DIET AND LONGEVITY STUDY

2. Study Design

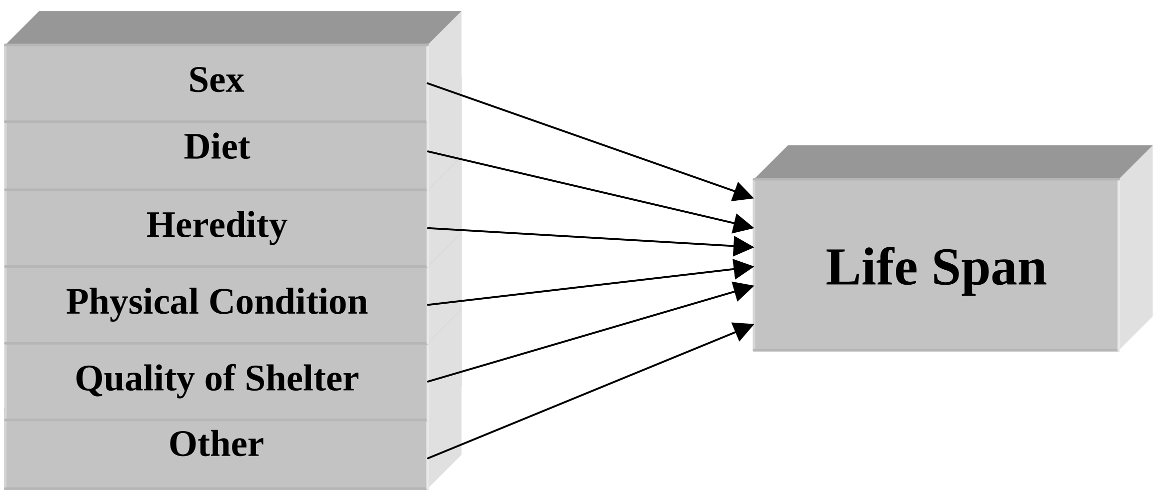
The inferences we may draw from the data depend crucially on the study's design and the way the data were collected. We will describe the study design by answering the following questions:

2.1 What factors affect the life span of mice?
2.2 What is the purpose of the experiment?
2.3 How to isolate and measure the effects of diet restriction on life span?
2.4 How to carry out randomization?
2.5 What is the scope of inferences in the experiment?

Before we will describe random mechanisms used in the experiment, we have to identify important variables affecting the life span of mice kept in laboratory conditions.

2.1 What factors affect the life span of mice?

The life span of a mouse kept in captivity is determined by several factors, among them diet, quality of the shelter, physical condition (disease, accidents), and heredity (offspring from long-lived parents have a longer life expectancy than those from short-lived parents). The life span is also affected by some environmental factors such as the level of pollution in the area, stress level (noise), and so on. In general, it would be difficult or even impossible to consider all variables affecting the life span. Some variables may not be recognized or measured.



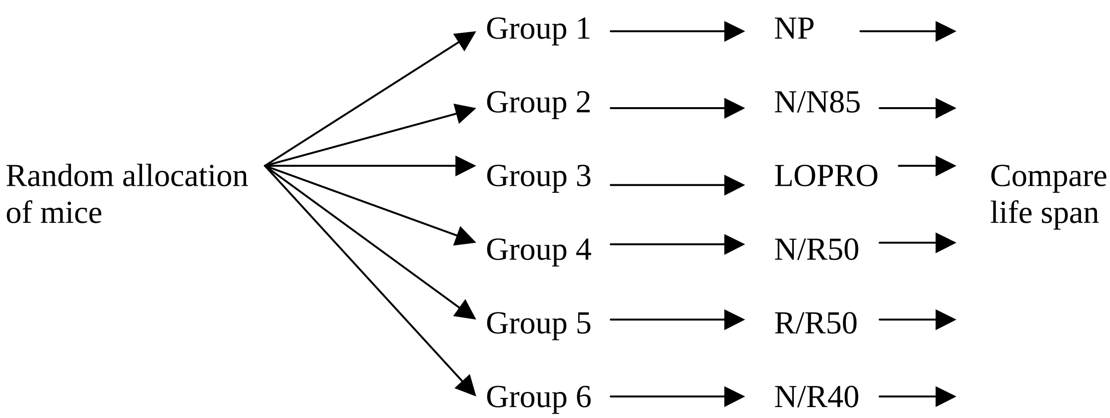
2.2 What is the purpose of the experiment?

The goal of the experiment is to establish the cause-and-effect relationship between diet restriction and longevity of mice. The experiment was restricted to mice but the researchers treated the study as the starting point to examine the relationship between the diet restriction and longevity for humans. The purpose of the experiment is not to explain the mechanism by which dietary restriction retards longevity.

Let us analyze now the way the experiment was conducted. In any experiment it is necessary to define the experimental unit upon which a treatment may be applied and the appropriate measurement is to be taken.

The experimental units are 349 genetically similar female mice obtained in laboratory conditions from a long-lived strain. These mice were obtained by mating males from the strain with females. Female mice were weaned at 21-28 days of age, individually caged in plastic cages. The mice were maintained under conventional conditions with temperature between 20 and 24°C, humidity 50-60%, and constant lighting throughout the study.

The 349 mice were divided randomly into 6 groups and subjected to one of four different regimens of dietary restriction, or one of two more normal diets.



The observed response variable in the case study is the lifetime of mice. This variable was measured in months. The experimental factor was the degree of underfeeding measured in kcal.

2.3 How to isolate and measure the effects of diet restriction on life span?

Randomization produces groups of experimental units (mice) that should be similar in all respects before the treatments are applied. Randomization ensures that mice with different and possibly relevant features are mixed up between the six experimental groups. The mice are not exactly alike. Some mice will die sooner than others regardless of the diet, and by chance more of the mice may end up in one group than in the others. In view of randomization, however, there is no reason to expect that they would be placed disproportionately in one of the six experimental groups, since every mouse had the same chance of being placed in that group.

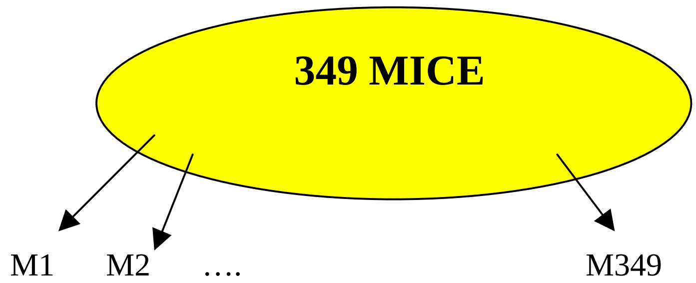
We cannot say that any difference in average life span among the six groups must be due to the diets. Some differences will appear even if the diets were identical, because of the natural differences among mice. Our goal is to use statistical tools to determine whether the observed differences among the groups are too large to be contributed to chance alone. If they are, we can claim that diet restriction had an effect on lifetime.

Note that the group sizes are relatively large, ranging from 49 for the NP group to 71 for the N/R50 group. This feature is important for the reliability of the conclusions drawn from the data.
Indeed, you would not trust the results of an experiment that fed each diet to only one mouse. The role of chance would be too large if we used only six mice and rolled a balanced die to decide which is fed which diet. The more mice used, the more likely that the randomization will create groups that are alike on the average. When differences among the mice are averaged out, only the effects of the different treatments will remain.

The control group is N/N85, the group that was fed normally both before and after weaning.

2.4 How to carry out the randomization?

We start with the group of the 349 mice used in the experiment.



Let's assign the successive integers to the six treatments as follows: NP=1, N/N85=2, LOPRO=3, N/R50=4, R/R50=5, N/R50=6. With this notation, we can assign each of the 349 mice randomly to the six treatment groups in the following way:

MICE	M1	M2	……	…..	M349
GROUP NUMBER	4	2			6

Roll a fair die in each case, or read numbers between 1 and 6 from a table of random digits or use the random number generation feature in a computer software

In a random process we expect that each of the six treatment groups will have approximately the same number of mice. Observe that in our experiment there are some significant differences among sample sizes. The differences are likely the result of losing mice to factors unrelated to the experiment. Our randomization process is complete. Now it is time to carry out the experiment and collect the data.

As all the experimental units (mice) were allocated at random among the 6 treatments, groups, the experimental design is *completely randomized*.

2.5 What is the scope of inferences in the experiment?

The 349 mice (experimental units) subjected to one of the diet restriction treatments or left as a control are not members of any well-defined population. They are not even selected randomly. Although a random mechanism was used to assign them to one of the six treatments, the mechanism used to obtain the mice was not random.

It might be tempting to extend the inferences to some general population. In this case, any inferences must be based on the assumption that these 349 mice are representative of the population.

Without the assumption, the observed pattern cannot be inferred to hold in some general population. The cause-and-effect conclusions can be drawn regarding the effect on the particular mice selected and the particular food used in the experiment.

As we don't have any population here and there is no random sampling involved, it makes no sense to test hypotheses about a population parameter. Any inferences in this case should be stated in terms of treatment effects and causation, rather than differences in population means and association.