

Contemporary Issues in Clinical Trials Methods

Longitudinal Data Analysis Part I

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Repeated Measures/Longitudinal Data

- In Alzheimer's Disease (AD) clinical trials we typically assess study participants *longitudinally* or *repeatedly* over time
- This gives rise to *serial observations* for each participant at various time points post baseline
- We cannot treat these serial observations as if they came from different people
- Our analysis methods must account for *within-subject correlation*
- There are specialized statistical methods to help accomodate many varieties of *correlated* or *clustered* data

Repeated Measures/Longitudinal Data

- We will explore *longitudinal data analysis* approaches commonly employed in AD clinical trials
- We will demonstrate these methods on a *simulated* clinical trial dataset
- Data is simulated and analyzed using (cran.r-project.org; rstudio.com)
- All of the code for this session is available from github.com/atrihub/AAIC2017ClinicalTrialMethods (see 01LDA.R file)

Let's simulate a hypothetical clinical trial...

- Two groups: placebo vs active (hypothetical)
- $n = 200$ mild to moderate dementia subjects per group
- Alzheimer's Disease Assessment Scale (ADAS-Cog) assessed at 0, 6, 12, 18 months
- Placebo group behaves like ADNI participants
- Weak effects for age and sex (based on ADNI pilot estimates)
- A treatment which slows ADAS-Cog progression by 12.5%
- Typical attrition ($\approx 30\%$)

Let's simulate a hypothetical clinical trial...

Simulation *reverses* the usual process of statistical modeling/estimation

- Model fitting: Data + Model \rightarrow Parameter Estimates
- Model simulation: Model + Parameter Estimates \rightarrow Pseudo Data
- Given a reasonable model, everything can be simulated: mean, variance, missingness, etc.
- CAUTION: Simulations can only provide information about *models*, but they cannot provide information about *reality*. *Real data* is required for the latter.

Let's simulate a hypothetical clinical trial...

These are all estimates required:

```
# fixed effects parameters estimated from ADNI
Beta <- c(
  '(Intercept)'=19.60, # mean ADAS at baseline
  'female'=-0.78, # better scores for females
  'age'= 0.01, # worse per year of age at baseline
  'month'= 0.40, # worse per month post baseline
  'month:active'=-0.05) # improvement per month with treatment

# standard deviations for random effects
sigma_random_intercept <- 6.0
sigma_random_slope <- 0.42
sigma_residual <- 3.1

# other design parameters
months <- c(0, 6, 12, 18)
n <- 200 # per group
attrition_rate <- 0.40/18 # approx per month
```

The pseudo data snapshot

	id	active	female	age	ensor	ran.intercept	ran.slope	month	residual	ADAS11
1	1	0	1	81.0	28.7	-6.57	0.670	0	3.31	16
2	1	0	1	81.0	28.7	-6.57	0.670	6	-4.24	15
3	1	0	1	81.0	28.7	-6.57	0.670	12	4.41	30
4	1	0	1	81.0	28.7	-6.57	0.670	18	2.62	35
5	2	1	0	75.6	72.1	-9.94	-0.463	0	2.10	13
6	2	1	0	75.6	72.1	-9.94	-0.463	6	-3.31	6

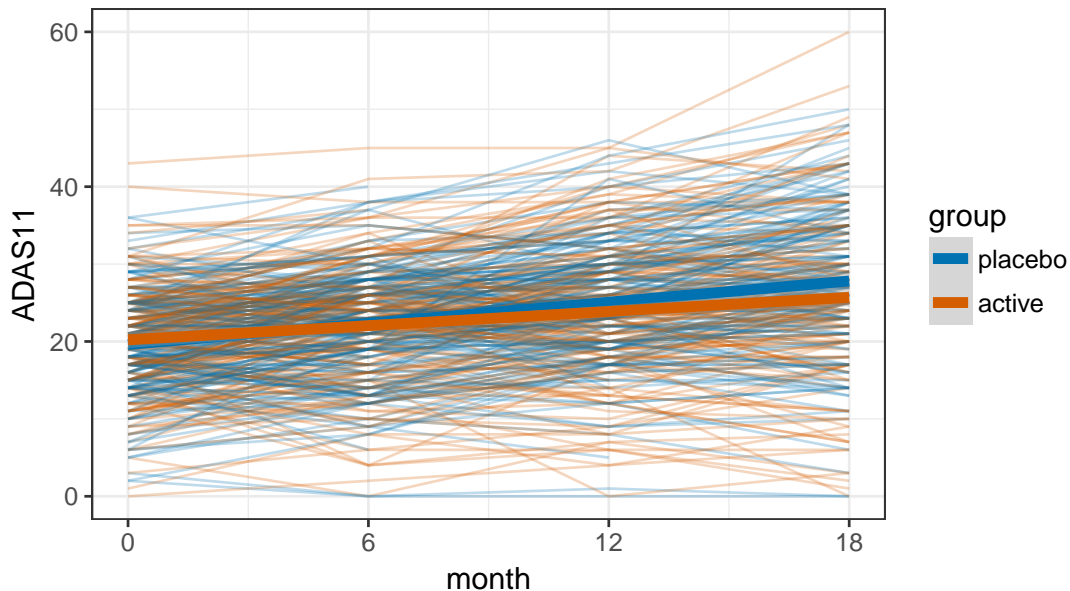
The pseudo data: Baseline characteristics

Table: Descriptive Statistics by group

	placebo <i>N</i> = 200				active <i>N</i> = 200			
female	49% (98)				55% (109)			
age	68.36	75.19	79.85	(74.58 ± 8.43)	70.33	75.59	80.80	(75.69 ± 7.78)
ADAS11	15.00	19.50	25.00	(19.73 ± 6.62)	15.00	21.00	25.00	(20.16 ± 6.98)

a b c represent the lower quartile *a*, the median *b*, and the upper quartile *c* for continuous variables. $x \pm s$ represents $\bar{X} \pm 1$ SD. Numbers after percents are frequencies.

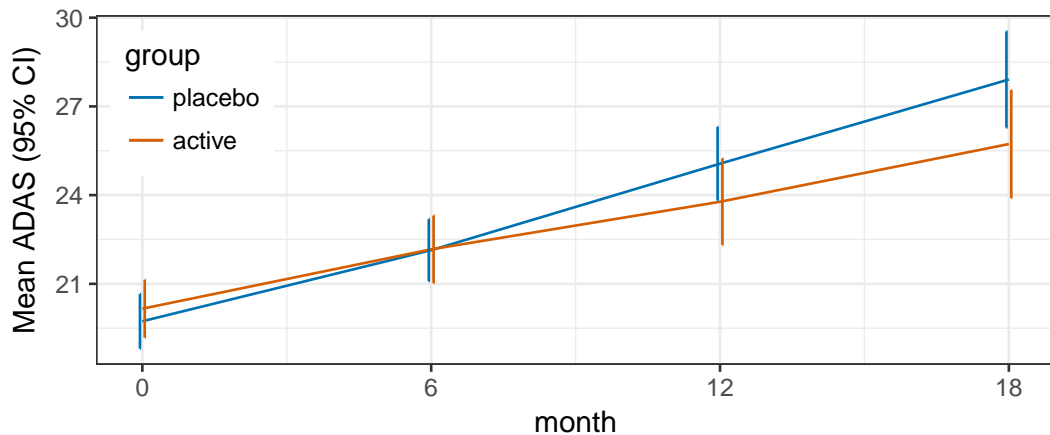
The pseudo data: Spaghetti plot



Basic longitudinal summaries of ADAS11

group	month	n	mean	sd	lower95	upper95	min	max
placebo	0	200	19.7	6.62	18.8	20.7	2	36
placebo	6	181	22.1	7.15	21.1	23.2	0	40
placebo	12	159	25.1	7.93	23.8	26.3	0	46
placebo	18	144	27.9	9.87	26.3	29.5	0	50
active	0	200	20.2	6.98	19.2	21.1	0	43
active	6	179	22.2	7.73	21.0	23.3	0	45
active	12	160	23.8	9.34	22.3	25.2	0	45
active	18	146	25.7	11.12	23.9	27.5	0	60

Mean ADAS

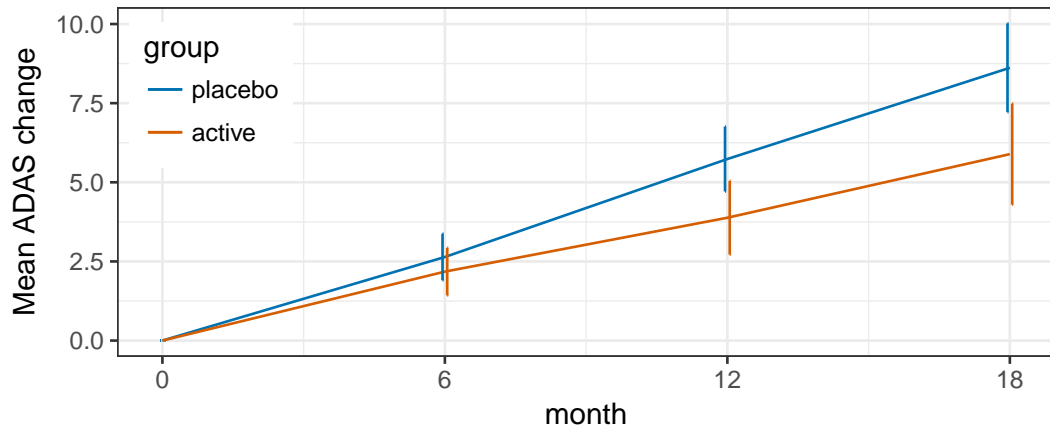


active	200	179	160	146
placebo	200	181	159	144

Basic longitudinal summaries of ADAS11 *change*

group	month	n	mean	sd	lower95	upper95	min	max
placebo	0	200	0.00	0.00	0.00	0.00	0	0
placebo	6	181	2.64	5.03	1.90	3.38	-9	16
placebo	12	159	5.74	6.54	4.71	6.76	-11	27
placebo	18	144	8.62	8.51	7.22	10.02	-13	31
active	0	200	0.00	0.00	0.00	0.00	0	0
active	6	179	2.18	5.09	1.43	2.93	-14	14
active	12	160	3.88	7.45	2.72	5.04	-14	20
active	18	146	5.89	9.75	4.30	7.49	-23	30

Mean ADAS change (95% CI)



active	200	179	160	146
placebo	200	181	159	144

Two sample *t*-test of mean change at month 18 (completers analysis)

- Difference between group means is $8.618 - 5.89 = 2.728$
- (pooled) standard deviation is 9.155
- $t = \frac{2.728}{9.155 \sqrt{\frac{1}{144} + \frac{1}{146}}} = 2.537$
- $144 + 146 - 2 = 288$ “degrees of freedom”

t-test

Two Sample t-test

data: ADAS11.ch by group

t = 2.54, df = 288, p-value = 0.0117

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

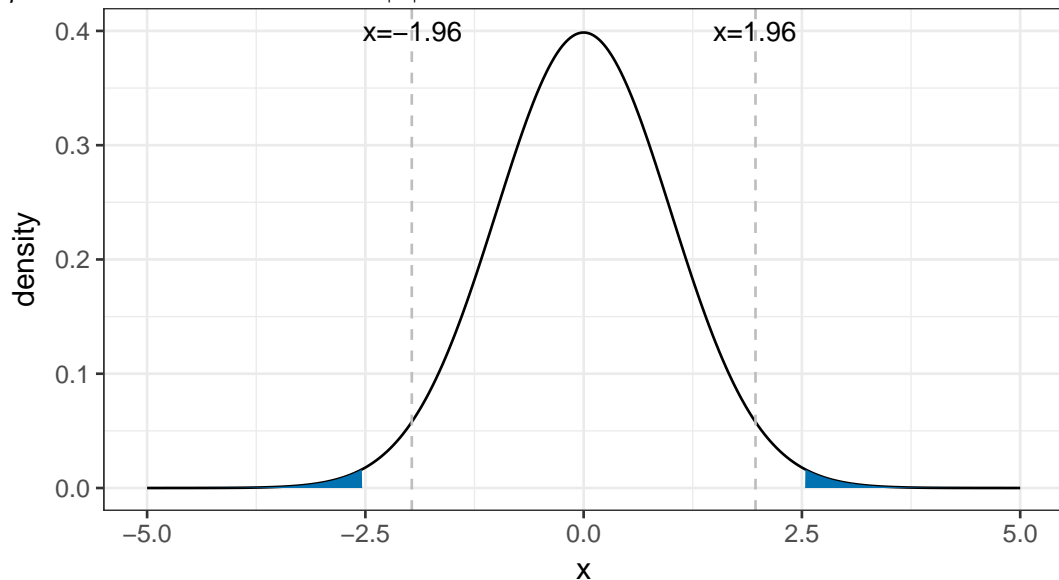
0.611 4.844

sample estimates:

mean in group placebo	mean in group active
8.61806	5.89041

The t_{288} -distribution

p -value is area under curve for $|x| > 2.537$, the value of the test statistic in this case.



Regression analysis

- “Regression” generally refers to a relationship between variables that is estimated by data
- “Ordinary Least Squares” regression, for example, describes a linear relationship between two continuous variables that is estimated by the line that minimizes the sum of squared “residuals”
- predictor/covariate → response/outcome
- “Residuals” are the differences between observations and values predicted by the regression

Ordinary Least Squares: minimizing the sum of squared residuals

Other types of regression

- “General linear models” can add multiple *covariates/predictors*
- “Generalized linear models” can accomodate other types of *outcome/response* variables (e.g. logistic regression can accomodate binary outcome variables)
- “Mixed-effects models” mix *random effects* with the standard *fixed effects* to account for complex correlation structures

All regression models share the common theme of estimating the best fit relationship between *outcome/response* variables and *covariates/predictors*

ANalysis of COVariance (ANCOVA) for “*pre-post*” data

- Very common for two groups, and one post- assessment
- $ADAS_{i1}$: baseline or pre- observation for subject i , $i = 1, \dots, 200$
- $ADAS_{i2}$: followup or post- observation for subject i , $i = 1, \dots, 200$
- $Active_i$: treatment group indicator (e.g. 1 if active, 0 if placebo)
- ANCOVA I: $ADAS_{i2} = \beta_0 + Active_i \beta_1 + ADAS_{i1} \beta_2 + \varepsilon_i$
 - β is the *intercept*
 - β_1 is the estimate of interest: group difference at 18 months
 - β_2 controls for baseline ADAS
 - ε_i is residual error
- ANCOVA II: $ADAS_{i2} = \beta_0 + Active_i \beta_1 + ADAS_{i1}^* \beta_2 + Active_i ADAS_{i1}^* \beta_3 + \varepsilon_i$
 - β_3 controls for interaction of treatment assignment and baseline ADAS
 - Need to *mean center* baseline covariates: $ADAS_{i1}^* = ADAS_{i1} - \bar{Y}_{.0}$

Yang & Tsiatis (2001). Efficiency Study of Estimators for a Treatment Effect in a Pretest-Posttest Trial. *The Am. Statistician*, 55(4) 314-321

ANCOVA I for effect of treatment on ADAS11 at 18 months

Call:

```
lm(formula = ADAS11.ch ~ active + ADAS11.m0, data = trial_mmrm)
```

Residuals:

	Min	1Q	Median	3Q	Max
	-26.193	-4.699	-0.012	4.376	28.312

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	9.6039	0.7317	13.12	< 2e-16 ***
active	-1.4640	0.4660	-3.14	0.0017 **
ADAS11.m0	-0.2151	0.0337	-6.38	2.8e-10 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 7.25 on 966 degrees of freedom

Multiple R-squared: 0.0512, Adjusted R-squared: 0.0493

F-statistic: 26.1 on 2 and 966 DF, p-value: 9.23e-12

ANCOVA II for effect of treatment on ADAS11 at 18 months

```
Call:
lm(formula = ADAS11.ch ~ active * center(ADAS11.m0), data = trial_mmrmm)

Residuals:
    Min       1Q   Median       3Q      Max
-26.25  -4.72  -0.06   4.28  28.11

Coefficients:
                Estimate Std. Error t value Pr(>|t|)
(Intercept)         5.3726     0.3298   16.29 < 2e-16 ***
active              -1.4631     0.4661   -3.14  0.0017 **
center(ADAS11.m0)    -0.2382     0.0495   -4.81  1.8e-06 ***
active:center(ADAS11.m0)  0.0431     0.0676    0.64  0.5240
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 7.25 on 965 degrees of freedom
Multiple R-squared:  0.0516, Adjusted R-squared:  0.0487
F-statistic: 17.5 on 3 and 965 DF, p-value: 4.44e-11
```

ANCOVA II with more covariates

Call:

```
lm(formula = ADAS11.ch ~ active * center(ADAS11.m0) + female +
    age, data = trial_mmrm)
```

Residuals:

Min	1Q	Median	3Q	Max
-26.005	-4.667	-0.201	4.166	28.166

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	10.0445	2.1898	4.59	5.1e-06 ***
active	-1.4053	0.4665	-3.01	0.0027 **
center(ADAS11.m0)	-0.2371	0.0496	-4.78	2.1e-06 ***
female	0.4820	0.4713	1.02	0.3067
age	-0.0660	0.0288	-2.30	0.0219 *
active:center(ADAS11.m0)	0.0467	0.0675	0.69	0.4887

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 7.23 on 963 degrees of freedom

Multiple R-squared: 0.058, Adjusted R-squared: 0.0531

F-statistic: 11.9 on 5 and 963 DF, p-value: 3.71e-11

ANCOVA & t -test summary

- Ubiquitous, simple, powerful framework
- ANCOVA more powerful/efficient than t -test
- Both are inherently **complete case** analyses!
- With missing data, not intention-to-treat (ITT) analysis
- Do not make use of **incomplete cases**!
- Might be biased and/or inefficient (low power) with missing data

Two-stage models

- *Subject-specific* longitudinal profiles can often be modeled with simple linear regression
- This leads to the 2-stage model:
 - Stage 1: Linear regression model for each subject separately
 - Stage 2: Model subject-specific regression coefficients with covariates of interest
- However, this is **NOT** a recommend analysis approach, but rather a means to introduce mixed-effect models.

Two-stage model example

- Stage 1:

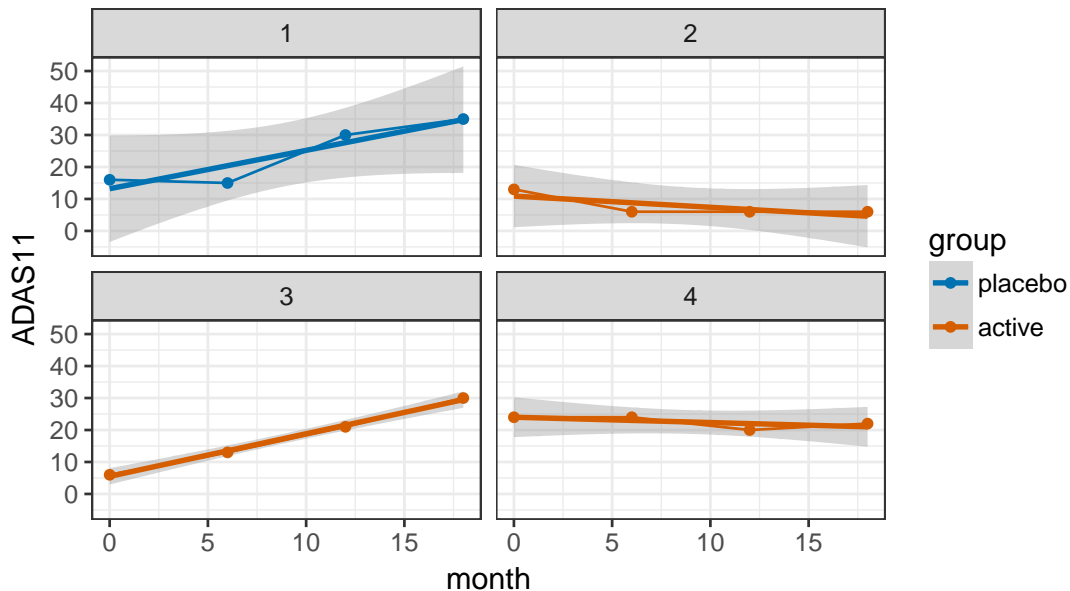
$$ADAS_{ij} = \beta_{0i} + t_{ij}\beta_{1i} + \varepsilon_i \quad (1)$$

for subject i at time t_{ij}

- Provides estimates of subject-specific intercepts, $\hat{\beta}_{0i}$ and slopes $\hat{\beta}_{1i}$
- $\varepsilon_i \sim \mathcal{N}(0, \sigma_i^2 I_{n_i})$ estimates *within*-subject variability
- *Between*-subject variability can now be modeled by treating $\hat{\beta}_i$ as “response variables”
- Stage 2:

$$\hat{\beta}_{1i} = X_i\beta + \varepsilon'_i \quad (2)$$

Stage 1 models of simulated trial



Stage 1 model of simulated trial

	id	beta.(Intercept)	beta.month	sigma	active	group	age	female
1	1	13.2	1.200	4.626	0	placebo	81.0	1
2	2	10.9	-0.350	2.711	1	active	75.6	0
3	3	5.5	1.333	0.707	1	active	70.0	0
4	4	24.0	-0.167	1.732	1	active	71.5	0
5	5	27.3	0.833	1.633	1	active	84.1	1
6	6	25.0	0.833	NaN	1	active	76.2	1

Stage 1 model of simulated trial

Call:

```
lm(formula = ADAS11 ~ month, data = trial_obs, subset = id ==
  1)
```

Residuals:

```
  1    2    3    4
2.8 -5.4  2.4  0.2
```

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	13.200	3.870	3.41	0.076 .
month	1.200	0.345	3.48	0.074 .

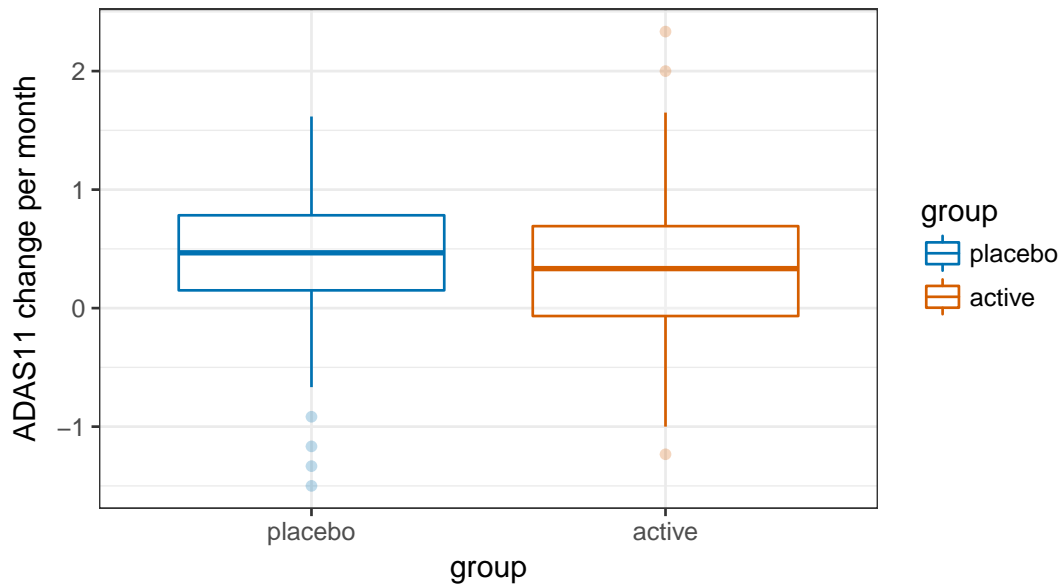
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 4.63 on 2 degrees of freedom

Multiple R-squared: 0.858, Adjusted R-squared: 0.787

F-statistic: 12.1 on 1 and 2 DF, p-value: 0.0736

Stage 2 model of simulated trial



Stage 2 model of simulated trial

Call:

```
lm(formula = beta.month ~ female + age + active, data = trial_stage1)
```

Residuals:

Min	1Q	Median	3Q	Max
-1.9180	-0.3578	0.0096	0.3602	2.0027

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.74667	0.27527	2.71	0.007 **
female	0.05687	0.05953	0.96	0.340
age	-0.00456	0.00363	-1.25	0.210
active	-0.10567	0.05960	-1.77	0.077 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.563 on 356 degrees of freedom

(40 observations deleted due to missingness)

Multiple R-squared: 0.0161, Adjusted R-squared: 0.00783

F-statistic: 1.94 on 3 and 356 DF, p-value: 0.122

Two-stage models

- In contrast to ANCOVA and t -test, two-stage models allow all randomized subject with at least one followup to be included into analysis (“modified intention-to-treat”)
- However, second stage models ignore the variability/uncertainty of the slope estimates from the first stage
- This means that p -values from second stage might be smaller than they should be and Type I error could be inflated

Linear mixed-effects model (LME)

Linear mixed-effects models provide a cleaner, more efficient, and more accurate one-step alternative to two-stage models

$$\left. \begin{array}{l} \text{Stage 1: } \text{ADAS}_{ij} = \beta_{0i} + t_{ij}\beta_{1i} + \varepsilon_{ij} \\ \text{Stage 2: } \hat{\beta}_{1i} = X_i\beta + \varepsilon'_{ij} \end{array} \right\} \rightarrow \text{ADAS}_{ij} = X_i\beta + b_{0i} + t_{ij}b_{1i} + \varepsilon_{ij}$$

- β : population level “fixed effects”
- $b_i \sim \mathcal{N}(0, D)$: subject-specific “random effects” for subject i
- $(\varepsilon_{i1}, \dots, \varepsilon_{i4}) \sim \mathcal{N}(0, \Sigma_i)$: vector of “residuals” for subject i
- D, Σ_i : “variance components”

$b_1, \dots, b_N, \varepsilon_1, \dots, \varepsilon_N$ are assumed independent

Linear mixed-effects models of simulated trial

```
Linear mixed-effects model fit by REML
Data: trial_obs
   AIC   BIC logLik
8571 8607 -4278

Random effects:
Formula: ~month | id
Structure: General positive-definite, Log-Cholesky parametrization
           StdDev Corr
(Intercept) 6.146  (Intr)
month       0.447 -0.051
Residual    3.121

Fixed effects: ADAS11 ~ month + month:active
           Value Std.Error   DF t-value p-value
(Intercept) 19.98     0.336  967   59.5  0.0000
month        0.45     0.039  967   11.4  0.0000
month:active -0.12     0.055  967   -2.3  0.0233
Correlation:
           (Intr) month
month      -0.135
month:active 0.001 -0.701

Standardized Within-Group Residuals:
   Min      Q1      Med      Q3      Max
-2.3435 -0.4831 -0.0143  0.5065  3.3294

Number of Observations: 1369
Number of Groups: 400
```

LME model with additional covariates

```

Linear mixed-effects model fit by REML
Data: trial_obs
   AIC   BIC logLik
8568 8615  -4275

Random effects:
Formula: ~month | id
Structure: General positive-definite, Log-Cholesky parametrization
          StdDev Corr
(Intercept) 6.061  (Intr)
month       0.448  -0.041
Residual    3.121

Fixed effects: ADAS11 ~ age + female + month + month:active
              Value Std.Error DF t-value p-value
(Intercept)  24.45    3.062 967    7.99  0.0000
age          -0.05    0.040 397   -1.14  0.2537
female       -1.98    0.653 397   -3.03  0.0026
month         0.45    0.039 967   11.41  0.0000
month:active -0.12    0.055 967   -2.23  0.0258
Correlation:
          (Intr) age    female month
age       -0.988
female    -0.125  0.015
month     -0.026  0.012  0.004
month:active 0.014 -0.013 -0.008 -0.701

Standardized Within-Group Residuals:
      Min      Q1      Med      Q3      Max
-2.3323 -0.4809 -0.0116  0.5043  3.3173

Number of Observations: 1369
Number of Groups: 400

```

Linear mixed-effects models (R code)

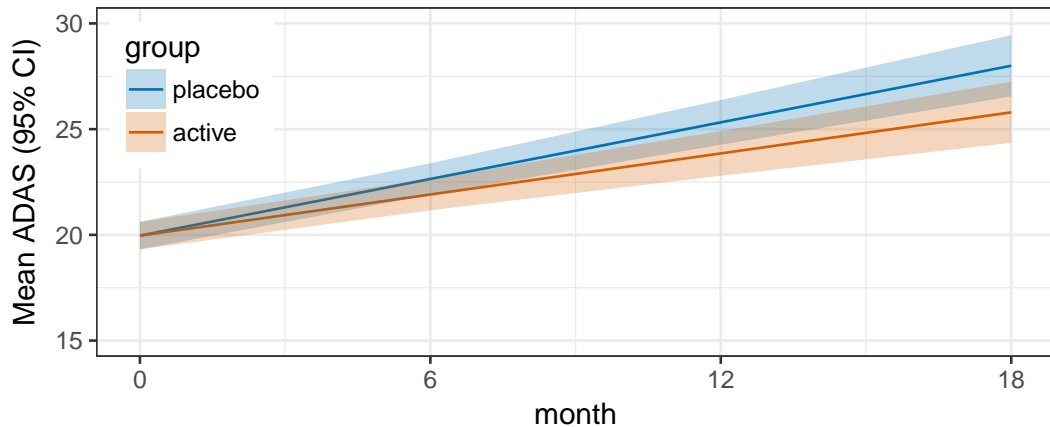
```
lme(ADAS11 ~ month + month:active,  
    data = trial_obs, random = ~month|id)
```

```
lme(ADAS11 ~ age + female + month + month:active,  
    data = trial_obs, random = ~month|id)
```

Mean profiles

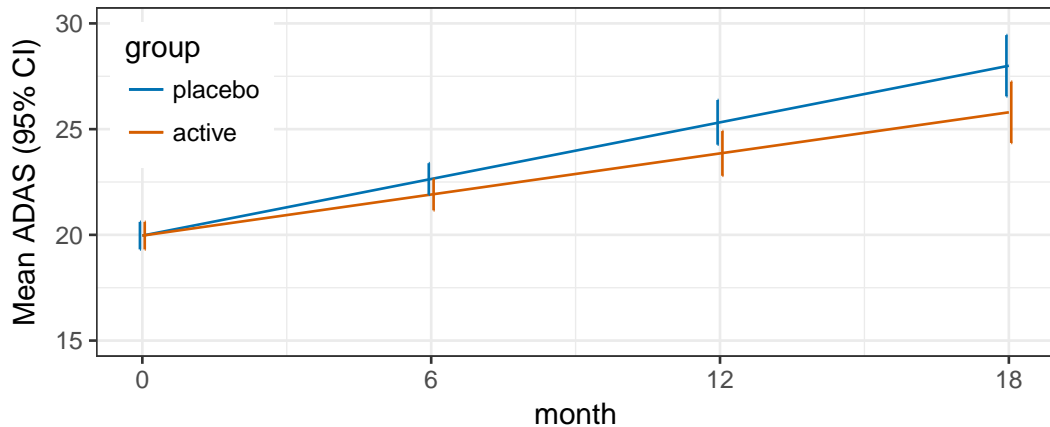
active	month.	active	month	Estimate	Lower	Upper	group
1		0	0	20.0	19.3	20.6	active
1		6	6	21.9	21.2	22.7	active
1		12	12	23.9	22.8	24.9	active
1		18	18	25.8	24.3	27.2	active
0		0	0	20.0	19.3	20.6	placebo
0		0	6	22.6	21.9	23.4	placebo
0		0	12	25.3	24.3	26.4	placebo
0		0	18	28.0	26.6	29.4	placebo

Modeled mean profiles (shaded CIs)



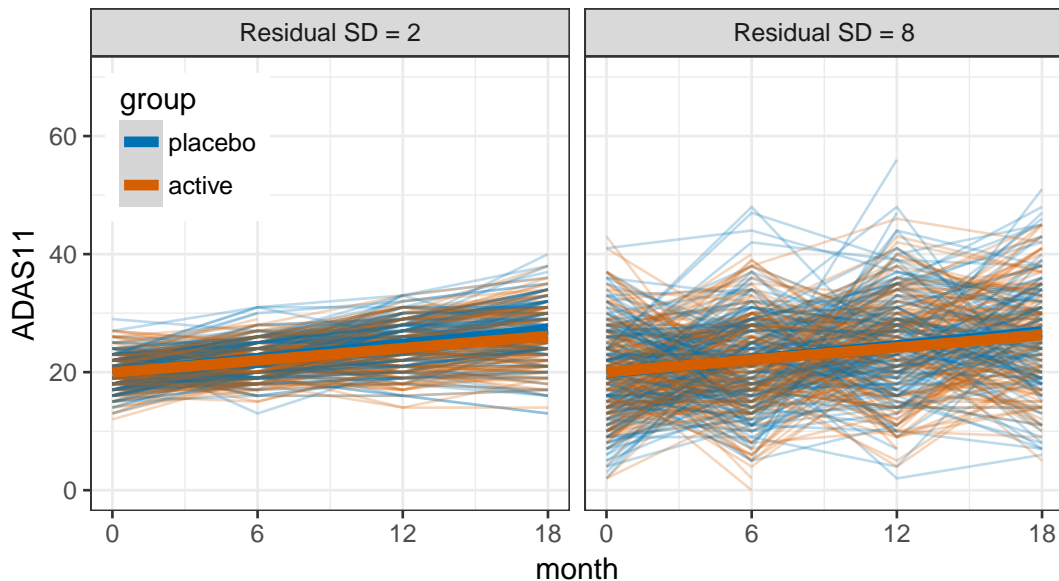
active	200	179	160	146
placebo	200	181	159	144

Plotting profiles (error bar CIs)

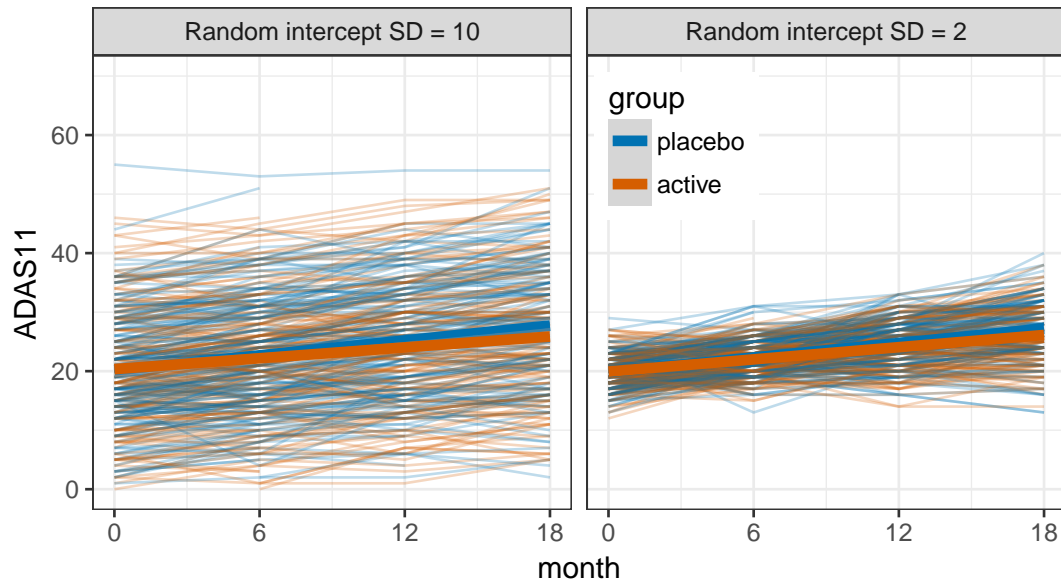


active	200	179	160	146
placebo	200	181	159	144

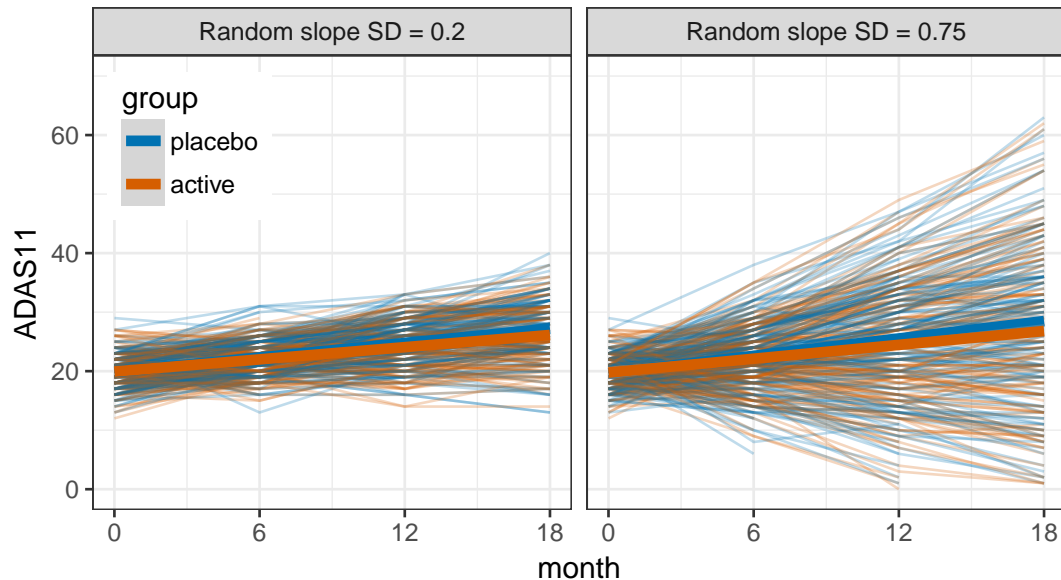
Mixed effect models: standard deviation of residuals



Mixed effect models: standard deviation of random intercepts



Mixed effect models: standard deviation of random slopes



Random intercepts model

- NOTE: With only two timepoints, it is impossible to fit a model with random slopes
- If we drop the *random slope* term, $t_{ij}b_{1i}$, what remains is called a *random intercepts* model:

$$\text{ADAS}_{ij} = X_i\beta + b_{0i} + \varepsilon_i$$

Random intercepts model

```

Linear mixed-effects model fit by REML
Data: trial_obs
   AIC   BIC logLik
8903 8929 -4446

Random effects:
Formula: ~1 | id
      (Intercept) Residual
StdDev:        6.75      4.58

Fixed effects: ADAS11 ~ month + month:active
              Value Std.Error   DF t-value p-value
(Intercept)  19.95    0.391 967    51.0   0e+00
month         0.46    0.026 967    17.4   0e+00
month:active -0.14    0.036 967    -3.8   2e-04
Correlation:
      (Intr) month
month      -0.271
month:active 0.001 -0.682

Standardized Within-Group Residuals:
      Min       Q1       Med       Q3       Max
-3.889850 -0.564081  0.000502  0.539757  3.577962

Number of Observations: 1369
Number of Groups: 400

```

Random intercepts model vs model with random slopes

```
anova(fit_lme_int, fit_lme)
```

	Model	df	AIC	BIC	logLik	Test	L.Ratio	p-value
fit_lme_int	1	5	8903	8929	-4446			
fit_lme	2	7	8571	8607	-4278	1 vs 2	336	<.0001

The model with random slopes is preferred (smaller AIC is better)

Further reading

- Fitzmaurice, G. M., Laird, N. M., Ware, J. H. (2012). *Applied Longitudinal Analysis*. Hoboken: Wiley.
- Diggle, P., Heagerty, P., Liang, K.-Y., Zeger, S. (2002). *Analysis of Longitudinal Data*. Oxford University Press
- Verbeke, G. and Molenberghs, G. (2000). *Linear Mixed Models for Longitudinal Data*. Springer Series in Statistics. New-York: Springer.
- Molenberghs, G. and Kenward, M.G. (2007). *Missing Data in Clinical Studies*. Wiley.