**Activity: Observational Studies versus Experiments**

**“Does the amount of caffeine you consume affect the amount of sleep you get?”**

**TASK 1**

We want to explore the relationship between the amount of caffeine each of you consumes and the amount of sleep you each get. Specifically, does more caffeine lead to less sleep?

To do this, we will obtain estimates of the amount of caffeine each of you consumed yesterday and compare it to your total hours of sleep last night.

To speed things up, here are some facts off the internet (<http://www.nutritionfoundation.org.nz/nutrition-facts/nutrition-a-z/Caffeine>)

to help you estimate how much caffeine you consumed yesterday:

1 cup (250ml) flat white or cappuccino or brewed coffee (single shot of coffee): 80mg

1 cup (250ml) of instant coffee (1teaspoon): 60mg

1 cup (250ml) tea : 55mg

1 can of Cola = 38mg

250ml of an energy drink e,g V, Red Bull = 80mg

50g milk chocolate bar (10 squares) = 2 teaspoons of unsweetened cocoa powder: 10mg

50mg dark chocolate (10 squares) : 35mg

(Note: the caffeine content of drinking chocolate and lemonade type soft drinks is minimal)

My estimate = \_\_\_\_\_\_\_\_\_\_\_\_\_mg

How much **sleep** (in hours) did you get last night (measure to the nearest quarter-hour)?

My estimate: \_\_\_\_\_\_\_\_\_\_\_ hrs (e.g. 6hrs means enter 6.25hrs etc on the spreadsheet)

**Quickly estimate and enter your results in the google sheet I have set up using google classroom. Then, answer the following questions:**

1. Is this an observational study or an experiment? Why?

Observational: No researcher intervention

1. Is it retrospective or prospective study? Why?

Retrospective: data from yesterday

1. How were the participants selected?

Volunteered (by teacher)

1. What is the **explanatory** variable?: Caffeine intake

and the **response** variable?:Hours of sleep

1. How should we display the results?: scatter plot

**We will now graph our class results using NZ Grapher**

1. From our study, does it appear that there is a relationship between caffeine consumption and sleep?

A weak, negative approx. linear association. The more caffeine consumed, a tendency for less sleep.

1. What is the scope of the conclusion from this type of study?

Non-Causal; We can only suggest an association between caffeine intake and amount of sleep

1. What are the limitations of this study? What improvements could we make?

A Very small study of a very narrow demographic (year 13 girls at WGHS)

Yesterday, Sunday, may not have been typical. More accurate estimates would be to average daily consumption and sleep over a week.

**To broaden the scope of our conclusion, we need to design an experiment.**

1. Design an experiment to test the effect of caffeine consumption on the amount of sleep a person gets.  *(You may ignore ethical concerns for this hypothetical experiment.-you may assume you have a group of willing volunteers who are aware of the potential harmful effects of caffeine and have agreed to participate.)* Think, instead, of the experimental design elements that will be needed in order to come to a meaningful conclusion.

Briefly describe how you would conduct the experiment and justify each of your decisions. (Bullet points with reasons are OK: **Work in a group with 2 or 3 other students**)

* The participants must be randomly allocated to a treatment (take caffeine) and control groups (no caffeine)-This is so that other factors affecting hours and quality of sleep are evenly distributed through both groups e.g. gender, age, amount of exercise they do, the type of work they do and their hours of work, whether they are heavy or light caffeine consumers normally. The only difference between the treatment and control groups should be the treatment i.e the amount of caffeine
* The experimental conditions must be the same for both groups e.g. take caffeine /placebo equivalent at same time each day. Eat the same food. Go to bed at the same time. Have exactly the same routine/activities. Be in the same environment.-The only difference between the two groups should be the treatment (whether they consume caffeine or not)

This could be arranged in a sleeping Laboratory

* There must be double-blinding so that neither the participants nor the people taking any measurements or observations are aware of which group each participant is in. (If the participants know which group they are in it can subconsciously affect their response and if the observers know it can subconsciously affect their observations).
* The amount and quality of sleep should be measured accurately (not self-reported) by an electronic device eg a fit-bit or similar sensor.

1. What is the scope of the conclusion that can be drawn from a properly designed experiment? : There is the potential for a causal conclusion: that the treatment caused the observed response (if a statistically significant difference is observed).

Why ? : Provided the only difference between the two groups is the treatment and that other factors have been successfully controlled by the random allocation.

1. Is there a factor which even the above type of study cannot control? Yes: the Hawthorne effect. Knowing that they are part of an experiment can also subconsciously alter a participant’s response and the ethical concerns here would make it impossible for that to be disguised.

**TASK 2: Evaluation of an actual study report**

Read **Report A** and **Report B** below and then answer the questions.

**Report A** is an extract from the actual study report, written by the researchers,

on the effects of caffeine on sleep. The researchers (Drake, C. et al) are from the Henry Ford Hospital and Wayne State College of Medicine in Detroit, and Zeo Inc, *a company that works in the area of sleep and produces sleep monitoring devices.* **Report A** was published in the [peer-reviewed](http://www.nhs.uk/news/Pages/Newsglossary.aspx#Peerreview) Journal of Clinical Sleep Medicine: *Vol. 9, No. 11, 2013* (Note: *Although the study was funded by Zeo Inc, it does not make any recommendations about the use of sleep monitoring devices, so there is no apparent direct conflict of interest.)*

**Report B** is an extract from an on-line news report about the same study.

**Report A**

**Subjects**

The participants comprised 12 healthy normal sleepers, as determined by a physical examination and clinical interview by a sleep specialist. Any individuals with reported current or previous history of any psychiatric illness or current medical disorder were excluded from participation. Individuals currently using oral contraceptives, hypnotics, or any central nervous system acting medications were also excluded.

Habitual sleep data was determined by self-report with a 1-week sleep diary the week prior to participation. Only individuals with habitual total sleep times between 6.5 and 9 h, with a sleep onset < 30 min were included. Habitual caffeine consumption was based on self-report and was calculated from the question “How much caffeine do you consume in an average day, including coffee, pop [*soft drinks*], tea, chocolate, or energy drinks? Please specify type and amount.” Daily and weekly servings (1 serve =100 mg) of caffeine were then estimated based on type and amount indicated. The amount of caffeine in specific beverages/sources was determined based on information provided at the brand website and published literature. Subjects were selected if they met either of the following criteria: (1) ≥ 3 servings of caffeine in any single day or (2) ≥ 5 caffeinated servings per week. Subjects who consumed > 5 caffeinated beverages per day were excluded from participation. There were no inclusion or exclusion criteria as to time of caffeine consumption.

A total of 16 healthy day-workers met initial screening criteria but data was not used from 4 subjects due to their violation of the study protocol.

Individuals were recruited from the Detroit area through local advertisements and were compensated for their participation.

**Procedures**

The study was a randomized, double-blind, placebo-controlled design.

For the experimental period, participants were instructed to maintain their normal sleep schedules, including a bedtime between 21:00 and 01:00, wake times between 06:00 and 09:00, time in bed of 6.5-9 h, and no habitual napping.

One week prior to the experiment, each participant completed a baseline sleep diary.

Each subject completed 4 treatment-condition nights which consisted of 400 mg of caffeine taken in pill form at either 6, 3, or 0 hours prior to scheduled bedtime, with identical placebo pills given at each of the other times. Thus, subjects were instructed to take 3 pills each study day with one of the pills being caffeine and the other 2 placebo. On one of the days, all 3 pills were placebo. Each participant completed all 4 caffeine condition days (none, 6hrs before bed, 3hrs before and at bed-time) in random order. Each condition day was preceded by 1 washout day where subjects did not wear any sleep sensors and did not take any study pills. Thus, experimental nights occurred every other night during the protocol. However, sleep diary data was collected in the morning for all nights (experimental and washout).

Subjects were given the pills in an alarm activated pill-case. Participants were required to maintain a fixed bedtime and wake time schedule based on [their habitual] sleep diaries throughout the protocol. Subjects were also given a sleep diary to complete each morning throughout the study. The pill-case alarms were set according to the subjects’ habitual bedtime, and the alarm was designed to sound until the subject manually turned it off. Study drug intake was monitored by having subjects call in to a time-stamped answering machine to verify that the study drug was taken at each predetermined time period (6 h prior, 3 h prior, and at bedtime).

In order to avoid any potential caffeine withdrawal effects, subjects were allowed to use caffeine during the study. However, subjects were instructed to refrain from consuming any alcohol or caffeine after 16:00 on study days.

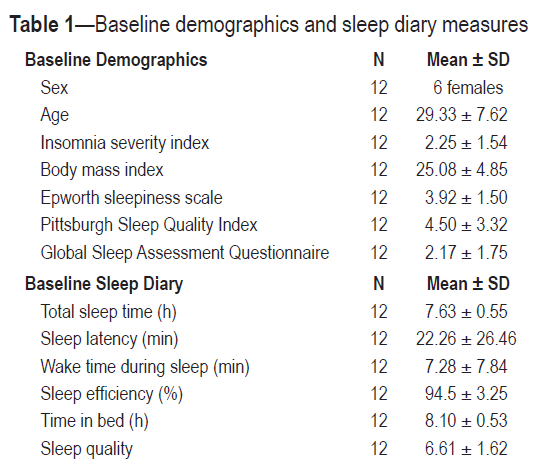
Adherence to a fixed bed-time and wake time schedule was likely to be dependent upon perceived sleep quality effects. Sleep disturbance (e.g. sleep quality, difficulty falling asleep) was therefore measured subjectively by means of the morning sleep diary and objectively using a widely available and previously validated in-home sleep monitor.

The monitor comprised a headband unit containing dry fabric sensors that wirelessly transmitted a single-channel EEG signal obtained from the forehead to a bedside device for processing. Subjects were instructed to put on the wireless system headband immediately upon going to bed with the intent to go to sleep and to keep the headband on all night long, placing it back on its bedside device upon rising from bed in the morning.

**Measures**

The head-band monitor provided objective measures of total sleep time (TST), latency to persistent sleep (*the time it took for the person to enter persistent sleep*) (LPS), wake time during sleep (WTDS) and sleep efficiency (*the number of minutes of sleep divided by the number of minutes in bed*) (SE). Although these were the primary measures of interest, the headband also enabled measures of combined stage 1 and 2 sleep, slow wave sleep, and REM sleep to be recorded.

**Results**

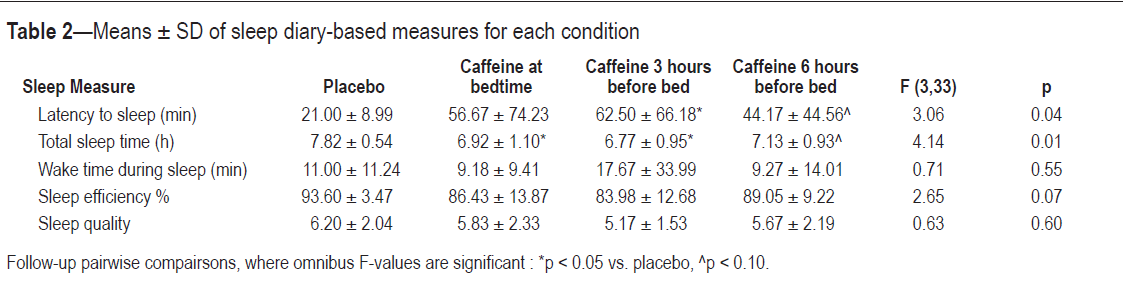


Data for the 7-day sleep diary taken during baseline as well as other self-report sleep-wake related measures are shown in **Table 1**.

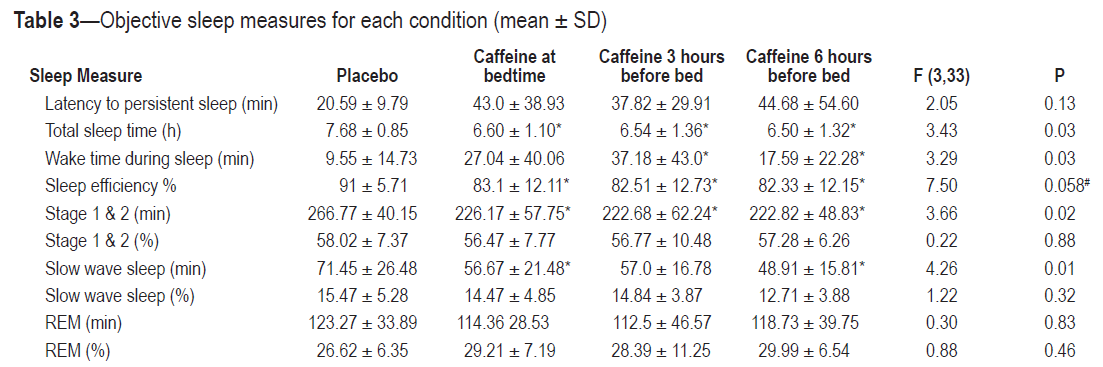
There were no differences in these parameters across the study conditions. There were no differences in non-study related caffeine intake between any of the 4 conditions.

Tables **2** and **3** below summarize, respectively, the **self-reported** and **objective** measures of sleep.

**Table 2:**The **self-reported** diary measures of sleep



**Table 3**: Means and standard deviations of **objective measures** of sleep (i.e., latency to sleep, total sleep time, and wake time during sleep)



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**Report B:** <http://www.nhs.uk/news/2013/11November/Pages/Even-afternoon-coffee-disrupts-sleep-study-finds.aspx>

Six men and six women (average age 29.3 years) completed the study correctly and their data was analysed. On average, these participants usually consumed 115mg caffeine a day. The amount of caffeine they consumed did not vary significantly on the different study days.

Based on the objective (headband) measurements, caffeine taken at bedtime, or three or six hours before bedtime significantly reduced total sleep time compared with placebo. The reduction in sleep time with caffeine was about an hour. Caffeine did affect some of the other objective sleep measures, but these differences were not always statistically significant. [*A result is statistically significant if it has a p value less than 0.05: shown by a \* in the results tables above*]

Compared with placebo, caffeine had the greatest effect on self-reported sleep if taken at bedtime or three hours before bedtime. Taken at these time points, caffeine:

* significantly reduced total sleep time
* significantly increased the time taken to fall asleep

When people took caffeine six hours before bedtime, they reported that:

* they slept on average 41 minutes less than with placebo
* they took about twice as long to fall asleep compared with placebo

However, these differences were not as large as those seen when caffeine was taken closer to bedtime, and were not large enough to be statistically significant. Caffeine did not affect self-reported waking time during the night, sleep quality or sleep efficiency.

**How did the researchers interpret the results?**

The researchers concluded that even if taken six hours before bedtime, a moderate dose of caffeine has important disruptive effects on sleep. They say that this supports recommendations to avoid substantial caffeine consumption for a minimum of six hours before going to bed.

**Limitations to the study:**

* The study was very small and included a very select group of participants. It analysed data from only 12 healthy young to middle-aged adults who took each of the timed test doses of caffeine on one night only. Larger studies in more mixed populations would be needed to confirm the findings and see if they apply to other groups.
* Not all of the self-reported and objective (headband) measurements of sleep completely agreed. For example, caffeine taken six hours before sleep only had a statistically significant effect on the objective measure of total sleep time, but not self-reported sleep time. The researchers suggest that this difference may be as a result of people having broken sleep, which they notice less than if they take longer to fall asleep. Larger studies where people undergo more extensive measurements in a sleep lab may help confirm the effects.
* The average caffeine consumption of participants was about 115mg per day. Other studies would be needed to see if the effect of the caffeine dose used in the study (400mg) differed in people who were used to consuming more or less caffeine.

**QUESTIONS**

1. Is the study an observational study or an experiment?

State the evidence from Report A which enabled you to determine this.

Experiment: It is a randomized, double-blind, placebo-controlled design.

1. Who were the participants and how were they selected?:

12 healthy normal sleepers. There were advertisements for volunteers in the local papers in the Detroit area of the USA. Volunteers were then screened to see if they were suitable. To be suitable, the participants had to usually sleep for between 6.5 and 9hours, fall asleep within 30 minutes, consume either at least 3 servings of caffeine in a single day or at least 5 servings over the course of a week. However people who had more than 5 servings of caffeine a day were screened out as were also people who had a history of psychiatric illness, had a current medical disorder or who took medications affecting the central nervous system eg oral contraceptives.

1. Report A describes the study as “double blind”. What does this mean and why was it important? Neither the participants nor the people observing the participants or taking measures know what group the participants have been allocated to. This is important because people who know what group they are in may behave differently (Hawthorne effect) and people taking measurements may be looking for specific effects and see what they expect rather an make an objective observation.
2. In this study, what are the (i) explanatory variable(s)?4 levels of treatment: 0 caffeine, or 400mg in pill form taken at bedtime, 3 hours before bed or 6 hours before bed.

(ii) the response variable(s)? Total sleep time, time taken to fall asleep, wake time during sleep and sleep efficiency (time asleep /number of minutes in bed). They also measured combined 1 and 2 stage sleep (slow wave sleep , REM).

1. Report A states that the participants kept a diary of their sleeping and waking habits for one-week prior to the commencement of the study. This diary served TWO key purposes. What were they?

(i)It provided baseline measures for of each participant’s habitual sleep pattern prior to the experiment.

(ii)The diary also supplied the habitual bed-time and wake-time for each participant

which was needed for setting the alarms on the pill-cases and for monitoring the fact that each participant was keeping to the same schedule as in their sleep diary.

1. The researchers (Report A) state that there was no difference across the different treatment conditions either for the baseline measures shown in Table 1 or for the non-study related caffeine intake of the participants.
   1. What aspect of the study design ensured that that was the case?

Random allocation of participants to the 4 treatment groups.

* 1. Why was it important for this to be the case?

Baseline measures are then the same across all groups. This enables any measured change to be attributed to the treatment since that is the only difference between the groups.

1. The participants had a washout day (where no pills were taken) between each treatment day. Suggest a reason why this was necessary. This was to ensure that caffeine levels in a person’s body returned to normal before they had the next treatment.

People with certain conditions were excluded from the study before it started. Who was excluded and why do you think it was necessary to do this? People who had more than 5 servings of caffeine a day were screened out as were also people who had a history of psychiatric illness, had a current medical disorder or who took medications affecting the central nervous system eg oral contraceptives.

If disturbed sleep is likely due to being on other medication the effect of this will be confounded with the effect of the caffeine and then a causal claim could not be made.

1. **Report B** states that :

“Compared with placebo, caffeine had the greatest effect on self-reported sleep if taken at bedtime or three hours before bedtime. Taken at these time points, caffeine:

* significantly reduced total sleep time
* significantly increased the time taken to fall asleep”

Use **Table 2** in report A to explain the evidence which supports these conclusions.

* Mean TTS with placebo : 7.82 hours. Mean TTS 3 hours before bed = 6.77 hours. Difference was almost an hour with a p value <0.05 indicating it was significant and similarly for caffeine at bedtime Mean TTS = 6.92 hours and also had a p value <0.05.
* For latency to sleep: Placebo 21mins, 3hours before bed: 62.5 mins , a difference of over 40 mins and was found to be significant (p <0.05) but although lower also for “at bedtime” at 56.67 mins this was not enough difference for p<0.05, hence Report B’s statement cannot be wholly supported.

1. **Report B** states that two limitations of this study were the small size (12) and the “very select” group of participants. Explain the way in which these two aspects limit the study and suggest how it could be improved.

* A small group of people will produce larger standard errors around the mean. More differences may have been significant with a larger group of people.
* This study was only with healthy and young to middle–aged participants and hence the findings may not be applicable to older and/or less healthy people.
* Improvement would be a larger study involving a wider age–range .

1. Can you think of any potential **confounding variables** for this study that you have not already discussed? How might this affect the outcome of the study?

People were allowed to follow normal routines in this study. Using a computer or similar blue-screened electronic device immediately prior to bed is known to disrupt sleep and hence if participants did this we cannot be sure their sleep disruption is due to the caffeine. With only 12 participants, random allocation may not have completely controlled for this.