DIET AND LONGEVITY STUDY

16. Brief Version of the Case Study

16.1 Problem Formulation
16.2 Study Design
16.3 Displaying and Describing the Data
16.4 Comparing the Average Effects with the F-Test
16.5 Multiple Comparisons
16.6 The Kruskal-Wallis Test
16.7 Summary

16.1 Problem Formulation

The problem is based on the data from *Diet Restriction and Longevity* experiment discussed in your textbook, pages 109-111. These data are available in the Excel file Case0501.xls located on the FTP server.

A series of studies involving several species of animals found that restricting caloric intake can dramatically increase life expectancy. In one such study, female mice were randomly assigned to one of the following six treatment groups:

NUMBER	TREATMENT CODE	TREATMENT DESCRIPTION	GROUP SIZE
1	**NP**	Mice ate as much as they pleased of a nonpurified, standard diet.	49
2	**N/N85**	Mice fed normally before and after weaning. After weaning, the ration controlled at 85kcal/wk.	57
3	**N/R50**	Mice fed a normal diet before weaning and a reduced-calorie diet of 50 kcal/wk after weaning.	71
4	**R/R50**	Mice fed a reduced-calorie diet of 50 kcal/wk both before and after weaning.	56
5	**N/R50 lopro**	Mice fed a normal diet before weaning, a restricted diet of 50 kcal/wk after weaning, and had dietary protein content decreased with advancing age.	56
6	**N/R40**	Mice fed normally before weaning and were given a reduced diet of 40 kcal/wk after weaning.	60

The group N/N85 serves as the control group because caloric intake is held reasonably constant.

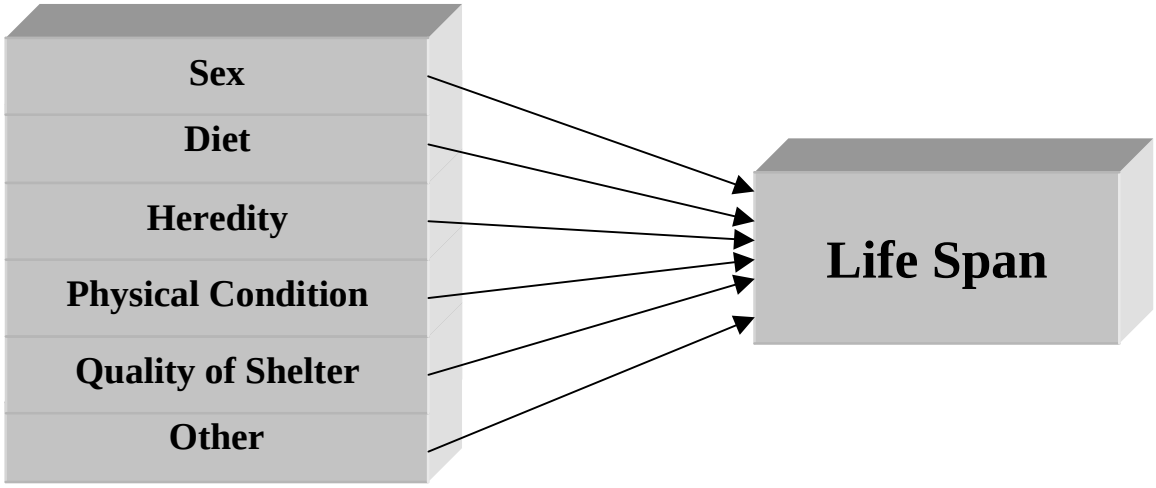
The following is a description of the variables in the data file:

Column	Name of Variable	Description of Variable
1	LIFETIME	Lifetime in months
2	TREATMT	Treatment Code

We will use SPSS to examine the effects of reduced diet on lifetime of mice in this experiment. Moreover, we will estimate the effects studied in the above questions using 95% confidence intervals.

16.2 Study Design and Data Collection

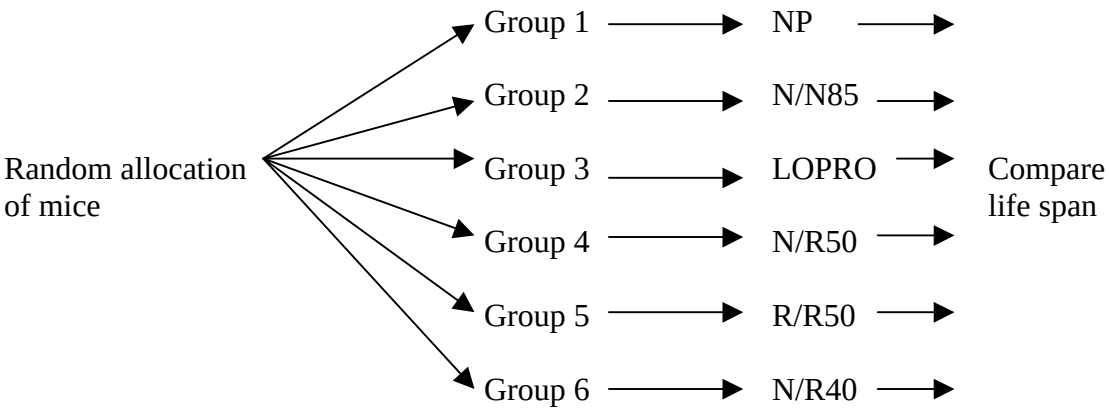
The life span of a mouse kept in captivity is determined by several factors, among them diet, quality of the shelter, physical condition (disease, accidents), and heredity (offspring from long-lived parents have a longer life expectancy than those from short-lived parents).



The goal of the experiment is to establish the cause-and-effect relationship between diet restriction and longevity of mice.

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.
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.

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.

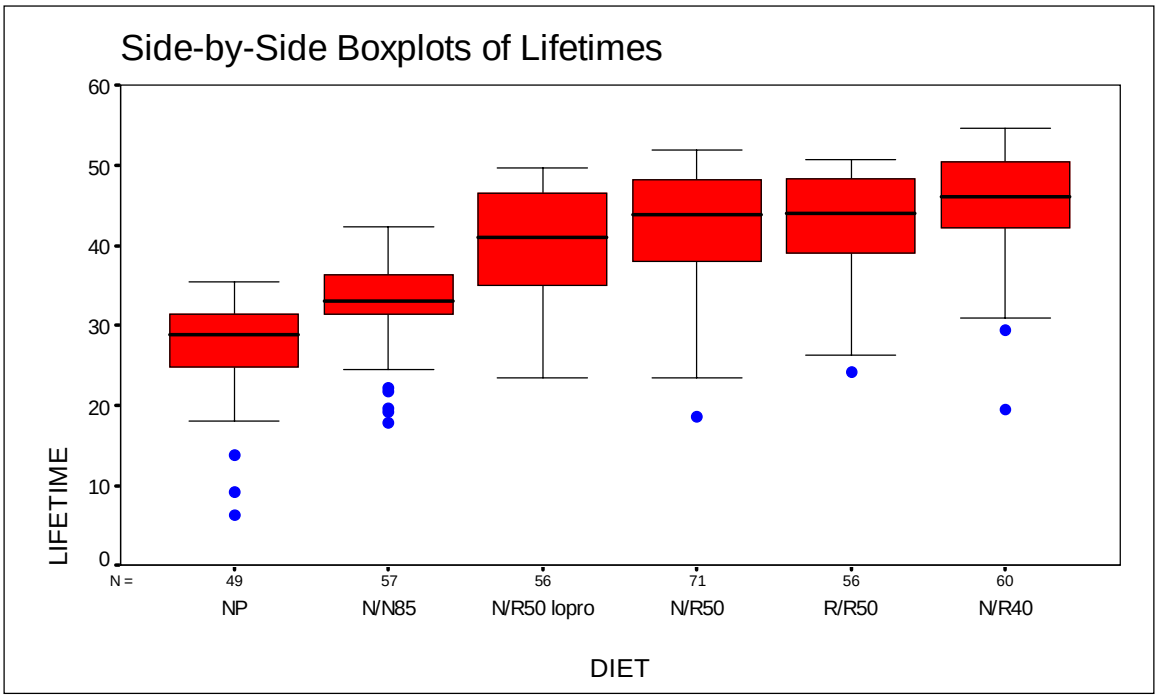
The 349 mice (experimental units) subjected to one of the diet restriction treatments or left as a control are not members of any well-defined population. They are not even selected randomly. Although a random mechanism was used to assign them to one of the six treatments, the mechanism used to obtain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

Therefore, 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.

16.3 Displaying and Describing the Data

SPSS produces the following side-by-side boxplots of lifetimes for the six treatment groups:



The positions of medians indicate that the median life span was shortest for NP mice, longer for N/N85 mice, even longer for N/R50 lopro mice and longest for the three other restricted groups N/R50, R/R50, and N/R40. The same conclusions can be reached about the maximum life span by examining the positions of the upper whiskers in the above boxplots. Therefore, it looks that appropriate dietary restriction of mice can increase mean and maximum life span.

Notice some differences in the variation of the lifetimes for the six groups. The variability is very small for the mice with no diet restrictions (NP) and the mice with normal diet (N/N85), but it is much larger for the remaining four diet restricted groups. It looks that diet restriction helped to extend the lifetime of some mice in the groups.

The side-by-side boxplots show that the distribution of lifetimes of mice are all negatively skewed. One possible explanation of the pattern is that there is something like an upper bound, a maximum possible lifetime for each group, and healthy mice all tend to get close to it. Unhealthy mice, however, die off sooner and at very different ages.

SPSS produces the following tables of descriptive statistics for the lifetimes of mice in the six treatment groups.

MEASURES OF	STATISTICS	DIET		
		NP	N/N85	LOPRO
CENTER	MEAN	27.4020	32.6912	39.6857
	MEDIAN	28.9000	33.1000	41.0500
	5% TRIM MEAN	27.9883	33.0096	39.9877
	95% CI FOR MEAN	(25.640, 29.164)	(31.331, 34.052)	(37.813, 41.558)
SPREAD	STANDARD DEV.	6.1337	5.1253	6.9917
	STD ERROR	0.8762	0.6789	0.9343
	VARIANCE	37.6223	26.2687	48.8838
	IQR	6.6500	5.2500	11.7500
	MINIMUM	6.4000	17.9000	23.4000
	MAXIMUM	35.5000	42.3000	49.7000
	RANGE	29.1000	24.4000	26.3000
SHAPE	SKEWNESS	-1.5499	-1.0961	-0.4638
	ST. ERROR SKEW	0.3398	0.3163	0.3190
	KURTOSIS	3.0443	1.4194	-0.7289
	ST. ERROR KURT	0.6681	0.6231	0.6283
COUNT		49	57	56

MEASURES OF	STATISTICS	DIET		
		N/R50	R/R50	N/R40
CENTER	MEAN	42.2972	42.8857	45.1167
	MEDIAN	43.9000	43.9500	46.0500
	5% TRIM MEAN	42.8568	43.4008	45.6407
	95% CI FOR MEAN	(40.458, 44.136)	(41.096, 44.676)	(43.385, 46.849)
SPREAD	STANDARD DEV.	7.7682	6.6832	6.7034
	STD ERROR	0.9219	0.8931	0.8654
	VARIANCE	60.3448	44.6645	44.9356
	IQR	10.6000	9.4000	8.2250
	MINIMUM	18.6000	24.2000	19.6000
	MAXIMUM	51.9000	50.7000	54.6000
	RANGE	33.3000	26.5000	35.0000
SHAPE	SKEWNESS	-1.0243	-0.9509	-1.2367
	ST. ERROR SKEW	0.2848	0.3190	0.3087
	KURTOSIS	0.4624	0.3965	2.5549
	ST. ERROR KURT	0.5625	0.6283	0.6085
COUNT		71	56	60

Mean life span was shortest for NP mice (~27 months), longer for N/N85 mice (~33 months), even longer for N/R50 lopro mice (~40 months) and longest for the three other restricted groups (42-43 for N/R50 and R/R50, ~45 for N/R40). The longest-lived individual mouse was from group N/R40 and lived 54.6 months.
It looks that appropriate dietary restriction of mice can increase mean and maximum life span. We can conclude that as the severity of dietary restriction increased, so did longevity. By comparing the descriptive statistics for N/R50 and R/R50 we conclude that food intake limited prior to weaning did not further increase longevity for mice subjected to postweaning dietary restrictions.

The groups N/R50 and N/R50 lopro differ in the protein content levels after weaning. Comparing the means for the two groups indicates that mice restricted in both calorie and

protein intake exhibited shorter mean life span than did mice fed the same number of calories of a high protein diet.

16.4 Comparing the Average Effects with the F-Test

In the experiment mice were divided at random into six experimental groups. We would like to know whether diet restriction had any effect on the life span of the mice. An appropriate statistical technique to examine the effect is one-way ANOVA. The purpose of ANOVA is to assess whether the observed differences among treatment groups are statistically significant. More precisely, the null hypothesis is that the treatments are not different on average, while the alternative hypothesis is that at least one of the treatments is different, on average, from the others (of course, they could all be different from each other).

SPSS produces the following output:

Analysis of Variance

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	Prob.
Between Groups	5	12733.9418	2546.7884	57.1043	.0000
Within Groups	343	15297.4150	44.5989		
Total	348	28031.3568			

Group	Count	Mean	Standard Deviation	Standard Error	95 Pct Conf Int for Mean
NP	49	27.4020	6.1337	.8762	25.6402 TO 29.1638
N/N85	57	32.6912	5.1253	.6789	31.3313 TO 34.0512
LOPRO	56	39.6857	6.9917	.9343	37.8133 TO 41.5581
N/R50	71	42.2972	7.7682	.9219	40.4585 TO 44.1359
R/R50	56	42.8857	6.6832	.8931	41.0960 TO 44.6755
N/R40	60	45.1167	6.7034	.8654	43.3850 TO 46.8483
Total	349	38.7971	8.9750	.4804	37.8522 TO 39.7420

The instructions how to obtain the above output are given in the *Computer Instructions* module (click on it to access them).

The value of the F statistic is 57.1043, and the p-value of the test is reported as zero. In fact, the p-value is an extremely small but positive number. Therefore, there is overwhelming evidence that mean lifetimes in the six treatments are different.

The output also provides the mean, standard deviation, and 95% confidence interval for the mean for each of the six treatment groups.

The above conclusions based on the ANOVA model are valid only if the underlying assumptions are satisfied. Specifically we assume that:

1. The lifetimes have normal distributions for each of the six treatments.
2. The treatment standard deviations are all the same.
3. Observations within each group are independent of each other.
4. Observations in any one group are independent of observations in other groups.

The assumptions are discussed in detail for the case study in Section 6.

16.5 Multiple Comparisons

SPSS has several multiple comparison procedures that should be run after the experiment has been conducted. The most important are Tukey's HSD method, Duncan's method, and the LSD (least significant difference) Fisher's method. The LSD method is the most liberal procedure (narrowest confidence intervals and low overall power of the test), whereas Tuckey's test is more powerful.

SPSS produces the following outputs for the LSD test:

Multiple Range Tests: LSD test with significance level .05

The difference between two means is significant if
MEAN(J)-MEAN(I) >= 4.7222 * RANGE * SQRT(1/N(I) + 1/N(J))
with the following value(s) for RANGE: 2.78

(*) Indicates significant differences which are shown in the lower triangle

		Group
		1 2 3 4 5 6
Mean	Group	
27.4020	1	
32.6912	2	*
39.6857	3	* *
42.2972	4	* * *
42.8857	5	* * *
45.1167	6	* * * *

In the above table the means are ordered and displayed from smallest to largest in the rows, and the asterisks in the lower part of the matrix indicate which pairs of groups differ significantly at the 5% level.

Variable LIFETIME
By Variable TREATMT

Multiple Range Tests: Tukey-HSD test with significance level .050

The difference between two means is significant if
MEAN(J)-MEAN(I) >= 4.7222 * RANGE * SQRT(1/N(I) + 1/N(J))
with the following value(s) for RANGE: 4.06

(*) Indicates significant differences which are shown in the lower triangle

Mean	Group	1 2 3 4 5 6
27.4020	1	
32.6912	2	*
39.6857	3	* *
42.2972	4	* *
42.8857	5	* *
45.1167	6	* * *

Multiple Range Tests: Duncan test with significance level .05

The difference between two means is significant if
MEAN(J)-MEAN(I) >= 4.7222 * RANGE * SQRT(1/N(I) + 1/N(J))
with the following value(s) for RANGE:

Step	2	3	4	5	6
RANGE	2.79	2.93	3.02	3.09	3.15

(*) Indicates significant differences which are shown in the lower triangle

Groups

Mean	TREATMT	1 2 3 4 5 6
27.4020	1	
32.6912	2	*
39.6857	3	* *
42.2972	4	* * *
42.8857	5	* * *
45.1167	6	* * * *

Note that the LSD and Duncan's procedures have detected more significant differences between the treatment means than Tukey's test.

16.6 The Kruskal-Wallis Test

The model presented in the above section has the underlying assumption of normality. However, in our case study the assumption is slightly violated because outliers are present in the data. In this case, the nonparametric Kruskal-Wallis test procedure provides a very good alternative.

The Kruskal-Wallis test output in SPSS for the diet restriction study is displayed below.

Kruskal-Wallis 1-Way Anova

LIFETIME
by GROUP

Mean Rank	**Cases**	
52.37	49	Group = 1
101.03	57	Group = 2
179.97	56	Group = 3
215.98	71	Group= 4
221.36	56	Group = 5
249.02	60	Group = 6

	349	Total

Chi-Square	D.F.	Significance
159.0128	5	.0000

The p-value of the test is reported as zero indicating strong evidence against the assumption of no treatment effects. This is consistent with the results obtained with the F-test.

16.7 Summary

The purpose of the experiment was to clarify the impact of dietary restriction (**under**nutrition without **mal**nutrition) on life span. Female mice from a long-lived strain were subjected to one four different regimens of dietary restriction, or one of two more normal diets. Experimental variables tested included the protein content of the restricted diet, and preweaning restriction. The response variable was the lifetime expressed in months.

The study found that as the severity of dietary restriction increased, so did longevity. Mean life span was shortest for the mice fed the normal unrestricted diet. Mice from the group fed with the lowest caloric intake lived longest of all. Moreover, extreme longevities attained by certain of the restricted mice were reported.

It was also found that food intake limited prior to weaning did not further increase longevity for mice subjected to postweaning dietary restriction. Mice restricted in both calorie and protein intake exhibited shorter mean life span than did mice fed the same number of calories of a high protein diet.

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. Therefore, any inferences to population must be based on the assumption that these 349 mice are representative of the population.

The cause-and-effect conclusions about the effect of dietary restriction on the life span of the particular mice used in the study can be drawn. The conclusions are valid only for the particular nutrient composition used in the experiment.

The findings call for examining the mechanism by which dietary restriction retards life span. It is also interesting to establish optimal nutrient composition and feeding strategies for these life span-extending diets.