Repeated measures analysis

Repeated measures by profile analysis

- More than one response *measurement* for each subject. Might be
  - measurements of the same thing at different times
  - measurements of different but related things
- Generalization of matched pairs (“matched triples”, etc.).
- Variation: each subject does several different treatments at different times (called *crossover design*).
- Expect measurements on same subject to be correlated, so assumptions of independence will fail.
- Called *repeated measures*. Different approaches, but *profile analysis* uses `Manova` (set up right way).
- Another approach uses *mixed models* (random effects).

Packages

```r
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 (some; there is a lot)

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

```
Type II Repeated Measures MANOVA Tests:

------------------------------------------

Term: (Intercept)

 Response transformation matrix:
    (Intercept)
lh0           1
lh1           1
lh3           1
lh5           1

Sum of squares and products for the hypothesis:
            (Intercept)
(Intercept)     285.366

Multivariate Tests: (Intercept)
                 Df test stat approx F num Df den Df    Pr(>F)   
Pillai            1  0.763467 19.36642      1      6 0.0045648 **
Wilks             1  0.236533 19.36642      1      6 0.0045648 **
Hotelling-Lawley  1  3.227738 19.36642      1      6 0.0045648 **
Roy               1  3.227738 19.36642      1      6 0.0045648 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

------------------------------------------

Term: drug

 Response transformation matrix:
    (Intercept)
```

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).

Univariate tests

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

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

```
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"
```

```
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 ***
---
```

Comments

- The sphericity test for the interaction is almost significant
- The H-F adjusted P-value for the interaction is a bit bigger than the univariate one, but still strongly significant.
- Therefore any lack of sphericity does not affect our conclusion: there is an interaction between drug and time
- ie that the effect of time on log-histamine is different for the two drugs.

Comments

- Here, univariate test with Huynh-Feldt correction to P-value for interaction was 0.00073.
- Significant interaction *is* the conclusion here.
- If the interaction had not been significant:
    - cannot remove interaction with time
    - so look at univariate (better, especially if adjusted for sphericity) tests of main effects in *this* model

Next

- Interaction significant. Pattern of response over time different for the two drugs.
- Want to investigate interaction.

The wrong shape

- But data frame has several observations per line ("wide format"):

```r
dogs %>% slice(1:6)
```

```
# A tibble: 6 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
```

- Plotting works with data in "long format": one response per line.
- The responses are log-histamine at different times, labelled `lh`-something. Call them all `lh` 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: for the times, we want just the numbers, not the letters `lh` every time. Want new variable containing just number in `time`: `parse_number`.

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

```
# A tibble: 32 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
 6 B     Morphine N     lh1   -2.81     1
 7 B     Morphine N     lh3   -3.91     3
 8 B     Morphine N     lh5   -3.91     5
 9 C     Morphine N     lh0   -2.66     0
10 C     Morphine N     lh1    0.34     1
```

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`.
- 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 the pipelined results

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

```
# A tibble: 32 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
 6 B     Morphine N     lh1   -2.81     1
 7 B     Morphine N     lh3   -3.91     3
 8 B     Morphine N     lh5   -3.91     5
 9 C     Morphine N     lh0   -2.66     0
```

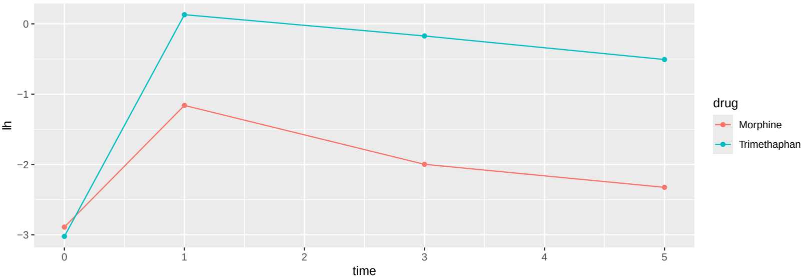
Comments

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 `lh` 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
```

```
              GG eps   Pr(>F[GG])     HF eps   Pr(>F[HF])
times      0.7022305 0.0003752847 0.8520467 0.0001117394
drug:times 0.7022305 0.1078608639 0.8520467 0.0942573437
attr(,"na.action")
(Intercept)        drug
          1           2
attr(,"class")
[1] "omit"
```

```
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 .
---
```

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

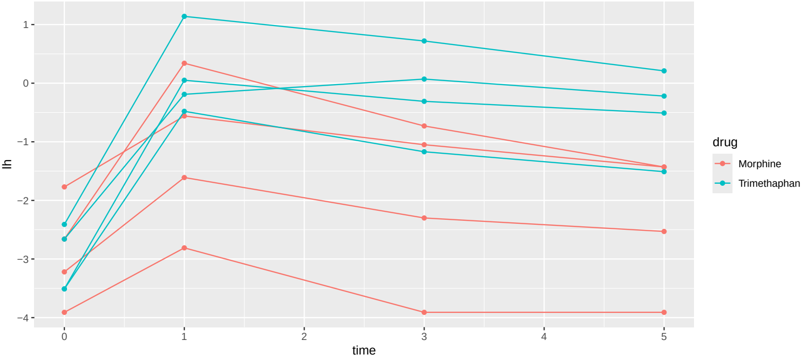
Is the non-significant drug effect reasonable?

- Plot *actual data*: `lh` against `days`, labelling observations by drug: “spaghetti plot”.
- Uses long data frame (confusing, yes I know):
- Plot (time,lh) points coloured by drug
- and connecting measurements for each *dog* by lines.
- This time, we want `group = dog` (want the measurements for each *dog* joined by lines), 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, there is a strong increase and gradual decrease in log-histamine. The gradual decrease explains the significant time effect after we took out time 0.
- The pattern is more or less the same for each dog, regardless of drug. This explains the non-significant interaction.
- Most of the trimethaphan dogs (blue) have higher log-histamine throughout (time 1 and after), and some of the morphine dogs have lower.
- *But* two of the morphine dogs have log-histamine profiles like the trimethaphan dogs. This ambiguity is probably why the `drug` effect is 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
dogs.3 <- lmer(lh~drug*time+(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 * time + (1 | dog)
          npar    AIC    LRT Pr(Chi)
<none>         113.26
drug:time    1 114.21 2.9534  0.0857 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
```

- Interaction again not significant, but P-value smaller than before

Re-fit without interaction

```r
dogs.4 <- update(dogs.3,.~.-drug:time)
drop1(dogs.4,test="Chisq")
```

```
Single term deletions

Model:
lh ~ drug + time + (1 | dog)
       npar    AIC    LRT Pr(Chi)  
<none>      114.21                 
drug      1 115.57 3.3560 0.06696 .
time      1 114.96 2.7501 0.09725 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '
```

- This time neither drug nor (surprisingly) time is significant.
- MANOVA and `lmer` methods won't agree, but both valid ways to approach problem.

The exercise data

- 30 people took part in an exercise study.
- Each subject was randomly assigned to one of two diets (“low fat” or “non-low fat”) and to one of three exercise programs (“at rest”, “walking”, “running”).
- There are $2 \times 3 = 6$ experimental treatments, and thus each one is replicated $30/6 = 5$ times.
- Nothing unusual so far.
- However, each subject had their 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
```

```
# A tibble: 90 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
 8     3 nonlowfat atrest      97 min15
 9     3 nonlowfat atrest      94 min30
10     4 nonlowfat atrest      80 min01
```

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.wide
exercise.wide %>% sample_n(5)
```

```
# A tibble: 5 x 6
     id diet      exertype min01 min15 min30
  <dbl> <chr>     <chr>    <dbl> <dbl> <dbl>
1     5 nonlowfat atrest      91    92    91
2    23 nonlowfat running     98   105    99
3    28 lowfat    running    103   124   140
4    14 nonlowfat walking     95    96   100
5    15 nonlowfat walking     89    96    95
```

- Normally `pivot_longer` `min01, min15, min30` into one column called `pulse` labelled by the number of minutes. But Manova needs it the other way.

Setting up the repeated-measures analysis

- Make a response variable consisting of `min01`, `min15`, `min30`:

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

- Predict that from `diet` and `exertype` and interaction using `lm`:

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

- 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

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

```
                    Sum Sq num Df Error SS den Df    F value    Pr(>F)    
(Intercept)         894608      1   2085.2     24 10296.6595 < 2.2e-16 ***
diet                  1262      1   2085.2     24    14.5238 0.0008483 ***
exertype              8326      2   2085.2     24    47.9152 4.166e-09 ***
diet:exertype          816      2   2085.2     24     4.6945 0.0190230 *  
times                 2067      2   1563.6     48    31.7206 1.662e-09 ***
diet:times             193      2   1563.6     48     2.9597 0.0613651 .  
exertype:times        2723      4   1563.6     48    20.9005 4.992e-10 ***
diet:exertype:times    614      4   1563.6     48     4.7095 0.0027501 ** 
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

- 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

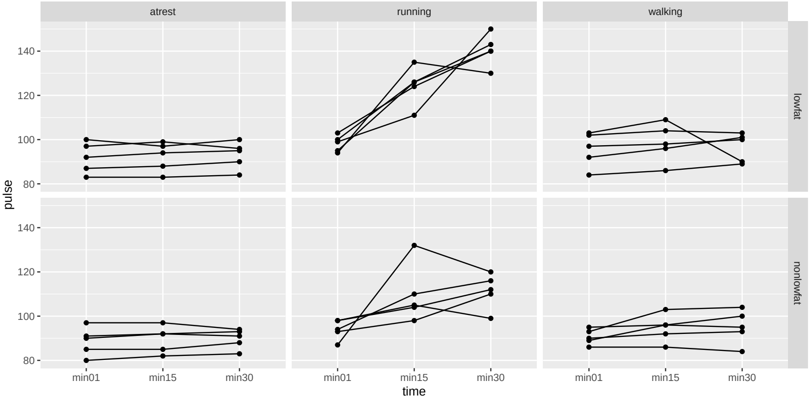
- Three-way interactions are difficult to understand. To make an attempt, look at some graphs.
- Plot time trace of pulse rates for each individual, joined by lines, and make *separate* plots for each `diet-exertype` combo.
- `ggplot` again. Using *long* data frame:

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

- `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.)

The graph(s)

```
g
```


atrest
running
walking
lowfat
nonlowfat
pulse
140
120
100
80
140
120
100
80
min01
min15
min30
min01
min15
min30
min01
min15
min30
time

Comments on graphs

- For subjects who were at rest, no change in pulse rate over time, for both diet groups.
- For walking subjects, not much change in pulse rates over time. Maybe a small increase on average between 1 and 15 minutes.
- For both running groups, an overall increase in pulse rate over time, but the increase is stronger for the `lowfat` group.
- No consistent effect of diet over all exercise groups.
- No consistent effect of exercise type over both diet groups.
- No consistent effect of 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
```

- 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

```r
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).

Understanding diet-time interaction

- The summary:

```
summm
```

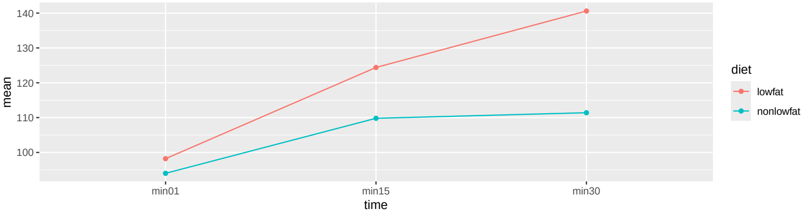
```
# A tibble: 6 x 4
# Groups:   time [3]
  time  diet       mean    sd
  <chr> <chr>     <dbl> <dbl>
1 min01 lowfat     98.2  3.70
2 min01 nonlowfat  94    4.53
3 min15 lowfat    124.   8.62
4 min15 nonlowfat 110.  13.1
5 min30 lowfat    141.   7.20
6 min30 nonlowfat 111.   7.92
```

- Pulse rates at any given time higher for `lowfat` (diet effect),
- Pulse rates increase over time of exercise (time effect),
- but the *amount by which pulse rate higher* for a diet depends on time: `diet` by `time` interaction.

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.