre>{r} model1 <- aov(Vigilance.Test.Improvement ~ factor(Sugar)*factor(Caffeine), data = data) summary(model1) ---</pre> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|--------|---------|---------|--------------|--------|---------------|---|--------|--------|--------|--------------|------------------|---|--------|--------|-------|-------|-------------------|---|--------|--------|-------|-------|--------------------------------|---|--------|--------|-------|-------|-----------|----|--------|--------|--|--|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|----|--------|---------|---------|--------|---------------|---|--------|--------|--------|-------------|------------------|---|--------|--------|-------|-------|--------------------------------|---|--------|--------|-------|-------|-----------|----|--------|--------|--|--|
| <table><tr><th></th><th>Df</th><th>Sum Sq</th><th>Mean Sq</th><th>F value</th><th>Pr(>F)</th></tr><tr><td>factor(Sugar)</td><td>1</td><td>1.4259</td><td>1.4259</td><td>43.104</td><td>3.37e-09 ***</td></tr><tr><td>factor(Caffeine)</td><td>1</td><td>0.0126</td><td>0.0126</td><td>0.381</td><td>0.539</td></tr><tr><td>factor(Age.Block)</td><td>3</td><td>0.0611</td><td>0.0204</td><td>0.616</td><td>0.686</td></tr><tr><td>factor(Sugar):factor(Caffeine)</td><td>1</td><td>0.0009</td><td>0.0009</td><td>0.028</td><td>0.867</td></tr><tr><td>Residuals</td><td>89</td><td>2.9443</td><td>0.0331</td><td></td><td></td></tr></table> | | Df | Sum Sq | Mean Sq | F value | Pr(>F) | factor(Sugar) | 1 | 1.4259 | 1.4259 | 43.104 | 3.37e-09 *** | factor(Caffeine) | 1 | 0.0126 | 0.0126 | 0.381 | 0.539 | factor(Age.Block) | 3 | 0.0611 | 0.0204 | 0.616 | 0.686 | factor(Sugar):factor(Caffeine) | 1 | 0.0009 | 0.0009 | 0.028 | 0.867 | Residuals | 89 | 2.9443 | 0.0331 | | | <table><tr><th></th><th>Df</th><th>Sum Sq</th><th>Mean Sq</th><th>F value</th><th>Pr(>F)</th></tr><tr><td>factor(Sugar)</td><td>1</td><td>1.4259</td><td>1.4259</td><td>43.650</td><td>2.5e-09 ***</td></tr><tr><td>factor(Caffeine)</td><td>1</td><td>0.0126</td><td>0.0126</td><td>0.386</td><td>0.536</td></tr><tr><td>factor(Sugar):factor(Caffeine)</td><td>1</td><td>0.0009</td><td>0.0009</td><td>0.029</td><td>0.866</td></tr><tr><td>Residuals</td><td>92</td><td>3.0054</td><td>0.0327</td><td></td><td></td></tr></table> | | Df | Sum Sq | Mean Sq | F value | Pr(>F) | factor(Sugar) | 1 | 1.4259 | 1.4259 | 43.650 | 2.5e-09 *** | factor(Caffeine) | 1 | 0.0126 | 0.0126 | 0.386 | 0.536 | factor(Sugar):factor(Caffeine) | 1 | 0.0009 | 0.0009 | 0.029 | 0.866 | Residuals | 92 | 3.0054 | 0.0327 | | |
| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| factor(Sugar) | 1 | 1.4259 | 1.4259 | 43.104 | 3.37e-09 *** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| factor(Caffeine) | 1 | 0.0126 | 0.0126 | 0.381 | 0.539 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| factor(Age.Block) | 3 | 0.0611 | 0.0204 | 0.616 | 0.686 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| factor(Sugar):factor(Caffeine) | 1 | 0.0009 | 0.0009 | 0.028 | 0.867 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Residuals | 89 | 2.9443 | 0.0331 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| factor(Sugar) | 1 | 1.4259 | 1.4259 | 43.650 | 2.5e-09 *** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| factor(Caffeine) | 1 | 0.0126 | 0.0126 | 0.386 | 0.536 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| factor(Sugar):factor(Caffeine) | 1 | 0.0009 | 0.0009 | 0.029 | 0.866 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Residuals | 92 | 3.0054 | 0.0327 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 | Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Figure 2. Analysis of Variance, With and Without Blocking respectively, for all 6 Runs

An analysis of variance of the experimental responses indicated that sugar with a p-value of 3.37×10^{-9} was the only significant factor at $\alpha = 0.05$. Both caffeine and their interaction factor were not significant with p-values of 0.539 and 0.867, respectively. Notably, the mean square of the age block was 0.0204, forming a very small ratio against the mean square error of 0.0311. This suggests that age was a poor choice of blocking. Within group and between group variation of age blocks were likely not significantly different. Choosing to block also lowered the degrees of freedom of the model, decreasing its sensitivity.

Hence, we re-ran test results removing the age block. Now, this turned the experimental design into simply a 2^2 factorial design, with each block being treated as a new replicate. In total, the data had 24 replicates of each treatment combination. Doing this increased the mean square error by a negligible amount while increasing the sensitivity of the test.

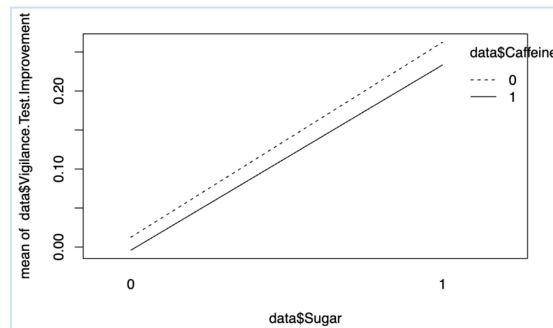


Figure 3. Interaction Plot of Sugar and Vigilance Test Improvement

Results proved to be similar. Again, sugar was the only factor having a significant effect on vigilance, with a p-value of 2.5×10^{-9} . Caffeine, with a p-value of 0.536, and their interaction, with a p-value of 0.866, were not significant. The interaction term plot confirmed this, showing nearly parallel lines indicating little to no interaction, as seen in Figure 3.

Estimated effects of the model also supported the same conclusions. Sugar had a positive estimated effect of 0.244, which was relatively large compared to the estimated effects of caffeine and their interaction of -0.023 and -0.006, respectively. Thus, the optimal treatment combination to improve vigilance was energy drinks with sugar and no caffeine.

Before accepting these results, we needed to check that the necessary model assumptions held. Looking at the graphs below, the normal Q-Q plot shows that residuals generally follow the theoretical normal Q-Q line in a roughly linear manner. The assumption of normality is thus satisfied. The residual plot is a little more tricky to interpret. First, the mean of the residuals is clearly 0, with points scattered evenly above and below the red line. Because of the limited values the two factors can take on, the range of fitted values is similarly limited to certain values. In turn, the residual plot looked a little unusual with two cluster-like gatherings. However, amongst those fitted values, points were evenly spread and variance remains constant. Therefore, the assumption of linearity and homoscedasticity still held, and the model results from above remained valid.

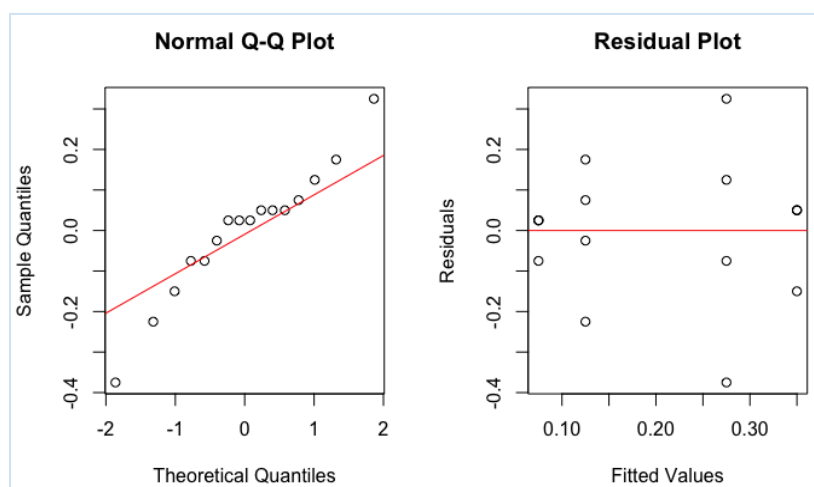


Figure 4. Model Assumption Plots- Normal Q-Q Plot and Residual Plot

Discussion

Our ANOVA results conclude that sugar had a statistically significant effect on a person's vigilance score, whereas caffeine did not have a statistically significant effect. These findings can be further confirmed by investigating our model assumptions of linearity and normality. We further tested the impact of sugar by assessing interaction plots between sugar and caffeine to ensure that its effects were not enhanced or masked by the effects of caffeine. Overall, our analysis confirmed that the effects of sugar, in the form of an energy drink, significantly affect an individual's mental awareness, tested through a vigilance test. Our results led us to conclude that there is a positive relationship between sugar consumption and cognitive performance.

The study "The Impact of Free and Added Sugars on Cognitive Function: A Systematic Review and Meta-Analysis" found that glucose has an immediate positive effect on free recall, although it may have detrimental effects if consumed excessively in the long term. Over a long period of time, continued consumption of added sugars, specifically sugar sweetened beverages such as the energy drinks we used during testing, was found to reduce cognitive function, leading to poor memory performance and a greater risk of cognitive impairment. In the short term, however, studies demonstrated the glucose facilitation effect, where the consumption of glucose led to short term improvements in cognitive function, tested through memory or problem solving cognitive tests. This effect was found to be the most observable in older participants, although our testing found that block by age was not significant.

Overall, in the short term, glucose and sugars have the facilitation effect where cognitive function may temporarily improve after consumption. However, in the long term, added sugars, such as those found in energy drinks and other sugar sweetened beverages, proved to be detrimental to cognitive function. To see an improvement in cognitive function, blood glucose and sugar consumption should be rigidly regulated with respect to individual physiology, age, and lifestyle factors (Gillespie, Kerri M, et al., 2023).

We acknowledge that the experimental design is constrained by multiple limitations. As sampling was limited to 6 random cities on the island of Bonne Sante rather than the entire island, the sample was not completely randomized. However, we believe that the size of the sample was ample enough to be a valid representation of Bonne Sante residents. Blocking by age in anticipation of potential age-related cognitive and reflex differences had little effect on the results and was a poor choice that did not significantly contribute to the analysis. This choice of block may have led us to miss another important confounding variable that may have affected the analysis of the treatments. Additionally, our research was limited only to short-term testing, where the effects of sugar and caffeine were collected on one day and only once per patient. Thus, we cannot make any conclusive determinations about the long-term or overall effect of sugar on cognitive function. Consequently, the difference in our conclusion and previous research may stem from the limitations of our research.

There are many possible adjustments that can be made to improve the design and quality of data. For one, increasing the sample size by expanding the pool of test subjects across additional cities and islands can increase the power of the test by capturing additional information of individual variation. As we concluded that age was not a significant block, we could further investigate other nuisance factors that may affect vigilance, such as weight, education, and gender. Moreover, vigilance is only one measure of mental alertness. Other types of cognitive tests can be conducted to verify the significance of our claim. Last but not least, though we concluded that sugar helps improve mental vigilance, that was only applicable to the form of energy drinks. Experiments testing other forms of sugar, such as Lollies or Sugar Tablets, on mental vigilance can further validate our conclusions.

References

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Appendix I: Code for Analysis

#Power Anova Test and Sample Size

```
sugar <- rep(c(-1, -1, 1, 1), 4)
caffeine <- rep(c(-1, 1), 8)
block <- c(rep(1, 4), rep(2, 4), rep(3, 4), rep(4, 4))

response <- c(-0.2, -0.1, -0.4, -0.6,
              0.1, 0, -0.2, -0.2,
              -0.1, -0.1, -0.4, -0.4,
              -0.3, -0.1, -0.4, 0.1)

response <- response * -1
model_no_block <- aov(response ~ factor(sugar)*factor(caffeine))
model_block <- aov(response ~ factor(sugar)*factor(caffeine) + factor(block))
summary(model_no_block)
summary(model_block)

#no block
d <- 0.2
MSE <- 0.03271
#install.packages("pwr")
library(pwr)
pwr.anova.test(k = 4, f = d/sqrt(MSE), power = 0.9)
```

#Interpreting Results Code - Main Effects, ANOVA (with/without Blocking), Interaction Plot

```
```{r}
data <- read.csv("-- csv pathway --")
head(data)
```

```{r}
model1 <- aov(Vigilance.Test.Improvement ~ factor(Sugar)*factor(Caffeine), data = data)

summary(model1)
```

```{r}
model2 <- aov(Vigilance.Test.Improvement ~ factor(Sugar)*factor(Caffeine)
```



```

+ factor(Age.Block), data = data)

summary(model2)
'''

'''{r}
main effects
lm_model_no_block <- lm(Vigilance.Test.Improvement ~ Sugar * Caffeine, data = data)
lm_model_block <- lm(Vigilance.Test.Improvement ~ Sugar * Caffeine + Age.Block, data =
data)

effect1 <- na.omit(2*coef(lm_model_no_block)[-1])
effect1

effect2 <- na.omit(2*coef(lm_model_block)[-1])
effect2
'''

'''{r}
library(dplyr)
'''

'''{r}
library(gplots)
'''

'''{r}
interaction.plot(data$Sugar, data$Caffeine, data$Vigilance.Test.Improvement)
'''

'''{r}
plot(interactionMeans(model1))
'''

#Model assumptions

```

```
``{r}
library(ggplot2)
par(mfrow = c(1, 2))
qqnorm(resid(model_no_block))
qqline(resid(model_no_block), col = "red")

plot(fitted(model_no_block), residuals(model_no_block),
 xlab = "Fitted Values", ylab = "Residuals",
 main = "Residual Plot")
abline(h = 0, col = "red")

``
```

## WHAT EVERYONE DID:

Janet:

- Brainstormed ultimate research question / topic of experiment
- Created “Interpreting Results” slides (4) with R Code (see above)
  - R Code included:
    - Importing dataset
    - Creating with & without block model
    - ANOVA
    - Interaction plots
    - Main effect calculation
    - Model Adequacy Check: Residual vs. Fitted & Normal Q-Q Plot
- Presented final presentation during lecture
- Attended group meetings

Katherine

- Attended group meetings
- Power of anova test + sample size
  - Filled out slides
  - + wrote description
- Went to Office Hour to ask questions for the group
- Created R markdown file for group
- Compiled all 6 replicates into one CSV for all of us to analyze

Audrey

- Improvements slide + reformatted some slides
- Presented final presentation in class
- Discussion section of project report
- Group meetings

Soomedha

- Model assumptions slide: Q-Q plot and residual vs fitted using R
- Limitations slide
- Attended group meetings
- Helped scrap initial blocking by weight idea since R results weren’t significant

Katinka

- Helped troubleshoot experimental design by discussing & emailing professor
- Wrote up report abstract
- Wrote report introduction & design of experiment sections
- Attended group meeting