Extended Essay in Biology HL

Topic:
Effect of different nuts on human sleeping pattern

Research Question: Is there a correlation between the ingestion of increasing quantities of dry fruits Prunus dulcis (almonds), Juglans regia (walnuts) and Pistacia vera (pistachios) with the average number of hours of sleep acquired, and is there a statistically significant difference between the average number of sleeping hours acquired by males and females of age group 16-17 years over a fifteen-day period?

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Section 1: Introduction

1.1: Research Question

Is there a correlation between the ingestion of increasing quantities of dry fruits *Prunus dulcis* (almonds), *Juglans regia* (walnuts) and *Pistacia vera* (pistachios) with the average number of hours of sleep acquired, and is there a statistically significant difference between the average number of sleeping hours acquired by males and females of age group 16-17 years over a fifteen-day period?

1.2: Context of the Essay

Sleep is a vital part of human lifestyle. A disturbance in sleep routine has been scientifically proven to have undesirable effects on body functioning. Last year, when I was studying for my finals, I attempted my exams with no more than 3-4 hours of sleep each day. This influenced my concentration and I tended to feel fatigued. As a result, I wondered whether getting enough sleep was as important in maintaining an ideal lifestyle as diet and exercise. My disturbed sleep cycle inspired me to choose a topic that's results could benefit others struggling with insomnia. Poor sleep has shown to have adverse effects on mental health, and can be correlated with psychological disorders such as depression and fatal illnesses including heart disease and type 2 diabetes.[1]

I wanted to improve my sleeping pattern using natural foods. During my Biology HL classes, I learned about the importance of melatonin in sleep-wake cycles. Melatonin ensures that our body follows the 24 hour cycle and that our sleeping habits fit this cycle.This led me to research to find healthy foods that contain melatonin. As a result, I came across a variety of dry fruits that had significant amounts of melatonin. I investigated dry fruits instead of other types of food because they are healthy, easily accessible to all participants and easy to ingest in large amounts without any adverse reactions on the human body. Additionally, they are easily quantifiable as compared to other foods or drinks that are known to improve sleep quality, such as chamomile tea or warm milk. This topic was selected after thoughtful planning, as I was able to use both

[1] Leech, Joe. "10 Reasons Why Good Sleep Is Important." *Healthline*, Healthline Media, 25 Feb. 2020, www.healthline.com/nutrition/10-reasons-why-good-sleep-is-important. Accessed July 5 2020.

primary and secondary data in this investigation due to the limitations of the global pandemic, during which this experiment was conducted. I acquired primary data by collecting participants, so that my data was authenticated. I used secondary data sources to create my hypotheses and confirm my findings.

1.3: Background Biological Information

Sleep is a natural state of altered consciousness. One's senses are relatively inhibited along with a significant decrease in the action of muscles and negligible function of voluntarily used muscles during the night. Inadequate and adequate sleep have significantly different effects on human health. Three to five hours of sleep may result in short term memory loss, accidents and impaired cognitive functioning. Seven to nine hours of sleep optimize productivity and focus, improve mood and reduce stress. Few practices that people can follow to improve sleep include avoidance of naps, regular exercise, avoidance of devices before sleeping[2] and setting a comfortable environment in their room.[3]

There are two types of sleep: REM (rapid-eye-movement) sleep and non-REM sleep.[4] One switches between the various stages of REM sleep and non-REM sleep during nighttime, ending with extended durations of REM towards the end of the night. REM sleep happens the first time nearly ninety minutes after drifting off to sleep. During this stage, breathing rate increases and blood pressure escalates near the magnitude of times we are awake. Non-REM sleep takes place in three separate stages. The first stage is the changeover from being awake to being asleep. Sleep is light, with heart and breathing rates along with any eye movements decelerate. Stage two is the changeover from light sleep to deeper sleep. Muscles ease up, body temperature falls to a minimum and eye movements stop completely. This stage of sleep is longer in comparison to others. Non-REM sleep's third stage is the stage wherein one sleeps the deepest. Heart and breathing rate drop to a minimum, and muscles are completely relaxed.

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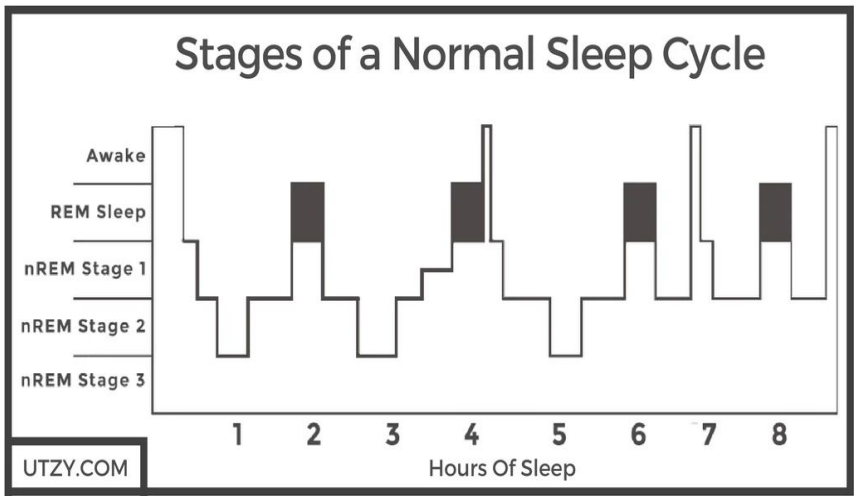
[2] Cherry, Kendra. "What Impact Does Sleep Have on Mental Health?" *Verywell Mind*, 24 Feb. 2020, www.verywellmind.com/how-sleep-affects-mental-health-4783067#:~:text=Lack of sleep is linked,, anxiety, and bipolar disorder, Accessed July 7 2020.

[3] Jenna Fletcher. "Why Is Sleep Important? 9 Reasons for Getting a Good Night's Rest." *Medical News Today*, MediLexicon International, 31 May 2019, www.medicalnewstoday.com/articles/325353, Accessed July 9 2020.

[4] "Brain Basics: Understanding Sleep." *National Institute of Neurological Disorders and Stroke*, U.S. Department of Health and Human Services, www.ninds.nih.gov/Disorders/patient-caregiver-education/Understanding-sleep, Accessed July 17 2020.

Figure 1. Graph showing stages of a sleep cycle

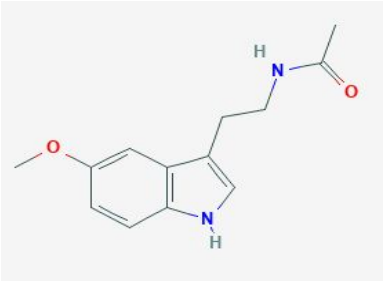


The dry fruits I am going to investigate all contain a similar composition of various nutrients, including high levels of carbohydrates, fats and proteins. *Prunus dulcis* have 22% carbohydrates, 21% protein and 50% fat per 100 grams. *Juglans regia* have 5% carbohydrates, 30% protein and 65% fat per 100 grams[5]. *Pistacia vera* have 10% carbohydrates, 40% protein and 59% fat per 100 grams.

The human body's 'clock' follows a 24-hour cycle, controlled by circadian rhythms. They depend on suprachiasmatic nuclei that are present within the hypothalamus. These cells regulate the levels of secretion of melatonin, also known as the sleep hormone. The production of melatonin increases during evening and decreases by morning, which is why we feel sleepy at night and awake by morning. High melatonin levels promote feelings of drowsiness and enable sleep. The IUPAC name of this hormone is *N*-[2-(5-methoxy-1*H*-indol-3-yl)ethyl]acetamide. Its chemical formula is $C_{13}H_{16}N_2O_2$.

[5] "Walnuts, NFS." *Nutrition Facts for Walnuts, NFS, Recommended Daily Values and Analysis.*, www.nutritionvalue.org/Walnuts,_NFS_nutritional_value.html, Accessed Aug. 5 2020.

Figure 2. Chemical structure of Melatonin[6]



Insomnia and sleep disorders are hypothesized to occur due to a lack of NAT(N-acetyltransferase) enzymes that are supposed to be present within the pineal gland. Melatonin's biosynthesis[7] is initiated with L-tryptophan[8], followed by hydroxylation by tryptophan-5-hydroxylase to the compound 5-hydroxy-L-tryptophan. This compound is then decarboxylated by 5-hydroxytryptophan decarboxylase enzyme to the neurotransmitter serotonin. Serotonin is changed to N-acetylserotonin by the enzyme serotonin arylalkylamine-N-acetyltransferase (AA-NAT). Finally, the enzyme acetylserotonin-O-methyltransferase converts N-acetylserotonin into melatonin through methylation.

The dry fruits chosen for this experiment also contain the melatonin precursor tryptophan. *Prunus dulcis* contain 0.28g per 100g of tryptophan, *Pistacia vera* contain 0.25g per 100g[9], and *Juglans regia* contain 0.17g per 100g grams of tryptophan.

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[6] "Melatonin." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Melatonin, Accessed June 29 2020.

[7] Alexei Evsikov. "Pathway: Serotonin and Melatonin Biosynthesis." *MetaCyc Serotonin and Melatonin Biosynthesis*, 9 Sept. 2008, metacyc.org/META/new-image?type=PATHWAY&object=PWY-6030&detail-level=2&ENZORG=TAX-9606 , Accessed Aug. 21 2020.

[8] Tordjman, Sylvie, et al. "Melatonin: Pharmacology, Functions and Therapeutic Benefits." *Current Neuropharmacology*, Bentham Science Publishers, Apr. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5405617/, Accessed Aug. 3, 2020.

[9] "Amount of Tryptophan (Trp) Standard Amino Acid in Nuts, Almonds, Honey Roasted, Unblanched." *Traditional Oven*, www.traditionaloven.com/foods/specific-nutrient/nuts-seeds/nuts-almonds-honey-roasted-unblanched/tryptophan-trp-standard-amino-acid.html, Accessed July 1 2020.

Section 2: Investigation

2.1: Variables

Independent Variables

1. The type of dry fruit (*Prunus dulcis, Pistacia vera* and *Juglans regia*).
2. The number of nuts consumed by the participants.
3. The number of days the nuts were given to each participant (males and females).

Dependent Variable

The number of hours of sleep each participant (male and female) gets each night during the experiment will be the dependent variable as it will be measured and is hypothesized to be influenced by the number of nuts eaten each night.

Control Variables

- Males and females of age group 16-17 years, as older individuals naturally have low levels of melatonin as compared to teenagers.
- Participants' relative health was normal, as most physiological diseases tend to upset the body's homeostasis, thus may influence sleep.
- The same number of nuts were taken by each participant for the same number of days.
- The participants were asked to avoid five types of foods that disrupt sleep[10] as mentioned below throughout the experiment five hours before bedtime to remove biases.
    1. Tomatoes
    2. Processed meats
    3. Carbonated drinks
    4. Coffee
    5. Fried food
- I also asked them to avoid foods that induced sleep, so that the effects of the nuts weren't masked. Those foods were:
    6. Warm milk
    7. Bananas
    8. Herbal tea
    9. Oats

[10] Simon, Nissa. "Insomnia? Can't Sleep? Foods That Harm Sleep." *AARP*, www.aarp.org/health/healthy-living/info-2014/foods-that-disrupt-sleep-photo.html#slide1, Accessed Aug. 27 2020.

10. Eggs

2.2: Hypothesis

A) I hypothesized that *Pistacia vera* will induce more sleep and give the highest Pearson's correlation value (*r* value) between the average number of hours of sleep and the number of nuts, followed by *Prunus dulcis* and finally *Juglans regia* based on their melatonin content as shown below. Since *Juglans regia*'s melatonin content is very low as compared to the other dry fruits, I predict that it will have negligible effect on sleep.

Table 1: Melatonin content of dry fruits

Dry fruit	Melatonin content (ng/g)
Pistacia vera	660
Prunus dulcis	39[11]
Juglans regia	2.5-4.5

B) For performing a t-test, the following hypotheses were made for each nut. I will investigate whether there is a statistically significant difference between average male and female sleeping hours in all three groups of dry fruits (*Prunus dulcis, Juglans regia* and *Pistacia vera*). I predict that the nuts will have a significantly higher influence on the sleep cycle of males as compared to females, based on a study[12] that's findings suggested that the associations between diet and sleep were prevalent in males but not in females.

Null hypothesis: There will be no statistically significant difference between average male and female sleeping hours.

Alternative hypothesis: There will be a statistically significant difference between average male and female sleeping hours.

[11] Meng, Xiao, et al. "Dietary Sources and Bioactivities of Melatonin." *Nutrients*, MDPI, 7 Apr. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5409706/, Accessed July 30 2020.
[12] St-Onge, Marie-Pierre, et al. "Effects of Diet on Sleep Quality." *Advances in Nutrition (Bethesda, Md.)*, American Society for Nutrition, 15 Sept. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC5015038/, Accessed Aug. 5 2020.

Subsequently, the same hypotheses were used in all three groups.

2.3: Materials

1. Each participant will be required to ingest 75 nuts in total throughout the experiment, depending on the group they were placed in (*Prunus dulcis, Pistacia vera* or *Juglans regia*).
2. Informed Consent Form explaining the rules of the experiment and to take prospective participants' consent to participate.
3. Each participant was asked to fill a Google document i.e. the Data Collection Form to record their sleeping and waking times to allow calculation and processing of data.
4. Microsoft Excel was used to calculate Pearson's correlation coefficient values, mean, standard deviation, error bars and make data tables for data presentation.
5. Microsoft Word was used to make raw data tables and some data processing tables.
6. The website https://www.socscistatistics.com/tests/studentttest/default2.aspx was used to conduct the t-tests.
7. The website https://miniwebtool.com/outlier-calculator/ was used to find outliers in raw data.

2.4: Method

1. Firstly, I formulated an Informed Consent Form to attract participants for the experiment. I circulated this form between my social groups, and finally was able to attract 30 participants (15 girls and 15 boys).
2. 15 females of age group 16-17 were gathered and randomly sorted into three groups of five females. The participants of each group were given one type of dry fruit (*Prunus dulcis, Pistacia vera* and *Juglans regia*) to consume for fifteen days.
3. The table below shows the number of dry fruits each participant will consume on each day of the experiment. Each participant was asked to keep track of the number of hours of sleep they get everyday over the course of the experiment.

Table 2: Instructions given to participants

Days of the experiment	Number of nuts to be consumed during that range of days
1-3	3
4-6	4
7-9	5
10-12	6
13-15	7

4. In order to record the data, participants filled their individual Data Collection Forms at the end of each day.
5. Raw data tables were constructed to collect information[13].
6. I used these tables to calculate the average number of hours of sleep acquired by participants each day. The standard deviation was also calculated.
7. A Pearson's test was performed separately for each of the three groups. I attempted to find the correlation between the average number of hours slept and the number of nuts consumed.
8. Steps 1-7 were repeated for males.
9. A t-test was carried out to investigate whether there is a statistically significant difference in the average number of hours of sleep acquired between males and females in each of the groups (*Prunus dulcis, Pistacia vera* and *Juglans regia*). I chose to do the independent samples t-test because it does not require a large amount of data. For other tests of significance such as the chi-square test, a minimum sample size of 50 is recommended.

[13] The Informed Consent Form, Data Collection Form and the Raw Data Tables can be found in the appendix.

2.5: Data Processing

Table 3: Average number of hours of sleep acquired by female participants in different dry fruit groups

		Average number of hours slept in different dry fruit groups (Y-Values)		
Day	No. of nuts eaten (X-Values)	*Prunus dulcis*	*Pistacia vera*	*Juglans regia*
1	3	4.09	6.78	6.68
2	3	4.93	6.72	7.85
3	3	5.82	6.89	7.88
4	4	7.00	8.37	7.20
5	4	6.16	8.02	7.73
6	4	7.93	8.76	6.82
7	5	5.40	7.15	7.28
8	5	4.43	7.17	6.03
9	5	5.86	6.60	7.03
10	6	5.43	7.68	6.12
11	6	5.90	8.86	7.77
12	6	6.27	8.17	6.88
13	7	8.85	8.27	6.15
14	7	7.45	7.05	6.35
15	7	6.60	7.53	6.27

Example calculation for average of *Prunus dulcis* eaten on Day 1 by five participants:

$$\frac{(5.20+1.92+5.67+2.00+5.67)}{5} = 4.09$$

Likewise, similar calculations were done for male participants as shown in Table 4. Values were taken from raw data tables in the appendices.

Table 4: Average number of hours of sleep acquired by male participants in different dry fruit groups

		Average number of hours slept in different dry fruit groups (Y-Values)		
Day	No. of nuts eaten (X-Values)	*Prunus dulcis*	*Pistacia vera*	*Juglans regia*
1	3	5.38	6.63	6.34
2	3	6.38	5.88	7.25
3	3	5.52	7.48	5.38
4	4	5.95	6.27	7.22
5	4	5.99	8.02	7.64
6	4	8.69	7.03	6.79
7	5	7.94	7.93	6.10
8	5	5.95	8.66	7.32
9	5	7.97	7.64	6.68
10	6	7.77	8.26	7.02
11	6	7.37	6.86	7.00
12	6	8.28	7.79	6.53
13	7	7.98	7.72	6.31
14	7	8.50	8.79	7.10
15	7	7.57	7.96	6.26

Table 5: Average and standard deviation for the average number of hours slept in different dry fruit groups (female participants)

		Prunus dulcis		*Pistacia vera*		*Juglans regia*	
Days	Total no. of nuts	Average	S.D.	Average	S.D.	Average	S.D.
1,2,3	9	4.95	0.71	6.80	0.07	7.47	0.56
4,5,6	12	7.03	0.72	8.38	0.30	7.25	0.37
7,8,9	15	5.23	0.60	6.97	0.26	6.78	0.54
10,11,12	14	5.87	0.34	8.24	0.59	6.92	0.67
13,14,15	21	7.63	0.93	7.62	0.50	6.26	0.08

The average was calculated by finding the sum of the values, then dividing the sum by the number of values in the sample. The aforementioned values were taken from Table 3.

Sample Calculation:

$$\frac{4.09+4.93+5.82}{3} = 4.95$$

Likewise, similar calculations were done for male participants as shown in Table 6. The values used for calculations in Table 6 were taken from Table 4.

Table 6: Average and standard deviation for the average number of hours slept in different dry fruit groups (male participants)

		Prunus dulcis		*Pistacia vera*		*Juglans regia*	
Days	Total no. of nuts	Average	S.D.	Average	S.D.	Average	S.D.
1,2,3	9	5.76	0.44	6.66	0.65	6.32	0.76
4,5,6	12	6.88	1.28	7.11	0.72	7.22	0.35
7,8,9	15	7.29	0.95	8.08	0.43	6.70	0.50
10,11,12	18	7.80	0.37	7.63	0.58	6.85	0.23
13,14,15	21	8.02	0.38	8.16	0.46	6.56	0.47

Table 7: Average standard deviation for each dry fruit group

	Prunus dulcis		*Pistacia vera*		*Juglans regia*	
Genders	Male	Female	Male	Female	Male	Female
Avg. S.D.	0.68	0.66	0.57	0.34	0.46	0.44

These results were calculated using the values from Tables 5 and 6.

Sample calculation for female *Prunus dulcis* group:

$$\frac{0.71+0.72+0.60+0.34+0.93}{5} = 0.66$$

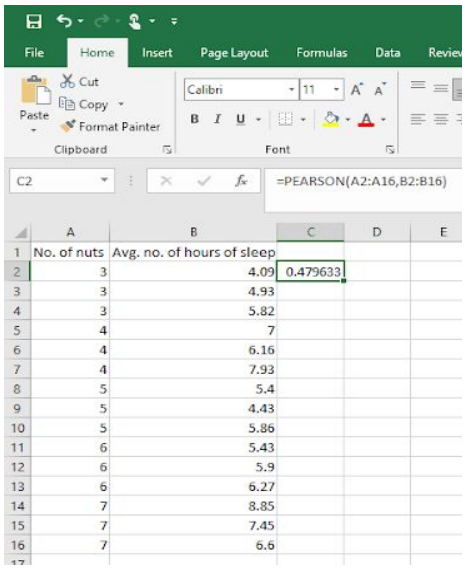
Section 3: Results and Data Presentation

3.1: Pearson's Test results

Figure 3. Formula for Pearson's correlation coefficient:

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[\, n\sum x^2 - (\sum x)^2 \,][\, n\sum y^2 - (\sum y)^2 \,]}}$$

Figure 4. Screenshot of sample Pearson's correlation calculation for female *Prunus dulcis* group



	A	B	C	D	E
1	No. of nuts	Avg. no. of hours of sleep			
2	3	4.09	0.479633		
3	3	4.93			
4	3	5.82			
5	4	7			
6	4	6.16			
7	4	7.93			
8	5	5.4			
9	5	4.43			
10	5	5.86			
11	6	5.43			
12	6	5.9			
13	6	6.27			
14	7	8.85			
15	7	7.45			
16	7	6.6			

Table 8: *r* values (X- no. of nuts eaten, Y- average no. of hours slept in each dry fruit group)

	Females	Males
Prunus dulcis	0.48	0.69
Pistacia vera	0.29	0.61
Juglans regia	-0.60	0.03

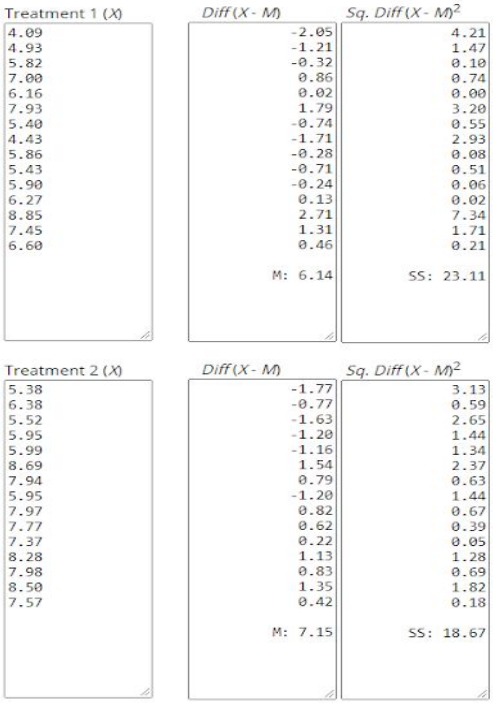
3.2: t-test results

Figure 5. Formula for t-test:

$$t = \frac{\overline{x}_1 - \overline{x}_2}{\sqrt{\left(s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)\right)}}$$

3.2.1: t-test Results for *Prunus dulcis* (almonds) group

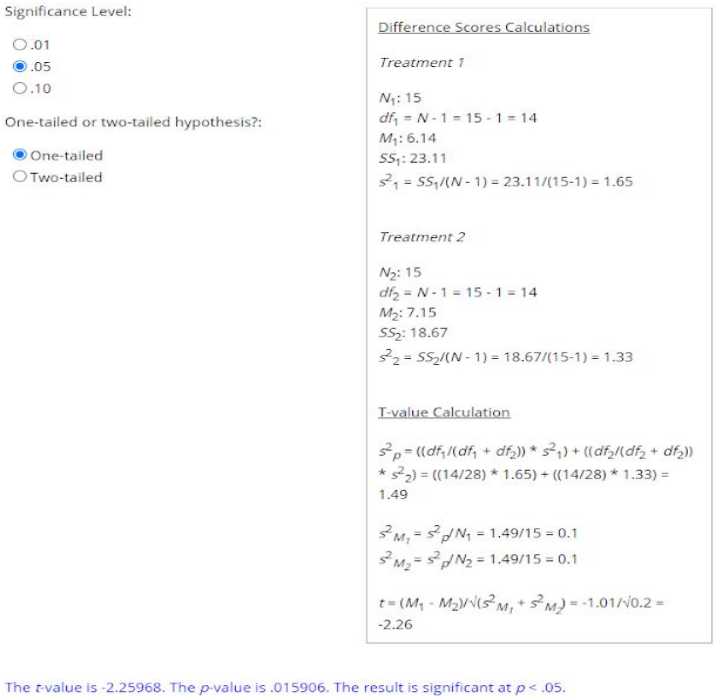
Figure 6. Screenshot of t-test calculator showing t-test statistics (Treatment 1: Females, Treatment 2: Males).



Treatment 1 (X)	Diff (X - M)	Sq. Diff (X - M)^2
4.09	-2.05	4.21
4.93	-1.21	1.47
5.82	-0.32	0.10
7.00	0.86	0.74
6.16	0.02	0.00
7.93	1.79	3.20
5.40	-0.74	0.55
4.43	-1.71	2.93
5.86	-0.28	0.08
5.43	-0.71	0.51
5.90	-0.24	0.06
6.27	0.13	0.02
8.85	2.71	7.34
7.45	1.31	1.71
6.60	0.46	0.21
	M: 6.14	SS: 23.11

Treatment 2 (X)	Diff (X - M)	Sq. Diff (X - M)^2
5.38	-1.77	3.13
6.38	-0.77	0.59
5.52	-1.63	2.65
5.95	-1.20	1.44
5.99	-1.16	1.34
8.69	1.54	2.37
7.94	0.79	0.63
5.95	-1.20	1.44
7.97	0.82	0.67
7.77	0.62	0.39
7.37	0.22	0.05
8.28	1.13	1.28
7.98	0.83	0.69
8.50	1.35	1.82
7.57	0.42	0.18
	M: 7.15	SS: 18.67

Figure 7. Screenshot of t-test calculator showing t-test statistics, results and significance level.



Significance Level:

○ .01
◉ .05
○ .10

One-tailed or two-tailed hypothesis?:

◉ One-tailed
○ Two-tailed

Difference Scores Calculations

Treatment 1

N_1: 15
$df_1 = N - 1 = 15 - 1 = 14$
M_1: 6.14
SS_1: 23.11
$s^2_1 = SS_1/(N - 1) = 23.11/(15-1) = 1.65$

Treatment 2

N_2: 15
$df_2 = N - 1 = 15 - 1 = 14$
M_2: 7.15
SS_2: 18.67
$s^2_2 = SS_2/(N - 1) = 18.67/(15-1) = 1.33$

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) = ((14/28) * 1.65) + ((14/28) * 1.33) = 1.49$

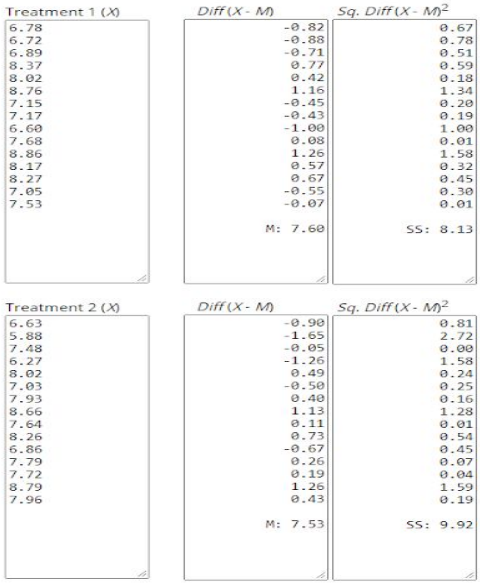
$s^2_{M_1} = s^2_p/N_1 = 1.49/15 = 0.1$
$s^2_{M_2} = s^2_p/N_2 = 1.49/15 = 0.1$

$t = (M_1 - M_2)/\sqrt{(s^2_{M_1} + s^2_{M_2})} = -1.01/\sqrt{0.2} = -2.26$

The *t*-value is -2.25968. The *p*-value is .015906. The result is significant at $p < .05$.

3.2.2: t-test Results for *Pistacia vera* (pistachios) group

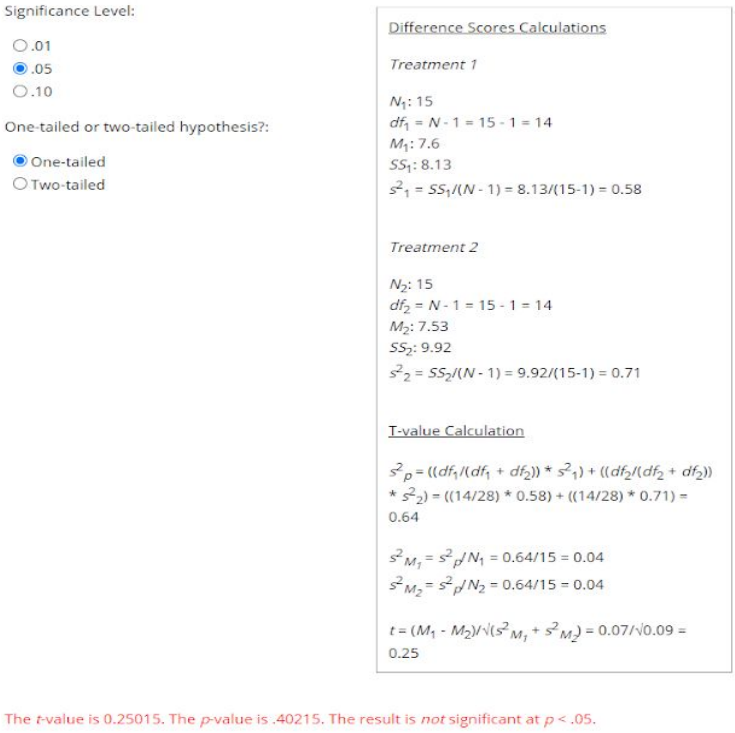
Figure 8. Screenshot of t-test calculator showing t-test statistics (Treatment 1: Females, Treatment 2: Males).



Treatment 1 (X)	Diff (X - M)	Sq. Diff (X - M)^2
6.78	-0.82	0.67
6.72	-0.88	0.78
6.89	-0.71	0.51
8.37	0.77	0.59
8.02	0.42	0.18
8.76	1.16	1.34
7.15	-0.45	0.20
7.17	-0.43	0.19
6.60	-1.00	1.00
7.68	0.08	0.01
8.86	1.26	1.58
8.17	0.57	0.32
8.27	0.67	0.45
7.05	-0.55	0.30
7.53	-0.07	0.01
	M: 7.60	SS: 8.13

Treatment 2 (X)	Diff (X - M)	Sq. Diff (X - M)^2
6.63	-0.90	0.81
5.88	-1.65	2.72
7.48	-0.05	0.00
6.27	-1.26	1.58
8.02	0.49	0.24
7.03	-0.50	0.25
7.93	0.40	0.16
8.66	1.13	1.28
7.64	0.11	0.01
8.26	0.73	0.54
6.86	-0.67	0.45
7.79	0.26	0.07
7.72	0.19	0.04
8.79	1.26	1.59
7.96	0.43	0.19
	M: 7.53	SS: 9.92

Figure 9. Screenshot of t-test calculator showing t-test statistics, results and significance level.



3.2.3: t-test Results for *Juglans regia* (walnuts) group

Figure 10. Screenshot of t-test calculator showing t-test statistics (Treatment 1: Females, Treatment 2: Males).

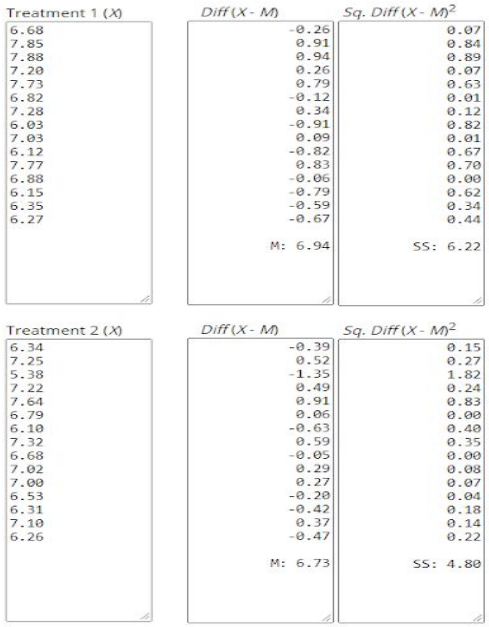


Figure 11. Screenshot of t-test calculator showing t-test statistics, results and significance level.

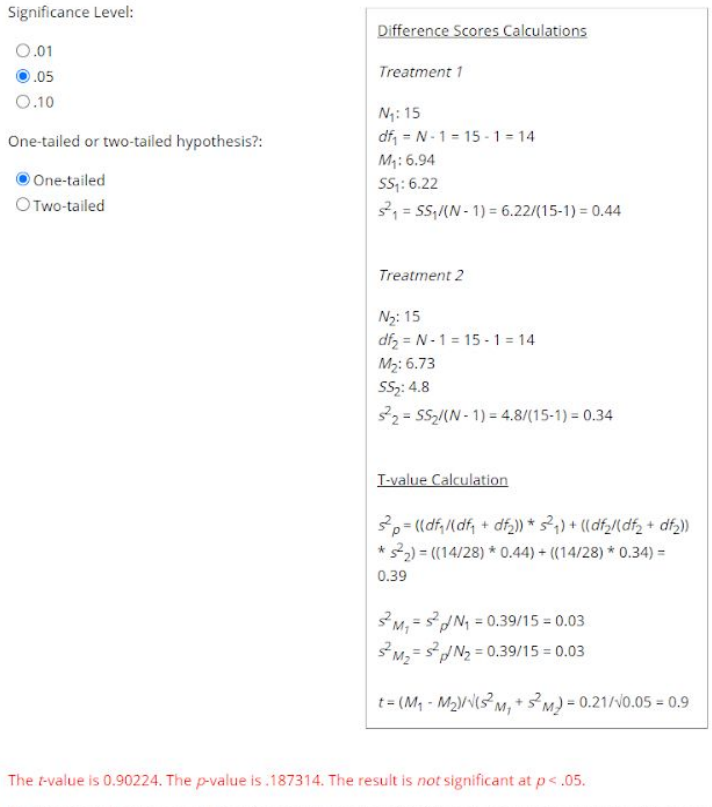


Table 9: T-test statistics as observed from the T-test Calculator

Key
F-Female
M-Male

	Average		Standard Deviation		Variance		N (sample size)		Degrees of Freedom	Critical t-value
Group	F	M	F	M	F	M	F	M		
Prunus dulcis	6.14	7.15	1.28	1.15	1.65	1.33	15	15	28	2.048
Pistacia vera	7.60	7.53	0.76	0.84	0.58	0.71	15	15	28	2.048
Juglans regia	6.94	6.73	0.67	0.59	0.44	0.34	15	15	28	2.048

Table 10: t-test results

Nut	t-value	Which hypothesis was accepted?	p-value
Prunus dulcis	-2.25968	Alternative	0.015906
Pistacia vera	0.25015	Null	0.40215
Juglans regia	0.90224	Null	0.187314

3.3: Data Presentation

Figure 12. Line graph comparing average number of hours slept by male and female participants in *Prunus dulcis* groups

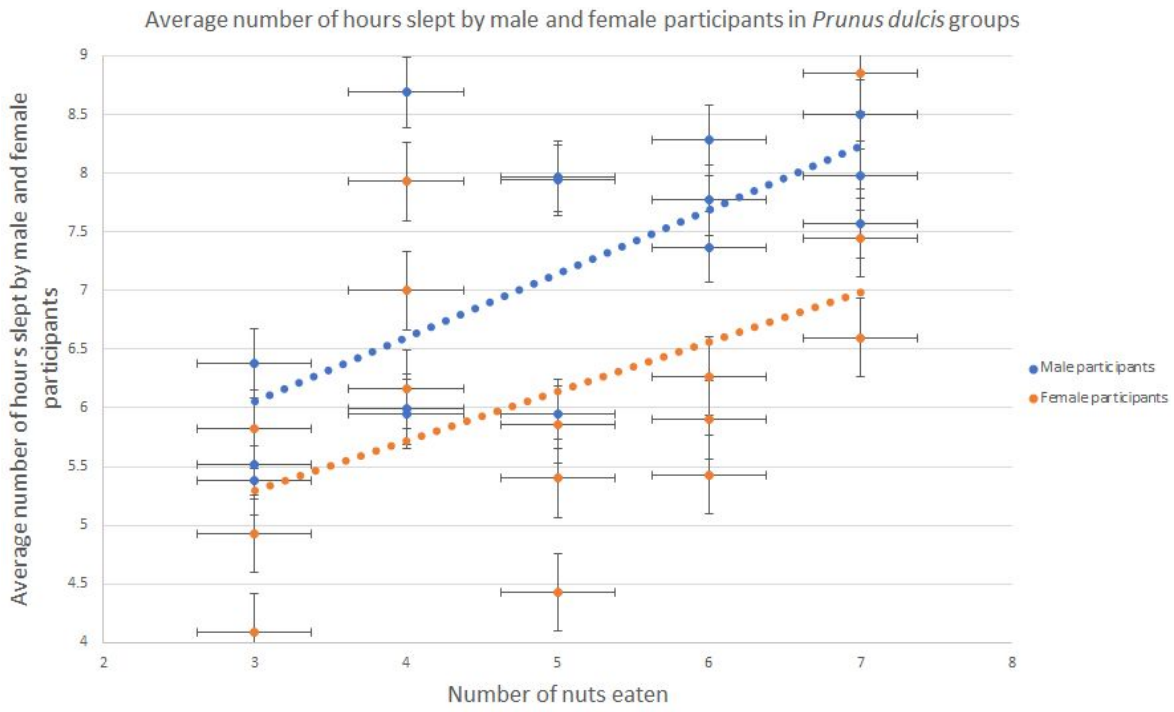


Figure 13. Line graph comparing average number of hours slept by male and female participants in *Juglans regia* groups

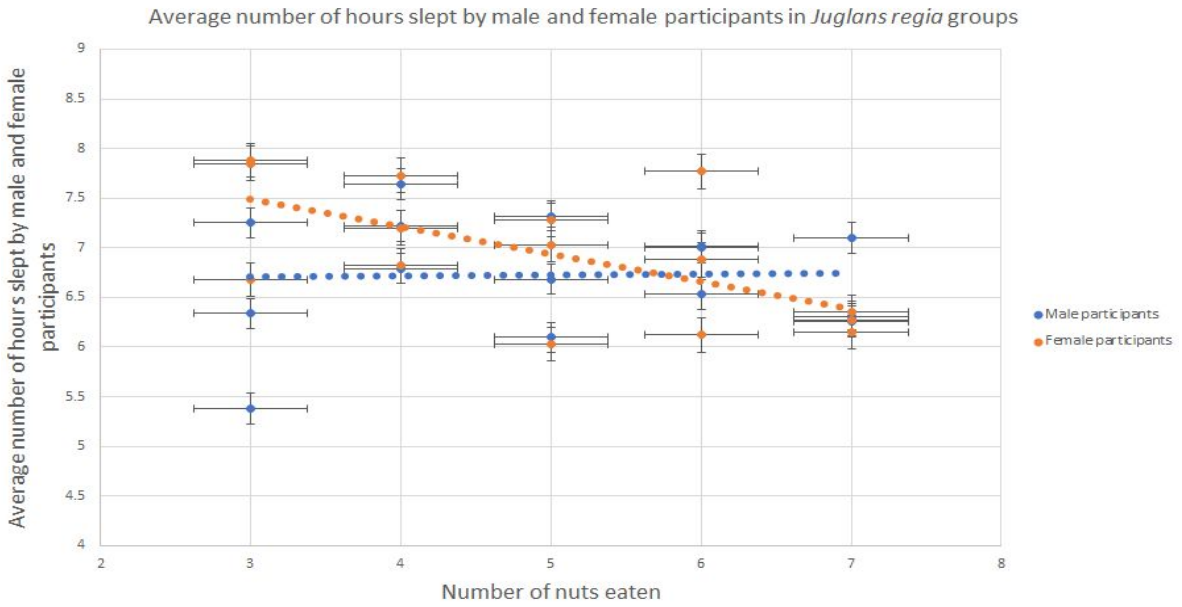


Figure 14. Line graph comparing average number of hours slept by male and female participants in *Pistacia vera* groups

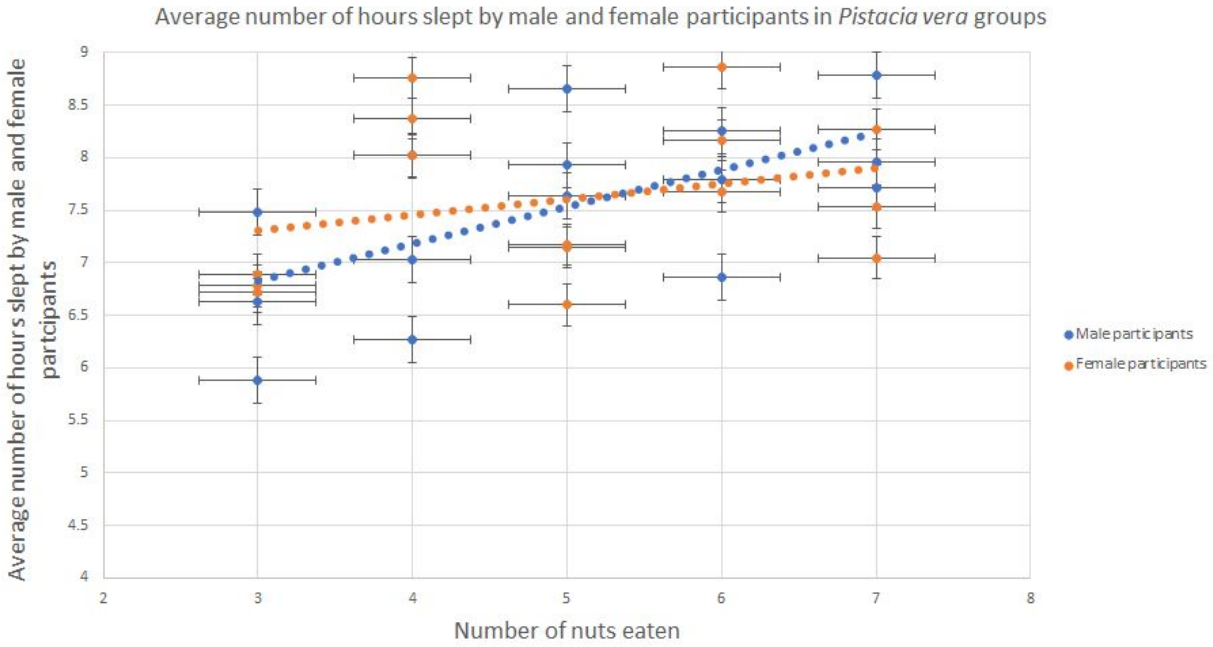


Figure 15. Line graph showing the average number of hours of sleep under the number of the three different types (*Prunus dulcis*, *Pistacia vera* and *Juglans regia*) of nuts eaten by female participants

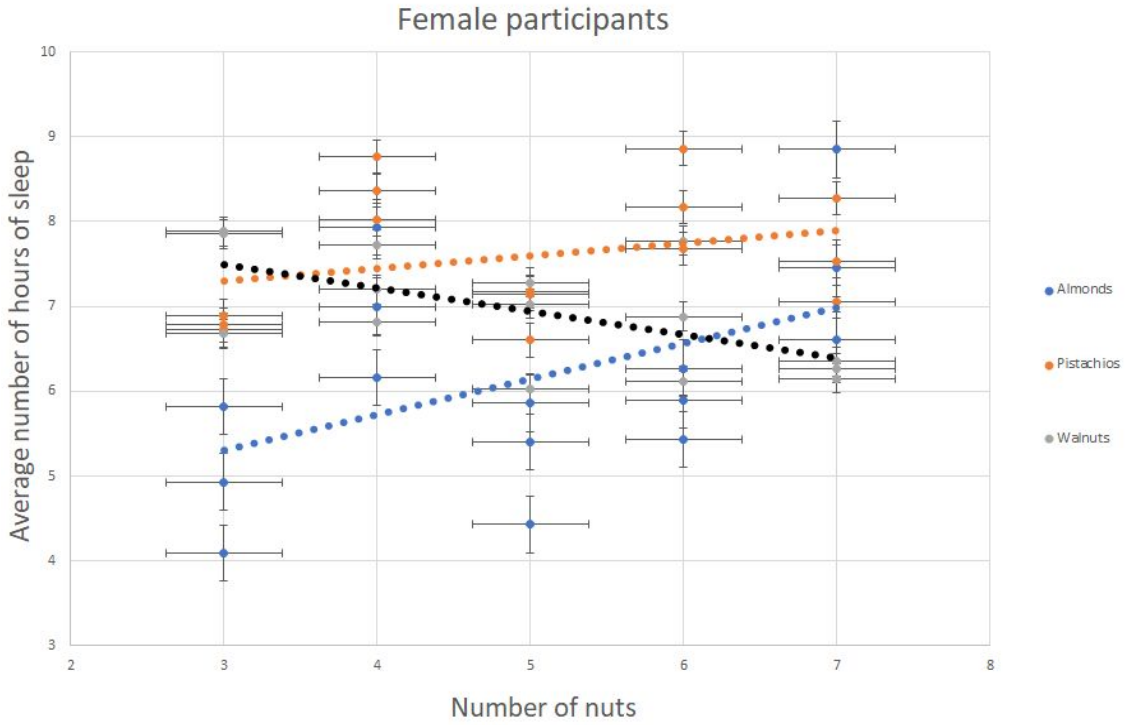


Figure 16. Line graph showing the average number of hours of sleep under the number of the three different types (*Prunus dulcis*, *Pistacia vera* and *Juglans regia*) of nuts eaten by male participants

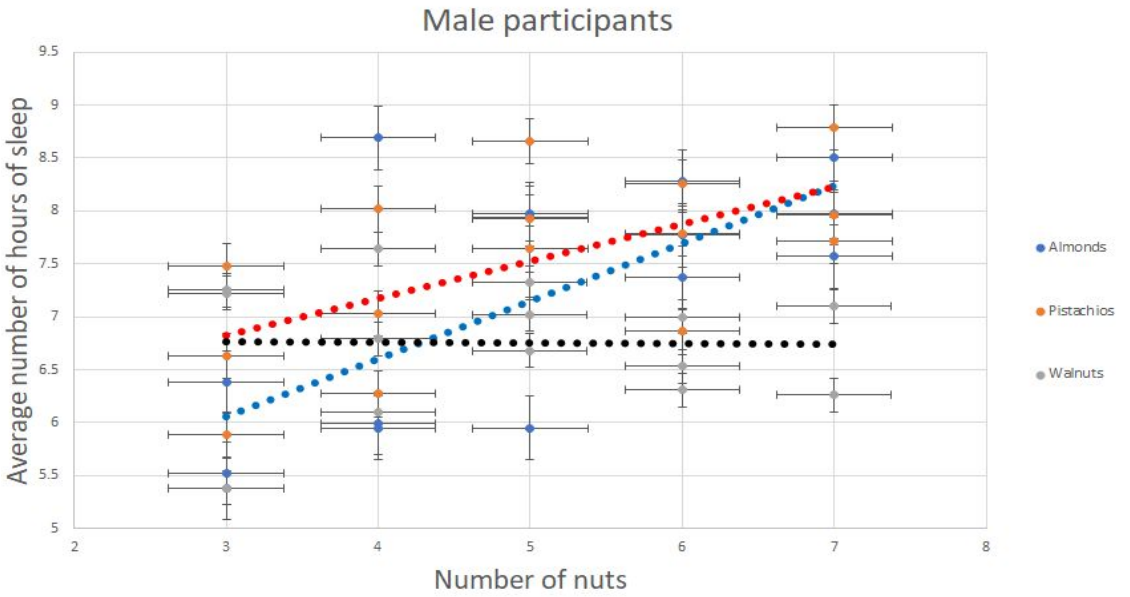
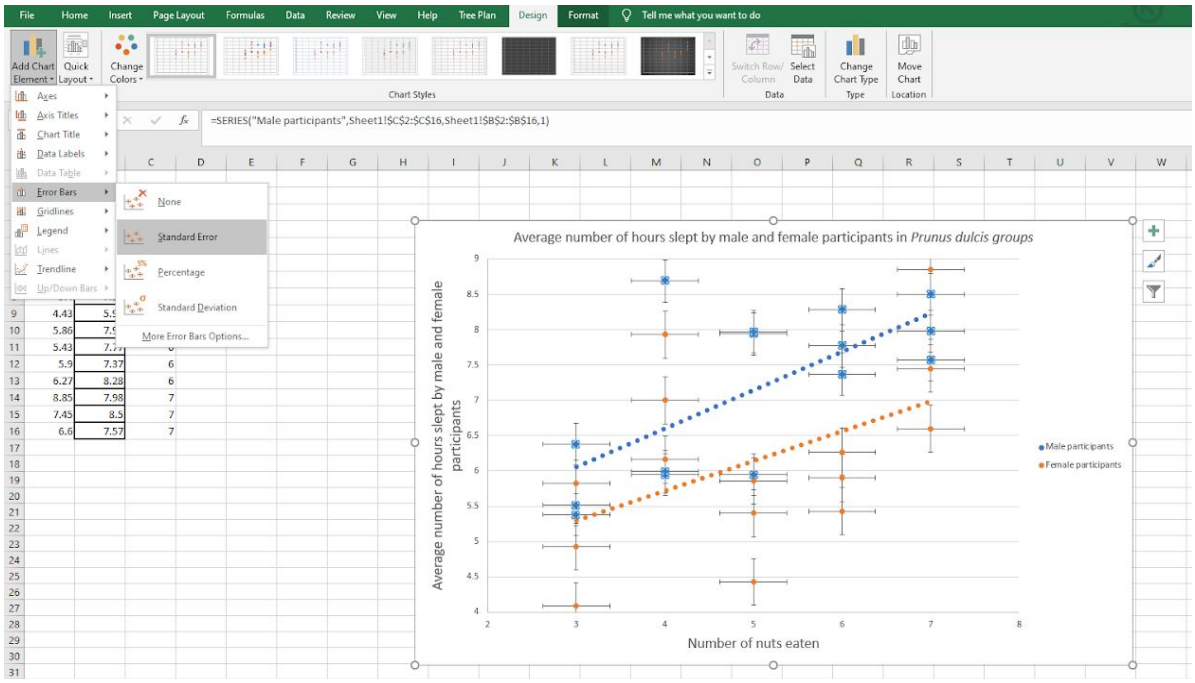


Figure 17. Screenshot of Excel spreadsheet showing the steps taken to display error bars according to standard error as shown in Figures 12, 13, 14, 15 and 16



Section 4: Discussion

4.1: Analysis

From Figure 12, we can observe that most blue data points (male participants) fall above an average of 6.5 hours while most orange data points (female participants) fall below 6.5 hours. One of the exceptions was the average number of hours slept when four nuts were eaten, wherein two of the three orange data points were above 6.5 hours. Unexpectedly, this means that two out of the three days that four nuts were eaten, the average number of hours slept by the female participants was relatively higher than the days that five and six nuts were eaten.

The correlation coefficient value *r* will be higher only if the average number of hours of sleep are less when the quantity of nuts is low. As evident by Table 8, the quantity of *Pistacia vera* has a lower correlation (0.29 and 0.61) with the average number of hours slept as compared to *Prunus dulcis* (0.48 and 0.69) in both female and male participants, respectively. This may be due to the fact that the average number of hours

of sleep induced by *Pistacia vera* was adequate even when the number of nuts ingested were less. For example, from Figure 14, the average number of hours slept ranged from nearly 5.9 to 7.5 hours when only three *Pistacia vera* nuts were eaten by participants. On the other hand, from Figure 12, the average number of hours slept ranged from 4 to nearly 6.5 hours when only three *Prunus dulcis* nuts were eaten by participants. This suggests that the effect of *Pistacia vera* on sleep duration may be less dependent on the quantity of intake as compared to *Prunus dulcis*.

As can be seen from Table 8, the *r* values were higher in the male groups (0.69, 0.61 and 0.03) than female groups (0.48, 0.29 and -0.60) in *Prunus dulcis*, *Pistacia vera* and *Juglans regia*, respectively. An anomaly in these results was the only negative correlation in the female-*Juglans regia* group (-0.60). This allowed me to interpret that *Juglans regia* had absolutely no effect on the sleep cycle of women but they did have some albeit minimal effect on men, reinforcing my prediction in hypothesis B i.e. nuts would induce more sleep in males than females. Since *Juglans regia* has the highest fat composition and the lowest tryptophan content out of the three nuts, the effect of melatonin may have become masked by the energy content.

The results showed that the quantity of *Prunus dulcis* had the highest correlation with average number of hours of sleep in both genders. I had initially predicted that *Pistacia vera* would give the highest *r* values in both genders, but *Prunus dulcis* significantly overtook *Pistacia vera*. These results can be loosely supported by a study conducted by students of Tehran University of Medical Sciences in 2019[14]. It investigated the effect of sweet almonds on the quality of sleep. The results showed that consuming the dry fruit for two weeks reduced insomnia at $P<0.05$.

An explanation for these results is that *Prunus dulcis* had the highest tryptophan levels. A higher amount of tryptophan would have allowed larger amounts of melatonin to build. Moreover, *Prunus dulcis* has a higher content of magnesium and calcium than *Pistacia vera*. These minerals enable muscle relaxation, which promotes sleep. Calcium is required to break the link between the muscle filaments actin and myosin when it is pumped back into the sarcoplasmic reticulum in sarcomeres, the contractile unit of muscle fibres.

[14] Ghafarzadeh J;Sadeghniiat-Haghighi K;Sadeghpour O;Akbarpour S;Amini-Behbahani F; “Investigating the Prevalence of Sleep Disorder and the Impact of Sweet Almond on the Quality of Sleep in Students of Tehran, Iran.” *Iranian Journal of Public Health*, U.S. National Library of Medicine, June 2019, pubmed.ncbi.nlm.nih.gov/31341858/, Accessed Oct. 1 2020.

Table 11: Mineral content of *Prunus dulcis* and *Pistacia vera*

	Prunus dulcis	*Pistacia vera*
Magnesium (mg per 1 oz)	76.0	34.3
Calcium (mg per 1 oz)[15]	75.0	29.8

With reference to Table 10, the t-test value (-2.26) which compared the average sleeping hours of males and females that ate *Prunus dulcis* had surpassed the critical value (2.048), so the alternative hypothesis was accepted. However, the t-test values that compared the average sleeping hours between males and females that ate *Juglans regia* (0.902) and *Pistacia vera* (0.250) did not surpass the critical value (2.048). Several perspectives can be taken into account. Firstly, it is proven that *Prunus dulcis* have the highest correlation with sleep in both genders, thus only the t-value calculated for the *Prunus dulcis* groups should be considered. On the other hand, the results of two out of three of the dry fruit t-tests showed acceptance of the null hypothesis. Therefore, it can be argued that the majority should be sided with. However, I sided with the former argument since it was supported by a study[16] that proved that males have longer non-REM sleep stage 1 sleep than females. Hence, the difference between average male and female sleeping hours is statistically significant.

From Table 7, we can observe that the average standard deviation is lower in females (0.66, 0.34 and 0.44) in *Prunus dulcis*, *Pistacia vera* and *Juglans regia* respectively as compared to males (0.68, 0.57 and 0.46). This implies that males have more trouble maintaining a consistent sleep routine as compared to females. Obstructive sleep apnea (OSA) is a condition that's symptoms include excessive daytime sleepiness. This may result in taking a larger number of naps, which can cause inconsistencies in nighttime sleep. A study[17] shows that OSA occurs in males more than it does in females.

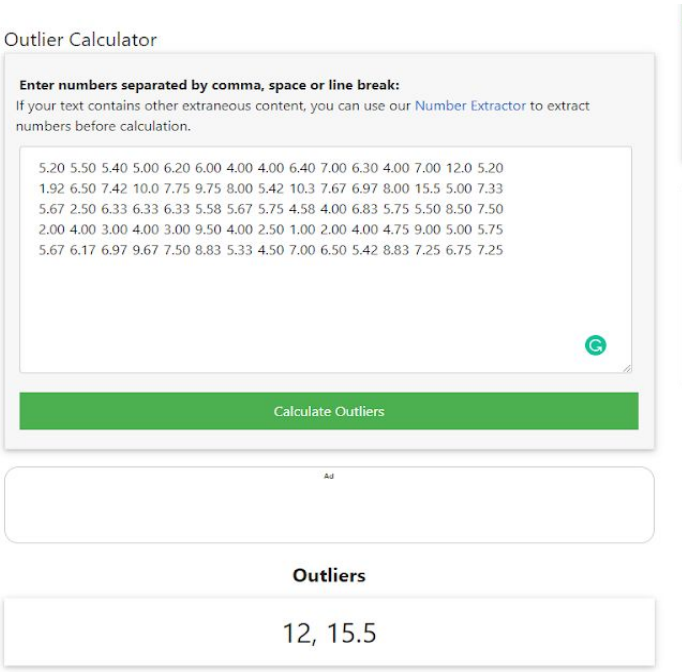
[15] Sources include: USDA

[16] Mallampalli, Monica P, and Christine L Carter. "Exploring Sex and Gender Differences in Sleep Health: a Society for Women's Health Research Report." *Journal of Women's Health (2002)*, Mary Ann Liebert, Inc., 1 July 2014, www.ncbi.nlm.nih.gov/pmc/articles/PMC4089020/, Accessed Sept. 15 2020

[17] V;, Mohsenin. "Effects of Gender on Upper Airway Collapsibility and Severity of Obstructive Sleep Apnea." *Sleep Medicine*, U.S. National Library of Medicine, Nov. 2003, pubmed.ncbi.nlm.nih.gov/14607346/, Accessed Dec. 3 2020.

Outliers were calculated using values from raw data. From the females-*Prunus dulcis* group, the outliers were 12 hours slept by Participant 1 on day 14 and 15.5 hours slept by Participant 2 on day 13. From the females-*Juglans regia* group, the outliers were 3 hours slept by Participant 4 and Participant 5 on day 13 and day 10 respectively, 3.5 hours slept by Participant 4 on day 14 and 10.8 hours slept by Participant 5 on day 6. From the male-*Pistacia vera* group, the outliers were 12 hours slept by Participant 1 on day 14 and 12.3 hours slept by Participant 5 on day 8. The data from the remaining three groups presented no outliers.

Figure 18. Screenshot of outlier calculator



4.2: Evaluation

Participant limitations

- ➢ In this experiment, no measuring instruments were used, hence there were no instrumental uncertainties. However, there can be methodological uncertainties in terms of the number of nuts eaten by participants and the questionable accuracy of their recorded sleeping and waking times in the Data Collection Forms. These uncertainties were discussed in the form of outliers in the Analysis.

- ➢ Participants may have forgotten to avoid the foods mentioned on the list, which could have influenced results.

- ➢ Some participants may not have been entirely truthful. For example, Participant 2 in the female-*Juglans regia* group filled six and a half hours everyday throughout an entire week of the experiment. This is not very probable, thus it may have resulted in a loss of accuracy in results.

- ➢ Participant 1 in the female-*Prunus dulcis* group was on a different clock because she was attending online classes set to the time zone of Canada. This may have influenced her results.

- ➢ In the middle of the experiment, Participant 5 in the male-*Pistacia vera* group had informed me that he was travelling abroad due to a family emergency. This may have resulted in jet lag.

Strengths

1. I supported my results with studies acquired from NCBI and PubMed, reliable databases that are a part of the National Institutes of Health (NIH). NIH is financially supported by the United States government.
2. I gathered five participants for each group, allowing me to have five repeats of the independent variable thus improving the accuracy of my results.
3. None of the statistical tests were calculated manually. Reliable softwares and websites were used to calculate and analyze the results, thus improving the validity of the study.

Methodological Limitations and Modifications

Limitation	**Modification**
There weren't enough controlled variables.	Controlling of extraneous variables such as naps, exercise and higher control over diet, such as the assigning of breakfast, lunch and dinner foods.
There were not enough variations of the independent variable.	A modification could be investigating other nuts with high amounts of melatonin, such as cashews or investigation of other foods such as vegetables and fruits.
The number of nuts might have been too less.	Increasing the number of nuts to either 8 or 9. The numbers of 3-7 might not have been enough to contribute to the participants' sleep routines significantly.
The experiment was too short.	The experiment could have been conducted over a longer period of time, perhaps a month.
The method of data collection might have been redundant.	Instead of a form, the use of a sleep tracker app or some form of technology that would remind them to consistently fill their forms and eat their dry fruits might have increased the efficiency of their participation and saved time, as it was a requirement to send them daily reminders to eat their dry fruits and fill out their forms.

4.3: Conclusion

As per my research question and Hypothesis A, there is a positive correlation (with the exception of female-*Juglans regia* group, which showed a negative correlation) between the ingestion of increasing quantity of dry fruits and average sleeping hours in males and females dry fruit groups. However, my research findings show that *Prunus dulcis* give the highest *r* values in both males and females, unlike my prediction of *Pistacia vera*.

As per my research question and Hypothesis B, there is a statistically significant difference between males and females sleeping hours of the *Prunus dulcis* groups. However, there seems to be no statistically significant difference between male and female sleeping hours in the other two groups (*Pistacia vera* and *Juglans regia*). My prediction that the nuts will have a higher influence on male sleeping patterns as compared to females stands true, as the male groups gave higher *r* values.

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Appendices

Raw Data Tables (FEMALE PARTICIPANTS)

Raw Data Table 1: No. of hours slept by participants in *Prunus dulcis* group

	Day of the experiment														
Participant No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	5.20	5.50	5.40	5.00	6.20	6.00	4.00	4.00	6.40	7.00	6.30	4.00	7.00	12.0	5.20
2	1.92	6.50	7.42	10.0	7.75	9.75	8.00	5.42	10.3	7.67	6.97	8.00	15.5	5.00	7.33
3	5.67	2.50	6.33	6.33	6.33	5.58	5.67	5.75	4.58	4.00	6.83	5.75	5.50	8.50	7.50
4	2.00	4.00	3.00	4.00	3.00	5.50	4.00	2.50	1.00	2.00	4.00	4.75	9.00	5.00	5.75
5	5.67	6.17	6.97	9.67	7.50	8.83	5.33	4.50	7.00	6.50	5.42	8.83	7.25	6.75	7.25
Average	4.09	4.93	5.82	7.00	6.16	7.93	5.40	4.43	5.86	5.43	5.90	6.27	8.85	7.45	6.60

Raw Data Table 2: No. of hours slept by participants in *Pistacia vera* group

	Day of the experiment														
Participant No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	8.75	9.00	8.58	8.33	9.25	9.83	8.17	8.58	8.00	7.67	7.75	7.67	7.50	7.17	7.50
2	5.33	5.33	7.12	7.67	8.00	7.33	4.83	5.50	5.50	9.00	8.67	6.67	6.50	5.83	5.83
3	5.97	3.25	4.33	9.75	6.50	9.00	4.42	6.97	7.50	4.17	9.83	8.33	5.75	6.50	7.00
4	8.42	9.92	8.42	7.00	9.00	11.3	9.97	10.2	8.00	9.33	10.4	10.7	10.3	9.25	11.0
5	5.42	6.08	6.00	9.08	7.33	6.33	8.33	4.58	4.00	8.25	7.67	7.50	11.3	6.50	6.33
Average	6.78	6.72	6.89	8.37	8.02	8.76	7.15	7.17	6.60	7.68	8.86	8.17	8.27	7.05	7.53

Raw Data Table 3: No. of hours slept by participants in *Juglans regia* group

	Day of the experiment														
Participant No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	4.83	9.83	8.83	7.67	6.83	8.50	9.50	5.58	6.83	6.33	8.50	7.75	7.08	8.00	6.83
2	8.50	9.00	9.00	9.00	6.00	6.50	9.00	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50
3	5.75	7.25	7.00	7.33	7.50	7.25	6.75	7.58	7.00	8.00	7.83	7.97	7.33	6.75	6.50
4	6.50	6.00	9.00	6.25	7.50	6.00	5.67	5.50	7.50	6.75	6.50	4.00	3.00	3.50	4.50
5	7.83	7.17	5.58	5.75	10.8	5.83	5.50	5.00	7.33	3.00	9.50	8.17	6.83	7.00	7.00
Average	6.68	7.85	7.88	7.20	7.73	6.82	7.28	6.03	7.03	6.12	7.77	6.88	6.15	6.35	6.27

MALE PARTICIPANTS

Raw Data Table 4: No. of hours slept by participants in *Prunus dulcis* group

	Day of the Experiment														
Participant No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	6.20	5.40	6.75	3.00	7.67	7.20	7.97	4.20	6.50	6.67	5.42	7.33	8.67	8.40	6.42
2	5.75	6.67	5.50	4.00	3.50	12.0	7.20	8.67	6.33	10.4	8.50	9.20	9.42	4.97	11.0
3	4.97	5.75	4.67	6.42	3.67	9.50	7.50	4.50	10.6	6.20	5.67	9.00	7.42	10.3	7.67
4	7.33	8.67	6.50	8.75	9.42	6.33	10.7	7.42	8.58	6.25	9.58	8.67	8.42	9.00	6.20
5	2.67	5.42	4.20	7.58	5.67	8.40	6.33	4.97	7.83	9.33	7.67	7.20	5.97	9.83	6.58
Average	5.38	6.38	5.52	5.95	5.99	8.69	7.94	5.95	7.97	7.77	7.37	8.28	7.98	8.50	7.57

Raw Data Table 5: No. of hours slept by participants in *Juglans regia* group

	Day of the Experiment														
Participant No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	5.25	6.67	4.33	7.42	5.83	3.50	4.97	8.58	6.75	5.67	9.97	6.50	2.97	8.33	6.83
2	7.33	8.58	7.00	6.20	9.50	4.67	5.75	6.25	5.83	7.25	5.42	4.58	8.67	6.00	5.75
3	6.20	8.50	5.67	7.25	8.50	8.97	6.83	7.75	5.50	4.00	6.67	7.33	9.67	6.83	4.67
4	7.58	4.83	5.50	8.75	3.97	9.00	7.75	4.33	4.33	10.5	6.25	8.67	5.42	7.75	6.83
5	5.33	7.67	4.42	6.50	10.4	7.83	5.20	9.97	11.0	7.67	6.67	5.58	4.83	6.58	7.20
Average	6.34	7.25	5.38	7.22	7.64	6.79	6.10	7.32	6.68	7.02	7.00	6.53	6.31	7.10	6.26

Raw Data Table 6: No. of hours slept by participants in *Pistacia vera* group

	Day of the Experiment														
Participant No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	8.20	7.33	8.58	6.97	9.50	7.67	10.6	8.33	8.00	6.67	7.42	9.50	6.25	12.0	9.83
2	6.42	6.00	7.50	5.20	8.67	6.33	9.42	6.58	8.83	11.3	7.67	7.50	8.00	9.20	7.58
3	5.33	4.67	6.42	7.33	5.50	8.33	4.50	8.67	6.97	7.42	5.20	8.00	7.50	6.20	8.50
4	7.25	7.00	8.42	6.58	7.67	5.83	9.50	7.42	5.97	6.33	8.50	7.00	8.50	9.20	7.20
5	5.97	4.42	6.50	5.25	8.75	7.00	5.67	12.3	8.42	9.58	5.50	6.97	8.33	7.33	6.67
Average	6.63	5.88	7.48	6.27	8.02	7.03	7.93	8.66	7.64	8.26	6.86	7.79	7.72	8.79	7.96

Informed Consent Form

Study Title: Effect of foods on sleep

Researcher: Rakshita Rupani

Questions you may have:

What is the purpose of this study?
You are being asked to participate in this study so that the researcher can investigate the effects of particular foods on sleep quality and habits.

What is the eligibility criteria for this study?
Participants must be between 15-18 years old, and should not be suffering from any health conditions.

What do I have to do?
You will be asked to consume a particular dry fruit over the course of fifteen days, and to keep a record of the number of hours of sleep you receive each day during the study. You may be asked to eat either walnuts, almonds or pistachios. The table below gives instructions on how many nuts you will have to ingest for each of the days of the experiment. Please read them carefully.

Days of the experiment	Number of nuts to be consumed during those days
1-3	3
4-6	4
7-9	5
10-12	6
13-15	7

You will be asked to avoid the following foods five hours before your bedtime to produce fair results:

1. Tomatoes

2. Processed meats
3. Carbonated drinks
4. Coffee
5. Fried food
6. Warm milk
7. Bananas
8. Herbal teas, such as chamomile
9. Oats
10. Eggs

Will the researcher buy the dry fruits for the experiment?
No, you will have to acquire them on your own in light of the pandemic.

How will I keep track of my sleeping habits?
The third page of this document contains a table that you will have to fill on a daily basis.

How can I contact the researcher for queries?
Email- rakshitarupani@gmail.com
Phone number- 9667225702
Instagram handle- @rakshita14

There are no anticipated risks or benefits to your participation in this study. Your participation is voluntary, and you may decide to discuss your participation with family or friends. You may keep a soft copy of this form if you wish to do so. The information you provide will remain confidential and will not be shared with anyone outside the study.

I, (type your name here), give my consent to participate in this research study. I will abide by the rules the researcher has put forth for all fifteen days to maintain the integrity of the study.

Signature of participant

Data Collection Form Exemplar
(Filled by Participant 3 in female-Almonds group)

Day of the experiment	Time of waking, in the form (00:00)	Time of sleeping, in the form (00:00)
1	7:40 AM	2:00 AM
2	7:40 AM	3:30 AM
3	6:00 AM	2:00 AM
4	8:20 AM	1:00 AM
5	7:20 AM	1:00 AM
6	7:20 AM	1:25 AM
7	7:00 AM	2:00 AM
8	7:40 AM	2:15 AM
9	8:00 AM	3:30 AM
10	8:02 AM	4:00 AM
11	8:00 AM	1:00 AM
12	7:48 AM	2:00 AM
13	7:45 AM	3:00 AM
14	8:30 AM	11:30 AM
15	8:00 AM	10:50 PM

Note: Ensure that your input is as accurate to your real sleeping and waking times as possible.