

## The Impact of Caffeine on Dehydration in Naked Eggs

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### **Abstract:**

Dehydration can cause undesired side effects, especially when it is paired with an intensive exercise such as weightlifting. While weightlifters often drink water along with their workout, pre-workout supplements with large amounts of caffeine have become a staple in weightlifting. This study aimed to investigate the impact of caffeine on dehydration in naked eggs as human trials were not feasible at the time of this study. Dehydration was attempted to be measured as the percent change in weight experienced by the naked eggs. A one-way ANOVA test was conducted, which yielded a p-value of 0.5232. This is far greater than this study's significance level of 0.05, elucidating that there was no statistically significant difference in dehydration between the treatment groups. Errors in the experiment set up did not allow for the impact of caffeine on dehydration to be investigated as intended. This study will also provide suggestions on how this error can be avoided in future research.

### **Introduction:**

Pre-workout supplements (referred to as PWS hereafter) are a common addition to a weightlifter's pre-workout routine that promote elevated levels of energy as a result of their high caffeine content. The global PWS market is valued at 12.6 billion USD and is expected to grow at an annual rate of 8.3% from 2020 to 2027 (Grandview research). In Canada it was found that it was found that 86% of PWS contain caffeine (Jagim et al. 254) with the average dose being 150-300mg per serving (Hollowc2) while some PWS can reach up to 600mg per serving ("HOOLIGAN Pre Workout..."). As a result of having large amounts of caffeine in these supplements, their safety is often debated. Dehydration is an accompanying risk that users of

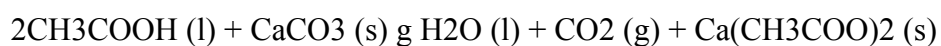
these supplements face and is listed as a side effect for many PWS (Weik). The impact that caffeine has on dehydration is a topic that has been well researched and has even been correlated to exercise. A previous paper looked at the effect of espresso with  $171 \pm 8.9$  mg of caffeine and found that dehydration occurred to 1.2% of the individual's body weight (Zavvos et al. 288). Another paper done by Zhang et al. specifically looked at caffeine ingestion prior to exercise and they found that at a median caffeine dosage of 300mg the diuretic effect caused by caffeine was negated during exercise so excess fluid loss was not exhibited in the 379 participants while exercising (570-573). However, both of these studies leave gaps on what happens if caffeine is taken in excess of the 400mg daily allowance (Food and Drug Administration), which can be the case with these pre workout supplements as they can be taken in 600mg doses. Since human trials were not feasible, this study will attempt to mimic the impact that caffeine has on dehydration through observing eggs with a dissolved shell (hereafter referred to as naked eggs). The weight loss that occurs in the naked eggs will be a result of osmosis, where water moves from the egg to the surrounding hypertonic environment. This is intended to mimic caffeine entering the bloodstream and drawing water out of muscles because as Hill et al. state, muscles can retain water as a result of increased levels of cortisol due to medium to high intensity exercise (587), therefore increasing the water content in the muscles and helping establish an osmotic gradient.

This study will attempt to answer if caffeine impacts dehydration in naked eggs. If caffeine causes dehydration, then there should be a greater weight loss in naked eggs that have been immersed in the solution containing 600mg of caffeine when compared to those that have

been immersed in solutions containing lower levels of caffeine. If the results found are significant then the null hypothesis which states there is no difference in egg weight loss through dehydration when submerged in solutions with varying caffeine concentrations will be rejected and the alternate hypothesis that there is a difference in egg weight loss via dehydration when submerged in solutions with varying caffeine concentrations will be supported.

### **Methods:**

12 clear plastic cups were filled with 300mL of vinegar each and one egg was submerged in each cup. The eggs were left to sit in the vinegar solution for 24 hours so that the eggshell would dissolve completely. The formation of bubbles on the eggshells indicated that the reaction had started. As seen in equation 1, these bubbles are carbon dioxide bubbles that formed as a result of the reaction between the calcium carbonate eggshells and acetic acid in the vinegar.



Equation 1: This equation shows the balanced reaction between the calcium carbonate in the eggshells and the surrounding vinegar environment to create carbon dioxide and calcium acetate (“Naked Eggs”).

After the 24 hours the eggs were removed from the cup and dried using paper towels. A clear plastic cup was then placed on the digital scale and the scale was zeroed with the cup on it to allow the mass of the eggs to be measured without having to subtract the mass of the cup after

each measurement. Each egg was placed into the cup one at a time and the mass was recorded as the initial weight. The plastic cups were emptied, and the eggs were placed into a temporary storage container (Figure 1). As shown in Figure 1 below, the eggs had left over eggshell residue, which according to equation 1 was calcium acetate, this residue was wiped off the egg using paper towels.



Figure 1: The eggs after their eggshells were dissolved in a vinegar solution placed in their temporary storage container. The white dots on the eggs are left over pieces of calcium acetate, this was wiped off prior to placing them in their respective treatment.

12 empty plastic cups were labelled with the sample number and the solution they were going to contain. Cups 1-3, were labelled as 500mL of vinegar, 4-6 were labelled as 200mg of caffeine, 7-9 were labelled as 400mg of caffeine and 10-12 were labelled as 600mg of caffeine. A

tinfoil piece was folded on three edges to make a boat to weigh the caffeine. 50 Allmax Caffeine pills containing 200mg of caffeine each were crushed in a mortar and pestle to turn them into a fine powder. The tinfoil boat was placed on the digital scale and the scale was zeroed to account for the mass of the tinfoil boat. 600mg of the powdered caffeine was weighed out onto the tinfoil boat using a teaspoon and the caffeine was then put into a clear plastic cup that was labelled with the sample number and amount of caffeine. This was done for the 200mg and 400mg caffeine samples as well and was repeated three times for each caffeine quantity. In total, there were 3 cups containing 600mg of caffeine, 3 cups containing 400mg of caffeine, and 3 cups containing 200mg of caffeine. Afterwards 500mL of water was added to each cup with caffeine, cups 4-12. The final concentrations of the caffeine containing solutions were 1.2mg/mL for the cups containing 600mg of caffeine, 0.8mg/mL for the cups containing 400mg of caffeine and 0.4mg/mL for the cups containing 200mg of caffeine. Afterwards, 500mL of vinegar was added to cups 1-3. The vinegar only treatment acted as the control for the study as the vinegar was intended to be isosmotic with the eggs therefore there should not have been weight loss or weight gain observed in the naked eggs in these cups. By having the vinegar only solution as the control, the amount of caffeine was intended to be the only variable that would impact the weight of the naked eggs so any statistically significant differences in the weight of the eggs would be a result of the caffeine content in each treatment. The cups with caffeine and water were stirred with a teaspoon to help dissolve the caffeine powder. After the caffeine had dissolved into the solutions (Figure 2), the eggs were left in their respective solutions for 24 hours and the cups were placed in a storage container so they would not be disturbed.



Figure 2: This figure shows naked eggs numbered 8-10 and the amount of caffeine in that treatment after being placed in their storage container for 24 hours. The solutions have been created using caffeine and water, sample 8 and 9 have a caffeine concentration of 0.8mg/mL while sample 10 has a caffeine concentration of 1.2mg/mL. The naked eggs were visibly larger than they were in Figure 1 prior to being subjected to their respective treatments.

After 24 hours, the cups were removed from the storage container. One clear and empty plastic cup was placed on the digital scale and the scale was zeroed. The naked eggs were removed from the solution one at a time and their exterior was dried with paper towels. The dried eggs were then placed in the plastic cup on the scale and the weight was recorded as the final weight. This was done for all 12 eggs. After the weight of all the eggs was recorded, the

solutions, plastic cups, and eggs were disposed of. After compiling the data, the percent change in weight was calculated and this data was used for the statistical tests.

**Results:**

Each treatment group had a sample size of 3 ( $n=3$ ), for a total of 12 samples. Each sample gained weight and the mean percent change in egg mass was 12.2% for the control group, 15.1% for the 200mg group, 14.9% for the 400mg group and 15.8% for the 600mg group. The 95% confidence interval for the control group was 9.0% to 15.4%, for the 200mg group was 10.3% to 19.9%, for the 400mg was 13.2% to 16.6% and for the 600mg group was 12.7% to 18.9%. Sample 3, which had a percent change in weight of 8.9% did not fit into the confidence interval for the control group and sample 12, which had a percent change in weight of 12.6% did not fit into the confidence interval for the 600mg group as seen in figure 3.

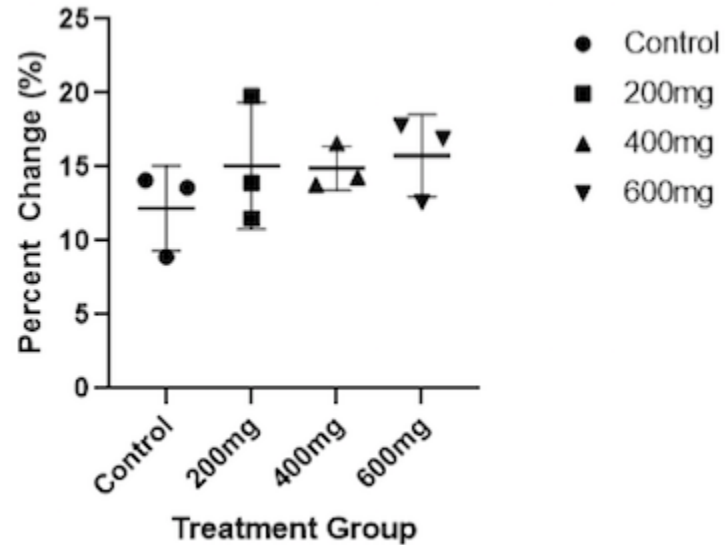


Figure 3: Percent change for each naked egg sample and mean percent change for each treatment group. The sample size is  $n=3$  for each treatment group. The mean percent change in weight for the control group is observed to be lower than the caffeinated samples. The largest confidence intervals are seen in the 200mg group and the smallest confidence intervals are seen in the 400mg group indicating that the 400mg group measurements were more precise than those of the other groups. This data was used to conduct a one-way ANOVA test which returned a p-value of 0.5232.

The alpha/significance level for this study was set at 0.05 because any results obtained with a p-value below this had a greater than 95% probability that the null hypothesis is incorrect and that the results obtained are statistically significant. This was also chosen as the significance level because previous literature done by Zavvos as well as Zhang used 0.05 as the significance



level (Zavvos et al. 288; Zhang et al. 570), so by using 0.05 in this study, it would be easier to directly compare results. As a result, the p-values obtained from the statistical tests must be less than 0.05 for the null hypotheses of those tests to be rejected and the alternate hypotheses supported. A Shapiro-Wilk test was conducted to see if the samples came from a normal distribution and it provided four p-values, one for each treatment. The p-value for the vinegar only solution was 0.1666, for the 200mg caffeine solution was 0.5439, for the 400mg solution was 0.3212 and for the 600mg solution was 0.3106. A one way ANOVA was conducted on the percent change data to determine if the data means of the groups were significantly different from each other and a p-value of 0.5232 was observed.

### **Discussion:**

In this study, the impact of caffeine on dehydration was tested in naked eggs. It was found that as a result of the experimental setup, weight loss in naked eggs was unable to be recorded as the eggs gained weight and yielded data which were not significant according to the statistical tests conducted.

Ten out of the twelve percent change values were within their treatment group's 95% confidence intervals but sample 3, part of the control group, and sample 12, part of the 600mg group, were not so there is a greater than 95% probability that these values are not indicative of the true mean of their groups. The Shapiro-Wilk test p-values were greater than the significance level of 0.05 used in this paper, therefore the null hypothesis of the Shapiro-Wilk test, that the

data come from a normal distribution, was unable to be rejected and the alternate hypothesis, that says the data does not come from a normal distribution, was unable to be supported by these results. This allowed for a one-way ANOVA test to be conducted on the normally distributed data for percent change in egg mass as the assumptions of the one-way ANOVA were met. The one-way ANOVA yielded a p-value of 0.5232 which is greater than the significance level of 0.05 therefore the null hypothesis that there is no difference in egg weight loss through dehydration when submerged in solutions with varying caffeine concentrations, was not rejected. This also meant that the alternate hypothesis that there is a difference in egg weight loss via dehydration when submerged in solutions with varying caffeine concentrations was unable to be supported. As a result this disproves the original hypothesis which was if caffeine has an impact on dehydration, then there should be a greater weight loss in the naked eggs submerged in the 600mg caffeine solution than the other treatment groups. This demonstrates that there was not a significant difference seen between the means of the treatment groups so it could not be concluded that one group has a greater impact on dehydration than the other groups.

This experiment was intended to demonstrate weight loss in the naked eggs however, no weight loss was observed in the naked eggs. Instead all of the naked eggs ended up gaining weight. This could be a result of incorrectly assuming that the naked eggs would be isotonic or hypotonic to their environment. Since the pure white vinegar was 93-96% water (Hill) and the eggs are 74% water (Johnson and Ridlen), it was assumed that this would be enough to change the osmolarity of the eggs to the point that they would be hypotonic or isotonic compared to their environment. However, it was evident that this was not the case and that this was an incorrect

assumption to make. A previous experiment found that naked eggs increased in size when they were placed in their initial vinegar solution as a result of water moving from the vinegar into the egg (Science World). This movement of water would also result in weight gain which is similar to what was observed in the results for this study. Another paper by Cocanour and Bruce also found that naked eggs gained weight when submerged in water, they also found that eggs placed in a glucose solution lost weight as water moved out of the egg and into the surrounding environment (127-130). Previous literature does not discuss the impact of caffeine on naked eggs and whether that could have influenced the obtained results. However, since weight loss was observed when the egg was placed in a glucose solution, it is possible that submerging naked eggs in a solution containing caffeine would also result in water moving out of the eggs and therefore causing dehydration to occur in the eggs.

To truly test if caffeine impacts dehydration through weight loss in naked eggs, future research must have a 48 hour period of submerging the naked eggs in distilled water prior to the caffeine treatment to ensure that the eggs will be hypotonic to their environment. This may have prevented the unexpected weight gain that was observed in this experiment and could have led to more significant results on the impact of caffeine on dehydration. This experiment could be repeated by just making this alteration but the sample size could also be increased to raise the statistical significance of the results obtained by the scientists doing this experiment in the future.

**Conclusion:**

This experiment attempted to demonstrate the impact that caffeine has on dehydration in naked eggs but as a result of errors in the methods this experiment was unsuccessful in demonstrating that a link between caffeine and dehydration measured through the percent change in weight exists in naked eggs. The p-values obtained from the one-way ANOVA were greater than the significance level of 0.05 therefore, the mean percent change in weight of the treatment groups were not significantly different from one another.

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**Appendix:**

Table 1: Raw Data for the Study

Sample Number	Caffeine Content (mg)	Caffeine Concentration (mg/mL)	Initial Weight of the Naked Egg (mg)	Final Weight of the Naked egg (mg)	Percent change in weight
1	0	0	87.3	99.2	13.6
2	0	0	92.2	105.2	14.1
3	0	0	89.4	97.4	8.9
4	200	0.4	88.7	98.9	11.5
5	200	0.4	82.2	93.6	13.9
6	200	0.4	90.9	108.9	19.8
7	400	0.8	85.8	100.0	16.6
8	400	0.8	87.0	99.4	14.3
9	400	0.8	92.3	105.0	13.8
10	600	1.2	83.9	98.8	17.8
11	600	1.2	87.3	102.1	16.9
12	600	1.2	84.4	95.0	12.6

Table 1: This table shows the raw data collected in the lab for each trial as well as the percent change in weight in the naked eggs.



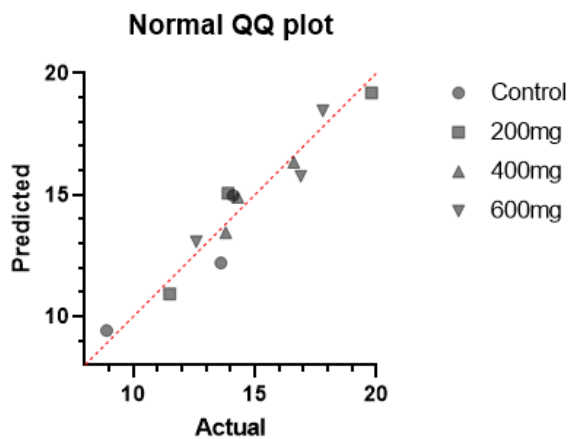


Figure 4: This figure shows the QQ plot of the raw percent change data from table 1. The data did not undergo any transformation so the percent change was plotted just as it was shown in table 1. The data fits the line of best fit and is therefore normally distributed.

The screenshot shows the GraphPad Prism software interface. The title bar reads 'Project1: Ordinary one-way ANOVA of Col: One-way ANOVA - GraphPad Prism 9.1.0 (221)'. The menu bar includes File, Edit, View, Insert, Analyze, Change, Arrange, Family, Window, and Help. The toolbar contains various icons for file operations, analysis, and formatting. The sidebar on the left has a search bar and several expandable sections: Data Tables (with 'Col: One-way ANOVA' selected), Info, Results (with 'Ordinary one-way ANOVA of Col...' selected), Graphs, and Layouts. The main window displays a table of results for the 'Ordinary one-way ANOVA' test.

Ordinary one-way ANOVA					
1	Table Analyzed	Col: One-way ANOVA			
2	Data sets analyzed	A-D			
3					
4	<b>ANOVA summary</b>				
5	F	0.8098			
6	P value	0.5232			
7	P value summary	ns			
8	Significant diff. among means (P < 0.05)	No			
9	R squared	0.2329			
10					
11	<b>Brown-Forsythe test</b>				
12	F (DFn, DFd)	0.3070 (3, 8)			
13	P value	0.8199			
14	P value summary	ns			
15	Are SDs significantly different (P < 0.05)	No			
16					
17	<b>Bartlett's test</b>				
18	Bartlett's statistic (corrected)				
19	P value				
20	P value summary				
21	Are SDs significantly different (P < 0.05)				
22					
23	<b>ANOVA table</b>	SS	DF	MS	F (DFn, DFd) P value
24	Treatment (between columns)	22.12	3	7.374	F (3, 8) = 0.8098 P=0.5232
25	Residual (within columns)	72.85	8	9.107	
26	Total	94.98	11		
27					
28	<b>Data summary</b>				
29	Number of treatments (columns)	4			
30	Number of values (total)	12			
31					
32					
33					
34					
35					
36					

Figure 5: This figure shows the p-value obtained from the one-way ANOVA test using graphpad prism 9. This p-value was greater than the significance level of 0.05 in the study so the data did not yield significant results and the null hypothesis could not be rejected and the alternate hypothesis could not be supported.

<b>Shapiro-Wilk test</b>				
W	0.8214	0.9440	0.8789	0.8753
P value	0.1666	0.5439	0.3212	0.3106
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns

Figure 6: Shows the p-values obtained from the Shapiro-Wilk test using graphpad prism 9. The p-values are greater than the significance level of 0.05 therefore, the data obtained followed a normal distribution.

$$\text{Percent Change in Weight} = (\text{Final Weight (g)} - \text{Initial Weight (g)}) / \text{Initial Weight} \times 100\%$$

Equation 2: The percent change in weight experienced by the naked eggs was calculated using the above formula.

$$\text{Concentration (mg/mL)} = \text{Amount of Caffeine in Solution(mg)} / \text{Volume of the Solution(mL)}$$

Equation 3: This equation shows how the caffeine concentrations used in this study were calculated.