

Repeated measures analysis

Repeated measures

- More than one response *measurement* for each subject, same thing at different times
- Generalization of matched pairs (“matched triples”, etc.).
- Expect measurements on same subject to be correlated, so assumptions of independence will fail.
- *Repeated measures*. *Profile analysis* uses Manova (set up).
- Another approach uses *mixed models* (random effects).
- Variation: each subject does all treatments at different times (called *crossover design*).

Packages

```
library(car)  
library(tidyverse)  
library(lme4) # for mixed models later
```

Example: histamine in dogs

- 8 dogs take part in experiment.
- Dogs randomized to one of 2 different drugs.
- Response: log of blood concentration of histamine 0, 1, 3 and 5 minutes after taking drug. (Repeated measures.)
- Data in `dogs.txt`, column-aligned.

Read in data

```
my_url <- "http://ritsokiguess.site/datafiles/dogs.txt"
dogs <- read_table(my_url)
dogs
```

```
# A tibble: 8 x 7
```

	dog	drug	x	lh0	lh1	lh3	lh5
	<chr>	<chr>	<chr>	<dbl>	<dbl>	<dbl>	<dbl>
1	A	Morphine	N	-3.22	-1.61	-2.3	-2.53
2	B	Morphine	N	-3.91	-2.81	-3.91	-3.91
3	C	Morphine	N	-2.66	0.34	-0.73	-1.43
4	D	Morphine	N	-1.77	-0.56	-1.05	-1.43
5	E	Trimethaphan	N	-3.51	-0.48	-1.17	-1.51
6	F	Trimethaphan	N	-3.51	0.05	-0.31	-0.51
7	G	Trimethaphan	N	-2.66	-0.19	0.07	-0.22
8	H	Trimethaphan	N	-2.41	1.14	0.72	0.21

Setting things up

```
response <- with(dogs, cbind(lh0, lh1, lh3, lh5))  
response
```

	lh0	lh1	lh3	lh5
[1,]	-3.22	-1.61	-2.30	-2.53
[2,]	-3.91	-2.81	-3.91	-3.91
[3,]	-2.66	0.34	-0.73	-1.43
[4,]	-1.77	-0.56	-1.05	-1.43
[5,]	-3.51	-0.48	-1.17	-1.51
[6,]	-3.51	0.05	-0.31	-0.51
[7,]	-2.66	-0.19	0.07	-0.22
[8,]	-2.41	1.14	0.72	0.21

Another way to make response

```
dogs %>% select(starts_with("lh")) %>%  
  as.matrix() -> response  
response
```

	lh0	lh1	lh3	lh5
[1,]	-3.22	-1.61	-2.30	-2.53
[2,]	-3.91	-2.81	-3.91	-3.91
[3,]	-2.66	0.34	-0.73	-1.43
[4,]	-1.77	-0.56	-1.05	-1.43
[5,]	-3.51	-0.48	-1.17	-1.51
[6,]	-3.51	0.05	-0.31	-0.51
[7,]	-2.66	-0.19	0.07	-0.22
[8,]	-2.41	1.14	0.72	0.21

The repeated measures MANOVA

Get list of response variable names; we call them `times`. Save in data frame.

```
times <- colnames(response)
times
```

```
[1] "lh0" "lh1" "lh3" "lh5"
```

```
times.df <- data.frame(times=factor(times))
times.df
```

```
      times
1    lh0
2    lh1
3    lh3
4    lh5
```


Fitting the model

```
dogs.1 <- lm(response ~ drug, data = dogs)
dogs.2 <- Manova(dogs.1,
  idata = times.df,
  idesign = ~times
)
```

The output (there is a lot)

- normally you just run

```
summary(dogs.2)
```

and pull out what you need to answer the question.

- But you can grab just individual pieces as shown below:

```
names(summary(dogs.2))
```

```
[1] "type"                "repeated"            "multivariate.test"
[4] "univariate.tests"    "pval.adjustments"    "sphericity.test"
[7] "SSPE"
```

What there is here

- three sets of tests, for
 - ▶ times; drug; their interaction
- two *types* of test for each of these:
 - ▶ univariate; multivariate
- univariate is more powerful *if* it applies; if it doesn't, can make adjustments to it

Sphericity

- The thing that decides whether the univariate tests apply is called “sphericity”.
- This holds if the outcomes have equal variance (to each other) and have the same (positive) correlation across subjects.
- Tested using Mauchly's test (part of output)
- If sphericity rejected, there are adjustments to the univariate P-values due to Huynh-Feldt and Greenhouse-Geisser. Huynh-Feldt better if responses not actually normal (safer).

Sphericity tests

```
summary(dogs.2)$sphericity.tests
```

	Test statistic	p-value
times	0.12334	0.084567
drug:times	0.12334	0.084567

Sphericity is not rejected; proceed to univariate tests.

Univariate tests

```
summary(dogs.2)$univariate.tests
```

	Sum Sq	num Df	Error SS	den Df	F value	Pr(>F)	
(Intercept)	71.342	1	22.1026	6	19.3664	0.004565	**
drug	11.520	1	22.1026	6	3.1272	0.127406	
times	26.160	3	2.2534	18	69.6546	4.215e-10	***
drug:times	5.111	3	2.2534	18	13.6095	7.050e-05	***

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

- Significant interaction between drug and time: the pattern of log-histamine over time is different for the different drugs.

If sphericity had been rejected

then we would use the H-F adjusted P-values:

```
summary(dogs.2)$pval.adjustments
```

```
              GG eps    Pr(>F[GG])      HF eps    Pr(>F[HF])
times          0.5261798 3.744618e-06 0.6822614 1.843418e-07
drug:times      0.5261798 2.348896e-03 0.6822614 7.307096e-04
attr(,"na.action")
(Intercept)          drug
              1          2
attr(,"class")
[1] "omit"
```

In this case (because sphericity was not rejected), these are very similar to the ones from the univariate tests, and the conclusion (significant interaction) was the same.

Comments

- If the interaction had not been significant:
 - ▶ cannot remove interaction with time
 - ▶ so look at univariate (or adjusted for sphericity) tests of main effects in model with non-significant interaction

Next

- investigate interaction with graph
- but dataframe has several observations per line (“wide”).
- Plotting works with data in “long format”: one response per line.
- The responses are log-histamine at different times, labelled 1h-something. Call them all 1h and put them in one column, with the time they belong to labelled.

Running pivot_longer, try 1

```
dogs %>% pivot_longer(starts_with("lh"),  
                       names_to = "time", values_to = "lh")
```

```
# A tibble: 32 x 5
```

	dog	drug	x	time	lh
	<chr>	<chr>	<chr>	<chr>	<dbl>
1	A	Morphine	N	lh0	-3.22
2	A	Morphine	N	lh1	-1.61
3	A	Morphine	N	lh3	-2.3
4	A	Morphine	N	lh5	-2.53
5	B	Morphine	N	lh0	-3.91
6	B	Morphine	N	lh1	-2.81
7	B	Morphine	N	lh3	-3.91
8	B	Morphine	N	lh5	-3.91
9	C	Morphine	N	lh0	-2.66
10	C	Morphine	N	lh1	0.34

```
# i 22 more rows
```

Getting the times

Not quite right: want new variable containing just number in time: `parse_number`. (Top 5 rows shown.)

```
dogs %>%  
  pivot_longer(starts_with("lh"),  
               names_to = "timex", values_to = "lh") %>%  
  mutate(time = parse_number(timex))
```

A tibble: 5 x 6

	dog	drug	x	timex	lh	time
	<chr>	<chr>	<chr>	<chr>	<dbl>	<dbl>
1	A	Morphine	N	lh0	-3.22	0
2	A	Morphine	N	lh1	-1.61	1
3	A	Morphine	N	lh3	-2.3	3
4	A	Morphine	N	lh5	-2.53	5
5	B	Morphine	N	lh0	-3.91	0

What I did differently

- I realized that `pivot_longer` was going to produce something like `lh1`, which I needed to do something further with, so this time I gave it a temporary name `timex` (which we actually *do* use later).
- This enabled me to use the name `time` for the actual numeric time.
- This works now, so next save into a new data frame `dogs.long`.

Saving

```
dogs %>%  
  pivot_longer(starts_with("lh"),  
               names_to = "timex", values_to = "lh") %>%  
  mutate(time = parse_number(timex)) -> dogs.long
```

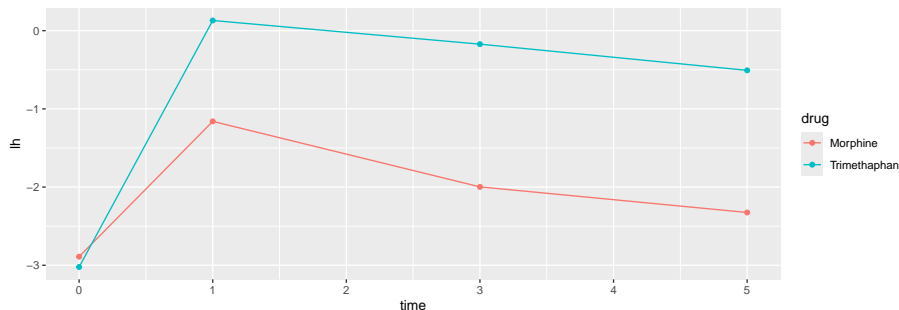
Comments

This says:

- Take data frame `dogs`, and then:
- Combine the columns `lh0` through `lh5` into one column called `lh`, with the column that each `lh` value originally came from labelled by `timex`, and then:
- Pull out numeric values in `timex`, saving in `time` and then:
- save the result in a data frame `dogs.long`.

Interaction plot

```
ggplot(dogs.long, aes(x = time, y = lh,  
                      colour = drug, group = drug)) +  
  stat_summary(fun = mean, geom = "point") +  
  stat_summary(fun = mean, geom = "line")
```



Comments

- Plot mean 1h value at each time, joining points on same drug by lines.
- drugs same at time 0
- after that, Trimethaphan higher than Morphine.
- Effect of drug not consistent over time: significant interaction.

Take out time zero

- Lines on interaction plot would then be parallel, and so interaction should no longer be significant.
- Go back to original “wide” dogs data frame.

```
response <- with(dogs, cbind(lh1, lh3, lh5)) # excl time 0
dogs.1 <- lm(response ~ drug, data = dogs)
times <- colnames(response)
times.df <- data.frame(times=factor(times))
dogs.2 <- Manova(dogs.1,
  idata = times.df,
  idesign = ~times
)
```

Results (univariate)

```
summary(dogs.2)$sphericity.tests
```

	Test statistic	p-value
times	0.57597	0.25176
drug:times	0.57597	0.25176

```
# summary(dogs.2)$pval.adjustments
```

```
summary(dogs.2)$univariate.tests
```

	Sum Sq	num Df	Error SS	den Df	F value	Pr(>F)	
(Intercept)	24.2607	1	20.1874	6	7.2106	0.03628	*
drug	16.2197	1	20.1874	6	4.8207	0.07053	.
times	3.3250	2	0.7301	12	27.3251	3.406e-05	***
drug:times	0.3764	2	0.7301	12	3.0929	0.08254	.

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

Comments

- sphericity: no problem (P-value 0.25)
- univariate test for interaction no longer significant (P-value 0.082)
- look at main effects:
 - ▶ strong significance of time, even after taking out time 0
 - ▶ actually *not* significant drug effect, despite interaction plot

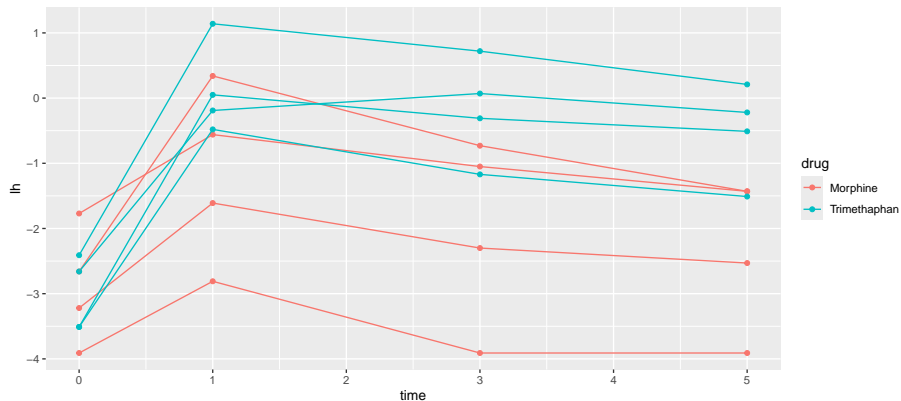
Non-significant drug effect reasonable?

- Plot *actual data*: lh against days, labelling observations by drug: “spaghetti plot”.
- Uses long data frame:
 - ▶ Plot (time, lh) points coloured by *drug*
 - ▶ connecting measurements for each *dog* by lines.
 - ▶ Hence, group = dog, but colour = drug:

```
ggplot(dogs.long, aes(x = time, y = lh,  
  colour = drug, group = dog)) +  
  geom_point() + geom_line() -> g
```

The spaghetti plot

g



Comments

- For each dog over time, gradual decrease in log-histamine from time 1: significant time effect after we took out time 0.
- Pattern about same for each dog, regardless of drug, hence non-significant interaction.
- Most trimethaphan dogs (blue) have higher log-histamine throughout (time 1 and after), some morphine dogs (red) have lower.
- *But* two morphine dogs have log-histamine profiles like trimethaphan dogs. This ambiguity probably why drug effect not quite significant.

Mixed models

- Another way to fit repeated measures
- Subjects (on whom repeated measures taken) are *random sample of all possible subjects* (random effects)
- Times and treatments are *the only ones we care about* (fixed effects)
- Use package lme4 function lmer (like lm in some ways)
- Uses long-format “tidy” data

Fitting the model (uses lme4)

```
# dogs.long including time zero with categorical timex  
dogs.3 <- lmer(lh ~ drug * timex + (1|dog), data=dogs.long)
```

- note specification of random effect: each dog has “random intercept” that moves log-histamine up or down for that dog over all times

What can we drop?

- using drop1:

```
drop1(dogs.3, test="Chisq")
```

Single term deletions

Model:

```
lh ~ drug * timex + (1 | dog)
```

	npar	AIC	LRT	Pr(Chi)
--	------	-----	-----	---------

<none>		62.167		
--------	--	--------	--	--

drug:timex	3	84.589	28.422	2.962e-06 ***
------------	---	--------	--------	---------------

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

- Interaction very significant. Including time zero, the pattern of log-histamine over time is different for the two drugs (as we found before).

Omitting time zero

Let's pretend we are working at $\alpha = 0.01$:

```
dogs.long %>% filter(timex != "lh0") -> dogs.long.no0
dogs.4 <- lmer(lh ~ drug * timex + (1|dog), data=dogs.long.no0)
drop1(dogs.4, test = "Chisq")
```

Single term deletions

Model:

lh ~ drug * timex + (1 | dog)

	npar	AIC	LRT	Pr(Chi)
<none>		42.119		
drug:timex	2	44.771	6.6518	0.03594 *

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

Interaction is not quite significant at $\alpha = 0.01$. So we could remove it.

Removing the interaction

```
dogs.5 <- update(dogs.4, . ~ . - drug:timex)
drop1(dogs.5, test = "Chisq")
```

Single term deletions

Model:

```
lh ~ drug + timex + (1 | dog)
```

	npar	AIC	LRT	Pr(Chi)
<none>		44.771		
drug	1	47.489	4.7176	0.02985 *
timex	2	62.972	22.2011	1.51e-05 ***

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

- Definitely an effect of time, but drug is not quite significant (at $\alpha = 0.01$).
- More or less same conclusions as from MANOVA.

The exercise data

- 30 people took part in an exercise study.
- Each subject randomly assigned to one of two diets (“low fat” or “non-low fat”) and to one of three exercise programs (“at rest”, “walking”, “running”).
- $2 \times 3 = 6$ experimental treatments, and thus each one replicated $30/6 = 5$ times. (Two-way ANOVA, so far?)
- However, each subject had pulse rate measured at three different times (1, 15 and 30 minutes after starting their exercise), so have repeated measures.

Reading the data

Separated by *tabs*:

```
url <- "http://ritsokiguess.site/datafiles/exercise2.txt"
exercise.long <- read_tsv(url)
exercise.long %>% slice(1:7) # top 7 rows
```

A tibble: 7 x 5

	id	diet	exertype	pulse	time
	<dbl>	<chr>	<chr>	<dbl>	<chr>
1	1	nonlowfat	atrest	85	min01
2	1	nonlowfat	atrest	85	min15
3	1	nonlowfat	atrest	88	min30
4	2	nonlowfat	atrest	90	min01
5	2	nonlowfat	atrest	92	min15
6	2	nonlowfat	atrest	93	min30
7	3	nonlowfat	atrest	97	min01

Comments

- “Long format”, usually what we want.
- But for repeated measures analysis, we want *wide* format!
- Keep track of which is which:
 - ▶ Manova analysis: wider
 - ▶ graphs and lmer analysis: longer.
- `pivot_wider`.

Making wide format

- `pivot_wider` needs: a column that is going to be split, and the column to make the values out of:

```
exercise.long %>% pivot_wider(names_from=time,  
                              values_from=pulse) -> exercise.w  
exercise.wide %>% sample_n(5) # random 5 rows
```

A tibble: 5 x 6

	id	diet	exertype	min01	min15	min30
	<dbl>	<chr>	<chr>	<dbl>	<dbl>	<dbl>
1	7	lowfat	atrest	87	88	90
2	5	nonlowfat	atrest	91	92	91
3	20	lowfat	walking	102	104	103
4	26	lowfat	running	95	126	143
5	9	lowfat	atrest	97	99	96

Setting up

- Make response variable from min01, min15, min30:

```
response <- with(exercise.wide, cbind(min01, min15, min30))
```

- Predict from diet, exertype, interaction using lm:

```
exercise.1 <- lm(response ~ diet * exertype,  
  data = exercise.wide  
)
```


... continued

- Run this through Manova:

```
times <- colnames(response)
times.df <- data.frame(times=factor(times))
exercise.2 <- Manova(exercise.1,
                     idata = times.df,
                     idesign = ~times)
```

Sphericity tests

```
summary(exercise.2)$sphericity.tests
```

	Test statistic	p-value
times	0.92416	0.40372
diet:times	0.92416	0.40372
exertype:times	0.92416	0.40372
diet:exertype:times	0.92416	0.40372

No problem with sphericity; go to univariate tests.

Univariate tests

```
summary(exercise.2)$univariate.tests
```

	Sum Sq	num Df	Error SS	den Df	F value
(Intercept)	894608	1	2085.2	24	10296.6595
diet	1262	1	2085.2	24	14.5238
exertype	8326	2	2085.2	24	47.9152
diet:exertype	816	2	2085.2	24	4.6945
times	2067	2	1563.6	48	31.7206
diet:times	193	2	1563.6	48	2.9597
exertype:times	2723	4	1563.6	48	20.9005
diet:exertype:times	614	4	1563.6	48	4.7095

Pr(>F)

(Intercept)	< 2.2e-16	***
diet	0.0008483	***
exertype	4.166e-09	***
diet:exertype	0.0190230	*
times	1.662e-09	***
diet:times	0.0613651	.
exertype:times	4.992e-10	***
diet:exertype:times	0.0027501	**

Comments

- The three-way interaction is significant
- the effect of diet on pulse rate over time is different for the different exercise types

Making some graphs

- Three-way interactions are difficult to understand. To make an attempt, look at some graphs.
- Plot time trace of pulse rates for each individual, joined by lines, and make *separate* plots for each diet-exertype combo.
- `facet_grid(diet~exertype)`: do a separate plot for each combination of diet and exercise type, with diets going down the page and exercise types going across. (Graphs are usually landscape, so have the factor `exertype` with more levels going across.)

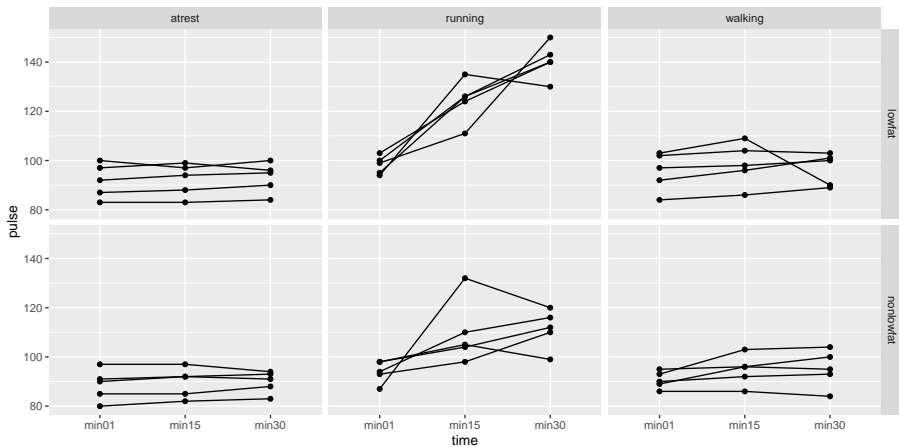
... continued

- ggplot again. Using *long* data frame:

```
g <- ggplot(exercise.long, aes(  
  x = time, y = pulse,  
  group = id  
)) + geom_point() + geom_line() +  
  facet_grid(diet ~ exertype)
```

The graph(s)

gg



Comments on graphs

- At rest: no change in pulse rate over time
- Walking: not much change in pulse rates over time.
- Running: overall increase in pulse rate over time, but increase stronger for lowfat group.
- No consistent effect of:
 - ▶ diet over all exercise groups.
 - ▶ exercise type over both diet groups.
 - ▶ time over all diet-exercise type combos.

“Simple effects” of diet for the subjects who ran

- Looks as if there is only any substantial time effect for the runners. For them, does diet have an effect?
- Pull out only the runners from the wide data:

```
exercise.wide %>%  
  filter(exertype == "running") -> runners.wide
```

... continued

- Create response variable and do MANOVA. Some of this looks like before, but I have different data now:

```
response <- with(runners.wide, cbind(min01, min15, min30))
runners.1 <- lm(response ~ diet, data = runners.wide)
times <- colnames(response)
times.df <- data.frame(times=factor(times))
runners.2 <- Manova(runners.1,
  idata = times.df,
  idesign = ~times
)
```

Sphericity tests

```
summary(runners.2)$sphericity.tests
```

	Test statistic	p-value
times	0.81647	0.4918
diet:times	0.81647	0.4918

- No problem, look at univariate tests.

Univariate tests

```
summary(runners.2)$univariate.tests
```

	Sum Sq	num Df	Error SS	den Df	F value	Pr(>F)	
(Intercept)	383522	1	339.2	8	9045.3333	1.668e-13	***
diet	1920	1	339.2	8	45.2830	0.0001482	***
times	4714	2	1242.0	16	30.3644	3.575e-06	***
diet:times	789	2	1242.0	16	5.0795	0.0195874	*

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

- Interaction still significant
 - ▶ dependence of pulse rate on time still different for the two diets

How is the effect of diet different over time?

- Table of means. Only I need long data for this:

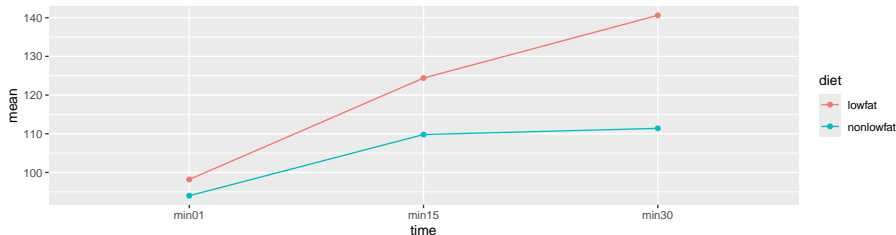
```
runners.wide %>%  
  pivot_longer(starts_with("min"),  
               names_to = "time", values_to = "pulse") %>%  
  group_by(time, diet) %>%  
  summarize(  
    mean = mean(pulse),  
    sd = sd(pulse)  
  ) -> summ
```

- Result of summarize is data frame, so can save it (and do more with it if needed).

Interaction plot

- We went to trouble of finding means by group, so making interaction plot is now mainly easy:

```
ggplot(summ, aes(x = time, y = mean, colour = diet,  
                 group = diet)) + geom_point() + geom_line()
```



Comment on interaction plot

- The lines are not parallel, so there is interaction between diet and time for the runners.
- The effect of time on pulse rate is different for the two diets, even though all the subjects here were running.