Sheffield Hallam University

Mathematics and Statistics Support

The following resources are associated: The dataset *'Cholesterol.csv'*

Paired t-test, one sample t-test and Wilcoxon signed rank in SAS

The **paired t-test** is used when two measurements of the same variable are collected for each individual. For example, assessing whether there is a change in weight before and after a diet or comparing taste scores for two products when each individual tastes both. For comparing three or more repeated measurements, use repeated measures ANOVA.

A **one sample t-test** is used when you are comparing the mean of one continuous variable to a given population mean e.g. is the mean exam score different to 60.

The **Wilcoxon signed rank test** is the non-parametric equivalent to both the one sample and paired t-test so it can be used when the dependent variable is ordinal or the assumptions of a t-test are not met. For a one sample t-test the dependent variable should be approximately normally distributed and for the paired t-test, the paired differences should be approximately normal.

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*' in SAS.

	Subject	Before	After4weeks	After8weeks	Group
1	2	6.76	6.2	6.13	A
2	4	4.8	4.27	4.15	A
3	6	7.49	7.12	7.05	A

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

Paired t-test

Dependent: Continuous (scale)
Independent: Binary (before/after or condition)

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

The paired t-test uses the differences for each person (Before – After4weeks). If there was no change in cholesterol between the two time points, the mean difference of the values would be close to 0. You can calculate this change by creating a new variable in the data step. Here the new variable is called meandiff_B4

```
data chol;
set dat.cholesterol;
meandiff_B4=Before-After4weeks;
label meandiff_B4='Change in cholesterol (Before - 4 weeks)'; run;
```

Before running procedures, turn the graphics on to get the automatic charts for checking assumptions. `ods graphics on;  run;`

To carry out the paired t-test, use the **paired** statement within the the `proc ttest` procedure specifying the two variables to be compared with a star in between **Before*After4weeks**.

```
proc ttest data=chol plots=box;
paired Before*After4weeks;    run;
```

DF	t Value	Pr > |t|
17	15.44	<.0001

The degrees of freedom (df), test statistic (t Value) and p-value are given in this table. A significant result indicates there has been a change but doesn't indicate which measurement is bigger.
Report: A paired t-test showed a statistically significant [t(17)=15.44, p<0.001)] change in cholesterol.

Following a significant result, use the mean difference and confidence interval (range of potential values for the population mean change) to describe the change. As before was given first in the procedure, the mean change is before – After4 weeks.

Mean	95% CL Mean	
0.5661	0.4887	0.6435

A boxplot of the paired differences is also useful as it visualises how many people had a positive change. The box contains the middle 50% of the paired differences (interquartile range) and the line in the centre is the median difference. You can get one by adding **plots=box** to the `proc ttest` line or produce a nicer one using the paired difference variable in `proc sgplot`.

Report: Participants in the trial had an average drop of 0.57 mmol/L [95% CI: 0.49, 0.64] after four weeks on the trial suggesting that the margarine is effective at reducing cholesterol. As Figure 1 shows, all participants reduced their cholesterol in the first four weeks.

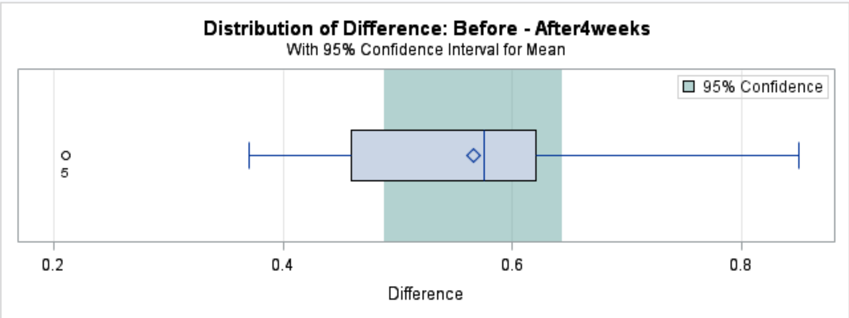


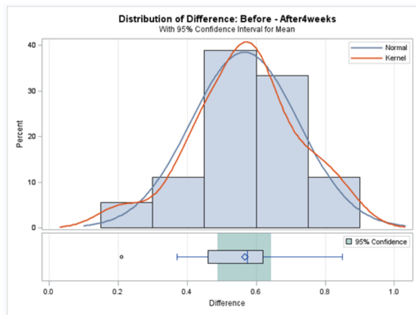
Figure 1: Boxplot of cholesterol differences (baseline - week 4)

PROC SGPLOT allows you to produce formatted plots with larger font for labels. The chart type is specified in the 2nd line with `vbox` indicating a boxplot is required.

```
proc sgplot data=chol;
vbox meandiff_B4;
/*** Adjust the size of the font for the variable and value labels**/
yaxis label='Difference in cholesterol (Baseline - week 4)'
labelattrs=(size=15pt weight=bold) valueattrs=(size=15pt);
```

Checking normality

In order for the p-value to be reliable, the paired differences should be approximately normally distributed. As long as the graphics are turned on, a histogram is produced automatically. The red curve is a smoothed out version of the histogram and the blue is a perfect normal distribution with the sample mean and standard deviation. Both are similar here so the assumption has been met.
If the paired differences are very skewed, a Wilcoxon signed rank test should be used.



Meaningful differences
Sample size impacts on statistical significance so the bigger the sample, the more likely a significant result is and large actual differences for small sample sizes may not be significant. Use the mean and CI of the difference to assess whether the observed difference is **practically important**, not just **statistically significant**.
Another method for reporting the magnitude of a difference is to 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.

Wilcoxon signed rank test for paired data

If your initial histogram of the paired differences is very skewed or you have ordinal data the non-parametric Wilcoxon signed rank test should be used which is available through the **proc univariate** procedure. You must calculate a new variable of the change for each person.

```
data chol;
set dat.cholesterol;
meandiff_B4=Before-After4weeks;
```

You must specify the variable for the change per person in the var line which will automatically compare to a hypothesised value of 0 `mu0=0` unless you specify a different value in the proc line.

```
proc univariate data=chol;
var meandiff_B4;        run;
```

Tests for Location: Mu0=0				
Test	**Statistic**		**p Value**	
Student's t	**t**	15.43887	**Pr > |t|**	<.0001
Sign	**M**	9	**Pr >= |M|**	<.0001
Signed Rank	**S**	85.5	**Pr >= |S|**	<.0001

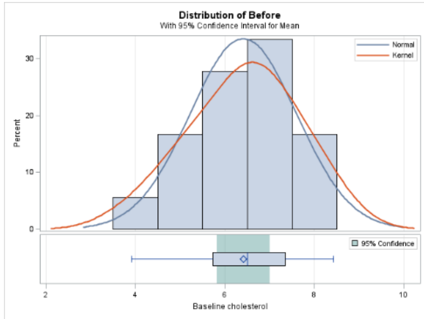
The Student's t-test on the top line is the same as the paired t-test above. Non-parametric tests are based on ranks rather than the values of the actual variable. The sign test is a more basic non-parametric test which compares the number of values above and below the specified hypothesised value of 0 here. For a Wilcoxon signed rank test, participants are ranked in order of absolute change from 0 and the sum of the positive (reduced cholesterol) ranks compared to the sum of the ranks for those who had an increase in cholesterol (negative change). Here everyone reduced their cholesterol so there are no negatives.

Summary statistics/chart: For skewed data, the median, quartiles and a box-plot should be used to summarise the difference.

Basic Statistical Measures			
Location		**Variability**	
Mean	0.566111	**Std Deviation**	0.15557
Median	0.575000	**Variance**	0.02420
Mode	0.560000	**Range**	0.64000
		Interquartile Range	0.16000

Report: A Wilcoxon signed rank test was used to test for a change in cholesterol as the assumptions of the paired t-test were not met. There was a statistically significant change in cholesterol (p< 0.0001) after four weeks of using the margarine. The median cholesterol reduction was 0.575 mmol/L and Figure 1 shows that all partcipants reduced their cholesterol.

One sample t-test



A one sample test for a mean is used to test whether a population mean has changed or a sample is different to a set population value. There is just one continuous variable which should be approximately normally distributed. If the variable is ordinal or very skewed, use the Wilcoxon signed rank test.

Research question: Is the mean starting cholesterol higher than 5.2?

Cholesterol under 5.2 mmol/L is within the normal range so participants need to be in a higher range than that and high cholesterol is greater than 6.2.

You can use `proc ttest` or `proc univariate` for a one sample t-test both with a **`var`** line specifying the one variable of interest. You must also state the hypothesised value of 5.2 using **`h0=`**`5` in the proc t-test line or **`mu0=`**`5.2` in the proc univariate line.

```
proc ttest data=chol plots=box h0=5.2;
var Before;
```

A significant result means that the sample mean is different to the hypothesised mean of 5.2.

DF	t Value	Pr > |t|
17	4.30	0.0005

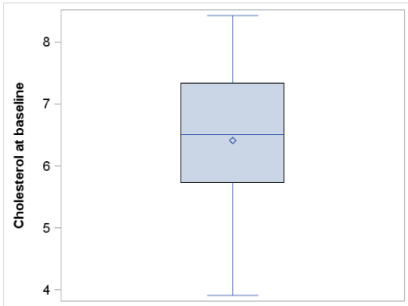


Figure 2: Boxplot of baseline cholesterol)

Mean	95% CL Mean	
6.4078	5.8155	7.0001

The sample mean is 6.4 and the confidence interval ranges from 5.8 to 7.

Report: A one sample t-test showed that the mean starting cholesterol level was significantly higher [t(17)=4.3, p=0.0005] than 5.2 with a mean of 6.4 [95% CI: 5.8, 7]. However, the mean starting cholesterol could be as low as 5.8 which is not classified as high (above 6.2) and the boxplot in Figure 2 shows that although most people were above the normal range, not all were classified as having high cholesterol.

Wilcoxon signed rank test for one sample

The Wilcoxon signed rank test is used when the variable of interest is ordinal or very skewed. It is the same as the test for the paired difference but you must specify the hypothesised value you are testing against in the proc line `mu0=5.2`. You must report the median rather than the mean when reporting a significant result along with a boxplot.

```
proc univariate data=chol mu0=5.2;
var before;
```

Basic Statistical Measures			
Location		Variability	
Mean	6.407778	Std Deviation	1.19109
Median	6.500000	Variance	1.41869
Mode	.	Range	4.52000
		Interquartile Range	1.61000

Tests for Location: Mu0=5				
Test	Statistic		p Value	
Student's t	t	5.01449	Pr > |t|	0.0001
Sign	M	7	Pr >= |M|	0.0013
Signed Rank	S	76.5	Pr >= |S|	0.0003

The first test is the same as the one sample t-test above. The sign test is a more basic non-parametric test which compares the number of values above and below the specified hypothesised value of 5.2 here. The signed rank test is preferable as it looks at the size of the ranking for ordinal or skewed data which has a p-value of 0.0003.

Report: A Wilcoxon signed rank test showed significant evidence (p=0.0003) that the starting cholesterol was above 5.2. However, the boxplot in Figure 2 shows that although most people were above the normal range, not all were classified as having high cholesterol.