American Society for Investigative Pathology
Investigating the Pathogenesis of Disease

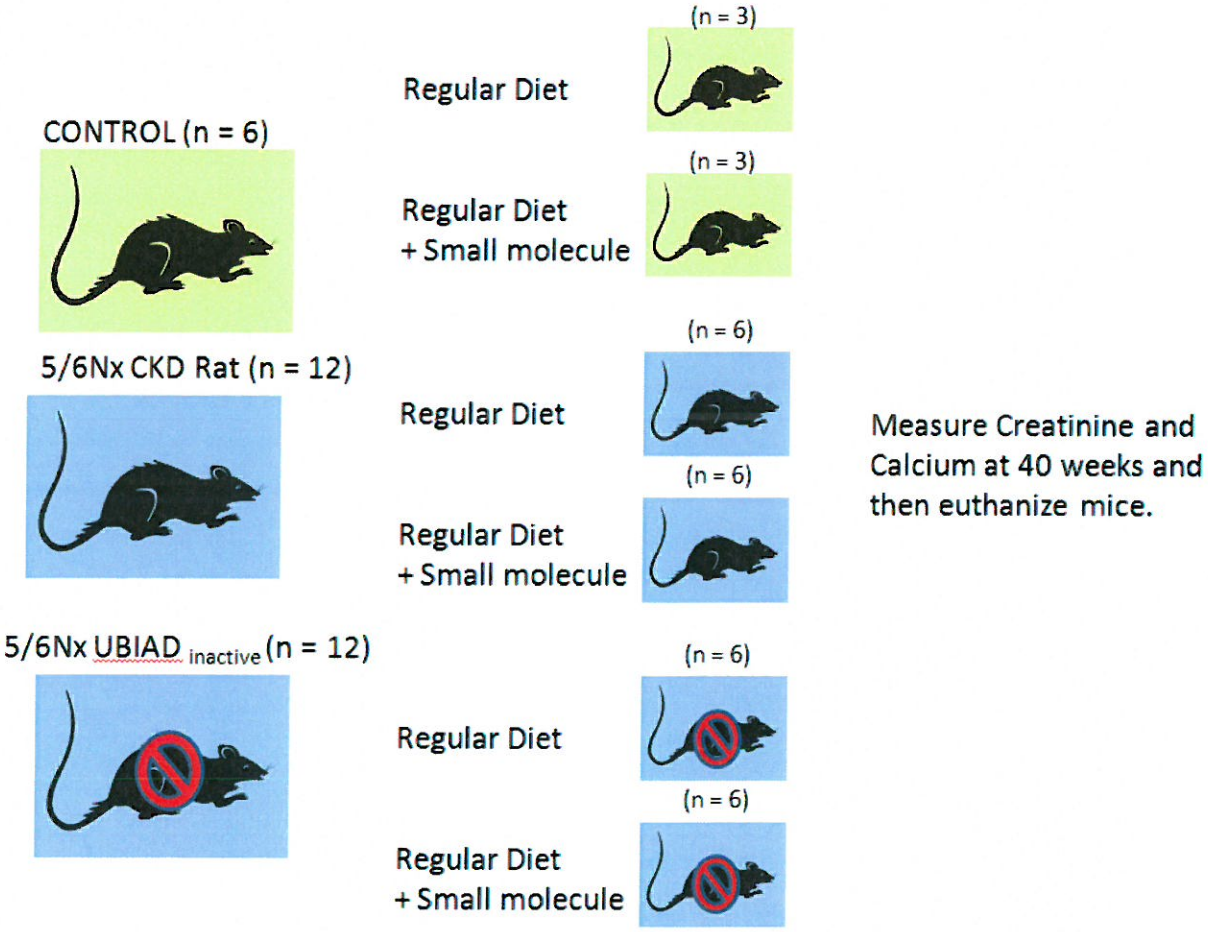
Lunch & Learn: Science, Statistics, and Getting it Right

ASIP 2018 Annual Meeting at Experimental Biology

Vignette 1

A small molecule screening program using human endothelial cell lines that develop calcification in the presence of calcification-inducing media identifies a molecule that drastically reduces the process. Interestingly, this molecule has extremely high oral bioavailability and is eliminated by direct excretion in the urine. The working biological hypothesis is that the molecule enhances the activity of UBIAD1 (an intracellular cholesterol regulator). Your laboratory has a working model of the 5/6Nx rat chronic kidney disease system and a collaborator happens to have a CRISPR/CAS9 tool to replace the UBIAD1 gene with an inactive form of the protein. In your system, the 5/6Nx rats develop chronic kidney disease including vascular calcifications and you monitor the disease using a peripheral blood measure of creatinine prior to euthanasia. In your collaborator's system, serum calcium is elevated in the UBIAD1 inactive form in normal rats.

You design an experiment to test the small molecule in your system as follows: At the end of 40 weeks, you measure the creatinine and calcium (see graph on the next page) of all of the rats and then sacrifice them. Using histology (H&E and Von Kassa stain) along with ImageJ (a free software program that allows you to do image based analyses, such as count cells or parse out a specific feature (nuclei, cytoplasm, etc) – download from http://imagej.nih.gov/ij/). You quantify the amount of calcification in the kidneys and the heart (see graph on the next page).



All rats are fed a 2% Ca, 1% P diet and followed for 40 weeks

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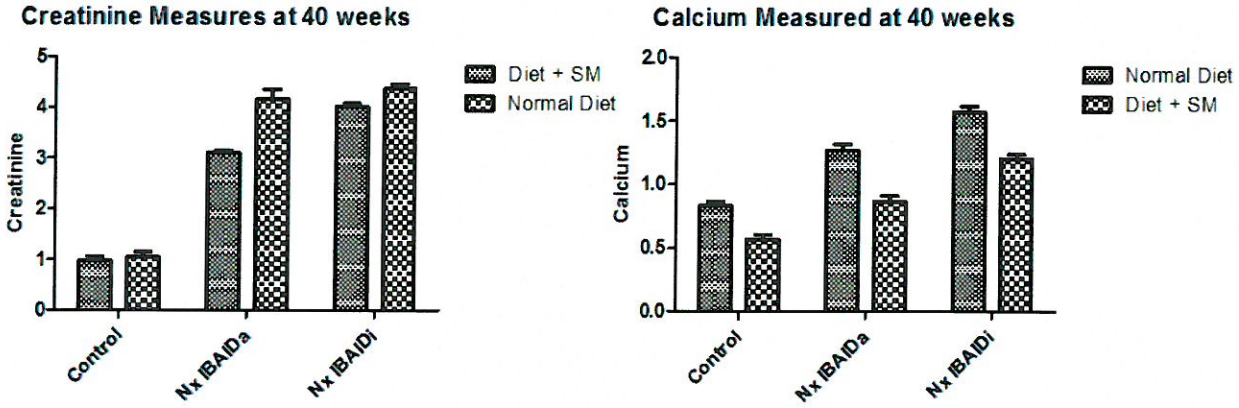

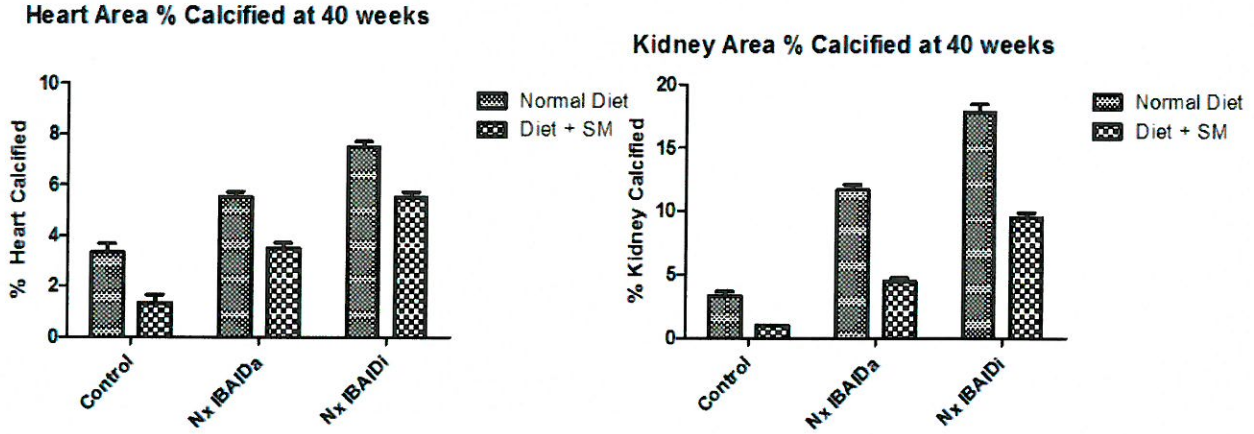

Questions:

1. Are the differences in measurements of creatinine, calcium, and tissue calcification different between the groups? Which ones?
2. When approaching data such as this, a few questions need to be answered prior to beginning any analysis (and should best be thought of before designing the experiment!). These include the following:
    i. What kind of variables do I have?
    ii. What kind of statistical test(s) can I perform?
    iii. What kind of result am I looking for?