statstutor

community project

encouraging academics to share statistics support resources

stcp-karadimitriou-pairedR

The following resources are associated: The dataset *'Cholesterol.csv'* and R script file 'Paired t-test in R' Checking normality in R, Wilcoxon signed rank test in R and Repeated measures ANOVA in R resources.

Paired t-test in R

Dependent: Continuous (scale)

Independent: Binary (before/after or condition)

Common Applications: Comparing means of data from two related samples; say, observations before and after an intervention on the same participant, or comparison of measurements from the same participant two different conditions.

Research question: Does using Clora margarine for four weeks change cholesterol?

Data: Participants used Clora margarine for 8 weeks. Their cholesterol (in mmol/L) was measured before the special diet, after 4 weeks and after 8 weeks. Open the csv file '*Cholesterol.csv'* and call it cholA, changing the command depending on where you have saved the file and what you called it, then use `attach (cholA)` so that the variable names can be used in future calculations.

```
cholA<-read.csv('D:\\stcp-Rdataset-cholesterol.csv',header=T)
attach(cholA)
```

Subject	Before	After4weeks	After8weeks	Margarine
1	6.42	5.83	5.75	B
2	6.76	6.20	6.13	A
3	6.56	5.83	5.71	B
4	4.80	4.27	4.15	A

There is one row per person with their cholesterol levels at the three time points in different columns e.g. The 'After4weeks' column contains the cholesterol measurements after 4 weeks on the diet.

Note: Although there are three time points which are all analysed together on the '*Repeated measures ANOVA in R sheet*', only the comparison of cholesterol before using the margarine and after 4 weeks is of interest here.

Summary Statistics

Since cholesterol level is continuous (scale) data, it can be summarised by producing the average (mean) and standard deviation. When comparing related samples data, either compare the means at the two time points or calculate the paired differences and summarise this with one mean. Both methods are described here.

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In order to calculate the mean and the standard deviation for the measurements of cholesterol before the diet and after 4 weeks on the diet, use the `mean()` and `sd()` commands respectively.

Calculate means and standard deviations for 'Before' and 'After4weeks' and combine in one table.`mu<-rbind(mean(Before),mean(After4weeks))`

Calculate the standard deviations of the two variables and combine.
`sds<-rbind(sd(Before),sd(After4weeks))`

Combine both results in one table called results1 and give rows and columns names.
```
results1<-cbind(mu,sds)
colnames(results1)<-c('Mean','SD')
rownames(results1)<-c('Before','After4weeks')
```

Round and display the results
`round(results1,2)`

The cholesterol level 4 weeks after the special diet is lower than before the diet with means of 5.84 and 6.40 respectively. The similar standard deviations suggest that the spread of the values at the two time points is similar.

```
> round(results1,2)
             Mean   SD
Before       6.41 1.19
After4weeks  5.84 1.12
```

Calculating and summarising differences

Subject	Before	After4weeks	Difference
1	6.42	5.83	-0.59
2	6.76	6.2	-0.56
3	6.56	5.83	-0.73
4	4.8	4.27	-0.53
5	8.43	7.71	-0.72
6	7.49	7.12	-0.37
7	8.05	7.25	-0.8
8	5.05	4.63	-0.42
9	5.77	5.31	-0.46
10	3.91	3.7	-0.21
11	6.77	6.15	-0.62
12	6.44	5.59	-0.85
13	6.17	5.56	-0.61
14	7.67	7.11	-0.56
15	7.34	6.84	-0.5
16	6.85	6.4	-0.45
17	5.13	4.52	-0.61
18	5.73	5.13	-0.6
	Mean difference		-0.57

The paired t-test calculates paired differences for each subject and calculates a test statistic from these differences. If there was no change in cholesterol between the two time points, the mean difference of the values would be close to 0.

To calculate the paired differences between the Cholesterol levels at the two time points:
`dif<-After4weeks-Before`

To calculate the mean change in cholesterol and the standard deviation of the differences:
```
mean(dif)
[1] -0.5661111
sd(dif)
[1] 0.1555687
```

Checking assumptions

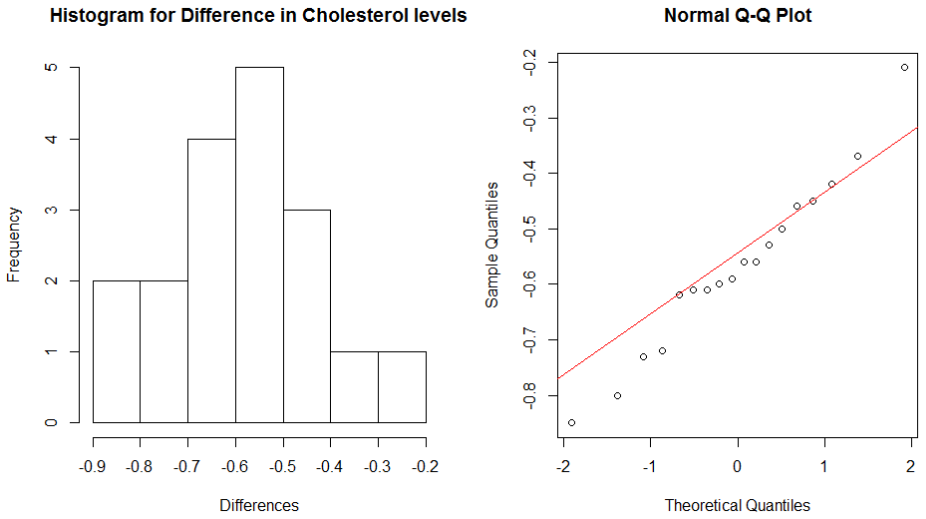
Before conducting the test, the assumption that the paired differences are normally distributed must be satisfied. This can be tested by looking at a histogram or QQ plot. Normality goodness of fit tests such as the Shapiro-Wilk can be used to test for normality but these are sensitive to sample size and outliers (see *Checking normality in R* resource for more details) so use a plot as well.

To plot a histogram of the paired differences:

```
hist(dif,main='Histogram for Difference in Cholesterol
Levels',xlab='Differences')
```

To draw a qq-plot and add a line where x=y to the qqplot to help assess normality

```
qqnorm(dif)
qqline(dif)
```



Both the graphs suggest that the data are normally distributed.

Histogram: The differences look approximately normally distributed.

QQ-plot: The points are fairly close to the line of x=y axis with no pattern pulling away from the line. Skewed data curves away from the line.

It is not necessary to carry out a test for normality as well as the charts but if you do wish to carry out a Shapiro – Wilk test to check normality use:

```
shapiro.test(dif)
```

```
        Shapiro-Wilk normality test

data:  dif
W = 0.97742, p-value = 0.9196
```

The Shapiro-Wilk Test tests the null hypothesis that the data are normally distributed versus the alternative that are not. For the Shapiro-Wilk Test, if the p-value is above 0.05 then we assume approximate normality. The test statistic is W= 0.97742 and the p-value = 0.9196 so normality can be assumed.

Data has to be very skewed to cause problems with t-tests. If the assumption of normality has not been met, use the non-parametric Wilcoxon signed rank test instead (see the *Wilcoxon signed rank test in R* resource).

Conducting the paired t-test

The paired t-test tests the null hypothesis

H_0: There is no difference in mean cholesterol before and after 4 weeks using Clora

versus the alternative hypothesis

H_1: There is a difference in mean cholesterol levels before and after 4 weeks using Clora.

To run a paired t-test, use the command `t.test(variable1, variable2,paired=T)`.

```
> t.test(After4weeks,Before,paired=T)

        Paired t-test

data:  After4weeks and Before
t = -15.4389, df = 17, p-value = 1.958e-11
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.6434736 -0.4887486
sample estimates:
mean of the differences
             -0.5661111
```

The test statistic is t=-15.439 and the p-value is very small (p < 0.001) so the null hypothesis is rejected, since $p < 0.05$ and evidence of a statistically significant difference is concluded.
The p-value is very small so if the null hypothesis of no difference is true, there is only a small probability of this result occurring by chance (type 1 error).

Reporting: A paired t-test was carried out to see if using Clora margarine for 4 weeks changed cholesterol levels. There was significant evidence ($t(17) = -15.439$, $p < 0.001$) of a change in cholesterol levels. On average, participants reduced their cholesterol by 0.566 mmol/L.

Confidence intervals and effect size
In this data set, using Clora for 4 weeks, improved cholesterol levels, on average, by 0.566 mmol/L. Of course, if we were to take other samples, we could get a 'mean paired difference in cholesterol levels' which is different from 0.566. This is why it is important to look at the 95% Confidence Interval (95%*CI*). The interpretation of the 95%*CI* is that if we were to do this experiment 100 times, 95 times out of the 100 the true value for the difference would lie in the interval. In our case, the 95%*CI* is (-0.643, -0.488) which indicates that although the difference in cholesterol levels is statistically significant, still the difference is actually relatively small. You would need to consider if this difference in cholesterol is **practically important**, not just **statistically important**.
To help decide, calculate the mean change as a percentage of the mean starting cholesterol:

$$\text{mean \% lost} = \frac{\text{mean difference}}{\text{mean before the diet}} = \frac{-0.566}{6.41} \times 100 = -8.8\%$$

The average person lost 8.8% of their starting cholesterol which seems practically important as well as statistically significant.
Some people like to calculate an effect size to assess the magnitude of the change. There are several types of effect size but eta squared will be calculated here using the test statistic t and the degrees of freedom (df) from the t-test output.

$$\text{Eta squared} = \frac{t^2}{t^2 + df} = \frac{-15.439^2}{-15.439^2 + 17} = 0.966$$

Cohen (1988) using the following guidelines for interpretation: 0.01 (small effect), 0.06 (moderate) and 0.14 (large effect). Here the magnitude of the change in cholesterol was large (eta squared = 0.966).