Experimental Design Module

Developed by: Kate Stuart and Ann Rundell

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Summary: This module is intended to refresh or introduce students to aspects of experimental design, enhance their understanding that the experiment design should support appropriate statistical analysis of the data, and promote the students' recognition that there is often more than one possible way to design an experiment. This module is easily adaptable for students of any background or any education level. Instructions for presenting the module are included here, an associated power point is available for modification and use, and possible modification and extensions are indicated.

This instructional teaching module was inspired and developed based upon the Experimental Design Assessment Tool (EDAT) developed by Karen Sirum from Bowling Green University and published in Bioscene: Journal of College Biology Teaching (2011).[1]

Module Design

Introduction to the module: This module is best used to motivate and engage the students so they are more interested and have an immediate context to learn about and practice elements of experiment design. No introduction or materials need to be provided to the students prior to the start of the module. Rather, at the beginning of the class period, students can simply be told that experimental design would be addressed that day, and the module can begin.

Prompt: Students are provided with a "prompt", a situation that requires the use of experimental design to resolve or address the issue. Students are provided with one half of a page of blank paper and then given approximately 10-15 minutes to write down their ideas for an experiment design based on the information provided in the prompt. Student names are not written on the paper.

An example prompt:

> The Sensa weight loss system claims to promote healthy eating habits. In particular, there are reports that Sensa promotes weight loss by enhancing the sense of smell of food. How would you design an experiment to determine the effects of Sensa on weight loss?

The instructor collects the written experimental design, randomizes them, and distributes them back to the class (students should not receive their own experimental design). Students are given about 2 minutes to read through the design they've been given.

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[1] Sirum, K., and J. Humburg (2011). The Experimental Design Ability Test (EDAT). *Bioscene: Journal of College Biology Teaching* 37(1), 8-16.

> Note: By eliminating names and randomizing the designs, the students have anonymity and are more likely to participate in discussion. They can speak about the experimental design they have been given (or their own) without fear of saying something incorrect.

Rubric: After exchanging the student experiment designs, the instructor provides the students with a rubric of elements that should be included in each design (see example rubric at the end). The instructor can either begin discussion immediately by walking through each element in the rubric, or could allow students time to assess the experimental design they have by grading it based on the rubric.

> Note: An annotated powerpoint of the experiment design rubric with embedded examples and details are provided in the associated materials, this is included as an image at the end of this document.

The instructor should lead the class through each element in the rubric in detail using the annotated powerpoint as a guide. This typically takes about 30 minutes to present and use the annotated powerpoint. Herein, each concept is defined and examples are given to ensure that the students understand the concept. Different possible ways to address each element for a particular prompt should be discussed, and examples solicited from the student responses to help address student questions and clarify concepts. (The powerpoint has appropriate rubric examples and responses for each of the example prompts listed below. These are just concatenated in the file.)

Assessment: After thoroughly discussing and reviewing each element in the rubric, students can grade the experimental design they have in front of them by simply adding up the number of elements in the rubric found in the design. These can then be collected for assessment of the class understanding before conducting the module.

Repeat the module: The entire process can then be repeated with a second prompt. Either this prompt could require the same level of experimental design to assess learning from the prior exercise, or the second prompt could emphasize different aspects of the experimental design and require additional detail. When performing this exercise a second time, students often follow the first rubric that they had diligently, but did not necessarily consider additional aspects of experimental design that may be necessary for a more complex experiment. Because of this, it may be best to inform students that a new prompt may result in a new rubric.

After completing the discussion on the second prompt and rubric (using the associated powerpoint), students can again grade the design in front of them. Even if the second prompt and rubric is more complex than the first, students will typically score higher on the second prompt because they are more tuned in to thinking about experimental design aspects.

As an alternative, the prompt could be altered to pose a question that the students need to actually design and conduct the experiment for. This is how it was used in Purdue's BME306 (Biotransport lab).

Modifications

The module can be modified for background and education level by tailoring the prompts and rubrics. Prompts can be very general such that anyone can understand the issue, or complex such that background knowledge in the field is needed to come up with a reasonable design. The rubrics can be as involved as appropriate for the student's level of experimental design and statistics background. For example, an element in the rubric discussing the need to repeat the experiment could be discussed in simple terms such that repetition reduces error, or more complexity can be added by discussing power equations.

This exercise could be performed at the beginning and end of a course, to determine improvements in the ability of students to design experiments.

Other example prompts:

> Its 3:30 pm and you're in lecture. But you've been in class since 8am, and you're exhausted. You can't concentrate on your professor. A friend tells you about 5 hour energy – the new energy drink that claims to increase your awareness and focus for a full 5 hours. Design an experiment to test the effects of 5-hour energy on your concentration/focus skills.
>
> You are designing a new molecule called CancerX that can kill cancer cells. Design a simple in vitro experiment to determine if it works to kill the cells.
>
> The media keeps talking about vitamin D. They say it's essential for our health in general, and in the winter we don't get enough of it. In particular, there are reports that vitamin D is necessary to maintain proper bone density. How would you design an experiment to determine the effects of vitamin D on bone density?
>
> Scientists have claimed that dogs can easily be trained to detect colon cancer by smelling a patient's stool sample. Assuming you had access to these trained dogs, as well as any other resources you need, how would you determine whether you believed this claim?

Example rubric elements:

- Recognition that an experiment can be done to test the claim
- Identification of a hypothesis
- Identification of an independent variable
- Identification of a dependent variable
- Description of how the dependent variable is measured
- Recognition to make a comparison between at least two different groups
- Recognition of the need for a placebo group
- Recognition of the need for a negative control
- Recognition of the need for a positive control
- Recognition that there are possible confounding factors
- Use of any form of randomization to eliminate confounding factors
- Demonstrate an understanding that the sample size (or replication) may influence the finding (how to power an experiment)
- Discussion of statistical significance (confidence interval)
- Discussion of clinical significance
- Discussion of what statistical tests will be used to analyze the data
- Recognition that there may be possible sources of error and that there may be limits to generalizing the conclusions

Part I: Introduction to Experimental Design

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Acknowledgements

- Development funded by *Howard Hughes Medical Institute* in grant awarded to Purdue University entitled “Deviating from the Standard: Integrating Statistical Analysis and Experimental Design into Life Science Education”
- Module rubrics were adapted from: Sirum, K., and J. Humburg (2011). The Experimental Design Ability Test (EDAT). Bioscene: Journal of College Biology Teaching 37(1), 8-16.

- Its 3:30 pm and you’re in lecture. But you’ve been in class since 8am, and you’re exhausted. You can’t concentrate on your professor. A friend tells you about 5 hour energy – the new energy drink that claims to increase your awareness and focus for a full 5 hours. Design an experiment to test the effects of 5-hour energy on your concentration/focus skills.

www.5hourenergy.com

Rubric I

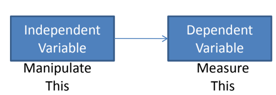
1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups
6. Recognition that there are possible confounding factors
7. Investigation of a placebo effect
8. Use of any form of randomization to eliminate confounding factors
9. Demonstrate an understanding that the sample size (or replication) may influence the finding
10. Recognition that there may be possible sources of error and that there may be limits to generalizing the conclusions

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable

Independent vs. Dependent Variable

- Independent – Change this to affect the dependent variable
- The dependent variable *depends* on the independent variable, and not vice versa.



- Independent Variable = 5 hour energy
- Dependent Variable = Focus/Concentration

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured

Measurement of Dependent Variable?

- How can you measure focus/concentration?
- How specific is the measurement?
  - Direct measurement?
  - Indirect quantity that relates to the measurement of interest?
- What is the uncertainty in the measurement?
  - Measurement noise?
  - Direct versus indirect?

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups?

Comparison of Groups Responses

- What constitutes a group?
  - Independent variable is different for each group
    - 5 hour energy vs. no 5 hour energy
  - Need a control group (no 5 hour energy)
    - show that the dependent variable changes due to changes in the independent variable
- Was there a change?
  - How much of a change is significant?
  - Statistics are your friend!
  - Is the change dependent on the factor level (how much 5 hour energy)?

Factorial Design

- What levels and how many levels should be tested?
  - Dose of 5 hour energy
  - Amount of times you take 5 hour energy
  - Pill vs liquid form
- Full factorial design: Evaluate all possible combinations
  - N factors at M levels = N^M
- Otherwise use fractional factorial design

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups
6. Recognition that there are possible confounding factors

Confounding Factors

- Things that can obscure the findings
- An extraneous variable that correlates with both the independent and dependent variable
- Examples:
  - Overall health
  - Diet
  - Metabolism
  - Activity level

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups
6. Recognition that there are possible confounding factors
7. Investigation of a placebo effect

Placebo: A Control

- A perceived (or actual) effect based on a sham medical intervention
- At a minimum need 2 groups
  - 5 hour energy
  - Water = control (placebo effect)

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups
6. Recognition that there are possible confounding factors
7. Investigation of a placebo effect
8. Use of any form of randomization to eliminate confounding factors

Randomization

- How do you assign groups?
- Simple randomization: all variables have an equal chance of being selected from the population for placement into each group
  - A purely random design gives the possibility that the groups are sufficiently different
- This method will work if you first eliminate many confounding factors
  - Use a population of males, 18-25 years old, that are physically fit, similar diets and activity levels

Randomization – A better way

- Stratified sampling: select independent samples from a number of subpopulations with the whole population
  - *Controls* for confounding factors, by spreading them across the groups
- Make sure each group has similar numbers of the subpopulations
  - Calcium intake, age, gender, physical activity, etc...

Rubric I

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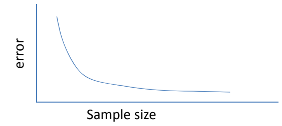
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups
6. Recognition that there are possible confounding factors
7. Investigation of a placebo effect
8. Use of any form of randomization to eliminate confounding factors
9. Demonstrate an understanding that the sample size (or replication) may influence the finding

Sample size

- The number of observations that are made
- There is inherent error in measurement
- Increasing the sample size reduces this error (to a degree)



Rubric I

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7. Investigation of a placebo effect
8. Use of any form of randomization to eliminate confounding factors
9. Demonstrate an understanding that the sample size (or replication) may influence the finding
10. Recognition that there may be possible sources of error and that there may be limits to generalizing the conclusions

Other considerations?

- How long does it take to see an effect?
- How long does it actually last?
- How much do you need to take?

Part II: More in-depth Experiment Design

- You are designing a new molecule called CancerX that can kill cancer cells. Design a simple in vitro experiment to determine if it works to kill the cells.

Rubric II

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Comparison between at least two groups used
6. Recognition of the need for a negative control
7. Recognition of the need for a positive control
8. Recognition of the need to use randomization or eliminate confounding factors?
9. Understanding that the sample size (or replication) may influence the finding?
10. Recognition that there may be possible sources of error and that there may be limits to generalizing the conclusions

Rubric II

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
   - CancerX
3. Identification of a dependent variable
   - killed cells
4. Description of how the dependent variable is measured
   - Count the cells, live/dead assay, etc...
5. Comparison between at least two groups used
   - Group with CancerX, group without CancerX
6. Recognition of the need for a negative control

Negative Control

- A negative control is known to give a negative result
  - Group receiving no treatment
  - Group receiving a similar agent known to not kill cells

Rubric II

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Comparison between more than two groups used
6. Recognition of the need for a negative control
7. Recognition of the need for a positive control

Positive Control

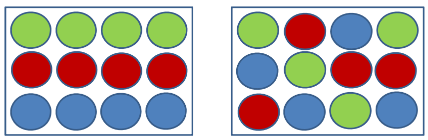
- Positive controls confirm that the procedure is competent in observing the effect
  - Use a molecule already known to kill cells
  - Shows that the experimental conditions and measurement techniques work, even if your treatment group doesn't work at all

Rubric II

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Comparison between more than two groups used
6. Recognition of the need for a negative control
7. Recognition of the need for a positive control
8. Recognition of the need to use randomization or eliminate confounding factors?

Randomization

- The order you perform the experiment
- The geometry of the experiment
  - Example 12-well plate



Rubric II

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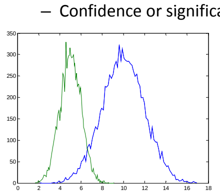
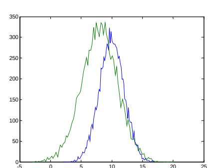
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Comparison between more than two groups used
6. Recognition of the need for a negative control
7. Recognition of the need for a positive control
8. Recognition of the need to use randomization or eliminate confounding factors?
9. Understanding that the sample size (or replication) may influence the finding?

Repeated Measurements vs. Replication

- Perform replicates in each experiment
  - Provides information on error within each experiment, reduces overall variability
- Repeat the experiment
  - Margin of error
  - Confidence or significance level



10,000 samples from normal distribution
Avg of 5 with std dev of 1
Avg of 10 with std dev of 2



10,000 samples from normal distributions
Avg of 8 with std dev 3
Avg of 10 with std dev of 2

Rubric II

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6. Recognition of the need for a negative control
7. Recognition of the need for a positive control
8. Recognition of the need to use randomization or eliminate confounding factors?
9. Understanding that the sample size (or replication) may influence the finding?
10. Recognition that there may be possible sources of error and that there may be limits to generalizing the conclusions

Part III: More complex Expt design

Biotransport Module 1 & 2

- An emerging area of biotechnology called "Tissue Engineering" develops new processes to grow organized living tissues of human or animal origin. A typical configuration is the engineered tissue bundle. Engineered tissue bundles have several potential biomedical applications, including the production of replacement body tissue (skin, bone marrow, etc.) for transplantation into the body, or for human-like tissues for in vitro drug testing.
- Living tissue requires nutrients to stay alive. The mass transport of the nutrients into the tissue is an important design consideration. These nutrients come from the blood vessels, and so you must include blood vessels into the engineered tissue.

Question

- Nutrients are delivered into the tissue space from the blood vessels by diffusion. Design an experiment to determine how closely the capillary vessels must be spaced to provide tissue with nutrients.
  - Propose a testing system (model)
  - Propose a testing plan (experimental plan with factors and potential factors explicitly recognized)
  - Next week we will construct a mathematical model to help refine the overall experiment design

Experimental Design for Module I

Combined Rubric (page 1 of 2)

Alternative Initial Prompts

Sensa®

- The Sensa weight loss system promotes a more healthy eating habits. In particular, there are reports that Sensa promotes weight loss by enhancing the sense of smell of food. How would you design an experiment to determine the effects of Sensa on weight loss?

- http://www.trysensa.com/

Rubric I

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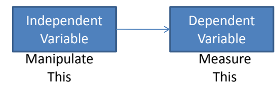
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups
6. Recognition that there are possible confounding factors
7. Investigation of a placebo effect
8. Use of any form of randomization to eliminate confounding factors
9. Demonstrate an understanding that the sample size (or replication) may influence the finding
10. Recognition that there may be possible sources of error and that there may be limits to generalizing the conclusions

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable

Independent vs. Dependent Variable

- Independent – Change this to affect the dependent variable
- The dependent variable *depends* on the independent variable, and not vice versa.



- Independent Variable = Sensa
- Dependent Variable = ΔWeight

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured

Measurement of Dependent Variable?

- How can you measure change in weight?
- How specific is the measurement?
  - Direct measurement?
  - Indirect quantity that relates to the measurement of interest?
- What is the uncertainty in the measurement?
  - Measurement noise?
  - Direct versus indirect?

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups?

Comparison of Groups Responses

- What constitutes a group?
  - Independent variable is different for each group
    - Sensa vs. no Sensa
  - Need a control group (no Sensa)
    - show that the dependent variable changes due to change in the independent variable
- Was there a change?
  - How much of a change is significant?
  - Statistics are your friend!
  - Is the change dependent on the factor level (amount of Sensa)?

Factorial Design

- What levels and how many levels should be tested?
- Full factorial design: Evaluate all possible combinations
  - N factors at M levels = M^N
- Otherwise use fractional factorial design

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups
6. Recognition that there are possible confounding factors

Confounding Factors

- Things that can obscure the findings
- An extraneous variable that correlates with the independent or dependent variable
- Examples:
  - Activity level
  - Innate olfaction sensitivity
  - Age
  - Health
  - Gender

Rubric I

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6. Recognition that there are possible confounding factors
7. Investigation of a placebo effect

Placebo: A Control

- A perceived (or actual) effect based on a sham medical intervention
- At a minimum need 2 groups
  - Sensa group
  - Inert powder group = control (placebo effect)

Rubric I

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6. Recognition that there are possible confounding factors
7. Investigation of a placebo effect
8. Use of any form of randomization to eliminate confounding factors

Randomization

- How do you assign groups?
- Simple randomization: all variables have an equal chance of being selected from the population for placement into each group
  - A purely random design gives the possibility that the groups are sufficiently different
- This method will work if you first eliminate many confounding factors
  - Use a population of males, 35-45 years old, 220-270 pounds, that are mostly sedentary...

Randomization – A better way

- Stratified sampling: select independent samples from a number of subpopulations with the whole population
  - *Controls* for confounding factors, by spreading them across the groups
- Make sure each group has similar numbers of the subpopulations
  - Beginning weight, age, gender, physical activity, etc...

Rubric I

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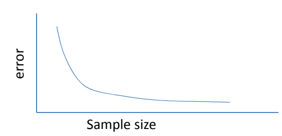
Sample size

- The number of observations that are made
- There is inherent error in measurement
- Increasing the sample size reduces this error (to a degree)



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8. Use of any form of randomization to eliminate confounding factors
9. Demonstrate an understanding that the sample size (or replication) may influence the finding
10. Recognition that there may be possible sources of error and that there may be limits to generalizing the conclusions

Other considerations?

- Subject compliance
- Do you use Sensa on ALL food?
- How much Sensa do you put on the food?
- How long does it take to have an effect?

Vitamin D

- The media keeps talking about vitamin D. They say its essential for our health in general, and in the winter we don't get enough of it. In particular, there are reports that vitamin D is necessary to maintain proper bone density. How would you design an experiment to determine the effects of vitamin D on bone density?

Rubric I

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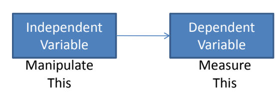

- Independent Variable = Vitamin D
- Dependent Variable = Bone Density

Rubric I

1. Recognition that an experiment can be done to test the claim
2. Identification of an independent variable
3. Identification of a dependent variable
4. Description of how the dependent variable is measured

Measurement of Dependent Variable?

- How can you measure bone density?
- How specific is the measurement?
  - Direct measurement?
  - Indirect quantity that relates to the measurement of interest?
- What is the uncertainty in the measurement?
  - Measurement noise?
  - Direct versus indirect?

Rubric I

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3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups?

Comparison of Groups Responses

- What constitutes a group?
  - Independent variable is different for each group
    - Vitamin D vs. no Vitamin D
  - Need a control group (no Vitamin D)
    - show that the dependent variable changes due to changes in the independent variable
- Was there a change?
  - How much of a change is significant?
  - Statistics are your friend!
  - Is the change dependent on the factor level (amount of Vitamin D)?

Factorial Design

- What levels and how many levels should be tested?
- Full factorial design: Evaluate all possible combinations
  - N factors at M levels = N^M
- Otherwise use fractional factorial design

Rubric I

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Confounding Factors

- Things that can obscure the findings
- An extraneous variable that correlates with both the independent and dependent variable
- Examples:
  - Activity level
  - Sunlight exposure
  - Age
  - Health
  - Gender

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7. Investigation of a placebo effect

Placebo: A Control

- A perceived (or actual) effect based on a sham medical intervention
- At a minimum need 2 groups
  - Vitamin D pill
  - Sugar pill = control (placebo effect)

Rubric I

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3. Identification of a dependent variable
4. Description of how the dependent variable is measured
5. Recognition to make a comparison between at least two different groups
6. Recognition that there are possible confounding factors
7. Investigation of a placebo effect
8. Use of any form of randomization to eliminate confounding factors

Randomization

- How do you assign groups?
- Simple randomization: all variables have an equal chance of being selected from the population for placement into each group
  - A purely random design gives the possibility that the groups are sufficiently different
- This method will work if you first eliminate many confounding factors
  - Use a population of males, 18-25 years old, that are physically fit, consume 300-600mg of calcium daily, and have 1-2 hours of outside exposure each day

Randomization – A better way

- Stratified sampling: select independent samples from a number of subpopulations with the whole population
  - *Controls* for confounding factors, by spreading them across the groups
- Make sure each group has similar numbers of the subpopulations
  - Calcium intake, age, gender, physical activity, etc...

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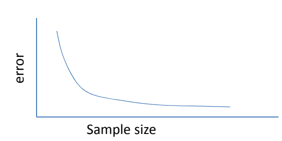
Sample size

- The number of observations that are made
- There is inherent error in measurement
- Increasing the sample size reduces this error (to a degree)



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9. Demonstrate an understanding that the sample size (or replication) may influence the finding
10. Recognition that there may be possible sources of error and that there may be limits to generalizing the conclusions

Other considerations?

- How often do you take the vitamin D?
- How much vitamin D at a time?
- How long does it take to have an effect?