

# SPSS Self-learning Practical

# Introduction to Longitudinal Data Analysis

A Biostatistics PGR Development Course FLHR 627

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# <u>Note</u>

The guide in **Sections 3 and 4** are based on SPSS Version 11 for Microsoft Windows. Although the university now uses SPSS version 24, the location of the analysis tools in the drop-down menus remains largely unchanged.

The screenshots in **Section 6** (Linear Mixed Models) are from SPSS Version 24 for Mac OSX.

## 1: Datasets for analysis

# weight.sav

A randomised controlled trial has been carried out to compare the efficacy of two weight management programs. Thirty individuals were randomised to each arm. The response variable is the subjects weight in pounds, measured at 14-day intervals for 18 weeks. There is also a baseline measure of pretreatment weight.

The variables included in the data set are:

a) subject Individual ID number

b) tmt Treatment Group

c) wgt0 Subject's weight at baseline (pre-treatment)

d) wgt14 to wgt126 Subject's weight at 14-day intervals

The aim of the analysis is to see if there is any significant difference in weight loss between the two treatment groups at day 126. There is also interest in any difference in rate of change of weight loss between the treatment groups across the period of the study.

# sleepstudy.sav

The average reaction time per day for subjects in a sleep deprivation study. On day 0 the subjects had their normal amount of sleep. Starting that night they were restricted to 3 hours of sleep per night. The observations represent the average reaction time on a series of tests given each day to each subject.

The variables included in the data set are:

a) Reaction Average reaction time (ms)

b) Days Number of days of sleep deprivation

c) Subject Subject number on which the observation was made

These data are from the study described in Belenky et al. (2003), for the sleep-deprived group and for the first 10 days of the study, up to the recovery period.

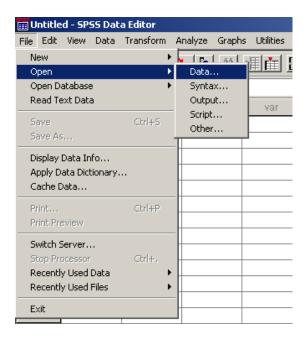
Gregory Belenky, Nancy J. Wesensten, David R. Thorne, Maria L. Thomas, Helen C. Sing, Daniel P. Redmond, Michael B. Russo and Thomas J. Balkin (2003) Patterns of performance degradation and restoration during sleep restriction and subsequent recovery: a sleep dose-response study. *Journal of Sleep Research* 12, 1–12.

## 2: How to access the data and access SPSS

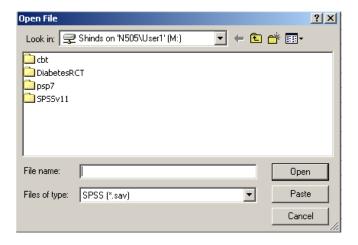
- 1) Open an internet web browser and go to https://github.com/graemeleehickey/FLHR627
- 2) Click on each data file (**weight.sav** and **sleepstudy.sav**) and click the download button.
- 3) Move the files to your own personal space (the **M: drive** if using a university a computer) by dragging them.
- 4) If you are using a university computer, then first check that SPSS is installed. If it is not, click on **Start All Programs Install University Applications Statistics SPSS24**. Wait whilst SPSS is installed and an icon appears on the desktop. Double click on the SPSS icon to start SPSS or **Start All Programs IBM SPSS Statistics SPSS Statistics 24**.
- 5) If you are using your own computer or laptop, the university allows you to download and install it (for both Windows and Mac OSX). Go to the following web site and follow the instructions there: <a href="https://www.liverpool.ac.uk/csd/software/">https://www.liverpool.ac.uk/csd/software/</a>.

## 3: Opening a data file in SPSS

If a dialogue box entitled "SPSS for Windows" appears when you open SPSS, providing options of files to open, move the cursor to 'Cancel' and click the left mouse button. Click the left mouse button on 'File' on the menu bar and move the cursor to 'Open': a new menu appears. Move the curser to 'Data' and left click on this selection.

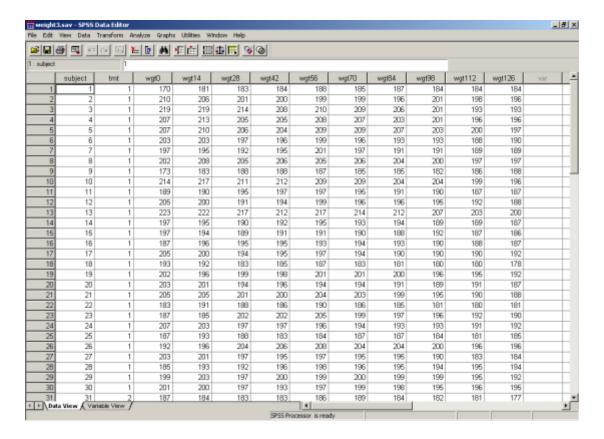


The 'Open File' dialogue box appears. Select the M: drive by clicking on the downward arrow in the 'Look in:' window and scrolling down the list. (Note that the dialogue box below shows folders and files for a personal space on the M: drive. Your own space may appear a little different.) If you are using your own computer instead of a university networked computer, then navigate to the folder where you saved the data from **Section 2** earlier.



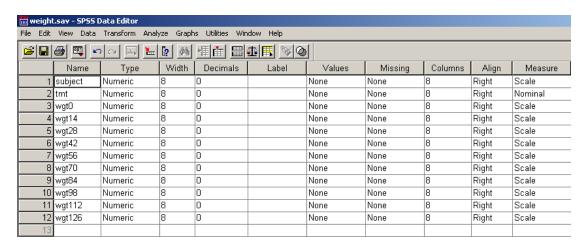
We will demonstrate this with the weight data. Select **weight.sav** by clicking on it and then click the 'Open' button. Alternatively, if using a university networked computer, in the File name window, type in **M:\weight.sav** and

click on 'Open'. The data will appear in the SPSS Data Editor window as below.



Column 1 contains the subject identifier, column 2 the treatment group identifier, column 3 the baseline (pre-treatment) weight and columns 4 to 12 (days 14 to 126) the weight for each subject at each fortnightly follow up.

The SPSS Data Editor contains another window called the 'Variable View'. Move the cursor to the 'Variable View' tab in the lower left corner and click the left button on the mouse to open the window.



This window shows the various attributes associated with each variable in the dataset. These attributes are defined by each of the columns.

- Name is the variable identifier. This cannot exceed eight characters in length.
- **Type** defines whether a variable is numeric or string, or one of a number of other data types (e.g. date).
- Width defines the maximum number of digits/characters that a variable can take. Decimals defines the number of decimal places assigned to a variable.
- **Label** is a descriptive identifier associated with a variable. This can be used to give a more meaningful variable description.
- Values is used to attach labels to the actual data values associated with a variable.
- Missing is used to identify missing data values.
- Columns defines the visible width of the columns in the data editor. If a
  character variable had long 'string' values (such as full names of
  subjects), then the column width may have to be made greater to see
  the whole string.
- **Align** is used to position the data values in the cells. In this case, all the variable values are placed at the right-hand side of the cells.
- Measure defines the type of variable. Possible types are Scale, Nominal and Ordinal. A Scale, or numerical, variable is recorded on an actual numeric scale, such as weight, and is also called continuous or discrete data. A Nominal variable is one that consists of one or more unordered categories (categorical data). Ordinal variables are also categorical data but the ordering has a definite meaning.

We will not spend any more time in the Data View and Variable View windows as we assume you are already familiar with this from other courses run by the Department of Biostatistics, where this is covered in greater detail.

Use Help from the main menu for more information on the Variable View options.

## 4: Methods of analysis

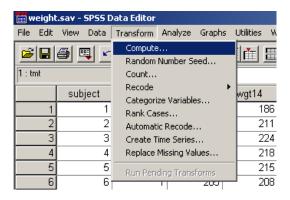
We will use three approaches to analysing these data:

- 1) A simple comparison between the groups of the change in weight from baseline to day 126 (Analysis of Change).
- 2) A comparison of the difference in average weight between the groups at day 126, controlling for any difference in average weight between the groups at baseline, using analysis of covariance (ANCOVA).
- 3) Use of the full eighteen weeks of data using Repeated Measures ANOVA to test for differences between the treatment groups.

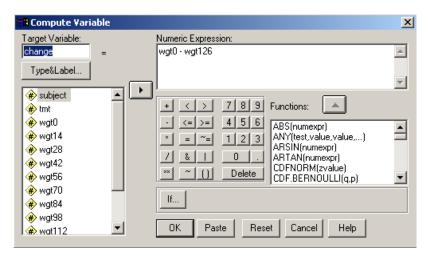
Methods 1) and 2) are reduced data methods, in that they do not use the full repeated measures data. Method 1) is also a simple form of summary measure. Method 3) is the only one of these that uses all the repeated measures data.

#### 4.1 Analysis of change

To obtain the change in weight from baseline to day 126 we need to select **Transform/Compute** from the menu bar in the Data View window.

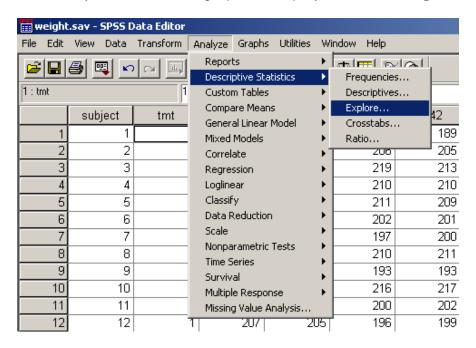


The 'Target Variable:' we will call **change** and the 'Numeric Expression:' is calculated as wgt0 - wgt126.

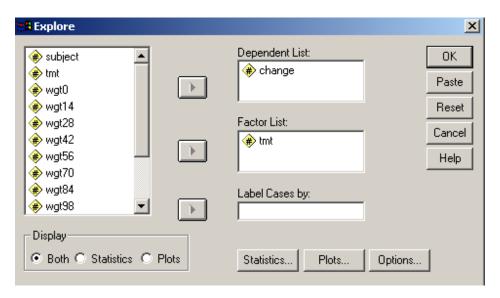


Click OK and the new variable will be appended to the right-hand side of the worksheet.

We can now use **Analyze/Descriptive statistics/Explore** from the menu bar to obtain summary measures and graphical displays for the **change** scores.



Move the **change** variable into the 'Dependent List:' window and put the **tmt** variable in the 'Factor List:' window to identify the treatment groups.



Click OK. If you now look at the SPSS Output window you will see the results of the data exploration procedure, shown below.

# **Explore**

# **TNM**

#### Case Processing Summary

			Cases					
		Va	lid	Miss	sing	Total		
	TMT	N	Percent	N	Percent	N	Percent	
CHANGE	1	30	100.0%	0	.0%	30	100.0%	
	2	30	100.0%	0	.0%	30	100.0%	

#### Descriptives

	TMT			Statistic	Std. Error
CHA NGE	1	Mean		8.28	1.865
		95% Confidence	Low er Bound	4.47	
		Interval for Mean	Upper Bound	12.10	
		5% Trimmed Mean		8.61	
		Median		10.50	
		Variance		104.322	
		Std. Deviation		10.214	
		Minimum		-16	
		Maximum		27	
		Range		42	
		Interquartile Range		14.13	
		Skewness		651	.427
		Kurtosis		.145	.833
	2	Mean		20.57	1.608
		95% Confidence	Low er Bound	17.28	
		Interval for Mean	Upper Bound	23.85	
		5% Trimmed Mean		20.44	
		Median		18.25	
		Variance		77.547	
		Std. Deviation		8.806	
		Minimum		4	
		Maximum		39	
		Range		35	
		Interquartile Range		13.38	
		Skewness		.478	.427
		Kurtosis		500	.833

Note there are no missing data in this analysis.

The descriptive statistics show there was a larger mean change in weight for group 2.

- On average, subjects in both groups lost weight.
- On average, the subjects in treatment group 2 lost 12.29 = 20.57 8.28) pounds more than those in group 1.

If we examine the Stem-and-leaf plots and Box plots (see below) it appears that the distribution of change is reasonably Normal in the two groups, so we can formally test the difference using the t-test.

#### **Stem-and-Leaf Plots**

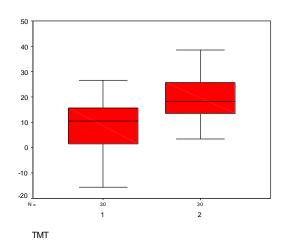
```
CHANGE Stem-and-Leaf Plot for
TMT= 1
```

Frequency	Stem	&	Leaf
1.00 1.00 1.00 2.00 4.00 5.00 7.00 7.00 1.00	-1 -1 -0 -0 0 0 1 1 2 2		5 3 9 34 0112 56789 0001333 5567789 3 6
Stem width:	1	10	) ase(s)

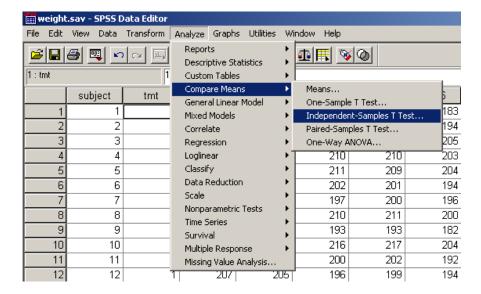
CHANGE Stem-and-Leaf Plot for TMT= 2

Frequency	Stem	&	Leaf
1.00 1.00 6.00 9.00 4.00 2.00 5.00 2.00	0 0 1 1 2 2 3 3		3 9 033333 556677799 0023 55 11234 68

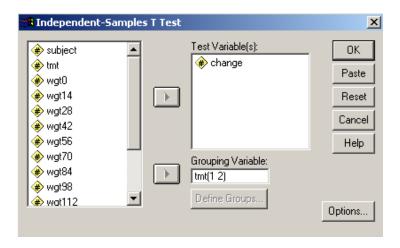
Stem width: 10 1 case(s) Each leaf:



To statistically test the difference between the two groups we will use the independent samples *t*-test. From the menu bar choose **Analyze/Compare Means/Independent Samples T Test**.



In the dialogue box place **change** in the 'Test Variable(s):' window and **tmt** in the 'Grouping Variable:' window, not forgetting to Define Groups (as 1 and 2).



Click OK to obtain the analysis output.

#### T-Test

 Group Statistics

 TMT
 N
 Mean
 Std. Deviation
 Std. Error Mean

 CHANGE
 1
 30
 8.28
 10.214
 1.865

 2
 30
 20.57
 8.806
 1.608

Independent Samples Test Levene's Test for Equality of Variances t-test for Equality of Means 95% Confidence Interval of the Difference Std. Error Mean CHANGE Equal variances -4.989 2.462 -17.212 -7.355 Equal variances -4.989 .000 -12.28 -7.352 56.770 2.462 -17.214 not assumed

This shows that the difference in weight loss between the groups is highly statistically significant. The mean difference in weight loss between treatment group 1 and treatment group 2 is -12.28 pounds (compare with descriptive statistics above), with a 95% confidence interval from -17.212 to -7.355 pounds; P<0.001.

Remember to save the results in the output window to the M: drive if you want to keep them.

#### 4.2 Analysis of covariance (ANCOVA)

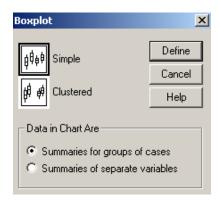
The simple analysis of change in weight between baseline and follow-up does not take account of whether there is a baseline difference in average weight between the groups. ANCOVA tests for a difference in average weight between the two groups at follow-up, controlling for weight at baseline. It is a regression technique that includes a continuous 'covariate' in the model. In this case, the covariate is the variable **wgt0** (weight at baseline).

Firstly, remove the **change** variable from the dataset. In the Data View window, click on the **change** variable name tab at the top of the column, then use **Edit/Clear** from the main menu.

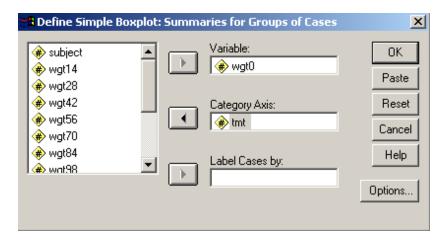
Alternatively, reset the data by selecting **File/Open/Data** from the main menu and re-opening the **weight.sav** dataset. You will be asked if you want to save the contents of the data editor. Click No.

This is merely to prevent the **change** variable from the appearing in the dialogue boxes of subsequent analyses.

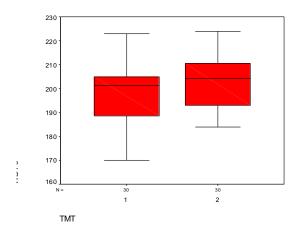
We begin with a graphical exploration of the data. First, we check for baseline balance between the two groups. Choose **Graphs/Boxplot** from the main menu. When the Boxplot dialogue box appears, click on Define.



Select **wgt0** and place in the 'Variable:' window and place **tmt** in the 'Category Axis:' window. Then click OK.

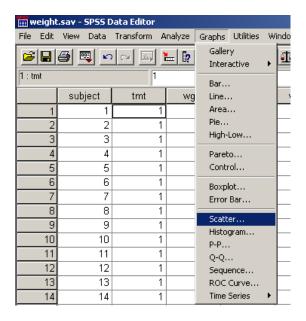


The resulting boxplot confirms that baseline weights are higher on average in treatment group 2. Thus, it may be that treatment group 2 have the potential to lose more weight because they are heavier, which could bias the results in the 'change' analysis above.

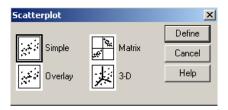


We need to adjust for imbalance to obtain unbiased estimates of differences between the treatment effects at day 126. This is what ANCOVA does.

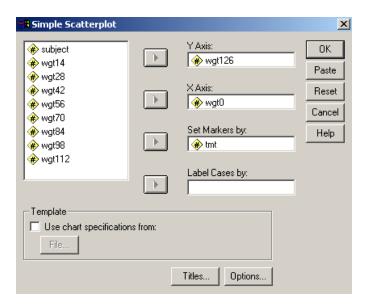
A useful plot when considering ANCOVA is a scatterplot of the outcome **wgt126** (weight at 126 days), against the baseline variable (covariate) **wgt0** (weight at baseline). Choose **Graphs/Scatter** from the main menu.



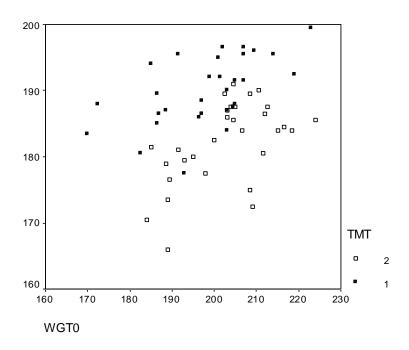
In the 'Scatterplot' dialogue box just click on the Define button. This selects the default 'Simple' scatterplot.



This brings up the 'Simple Scatterplot' dialogue box.



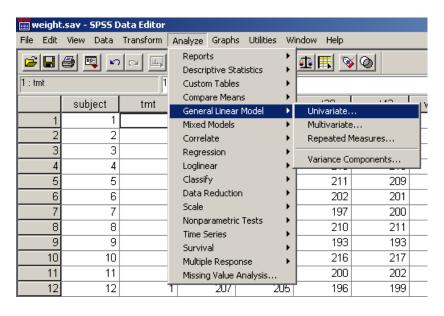
In the dialogue box place the outcome variable **wgt126** in the 'Y Axis:' window and the baseline variable **wgt0** in the 'X Axis:' window. Then place the **tmt** variable in the 'Set Markers by:' window. Click OK to obtain the scatterplot.



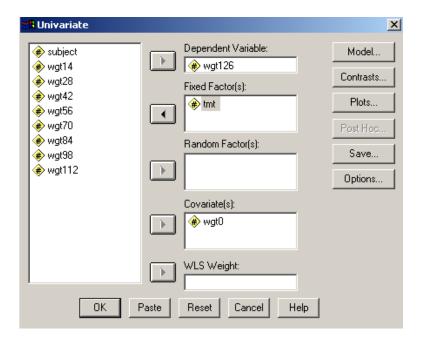
The scatterplot shows a positive correlation between weight at baseline (WGT0) and weight at day 126 (WGT126), i.e. those with higher baseline weight have higher weight at day 126. There is also evidence of a difference between the two treatment groups.

Try adding a title to the plot and labels for the two treatment groups using the Scatterplot 'Titles..' and 'Options..' buttons.

To carry out the ANCOVA, select **Analyze/General Linear Model/Univariate** from the main menu.

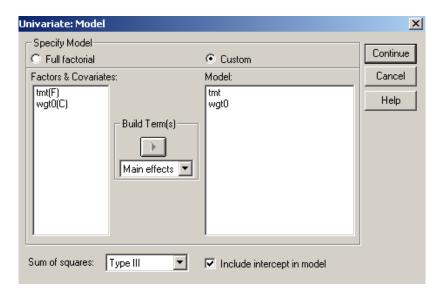


In the 'Univariate' dialogue box, place **wgt126** in the 'Dependent Variable:' window, **tmt** in the 'Fixed Factor(s):' window and **wgt0** in the 'Covariate(s):' window.



Click on the 'Model' button.

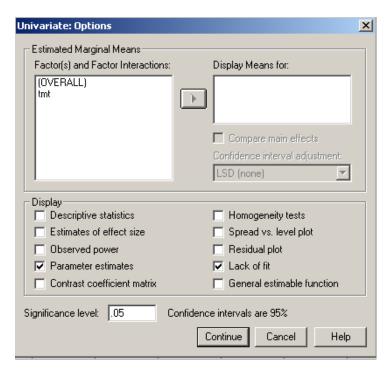
In the 'Univariate: Model' dialogue box, first click on the 'Custom' button. Then, in the 'Build Term(s)' window, select Main effects from the list of options. In the 'Factors & Covariates:' window, select **tmt** and **wgt0** and move across to the 'Model:' window. This defines the model to include main effects only, without interactions.



Note that the 'Sum of squares:' window contains Type III as the default. These represent the contribution of each term to the model when including all other possible terms. Other types represent the effect of adding model terms in different ways (e.g. Type I assesses the contribution by adding terms one at a time in sequence).

Click Continue.

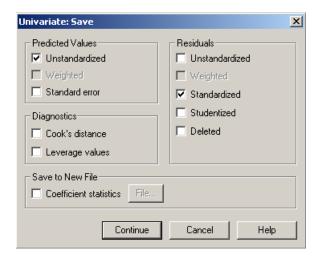
Click on the 'Options' button in the 'Univariate' dialogue box. This opens the 'Univariate: Options' dialogue box.



In the Display menu, select 'Parameter estimates' and 'Lack of fit'. Then click on the Continue button.

Click on the 'Save' button in the 'Univariate' dialogue box.

In the 'Univariate: Save' dialogue box, select 'Unstandardized' from the 'Predicted Values' list and 'Standardized' from the 'Residuals' list. This will save the predicted values from the model and the standardised residuals in two columns of the worksheet in the Data View window. These are used for checking the fit of the model.



Click 'Continue', then click on OK in the 'Univariate' dialogue box to carry out the analysis.

You should obtain the output as shown on the following pages.

# **Univariate Analysis of Variance**

Between-Subjects Factors

		N
TMT	1	30
	2	30

This box is a summary of the numbers included in the analysis for each treatment group. We see there are no missing data in either group and all subjects are included in the analysis.

Tests of Between-Subjects Effects

Dependent Variable: WGT126

Course	Type III Sum	alt	Maan Causes	F	 
Source	of Squares	df	Mean Square	Г	Sig.
Corrected Model	1491.751 <sup>a</sup>	2	745.876	32.019	.000
Intercept	3189.385	1	3189.385	136.915	.000
TMT	1176.607	1	1176.607	50.510	.000
WGT0	583.047	1	583.047	25.029	.000
Error	1327.795	57	23.295		
Total	2079881.750	60			
Corrected Total	2819.546	59			

a. R Squared = .529 (Adjusted R Squared = .513)

The 'Tests of Between-Subjects Effects' box shows that the treatment effect (TMT) is highly significant. R Squared shows that the model explains approximately 53% of the variation in the data, or 51% when adjusted for the number of parameters in the model.

Parameter Estimates

Dependent Variable: WGT126

					95% Confidence Interval		
Parameter	В	Std. Error	t	Sig.	Low er Bound	Upper Bound	
Intercept	125.878	11.286	11.154	.000	103.279	148.477	
WGT0	.278	.055	5.003	.000	.167	.389	
[TMT=1]	9.033	1.271	7.107	.000	6.488	11.578	
[TMT=2]	0 <sup>a</sup>						

a. This parameter is set to zero because it is redundant.

The 'Parameter Estimates' box shows that, at day 126, the weight of treatment group 1 [TMT=1] is, on average, 9.03 pounds greater than treatment group 2.

WGT0 is the parameter for the fitted linear relationship between weight at day 126 and weight at day 0 (baseline). This shows a positive linear association between weight at baseline and weight at day 126. We noted this earlier when examining the scatterplot of the **wgt126** variable against the **wgt0** variable.

Lack of Fit Tests

Dependent Variable: WGT126

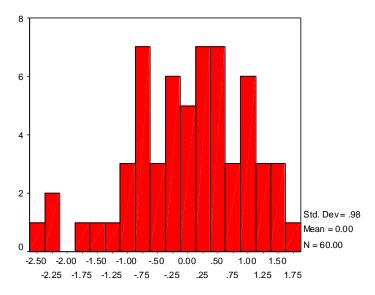
Source	Sum of Squares	df	Mean Square	F	Sig.
Lack of Fit	1128.670	46	24.536	1.355	.303
Pure Error	199.125	11	18.102		

In fitting any regression model, we need to assess the goodness-of-fit of the model.

The 'Lack of Fit Tests' box gives an *F-test* for whether the relationship between the dependent variable and the independent variables can be adequately described by the model. The null hypothesis for the F-test is that the model adequately fits the data. The test result gives a value of 1.355 for F, with a *P*-value of 0.303. The *P*-value is greater than 0.05 so we do not reject the null hypothesis of an adequate fit.

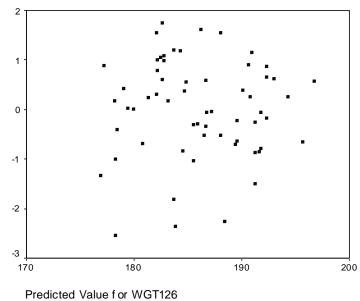
Model fit is also assessed by checking the distribution of the residuals, which should have a standard Normal distribution, with mean at 0 and SD close to 1.

From the main menu choose **Graphs/Histogram**. Place 'Standardized Residual (zre\_1)' in the 'Variable' window and click OK. The histogram of the residuals shows they are reasonably Normally distributed. This is another indication that the model is a reasonable fit to the data.



Standardized Residual for WGT126

Another useful plot to look at is a scatterplot of the standardised residuals (zre\_1) against predicted values (pre\_1). Try to create this scatterplot yourself- use the instructions provided on pages 13 & 14 if you need help.



In this plot, we are looking for a random scatter of data. There are no obvious patterns or trends in the plot, again confirming a reasonable fit to the data.

In addition to looking at the overall fit of the model, it is useful to identify particular data points (i.e. subjects) that may be exerting an undue influence on the fitted model.

Scatterplots of 'leverage' and 'Cook's distance' against 'subject' produce diagnostic plots which are useful for identifying outlying points.

We will not look into this here, but it may be worth noting that these issues are covered in more detail in the Logistic Regression workshop.

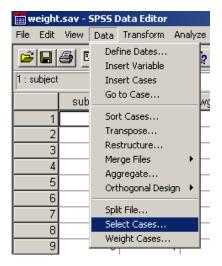
Save the results in the Output window to the M: drive if you want to keep them.

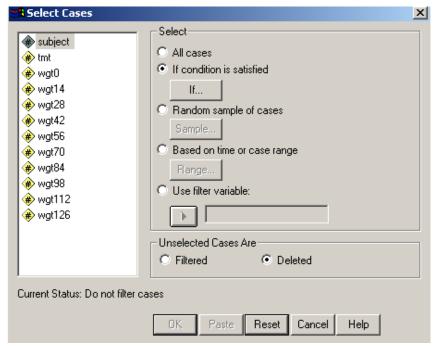
#### 4.3 Repeated Measures ANOVA

Before beginning this section, reset the data by selecting File/Open/Data from the main menu and re-opening the weight.sav dataset. This discards the variables created during the previous analysis. You will be asked if you want to save the contents of the data editor. Click No.

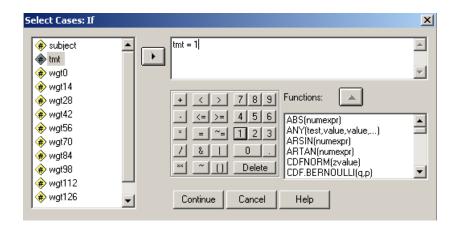
Note that, in comparison to the previous analyses 1) and 2), which are reduced data methods, this method uses all of the data at all of the time points. It does however make certain assumptions of the data, to be checked in the analysis.

A useful way to begin the analysis is to examine the data graphically. A common procedure is to produce a plot of individual profiles, sometimes called a 'Trellis plot'. This is an overlay line-plot of outcome versus follow-up time for each subject in the data. We will produce a Trellis plot for each of the treatment groups in turn. To do this, you need to use **Data/ Select Cases** from the main menu to select the treatment group.





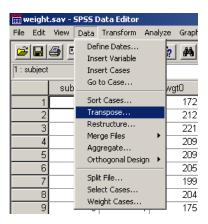
In the 'Select Cases' dialogue box, click on the 'If condition is satisfied' button. Then, in the 'Unselected Cases Are' box, click on the 'Deleted' button. Then click on the 'If...' button under the 'If condition is satisfied' radio button. This brings up the 'Select Cases: If' dialogue box.



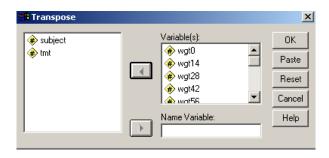
Enter **tmt = 1** in the upper right window. Click Continue to return to main dialogue box, then click OK to select the treatment group. Treatment group 2 will be excluded from any further analyses.

**IMPORTANT:** do not save the data at any time during these operations, as you may accidentally replace and lose your original **weight.sav** dataset.

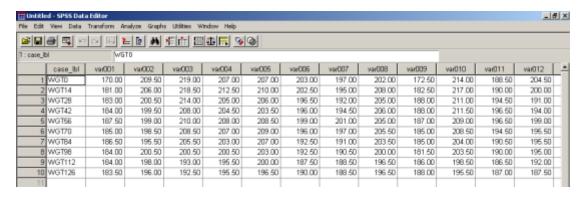
To produce a trellis plot in SPSS we first need to transpose the data, such that the variables in columns become rows and the cases in rows become columns. Choose **Data/Transpose** from the menu.



In the 'Transpose' dialogue box, move all the 'wgt' variables to the 'Variable(s):' window. To do this in one operation first select **wgt0**, then while simultaneously holding down the shift/control keys, click on **wgt126**. This selects all the 'wgt' variables which can then be moved across to the 'Variable(s):' window with one click on the arrow button.

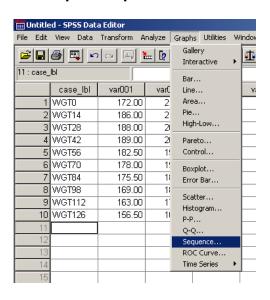


Click OK. A warning box may appear telling you that 'Some variables are not selected for transposition. Un-transposed variables will be lost' Click ok and the warning box will disappear. The dataset now appears as follows (note: only part of the dataset is in view).

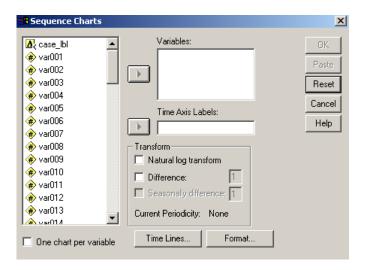


There is a variable (case\_lbl) identifying the follow-up occasions. The remainder of the variables correspond to each subject in the data, containing subject weight at each follow up time.

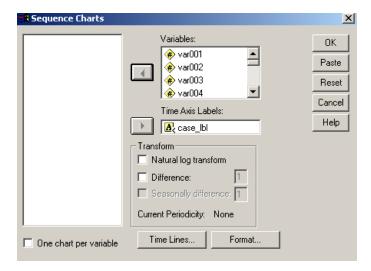
To create the plot, select **Graphs/Sequence** from the main menu.



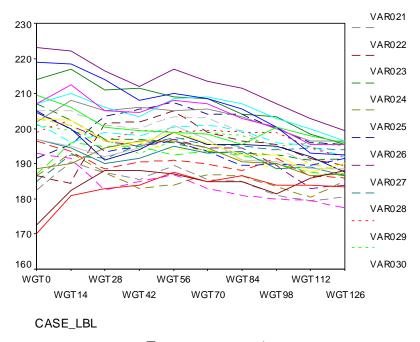
The Sequence Charts dialogue box appears as follows.



Move the 'case\_lbl' variable to the 'Time Axis' window and all other variables (var001 to var030) to the 'Variables' window. To move these in one operation first select **var001**, then while simultaneously holding down the shift/control keys, click on **var030**. This selects all the 'var' variables, which can be moved across to the 'Variables' window with one click on the arrow button. Click OK.



The Trellis plot for treatment group one appears in the Output window.



Treatment group 1

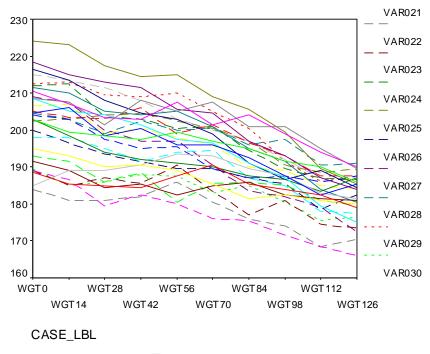
Although Trellis plots can appear rather messy, especially with many subjects, general trends in the data can be perceived. For very large numbers of subjects it might be more useful to take a random sample from them before producing a Trellis plot.

In this plot, there are various patterns of change over the first 42 days, followed by a downward trend.

Re-open the **weight.sav** dataset by using **File/Open/Data** from the main menu. You will be asked if you want to save the contents of the data editor. Click No.

Now select treatment group 2, using **Data/Select Cases** from the main menu as previously. Once the group has been selected, use **Data/Transpose** followed by **Graphs/Sequence**, as previously, to produce a Trellis plot for this group.

The Trellis plot for treatment group 2 is as follows.



Treatment group 2

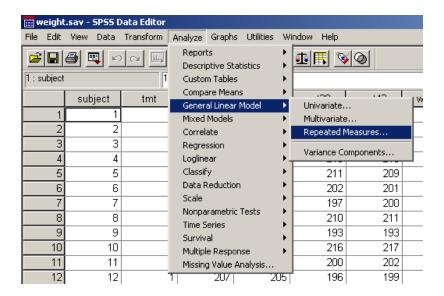
Different patterns of change are discernible between the treatment groups. Group 2 appears to show a downward trend immediately upon treatment.

Trellis plots are also useful for assessing variability in the data. For both treatment groups, there is less variability between subjects at the end of the trial than at the beginning, particularly in group 2.

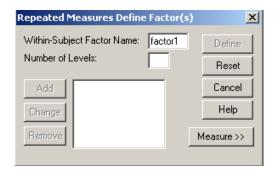
Re-open the **weight.sav** dataset by using **File/Open/Data** from the main menu. You will be asked if you want to save the contents of the data editor. Click No.

Save the results and graphs in the Output window if you wish.

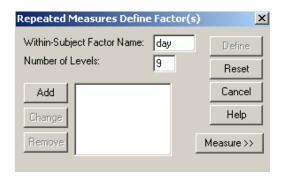
To carry out the repeated measures analysis, choose **Analyze/General Linear Model/Repeated Measures** from the main menu.



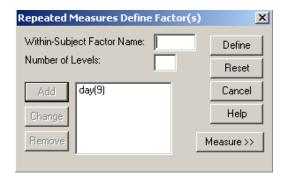
The Repeated Measures Define Factors dialogue box appears. There are two factors in the data: a between-subjects factor and a within-subjects factor. The between-subjects factor is the treatment group variable (tmt), the within-subjects factor is composed of the nine post-treatment 'wgt' variables. The within-subjects factor has to be defined in the Define Factors dialogue box.



Enter 'day' for the 'Within-Subject Factor Name'. This gives a meaningful identifier, since weight is measured at ten follow-ups defined in terms of number of days since start of trial. Enter 9 for the 'Number of Levels'. The number of levels in this case is the number of follow-ups from baseline since the start of the trial.



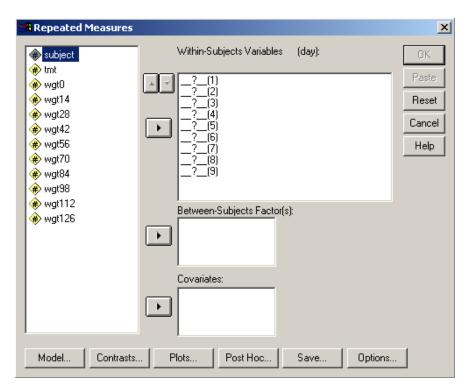
Click the Add button.



When the factor has been Added, click on the 'Define' button.

The Repeated Measures dialogue box appears.

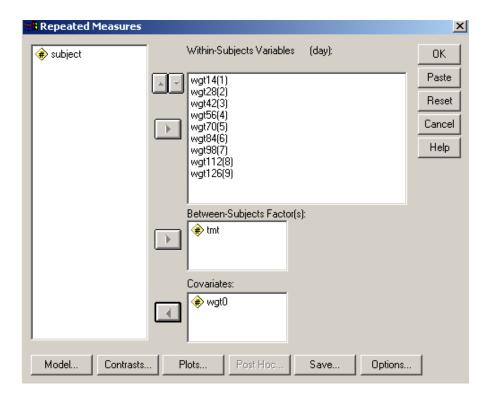
Note that the 'Within-Subjects Variables' are an ordered set of time points. The variables have to be entered in the correct time order. In the **weight.sav** dataset, the 'wgt' variables are already ordered by time (from 0 days to 126 days).



Select each of the 'wgt' variables from wgt14 to wgt126 and move across to the appropriate place in the 'Within-Subjects Variables' window. Because the 'wgt' variables are already time ordered, you can do this in one by first selecting **wgt14**, then while holding down the shift/control keys, click on **wgt126**. This selects the block of variables. Then click on the arrow to move the selected block to the 'Within-Subjects Variables:' window.

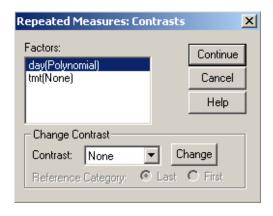
Next, click on **tmt** and move to the 'Between-Subjects Factors(s):' window. Then click on **wgt0** and move to the 'Covariates:' window.

The 'Repeated Measures' dialogue box should now appear as follows.

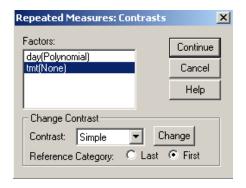


Click on the 'Contrasts' button.

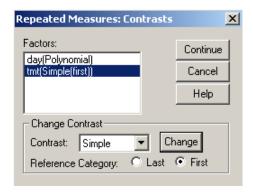
The 'Repeated Measures: Contrasts' dialogue box is used for defining how the levels of the factors in the model are to be compared.



We want to compare treatment 2 against treatment 1. Click on **tmt** in the 'Factors:' window. In the 'Change Contrast' box, click on the menu select arrow in the 'Contrast:' window and select Simple from the menu. Then click on the 'First' button.



Click on the 'Change' button to put the contrast into effect. You will see the selected contrast appear next to the **tmt** variable in the 'Factors:' window.

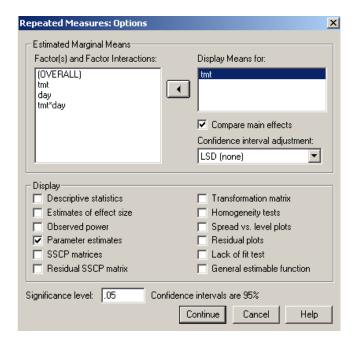


Click 'Continue' to return to the 'Repeated Measures' dialogue box.

Click on the 'Options' button.

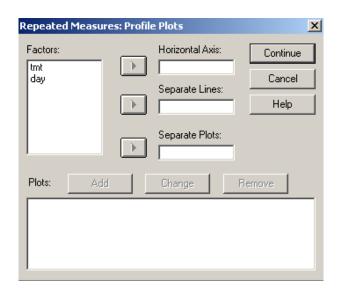
The 'Repeated Measures: Options' dialogue box appears. In the 'Estimated Marginal Means' box, select **tmt** from the 'Factor(s) and factor Interactions' window and move to the 'Display Means for' window. Select 'Compare main effects'.

Click on 'Parameter estimates' in the 'Display' menu box.

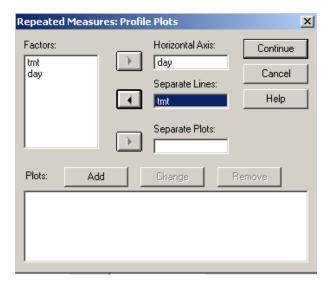


Note that the 'Repeated Measures: Options' dialogue box is the same as the 'Univariate: Options' dialogue box that we used for ANCOVA. This is because these methods all fall under the umbrella of what is called the **General Linear Model (GLM)**. Not all of the 'Display' options are necessary for all the types of possible analyses; Univariate, Multivariate and Repeated Measures. Click on the 'Help' button for more information on the options.

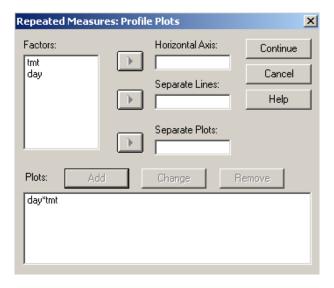
Click 'Continue' to return to 'Repeated Measures'. Then click on 'Plots'. The 'Repeated Measures: Profile Plots' box appears.



Select **day** from the 'Factors:' window and place in the 'Horizontal Axis:' window. Select **tmt** from the 'Factors:' window and place in the 'Separate Lines:' window.



Click on the 'Add' button to action the operation.



Click on 'Continue' to return to the 'Repeated Measures' dialogue box.

Click the OK button to carry out the analysis.

The output on the following pages should be obtained.

#### **General Linear Model**

#### Within-Subjects Factors

Measure: MEASURE 1

MCGSGIC. MID (OCIVE_1				
DAY	Dependent Variable			
1	WGT14			
2	WGT28			
3	WGT42			
4	WGT56			
5	WGT70			
6	WGT84			
7	WGT98			
8	WGT112			
9	WGT126			

#### Between-Subjects Factors

		N
TMT	1	30
	2	30

These boxes identify the variables associated with the Within-Subjects Factors and the Between-Subjects Factors.

#### Notes:

- MEASURE\_1 represents the set of outcome observations on each individual.
- ii. The Between-Subjects factors box shows there are no missing data in this study.

Multivariate Test\$

Effect		Value	F	Hypothesis df	Error df	Sig.
DAY	Pillai's Trace	.618	10.127 <sup>a</sup>	8.000	50.000	.000
	Wilks' Lambda	.382	10.127 <sup>a</sup>	8.000	50.000	.000
	Hotelling's Trace	1.620	10.127 <sup>a</sup>	8.000	50.000	.000
	Roy's Largest Root	1.620	10.127 <sup>a</sup>	8.000	50.000	.000
DAY * WGT0	Pillai's Trace	.673	12.845 <sup>a</sup>	8.000	50.000	.000
	Wilks' Lambda	.327	12.845 <sup>a</sup>	8.000	50.000	.000
	Hotelling's Trace	2.055	12.845 <sup>a</sup>	8.000	50.000	.000
	Roy's Largest Root	2.055	12.845 <sup>a</sup>	8.000	50.000	.000
DAY * TMT	Pillai's Trace	.464	5.416 <sup>a</sup>	8.000	50.000	.000
	Wilks' Lambda	.536	5.416 <sup>a</sup>	8.000	50.000	.000
	Hotelling's Trace	.867	5.416 <sup>a</sup>	8.000	50.000	.000
	Roy's Largest Root	.867	5.416 <sup>a</sup>	8.000	50.000	.000

a. Exact statistic

b.

Design: Intercept+WGT0+TMT Within Subjects Design: DAY

Multivariate tests are part of the GLM output, but are more appropriate and useful when fitting models for multivariate responses. The statistics show a highly significant effect of time (the DAY Effect), a highly significant interaction between the treatments and time (DAY\*TMT, i.e. the treatment effects differ over time) and a highly significant interaction between baseline-weight and weight over time (DAY\*WGT0, i.e. weight-change depends on baseline weight).

#### Mauchly's Test of Sphericity

Measure: MEASURE_1								
						Epsilon <sup>a</sup>		
		Approx.			Greenhous			
Within Subjects Effect	Mauchly's W	Chi-Square	df	Sig.	e-Geisser	Huynh-Feldt	Low er-bound	
DAY	.023	204.919	35	.000	.469	.524	.125	

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

b.

Design: Intercept+WGT0+TMT Within Subjects Design: DAY

An assumption underlying repeated measures ANOVA is that the repeated measures on an individual are equally correlated across time (sphericity). This is the null hypothesis for the Mauchly test. The test result rejects the null hypothesis. This means the assumption of sphericity required for the repeated measures ANOVA does not hold for this data set. Mauchly's test is known to be limited when the data are non-normally distributed and so it is always advised to consider the magnitude of 'epsilon' as well (particularly when Mauchly's test does not reject the null hypothesis).

Epsilon can be thought of as being similar to correlation. The closer that epsilon is to 1.00 the more homogenous the variances of the differences, and hence the closer the data are to being spherical. As you can see from the above table there are three types of 'epsilon' to choose from. The difference between these epsilons is the extent to which each assumes violation of sphericity.

The Lower-bound epsilon assumes maximum violation of sphericity and will always be lower than the other two epsilons followed by Greenhouse-Geisser, and then Huynh-Feldt. In the absence of a 'gold-standard' epsilon that is accurate across all degrees of sphericity we suggest the following simple rule: if the Greenhouse-Geisser epsilon is below 0.75, use this epsilon to correct the analysis, if the Greenhouse-Geisser epsilon is above 0.75 use the Huynh-Feldt epsilon.

Epsilons are not only used as an indication of the extent of violation of sphericity but are also used to correct for that violation. The following table shows the results of applying each epsilon to correct the *F*-test. The correction is important so our probability of a false positive result (Type 1 error) is not too high.

For our data, the Greenhouse-Geisser estimate of epsilon is 0.469. This is below 0.75 so we will use the *F*-test correction corresponding to Greenhouse-Geisser. This means we are only interested in the rows of the table labelled Greenhouse-Geisser.

a. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in t Tests of Within-Subjects Effects table.

#### Tests of Within-Subjects Effects

Measure: MEASURE\_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
DAY	Sphericity Assumed	1418.345	8	177.293	23.626	.000
	Greenhouse-Geisser	1418.345	3.755	377.704	23.626	.000
	Huynh-Feldt	1418.345	4.194	338.182	23.626	.000
	Low er-bound	1418.345	1.000	1418.345	23.626	.000
DAY * WGT0	Sphericity Assumed	1884.001	8	235.500	31.383	.000
	Greenhouse-Geisser	1884.001	3.755	501.708	31.383	.000
	Huynh-Feldt	1884.001	4.194	449.210	31.383	.000
	Low er-bound	1884.001	1.000	1884.001	31.383	.000
DAY * TMT	Sphericity Assumed	889.432	8	111.179	14.816	.000
	Greenhouse-Geisser	889.432	3.755	236.855	14.816	.000
	Huynh-Feldt	889.432	4.194	212.071	14.816	.000
	Low er-bound	889.432	1.000	889.432	14.816	.000
Error(DAY)	Sphericity Assumed	3421.875	456	7.504		
	Greenhouse-Geisser	3421.875	214.045	15.987		
	Huynh-Feldt	3421.875	239.060	14.314		
	Low er-bound	3421.875	57.000	60.033		

'Tests of Within-Subjects Effects' gives *F*-tests for the weight-change across time (the DAY factor), the interaction between treatment and weight-change across time (DAY\*TMT) and the interaction between weight-change and baseline-weight (DAY\*WGT0). Tests are given for 'Sphericity Assumed' and, where this cannot be assumed, the 'Greenhouse-Geisser', 'Huynh-Feldt' and Lower-bound corrections. In this case, we cannot assume sphericity and have to use the corrected tests. The *F*-tests using the Greenhouse-Geisser correction show that the factor effects are highly significant.

In fact, all the corrected *F*-tests show highly significant factor effects. The 'Lower-bound' test is the most stringent and the effects are still highly significant, so we can be confident that the effects have statistically significant contributions to the model.

Tests of Within-Subjects Contrasts

Measure: MEASURE 1

Measure: MEA	TOOKL_1	Type III Sum				
Source	DAY	of Squares	df	Mean Square	F	Sig.
DAY	Linear	1311.126	1	1311.126	54.604	.000
	Quadratic	19.803	1	19.803	1.388	.244
	Cubic	69.467	1	69.467	10.280	.002
	Order 4	13.729	1	13.729	3.757	.058
	Order 5	.514	1	.514	.144	.706
	Order 6	.511	1	.511	.226	.636
	Order 7	.417	1	.417	.138	.712
	Order 8	2.778	1	2.778	1.110	.297
DAY * WGT0	Linear	1775.648	1	1775.648	73.950	.000
	Quadratic	10.576	1	10.576	.741	.393
	Cubic	69.883	1	69.883	10.341	.002
	Order 4	22.890	1	22.890	6.264	.015
	Order 5	.973	1	.973	.273	.603
	Order 6	.312	1	.312	.138	.712
	Order 7	.842	1	.842	.279	.600
	Order 8	2.878	1	2.878	1.150	.288
DAY * TMT	Linear	841.212	1	841.212	35.034	.000
	Quadratic	2.246	1	2.246	.157	.693
	Cubic	22.224	1	22.224	3.289	.075
	Order 4	1.774	1	1.774	.485	.489
	Order 5	1.448	1	1.448	.406	.526
	Order 6	5.738	1	5.738	2.542	.116
	Order 7	3.036	1	3.036	1.005	.320
	Order 8	11.755	1	11.755	4.697	.034
Error(DAY)	Linear	1368.661	57	24.012		
	Quadratic	813.108	57	14.265		
	Cubic	385.190	57	6.758		
	Order 4	208.289	57	3.654		
	Order 5	203.127	57	3.564		
	Order 6	128.682	57	2.258		
	Order 7	172.161	57	3.020		
	Order 8	142.657	57	2.503		

This table gives results from an alternative procedure for testing effects, based on a set of transformed variables. However, these tests are associated with a multivariate approach to analysing repeated measures data and will not be pursued here.

Tests of Between-Subjects Effects

Measure: MEASURE\_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	12611.426	1	12611.426	82.047	.000
WGT0	19532.274	1	19532.274	127.072	.000
TMT	3825.443	1	3825.443	24.887	.000
Error	8761.467	57	153.710		

'Tests of Between-Subjects Effects' is a test of whether there is an overall difference between the treatments (TMT) across time. The P-value (Sig. = 0.000) shows there is a highly significant effect and we can reject the null hypothesis of no difference between the treatments.

#### Parameter Estimates

						95% Confide	ence Interval
Dependent Variable	Parameter	В	Std. Error	t	Sig.	Low er Bound	Upper Bound
WGT14	Intercept	25.245	7.930	3.184	.002	9.366	41.125
	WGT0	.867	.039	22.236	.000	.789	.945
	[TMT=1]	2.335	.893	2.615	.011	.547	4.124
	[TMT=2]	0 <sup>a</sup>					
WGT28	Intercept	59.997	11.669	5.142	.000	36.631	83.364
	WGT0	.679	.057	11.826	.000	.564	.793
	[TMT=1]	2.320	1.314	1.766	.083	311	4.952
	[TMT=2]	0 <sup>a</sup>		,			
WGT42	Intercept	75.681	11.750	6.441	.000	52.153	99.209
	WGT0	.601	.058	10.394	.000	.485	.716
	[TMT=1]	2.102	1.323	1.589	.118	547	4.752
	[TMT=2]	0 <sup>a</sup>		,			
WGT56	Intercept	80.867	12.260	6.596	.000	56.316	105.418
	WGT0	.570	.060	9.450	.000	.449	.690
	[TMT=1]	4.814	1.381	3.486	.001	2.049	7.579
	[TMT=2]	0 <sup>a</sup>		,			
WGT70	Intercept	81.202	12.049	6.740	.000	57.075	105.329
	WGT0	.559	.059	9.427	.000	.440	.677
	[TMT=1]	5.147	1.357	3.793	.000	2.430	7.864
	[TMT=2]	0 <sup>a</sup>					
WGT84	Intercept	93.257	12.376	7.535	.000	68.474	118.039
	WGT0	.480	.061	7.888	.000	.358	.602
	[TMT=1]	6.794	1.394	4.874	.000	4.003	9.585
	[TMT=2]	0 <sup>a</sup>					
WGT98	Intercept	99.493	11.353	8.764	.000	76.760	122.226
	WGT0	.436	.056	7.804	.000	.324	.547
	[TMT=1]	7.394	1.278	5.783	.000	4.834	9.954
	[TMT=2]	0 <sup>a</sup>		,			
WGT112	Intercept	111.821	11.255	9.935	.000	89.284	134.358
	WGT0	.353	.055	6.382	.000	.242	.464
	[TMT=1]	8.923	1.267	7.040	.000	6.385	11.461
	[TMT=2]	0 <sup>a</sup>					
WGT126	Intercept	125.878	11.286	11.154	.000	103.279	148.477
	WGT0	.278	.055	5.003	.000	.167	.389
	[TMT=1]	9.033	1.271	7.107	.000	6.488	11.578
	[TMT=2]	0 <sup>a</sup>					

 $<sup>\</sup>ensuremath{\text{a.}}$  This parameter is set to zero because it is redundant.

The Parameter Estimates table gives results of tests for each of the dependent variables at each time point. Note that WGT126 is the ANCOVA we produced earlier. The results of the tests for treatment (TMT=1) show a significant difference between the treatment groups apart from day 28 (WGT28) and day 42 (WGT42).

Note that these are separate tests carried out at each time point, so there is the issue of multiple testing to be considered. We can adjust by using the Bonferroni correction, which adjusts the significance level by dividing it by the number of tests. There are nine separate tests of treatment effect, so the adjusted significance level is 0.05/9 = 0.0056 to 4dp. Only *P*-values less than this are statistically significant. Using this correction, the difference at day 14 (WGT14) is not significant, so significant change only occurs after day 42 (WGT42). However, Bonferroni's correction is known to be conservative.

### **Custom Hypothesis Tests**

#### Contrast Results (K Matrix)

			Averaged Variable
TMT Simple Contrast <sup>a</sup>			MEASURE_1
Level 2 vs. Level 1	Contrast Estimate		-5.429
	Hypothesized Value  Difference (Estimate - Hypothesized)		0
			-5.429
	Std. Error		1.088
	Sig.		.000
	95% Confidence Interval	Low er Bound	-7.608
	for Difference	Upper Bound	-3.250

a. Reference category = 1

#### **Test Results**

Measure: MEASURE\_1

Transformed Variable: AVERAGE

Source	Sum of Squares	df	Mean Square	F	Sig.
Contrast	425.049	1	425.049	24.887	.000
Error	973.496	57	17.079		

The *F*-test in the 'Test results' table is the same as in the 'Tests of Between-Subjects Effects' table seen previously. It appears again here because we requested a specific contrast between the treatment groups. This is in the 'Contrast Results' table, which gives the result of comparing Treatment 2 (Level 2) against Treatment 1 (Level 1). On average, Treatment group 2 weigh 5.429 lb less than Treatment group 1 across the study period.

Contrasts become more useful when we wish to compare more than two treatments or groups.

Note that the F-test is based on means of the responses on each individual over the period of the study. The response of each individual is summarised by a mean response. This is essentially a one-way analysis of variance of the means of the responses on each individual and is essentially a summary measure approach.

# **Estimated Marginal Means TMT**

#### Estimates

Measure: MEASURE\_1

Micaedare: Mill reer te_1							
			95% Confidence Interval				
TMT	Mean	Std. Error	Low er Bound	Upper Bound			
1	196.539 <sup>a</sup>	.762	195.013	198.065			
2	191.110 <sup>a</sup>	.762	189.584	192.636			

a. Evaluated at covariates appeared in the model: WGT0 = 200.48.

#### Pairwise Comparisons

Measure: MEASURE\_1

		Mean Difference			95% Confiden Differ	
(I) TMT	(J) TMT	( <b>⊦</b> J)	Std. Error	Sig. <sup>a</sup>	Lower Bound	Upper Bound
1	2	5.429*	1.088	.000	3.250	7.608
2	1	-5.429*	1.088	.000	-7.608	-3.250

Based on estimated marginal means

- \*. The mean difference is significant at the .05 level.
- Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

The 'marginal means are the overall mean values for weight across the 126 days of the study following treatment intervention, obtained from the individual profile means mentioned previously. The 'Estimates' table gives the overall mean values for the two treatment groups. We see that the overall mean weight in treatment group 1 is higher than the overall mean weight in treatment group 2 (195.5 lb v 191.1 lb).

The 'Pairwise Comparisons' table gives the difference between the means of the two treatment groups, together with the significance of the difference and 95% confidence interval for the difference. Note that this is the same difference as in the 'Contrast results' table from the Custom Hypothesis Tests output.

#### **Univariate Tests**

Measure: MEASURE 1

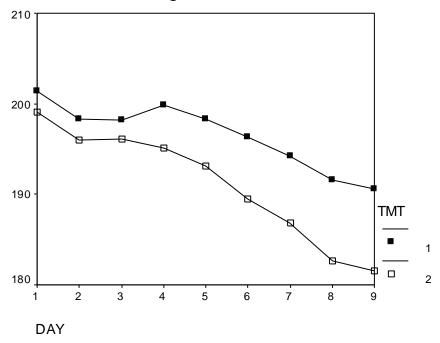
Weasure: WEASONE_1							
	Sum of Squares	df	Mean Square	F	Sig.		
Contrast	425.049	1	425.049	24.887	.000		
Error	973.496	57	17.079				

The F tests the effect of TMT. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

This also gives the result of the *F*-test for the significance of the overall mean difference between treatment groups, as in the custom hypothesis test and between-subjects tests seen earlier.

#### **Profile Plots**

# Estimated Marginal Means of MEASURE\_1



Estimated marginal means give estimates of predicted mean values from the model for each follow-up time, and the profile plot of these means allows you to visualize the relationships.

Note that the default output for the DAY axis only gives factor level identifiers and not actual day values. Also, note that day 0 (baseline) does not appear in the graph as it was fitted as a covariate in the model, not as a response.

From the graph, we can see how the rate of change of weight differs between the two groups, the rate of change being greater for treatment group 2 after the third follow-up (day 42).

The simple change analysis shows the largest difference between the two treatment groups. However, because the groups were imbalanced at baseline, the comparison is biased.

ANCOVA is a test for difference between the groups at day 126 that adjusts for baseline imbalance. The bias is removed and the treatment difference is adjusted.

Repeated Measures ANOVA tests for a difference between the average treatment effects across the whole-time period and gave a significant result. There is a significant interaction between DAY and TMT, which means that the rate of change (i.e. the slopes) of the two treatments differs across time. The statistical tests showed that the interaction is significant. Treatment group 2 loses weight at a faster rate than Treatment group 1. From analysis at each

time point, the mean weight in Treatment group 2 is significantly less than in Treatment group 1 after day 42.

### 5. Observations

When analysing change between baseline and final follow-up, ANCOVA is the generally preferred method. It adjusts for bias due to baseline imbalance and in most situations, has greater power for detecting a difference between groups.

Repeated measures ANOVA uses all the information in a follow-up study that has more than two visits, but the question it answers is rather different than ANCOVA. In repeated measures ANOVA, the focus is on the average effect across the whole follow-up period and differences between groups are tested in terms of the average effect.

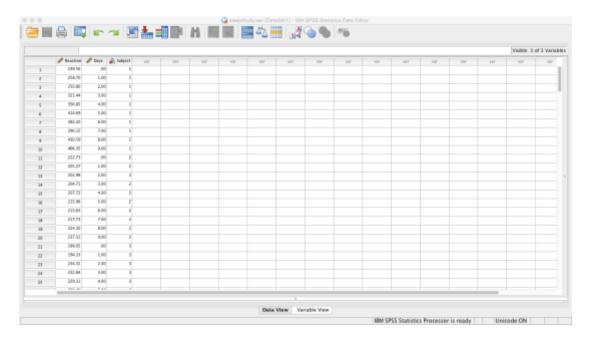
If the research question is focused on size of differences at the end of the follow-up period, then repeated measures ANOVA does not answer this and ANCOVA should be used. Note that the Parameter Estimates table on page 38 is effectively a set of separate ANCOVAs, each with one of the follow-up days as the outcome and each controlling for baseline weight. The final row of this table is the ANCOVA for day 126 and gives the same results as the ANCOVA we carried out in **Section 4.2**. However, the primary research question of interest should be decided at the study design stage and not based on a *post hoc* examination of analysis results.

Another question answered by repeated measures ANOVA is the rate-ofchange across time. For some group or treatment comparisons the rate-ofchange may be an important issue. In this analysis, we found that treatment group 2 lost weight at a greater rate than treatment group 1.

Repeated measures ANOVA requires complete data. Any subjects with <u>any</u> missing data are excluded from the analysis. Studies with missing data require more complex methods for their analysis.

## 6. Linear mixed effects models

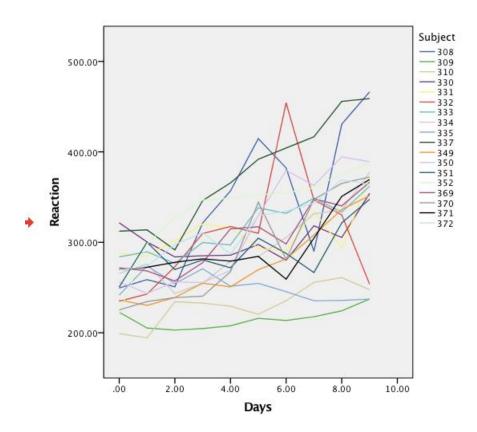
We will now fit a linear mixed effects model (LMM) to the sleep study data. We begin by opening the data **sleepstudy.sav** following the same instructions as given in **Section 3**. We will then see the following screen.



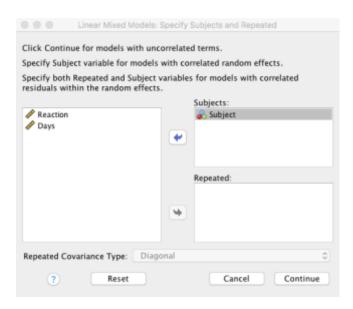
There are 3 columns and 180 rows:

- Reaction: the reaction time measured in milliseconds.
- **Days**: the number of days from follow-up, with 0 denoting baseline (the day before the first sleep deprivation).
- **Subject**: a subject ID number.

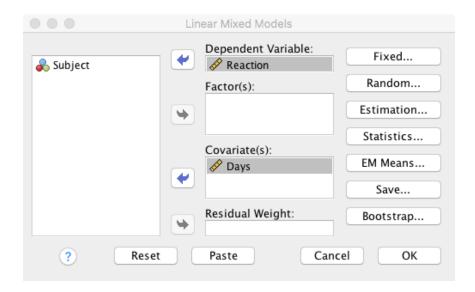
We can perform exploratory data analyses as before, including plotting individual subject profile plots.



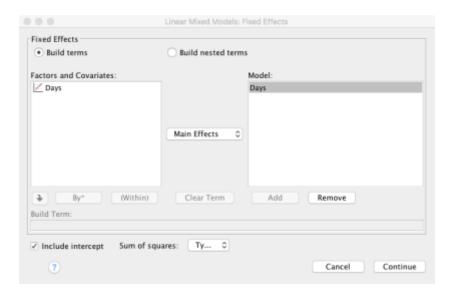
To fit a LMM we select **Analyze – Mixed Models – Linear**. On the Linear Mixed Models panel, we move Subject into the **Subjects:** box and click the **Continue** button. Note that we leave the **Repeated:** box empty (this is for specifying residual correlation structures).



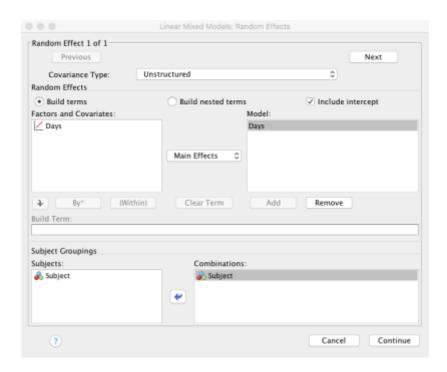
On the following screen we move Reaction into the **Dependent Variable:** box (this is our outcome), and move Days into the **Covariate(s):** box (this is out continuous time measure).



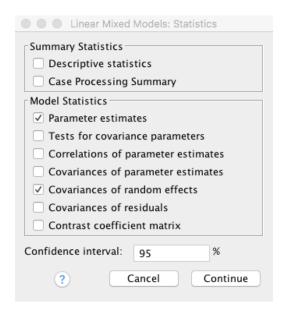
We next specify the fixed and random effects parts of the model. First, we click on **Fixed...** and add Days as main effect into the model.



After clicking **Continue**, we return back to the model screen where we click the **Random...** button. Here, we add Days into the model as a random effect (as we want random slopes) and tick the **'Include Intercept'** box (as we also want random intercepts). We want our random intercepts and slopes to be correlated, therefore we must also select **Covariance Type:** as 'Unstructured' from the drop-down menu. Finally, we need to specify the clustering of the random effects by adding Subjects from the **Subjects:** box into the **Combinations:** box. We can now click **Continue**.



On the Statistics... box we can select what we want SPSS to report. We will select Parameter estimates and Covariances of parameter estimates. We close the box by clicking **Continue**.



Clicking OK instructs SPSS to fit the LMM. The following output screen is then presented.

#### Estimates of Fixed Effectsa

						95% Confide	nce Interval
Parameter	Estimate	Std. Error	df	t	Sig.	Lower Bound	Upper Bound
Intercept	251.405105	6.824557	17	36.838	.000	237.006549	265.803661
Days	10.467286	1.545789	17.000	6.771	.000	7.205956	13.728615

a. Dependent Variable: Reaction.

#### **Covariance Parameters**

# Estimates of Covariance Parametersa

Parameter		Estimate	Std. Error
Residual		654.941027	77.185540
Intercept + Days [subject = Subject]	UN (1,1)	612.089939	288.782654
	UN (2,1)	9.604333	46.678471
	UN (2,2)	35.071661	14.782062

a. Dependent Variable: Reaction.

# Random Effect Covariance Structure (G)<sup>a</sup>

	Intercept   Subject	Days   Subject		
Intercept   Subject	612.089939	9.604333		
Days   Subject	9.604333	35.071661		

Unstructured

We conclude that the average reaction time before beginning sleep deprivation is 251.4 ms. However, there was subject variability about this mean, with a random intercepts standard deviation of 24.7 ms. For every day of sleep deprivation, reaction times increased (i.e. the patient became less reactive) by 10.5 ms (P < 0.001), with subject variation in the slopes of 5.9 ms.

a. Dependent Variable: Reaction.