Analysis of correlated data - repeated measures designs

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23 oktober 2018

What packages do you need?

```r
library(readxl) # Read data into R
library(tidyverse) # Data manipulation
library(lme4) # For fitting models
```

Hypothesis testing

- If we are interested in comparing means between groups or within groups (paired observations), the *t-test* can be used, however
- the t-test is limited to one dependent variable (continuous) and one independent variable (group/condition) and cannot be extended to multiple groups.
- Multiple testing of more than two groups would violate the specified false discovery rate (α)

We need something else!

Repeated measures designs

- Repeated measures designs were typically analyzed using **An**alysis **o**f **Va**riance (ANOVA).
- ANOVA models have a long history and can be used for hypothesis testing comparing means of more than two groups.
- The null-hypothesis of a simple ANOVA is that no group is different to the grand mean (μ):

$$\mu_a = \mu_b = \mu_c = \mu_n = \mu$$

- In the simple case (one-way ANOVA), variation between groups is compared to variations within groups
- In the more complex case (e.g. repeated-measures ANOVA), we can take advantage of variation that is due to the experimental unit (e.g. participant).
- We then partition variance into *known* (e.g. treatment) and *unknown* (residual error) sources.

ANOVA, linear regression and limitations

- The ANOVA model is a special case of linear regression, we can therefore test for specific effects after doing the initial test (post-hoc tests) by combining terms from the model
- In the ANOVA, all explaining variables are categorical, we are therefore limited to special study designs
- The typical ANOVA requires balanced data and no missing values.
  - Missing values will require the removal of all data from the specific participant in a repeated measures design
  - Unbalanced data will lead to biased estimates.

We need something else!

Extending the linear model - Linear Mixed Models (LMM)

- The LMM is an extension of the linear regression, with (quite a few!) exceptions
- The ordinary linear model assumes independent data
- An ANOVA can account for a single source of independent data, e.g. participants
- LMM is more flexible than the ANOVA/linear model when dealing with correlated data (e.g. repeated-measures designs).
- Additionally, we can include continuous covariates (e.g. time) analyse more complex designs and we do not have to worry about missing data in the LMM.
- An example of more a complex design could be multiple levels of "unexplained" variation due to e.g. different participants, located at different facilities, coached by different coaches.

What is a Linear Mixed Model

- The linear mixed model combines to types of **effects**, an effect being a change in the measured quantity due to some event or condition (continuous or categorical).
- A **Fixed effect** is an event/condition were we know all values of interest, this can be a grouping variable that we use in a study design.
- A **Random effect** is an effect that is sampled by random, we do not know all levels of this effect. This can be different participants recruited to a study.
- If we combine **Random** and **Fixed** effects, our model becomes **mixed**

What is a LMM

- The linear regression model can be written as

$$y = \beta_0 + \beta_1 X_1$$

- We can estimate this model by minimizing the distance from all observations to the "best fit line"
- The mixed model also includes *random effects* that can be thought of as effects specific to e.g. a certain participant

$$y_i = \beta_0 + \beta_1 X_1 + b_{i0} \qquad i = 1, ..., n$$

- Every participant gets their own intercept!

Comparing LM to LMM

Table 1: Regression coefficients from a linear model and a mixed linear model examining the effects of timepoint (within) and group (between) on 1RM strength

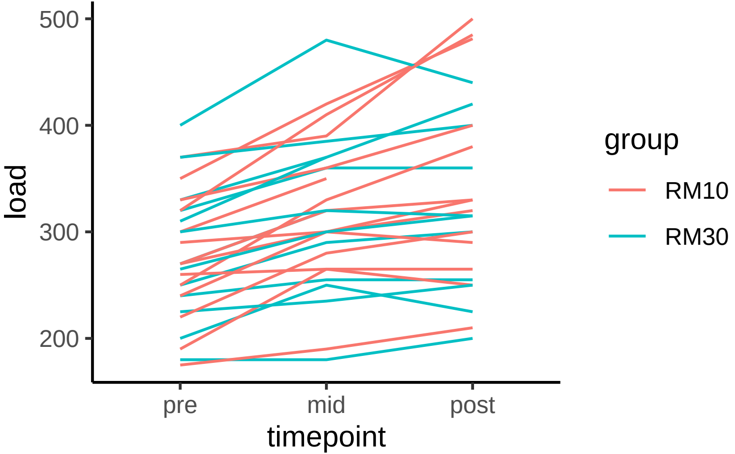
	Estimate	Std. Error	t value
Linear model			
(Intercept)	273.9	19.6	13.9
timepointmid	46.1	27.8	1.7
timepointpost	75.4	28.3	2.7
groupRM30	7.6	28.3	0.3
timepointmid:groupRM30	-11.1	40.0	-0.3
timepointpost:groupRM30	-40.6	41.3	-1.0
Mixed-linear model			
(Intercept)	273.9	19.4	14.1
timepointmid	46.1	8.6	5.3
timepointpost	77.4	8.9	8.7
groupRM30	7.6	27.9	0.3
timepointmid:groupRM30	-11.1	12.5	-0.9
timepointpost:groupRM30	-38.6	13.0	-3.0

Random intercepts and random slopes

- Random effects are used to account for dependence in the data, i.e. when data is not independent.
- This will generally improve precision in estimates from the erroneously fitted linear model (we violate assumptions)
- There are two ways we can include random effects, **random slopes** and **random intercepts**
- In a longitudinal design, random intercepts are the deviance for each participant from the over-all intercept, each participant gets their own starting point.
- Random slopes can be added if we think that individuals respond differently to treatments.
- To estimate random effects, we need data to do so. Random intercepts are straight forward, random slopes requires more than two data points to get the estimate.

Mixed linear models - Examples

- The 10 vs. 30RM-study also had a “mid-point” measurement after 12 weeks.



Mixed linear models - Examples

- The data-set contains missing values
- Groups are not balanced
- ANOVA is not an option, we should use mixed models!

Main effects and interactions

- We want to estimate the effect of time-point, and group.
- But we are mostly interested in the **interaction** between group and time-point.
- This is the main question in the study, do the groups deviate from each other after the training period?

How to fit LMM in R

```r
library(lme4)

# Fit model
m1 <- lmer(load ~ timepoint + group + timepoint:group + (1|subject),
           data = ten_vs_thirty)

# This is the same as
m1 <- lmer(load ~ timepoint * group + (1|subject),
           data = ten_vs_thirty)

# Retrieve model coefficients
summary(m1)$coef
```

Including random effects

- Random effects are included in the model using `(1|subject)`, this means that we have specified a random intercept for each participant.
- A random slope for time could be added to the model if we had more than one measurement at each time-point for each participant or if we treated time as continuous variable.
- A random slope could be added by `(1 + time|subject)`

How to interpret model coefficients

- Remember the linear regression model, the fixed effects part of an mixed model can be read as an ordinary regression model
- In the example `timepoint` and `group` are categorical variables (coded as 0 and 1)
- Fit the model, how do you read the coefficients?

Example

```r
ten_vs_thirty <- read_excel("./data/ten_vs_thirty_complete.xlsx",
                            na = "NA") %>%
        filter(exercise == "legpress") %>%
        spread(timepoint, load) %>%
        gather(timepoint, load, mid:pre) %>%
        mutate(timepoint = factor(timepoint,
                                  levels = c("pre", "mid", "post")))

# Fit model
m1 <- lmer(load ~ timepoint + group + timepoint:group + (1|subject),
           data = ten_vs_thirty)

# Retrieve model coefficients
summary(m1)$coef
```

What about baseline corrections?

- In the mixed model we just formulated, the `groupRM30` coefficient is difference between groups at the intercept.
- The difference between groups at the intercept is added to time-point and the effect seen in the interaction term
- Other options for baseline correction could be to model the change scores and include baseline as a covariate

Interactions

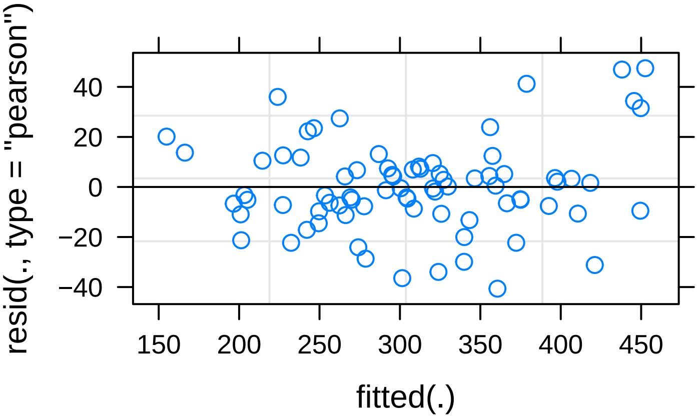
- How do you interpret the interaction term?
- Make a drawing of the of the model based on the output

Table 2: Regression coefficients

	Estimate	Std. Error	t value
(Intercept)	273.9	19.4	14.1
timepointmid	46.1	8.6	5.3
timepointpost	77.4	8.9	8.7
groupRM30	7.6	27.9	0.3
timepointmid:groupRM30	-11.1	12.5	-0.9
timepointpost:groupRM30	-38.6	13.0	-3.0

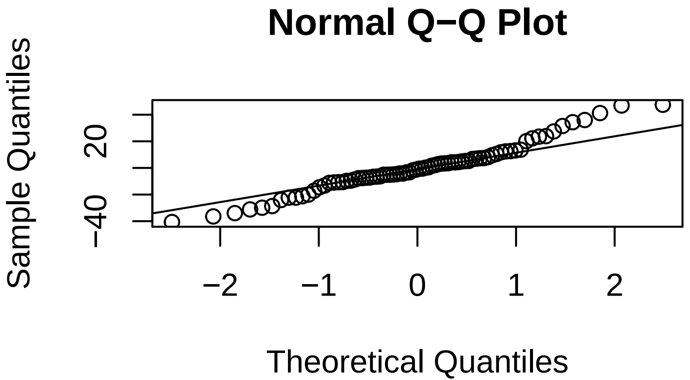
Model diagnostics

- As mixed linear models are very similar to regression models, we can use some of the same diagnostic tools
- For a first look use `plot(model)`



Model diagnostics (2)

```
qqnorm(resid(m1)); qqline(resid(m1))
```



Assumptions

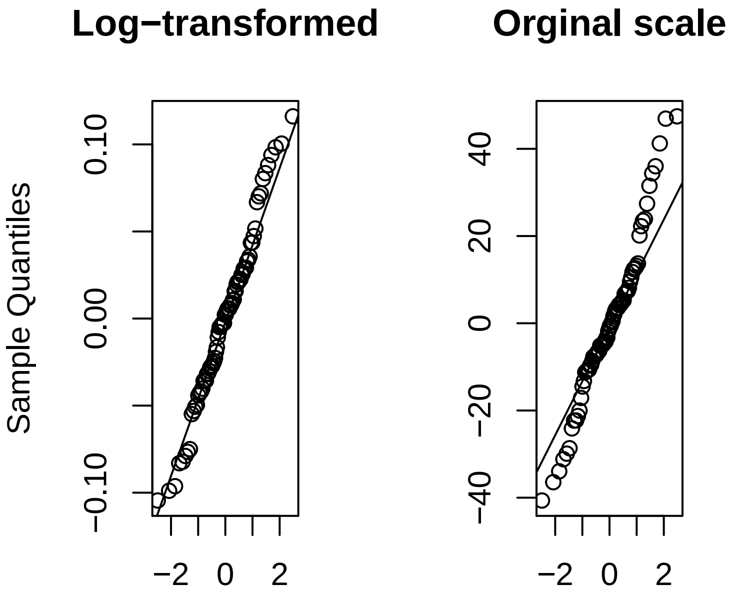
- We assume that the residuals from the model are equally scattered over the fitted data (homoscedasticity, check with `plot(model)`)
- We also assume that the residuals are normally distributes (check with `qqnorm(resid(model)); qqline(resid(model))`)
- If we have problems here, transformation of the dependent variable is a first resort
- A efficient transformation is the log transformation

Mixed models using log-transformed data

```
# calculating the log of the dependent variable
ten_vs_thirty <- read_excel("./data/ten_vs_thirty_complete.xlsx",
                            na = "NA") %>%
        filter(exercise == "legpress") %>%
        spread(timepoint, load) %>%
        gather(timepoint, load, mid:pre) %>%
        mutate(timepoint = factor(timepoint,
                                  levels = c("pre", "mid", "post"))) %>%
        mutate(log.load = log(load))

# Fitting the model
m1_log <- lmer(log.load ~ timepoint + group + timepoint:group + (1|subject),
           data = ten_vs_thirty)
```

Model diagnostics



Mixed models using log-transformed data

- The interpretation of the log-transformed dependent variable is different to the model with the dependent variable on the original scale.

$$log(A) - log(B) = log(\frac{A}{B})$$

- Back-transformed regression coefficients gives fold-changes, can be interpreted as percentage change.
- Do not back-transform standard errors, use point-estimates and confidence intervals

```
exp(confint(m1_log))

exp(summary(m1_log)$coef[,1])
```

Back-transformed estimates

Table 3: Regression coefficients

	estimate	2.5 %	97.5 %
(Intercept)	268.07	236.81	303.46
timepointmid	1.17	1.12	1.23
timepointpost	1.27	1.21	1.33
groupRM30	1.02	0.86	1.22
timepointmid:groupRM30	0.96	0.90	1.02
timepointpost:groupRM30	0.89	0.84	0.96

Inference from mixed models

- Confidence intervals can be calculated on model parameters using `confint(model)`, these can be used for hypothesis testing
- p-values are not provided in `lme4`, this is by design, but there are other ways to calculate p-values for specific effects

Calculating p-values in the mixed-effects model using likelihood ratio tests

- Likelihood ratio tests (LRT) can be used to calculate p-values.
- Here we compare a model with an effect, to a model without the effect, the test compares “goodness of fit”

```
# Fit a model with all effects
m1 <- lmer(load ~ timepoint + group + timepoint:group + (1|subject),
           data = ten_vs_thirty)
# Then fit a null model for comparison, we drop the higher order interaction
m0 <- lmer(load ~ timepoint + group + (1|subject),
           data = ten_vs_thirty)

# compare the models using the anova() function
anova(m0, m1)
```

LRT p-values

```
## Data: ten_vs_thirty
## Models:
## m0: load ~ timepoint + group + (1 | subject)
## m1: load ~ timepoint + group + timepoint:group + (1 | subject)
##    Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
## m0  6 813.37 827.51 -400.68   801.37
## m1  8 808.33 827.18 -396.16   792.33 9.039      2    0.01089 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Exercise / Assignment

- We will use the complete 10 vs 30RM data set `ten_vs_thirty_complete.xlsx`
- Compare your analyses of the pre-post data set, with the mixed model approach, do you reach the same conclusions?