Analysis of repeated measurements (KLMED8008)

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Repeated measurements

This term is more or less equivalent with ...

- Within subjects experiments
- Grouped/clustered data
- Hierarchical models
- Longitudinal data

… We will not consider

- Time series

Day 1

- Welcome. Introduction. Presentation.
- Practical issues about the course
  - Website: http://folk.ntnu.no/eiriksko/KLMED8008/KLMED8008v13.html
  - Lectures
  - Textbook
  - Software
  - Excercises
  - Exam
- Repeated measurements: advantages and problems
- One summary measure
- Review of variance, standard deviation, covariance and correlation
- Linear combination of random variables
- (Brief) review of linear regression (Textbook Chapter 1)

Repeated measurements

Advantages

- The individual acts as his own control → reduces total variance
- Effective (re-) use of recruited individuals/patients → economy

Problems

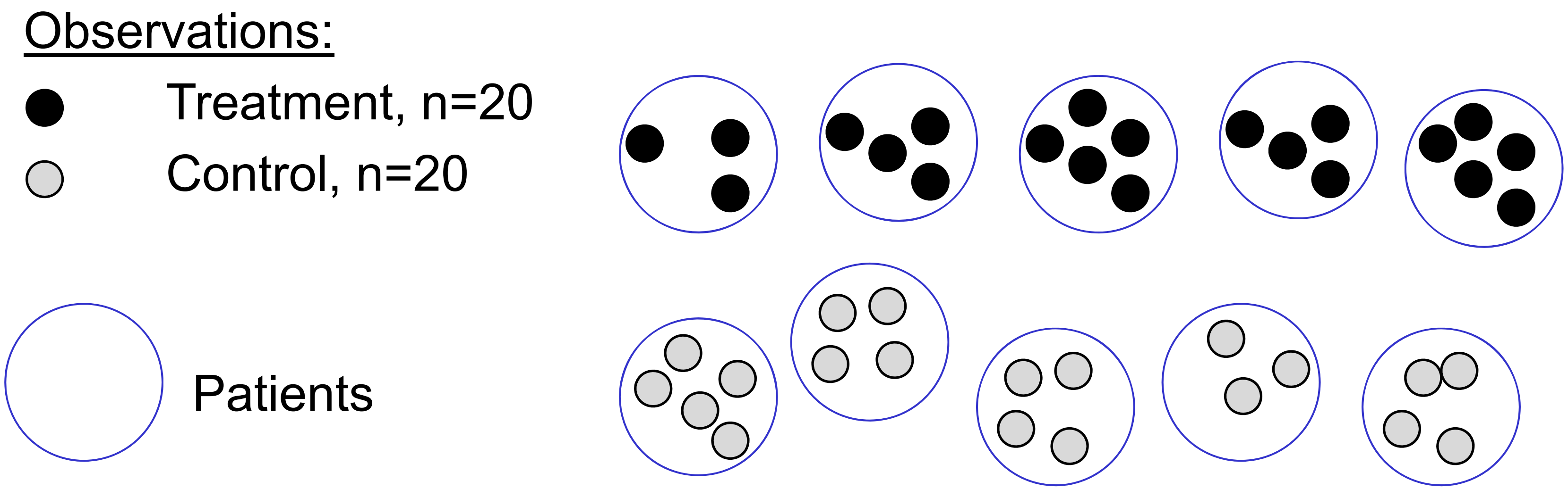
- Potential “carry-over” effect
- Spontaneous change may occur over time
- In general:
  “usual statistical procedures” (e.g. testing the null hypothesis) require independent observations!
- Special methods and software necessary

Repeated measurements

Ignoring dependency between observations may lead to…

- p-values becoming too small when doing between-patient comparisons (i.e. yield false positive results)

Textbook 3.10.2 (p. 167), *Veierød et al.* 7.1 (p. 231)

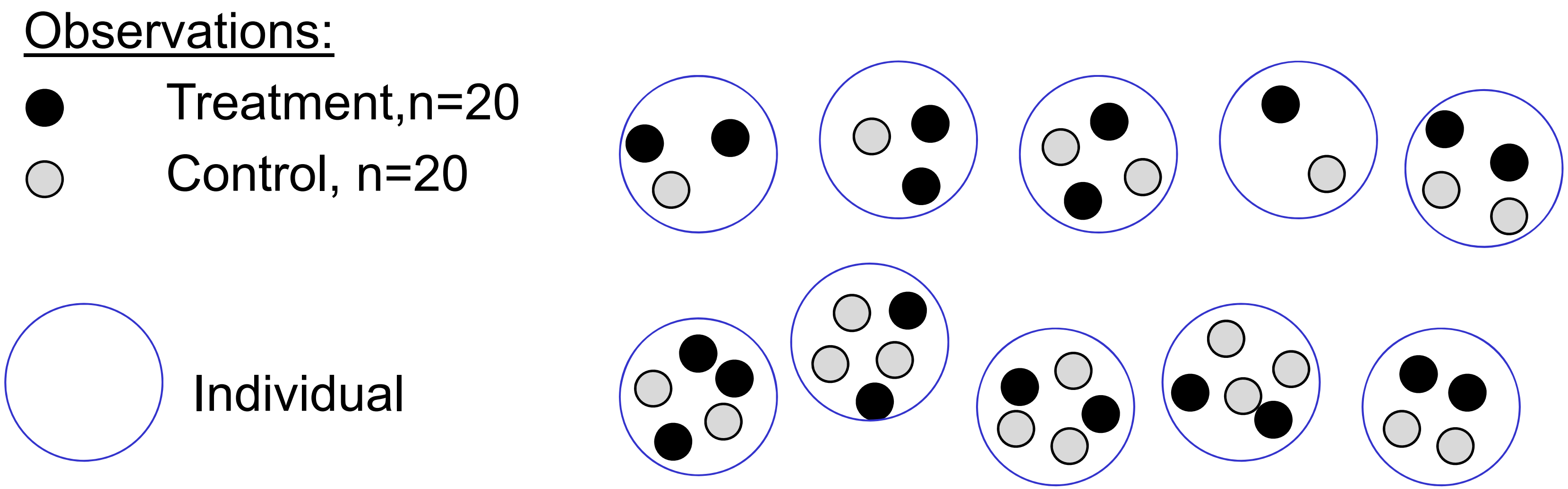


Repeated measurements

Ignoring dependency between observations may lead to…

- p-values becoming too large when doing within-patient comparisons (i.e. yield false negative results)

Textbook 3.10.2 (p. 167), *Veierød et al.* 7.1 (p. 231)



ONE SUMMARY MEASURE

Avoiding the problem: use <u>one</u> summary measure per individual

- Change (e.g. from baseline measurement)
- Mean or median
- Maximum
- Maximum change
- Area under the curve (AUC)
- Estimated slope (β) for each individual obtained by simple linear regression

$\rightarrow$ These outcomes may now be analyzed "as usual" (with t-tests etc.), as the observations are *now independent*

Matthews (1990), *Altman* (1991), *Davis* (2002)

Example:
gastric tube to reduce postoperative nausea

- Double blind randomized controlled study
- 30 and 29 patients, without or with gastric tube, respectively
- Nausea registered on an ordinal 5-point ordinal scale (0…4) six times postoperatively

- Research question:
  - does gastric tube affect nausea?

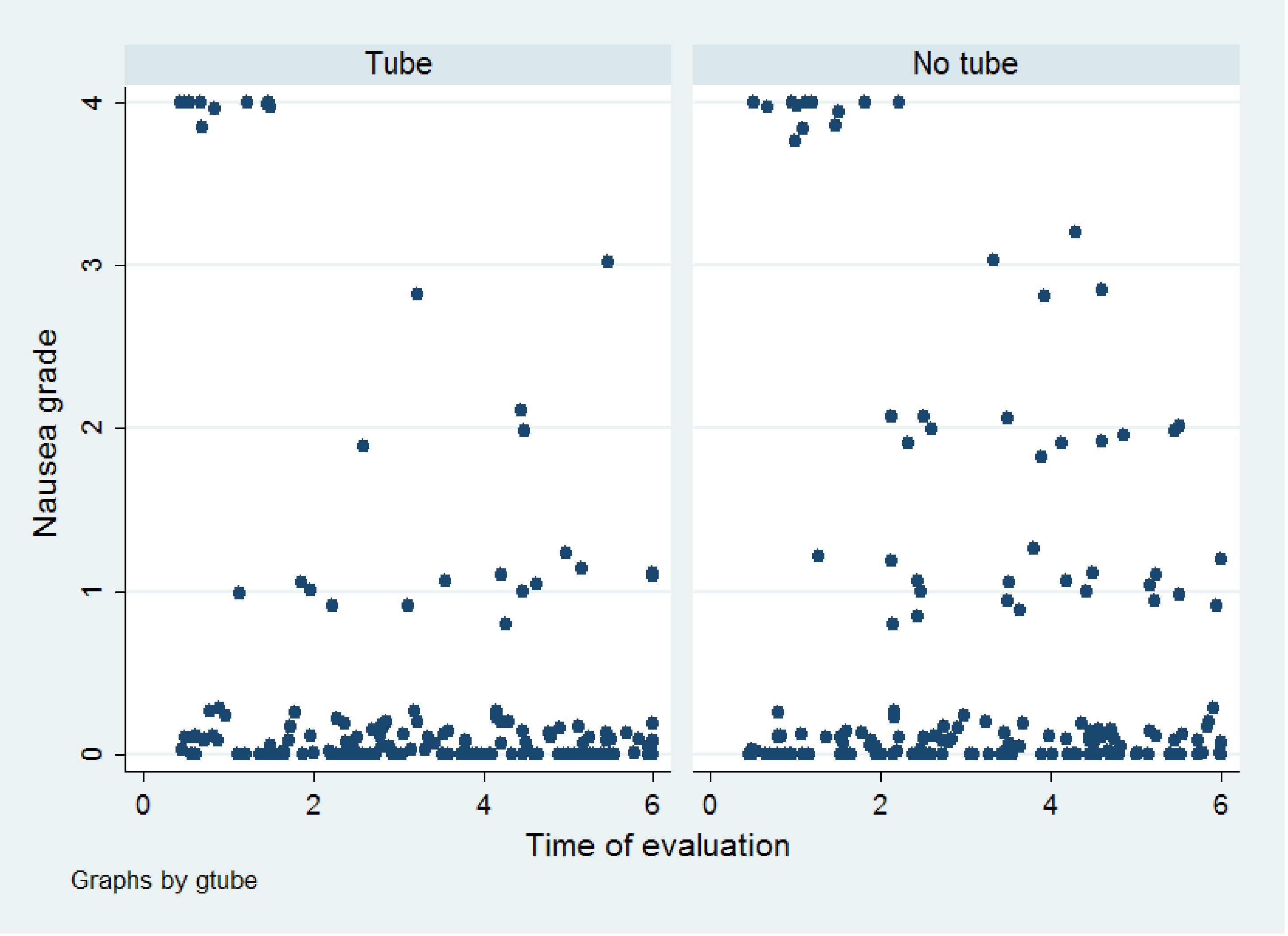
Results for the first 20 patients…

	grp	t1	t2	t3	t4	t5	t6
1.	No tube	0	0	3	3	3	0
2.	Tube	4	0	0	0	0	0
3.	Tube	0	0	2	0	0	0
4.	Tube	0	0	0	0	0	.
5.	No tube	4	0	0	0	0	0
6.	No tube	0	0	0	0	1	.
7.	Tube	0	0	0	0	0	0
8.	Tube	4	1	0	2	1	.
9.	No tube	0	0	0	0	0	0
10.	No tube	4	0	0	0	0	.
11.	Tube	4	0	0	0	0	.
12.	No tube	0	0	0	3	0	0
13.	Tube	0	0	0	0	0	0
14.	Tube	0	0	0	0	1	.
15.	Tube	4	1	0	2	3	1
16.	No tube	1	1	2	0	0	0
17.	Tube	4	4	0	0	1	0
18.	Tube	1	0	0	1	0	1
19.	Tube	4	0	0	0	0	0
20.	No tube	0	0	0	0	0	0

- Some missing observations
- Which summary measure to use?
  - Mean (t1..t6)
  - Median (t1..t6)
  - Max (t1..t6)
  - Does the maximum value of 4 occur at all?
  - Do any of the values 3 or 4 occur at all?

(should be clinically relevant, and decided on before results are known!)

One plot tells more than 1000 t-tests…



Adding summary measures

	grp	t1	t2	t3	t4	t5	t6	m	md	mx	m4	m34
1.	No tube	0	0	3	3	3	0	1.5	1.5	3	0	1
2.	Tube	4	0	0	0	0	0	.6666667	0	4	1	1
3.	Tube	0	0	2	0	0	0	.3333333	0	2	0	0
4.	Tube	0	0	0	0	0	.	0	0	0	0	0
5.	No tube	4	0	0	0	0	0	.6666667	0	4	1	1
6.	No tube	0	0	0	0	1	.	.2	0	1	0	0
7.	Tube	0	0	0	0	0	0	0	0	0	0	0
8.	Tube	4	1	0	2	1	.	1.6	1	4	1	1
9.	No tube	0	0	0	0	0	0	0	0	0	0	0
10.	No tube	4	0	0	0	0	.	.8	0	4	1	1
11.	Tube	4	0	0	0	0	.	.8	0	4	1	1
12.	No tube	0	0	0	3	0	0	.5	0	3	0	1
13.	Tube	0	0	0	0	0	0	0	0	0	0	0
14.	Tube	0	0	0	0	1	.	.2	0	1	0	0
15.	Tube	4	1	0	2	3	1	1.833333	1.5	4	1	1
16.	No tube	1	1	2	0	0	0	.6666667	.5	2	0	0
17.	Tube	4	4	0	0	1	0	1.5	.5	4	1	1
18.	Tube	1	0	0	1	0	1	.5	.5	1	0	0
19.	Tube	4	0	0	0	0	0	.6666667	0	4	1	1
20.	No tube	0	0	0	0	0	0	0	0	0	0	0

The summary measures – now independent – can be analyzed “as usual”:

```
. ttest m, by(grp)  // slide 13

Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    Tube |      29    .4287356    .1025633    .5523201    .2186443     .638827
 No tube |      30    .5755556    .0953575    .5222948    .3805275    .7705836
---------+--------------------------------------------------------------------
combined |      59    .5033898    .0700055    .5377224    .3632586    .6435211
---------+--------------------------------------------------------------------
    diff |           -.1468199    .1399091               -.426983    .1333432
------------------------------------------------------------------------------
    diff = mean(Tube) - mean(No tube)                             t =  -1.0494
Ho: diff = 0                                     degrees of freedom =       57

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.1492         Pr(|T| > |t|) = 0.2984          Pr(T > t) = 0.8508
```

```
. tabulate grp m34, chi // slide 13

           |  t1 t2 t3 t4 t5 t6 ==
           |          3 4
     gtube |         0          1 |     Total
-----------+----------------------+----------
      Tube |        19         10 |        29
   No tube |        18         12 |        30
-----------+----------------------+----------
     Total |        37         22 |        59

          Pearson chi2(1) =   0.1920   Pr = 0.661
```

Review of

VARIANCE AND COVARIANCE

Sample variance

<u>Definition (Rosner Def.2.7)</u>

$$S^2 = \frac{1}{n-1}\sum_{i=1}^{n}(x_i - \overline{x})^2$$

<u>Comment</u>

(n -1) is used rather than n in the denominator, as we already have made use of the sample mean value.
Thus one piece of information (one Degree of Freedom, DF) from the sample has been ”expended”.
This yields a slightly larger estimate of S^2. For large n, the difference is trivial.

Sample variance

$$S^2 = \frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2$$

Properties

- Always positive
- Extreme observations contribute more (squaring)
- Favorable mathematical properties
- *But* cannot be interpreted on original scale

(kg $\rightarrow$ kg^2, mg/dl $\rightarrow$ mg^2/dl^2, cm $\rightarrow$ cm^2 etc.)

Sample standard deviation (S, SD)

$$S = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \overline{x})^2}$$

Properties

- Always positive
- Extreme observations contribute more (squaring)
- Favorable mathematical properties
- May be interpreted on original scale!

Covariance

Def. Rosner6 5.12, p. 142

Cov $(X,Y) = E[(X-\mu_x)(Y-\mu_y)]$

More clearly ☺ :
"The expected product of the deviation of *X* and *Y* from their respective expected values"

- If *X* og *Y* are independent, then cov $(X,Y) = 0$
- The reverse does not hold!

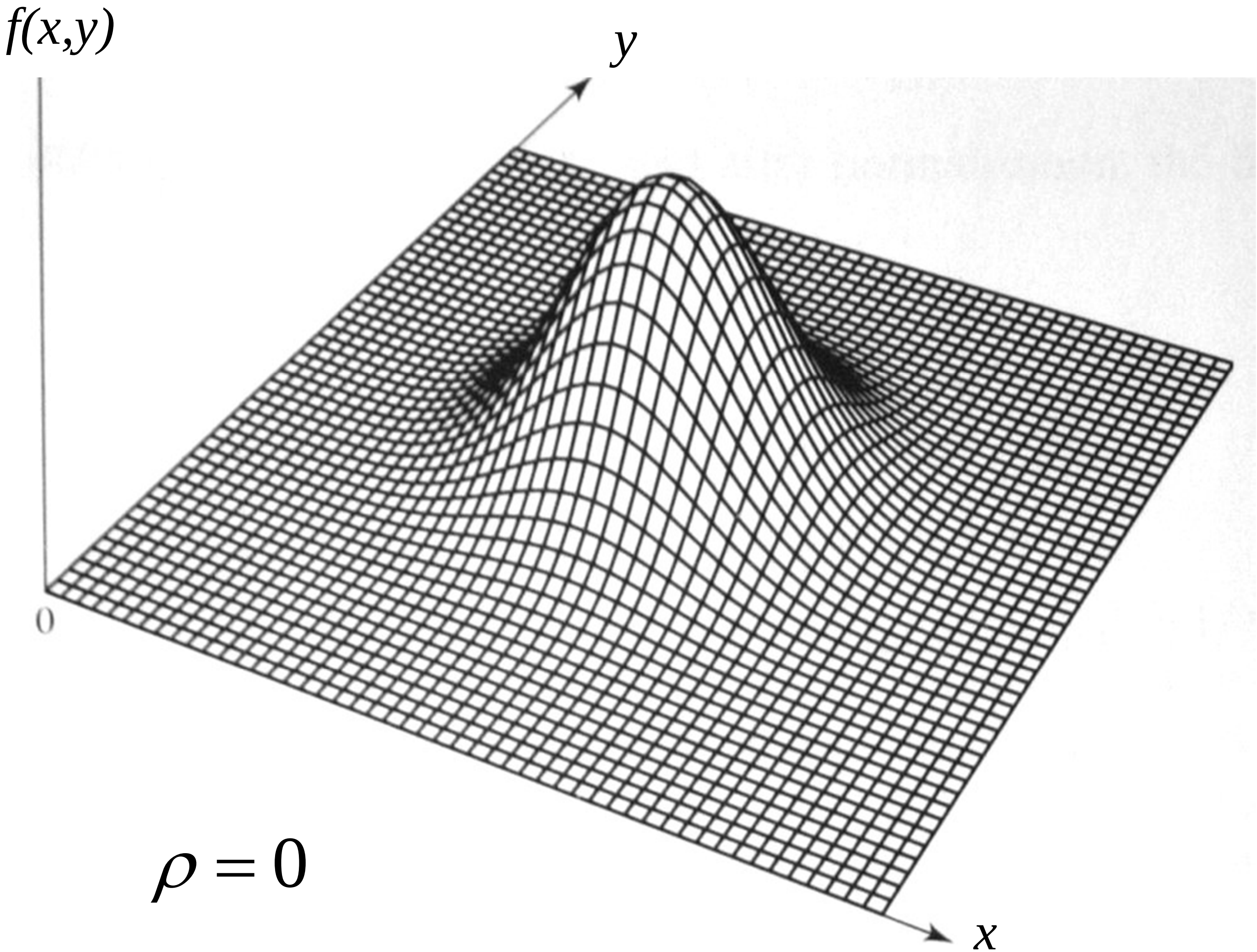
Covariance and correlation

What about the units …

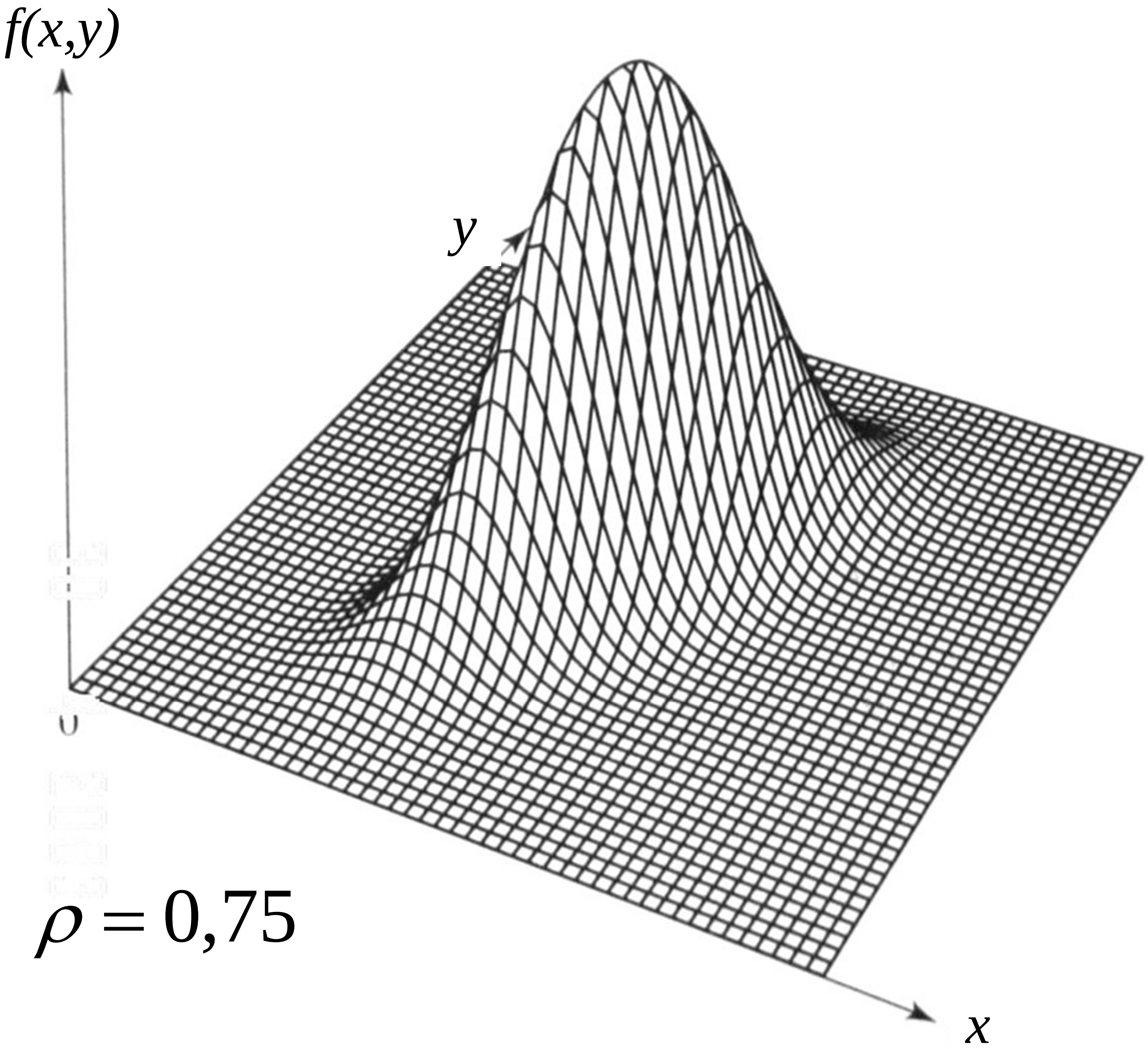
- If X measures FEV (liters, l) and Y measures height (meters, m) then their covariance has unit l*m
  (compare this with the variance, which has squared units; e.g. l^2, m^2)
- The problem is solved by dividing by the product of the respective standard deviations:

$$\mathrm{Corr}\,(X,Y) = \rho = \frac{\mathrm{Cov}\,(X,Y)}{\sigma_X \sigma_Y}$$

$$\rho \in (-1,1)$$

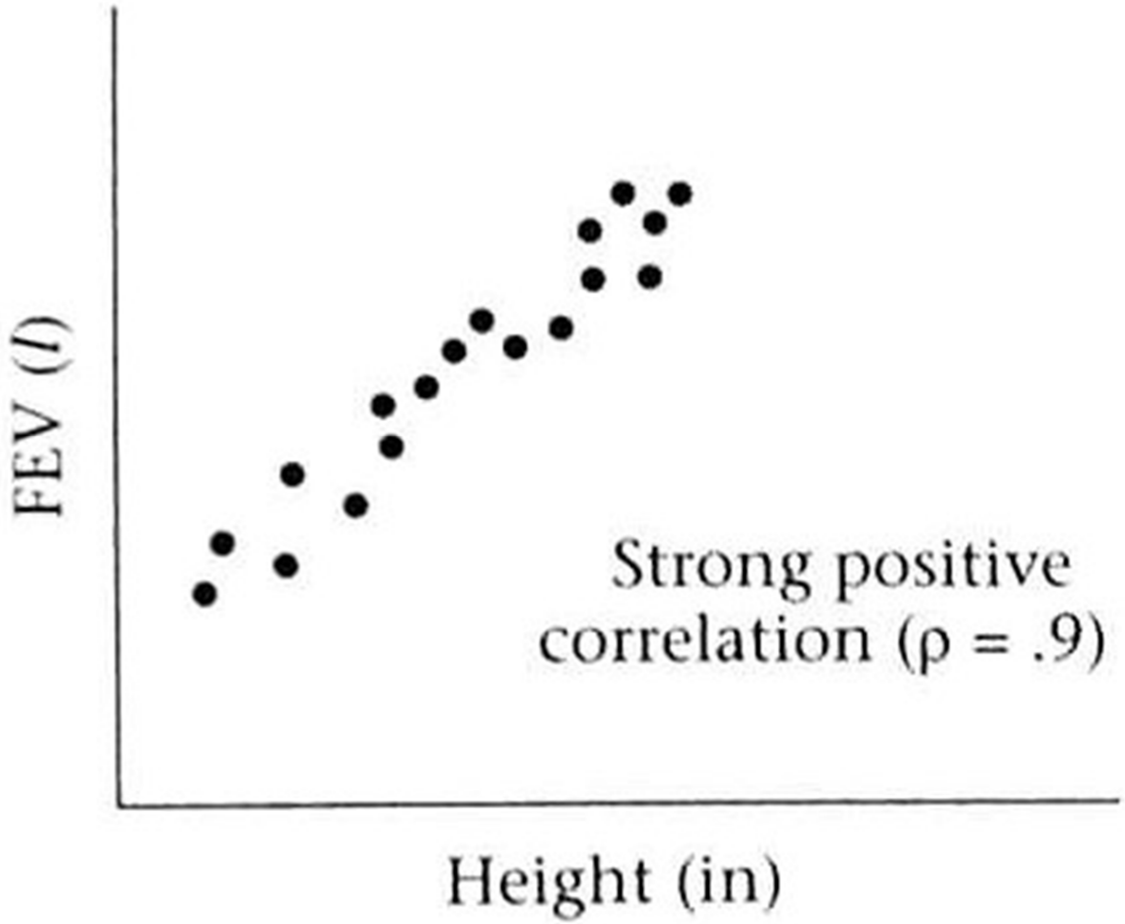


$\rho = 0$

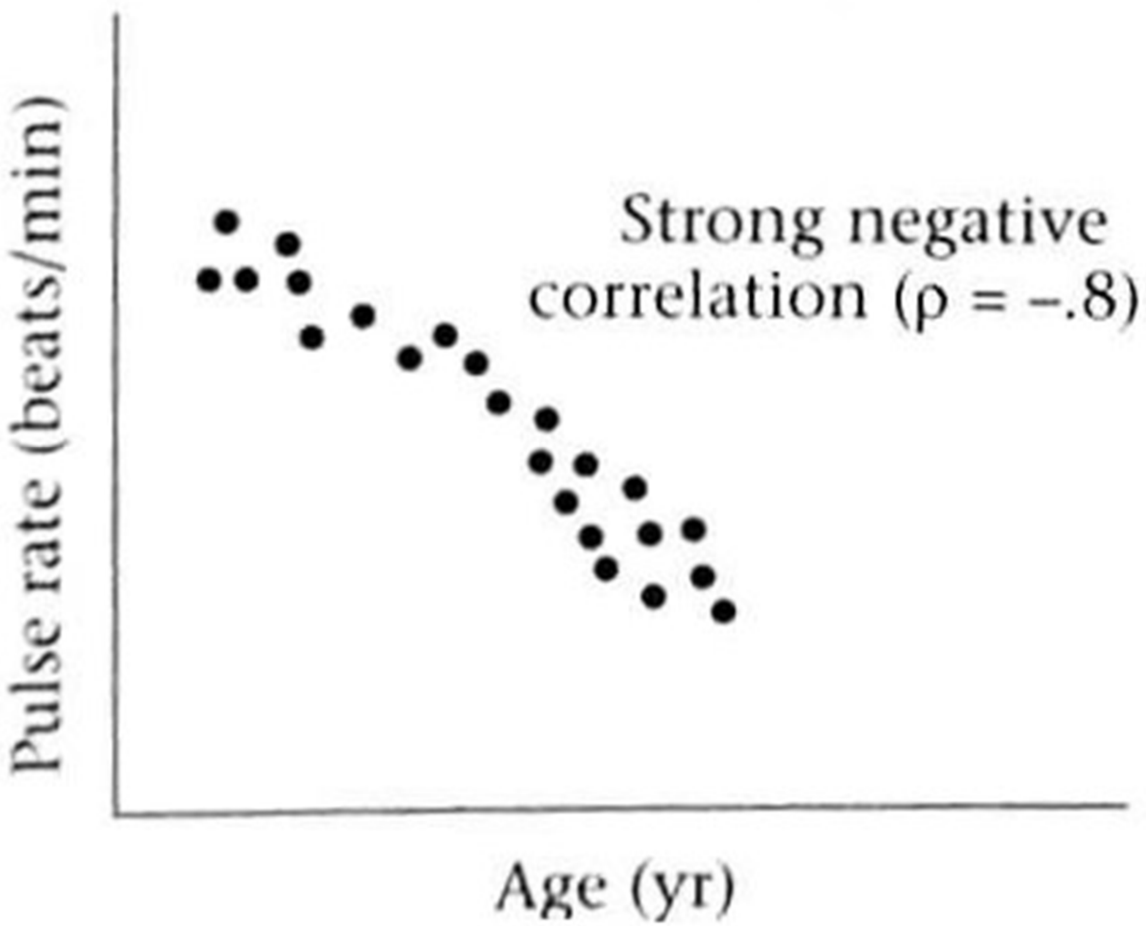


$\rho = 0{,}75$

Example: Rosner6, Fig. 5.16a

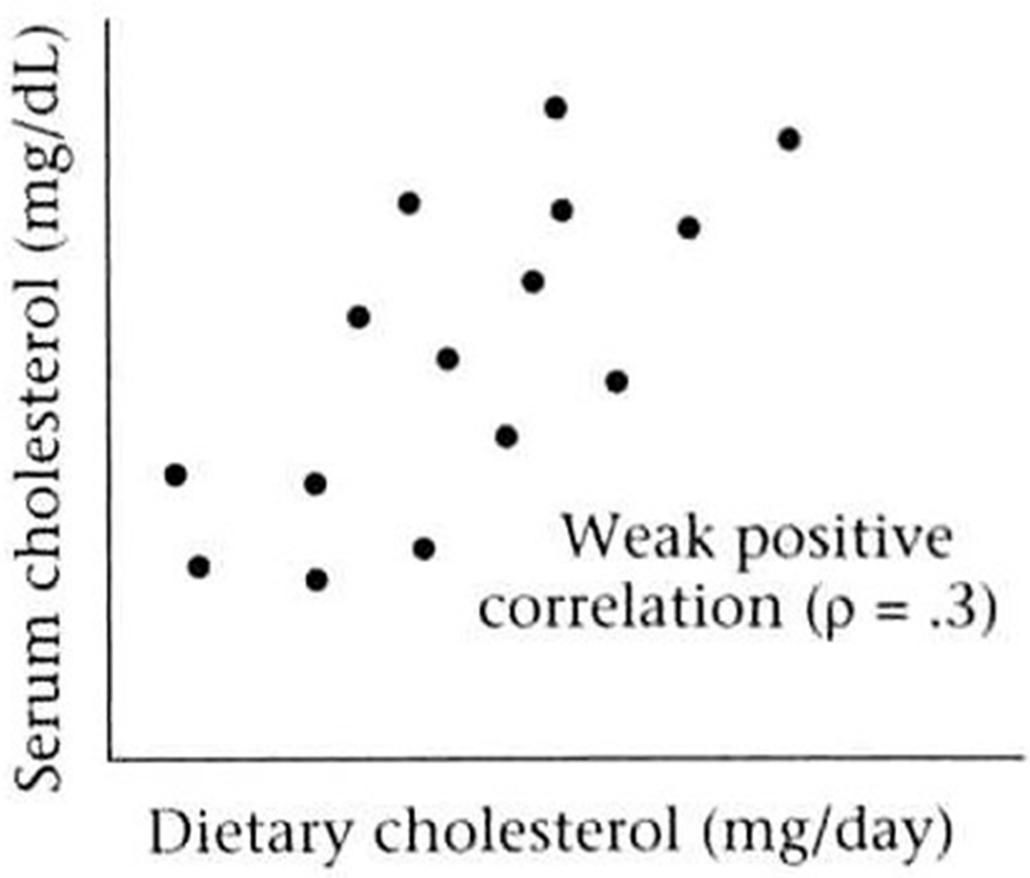


Example: Rosner6, Fig. 5.16c



A rather unusual situation!
Unlikely with repeated measurements.

Example: Rosner6, Fig. 5.16b



The usual situation with repeated measurements.

Sample covariance and sample correlation - Pearson's *r*

$$S_{X,Y} = \frac{1}{n}\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})$$

$$r = \frac{S_{X,Y}}{S_X S_Y}$$

$$r = \frac{\frac{1}{n}\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \overline{x})^2}\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \overline{y})^2}} = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \overline{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \overline{y})^2}}$$

Example

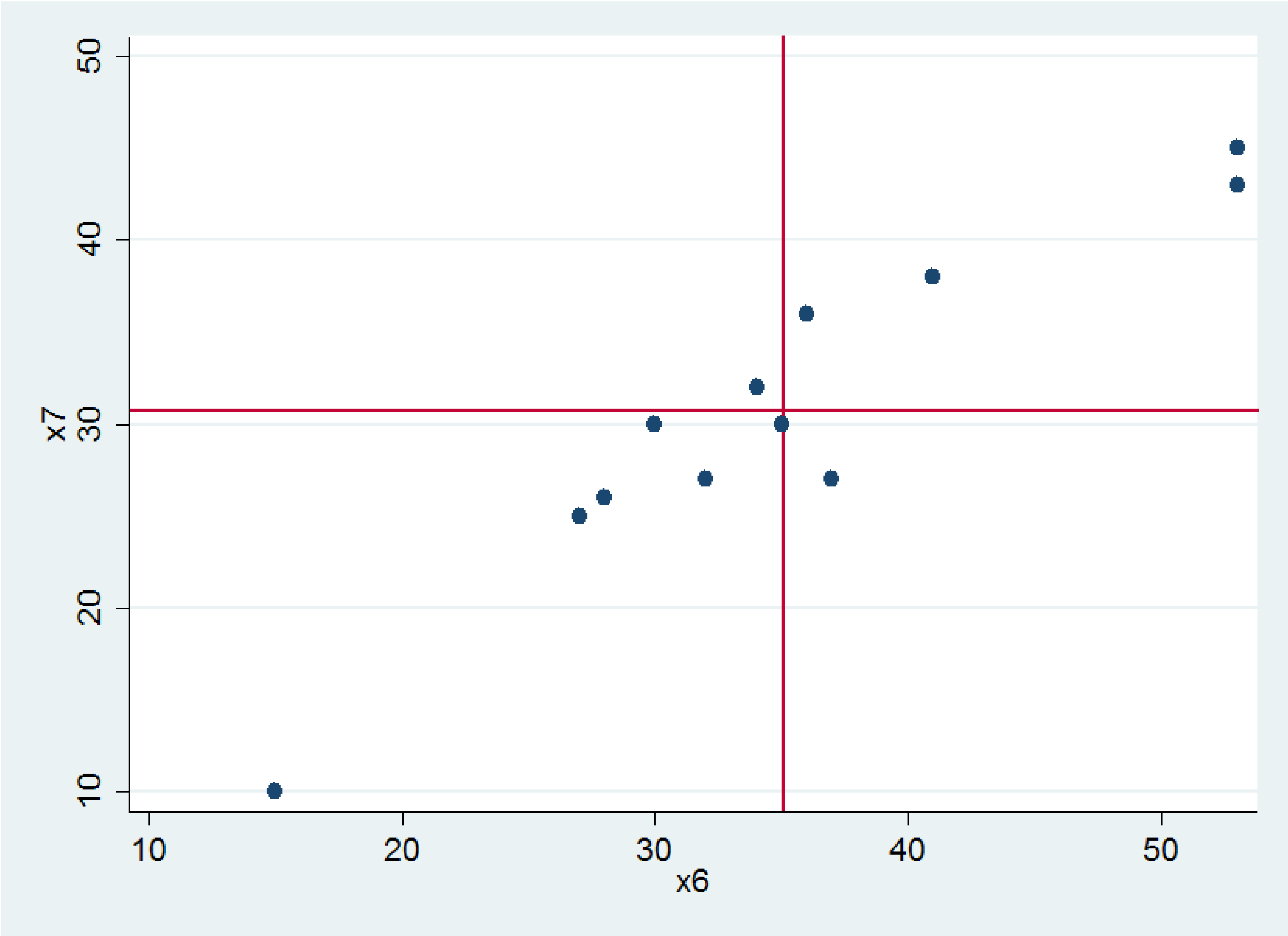
```
Central venous PO2
measured in 12
patients during
mobilisation after
cardiac surgery; in
two situations (x6
and x7)
```

pnr	x6	x7
301	32	27
302	30	30
304	34	32
306	36	36
307	28	26
308	41	38
309	35	30
310	53	43
311	27	25
312	37	27
313	15	10
314	53	45

Tasks

- Plot x6 and x7
- Calculate *by hand* the following sample statistics, using only the observations on the first 3 patients :
  - mean
  - variance
  - standard deviation
  - covariance
  - correlation coefficient

```
. twoway (scatter x7 x6,yline(30.75) xline(35.1))
```



Covariance matrix

```
. cor x6 x7, cov mean
(obs=12)

    Variable |      Mean   Std. Dev.         Min         Max
-------------+------------------------------------------------
          x6 |  35.08333    10.6041          15          53
          x7 |     30.75   9.294426          10          45


             |      x6       x7
-------------+------------------
          x6 |  112.447
          x7 |  93.2955  86.3864
```

Correlation matrix

```
. pwcorr x6 x7,  star(.05) sig

             |      x6       x7
-------------+------------------
          x6 |   1.0000
             |
             |
          x7 |   0.9466*  1.0000
             |   0.0000
             |
```

T-test for sample correlation coefficient

(Rosner6 Eq. 11.20, p. 496)

- Calculate Pearson's *r*
- Transform it to *T:*

$$T_{n-1} = \frac{\bar{x} - \mu}{\frac{s}{\sqrt{n}}} \quad \text{... in this case:}$$

$$T_{n-2} = \frac{r - 0}{\frac{\sqrt{1-r^2}}{\sqrt{n-2}}} = \frac{r}{\frac{\sqrt{1-r^2}}{\sqrt{n-2}}} = \frac{r(\sqrt{n-2})}{\sqrt{1-r^2}}$$

Example:

r = 0.95, n =12,

T_{10} = 0.95*sqrt(10)/sqrt(1-0.95^2) = 9.62

P(|T_{10}|>9.62) = 0.000

```
. pwcorr x6 x7,  star(.05) sig

             |      x6         x7
-------------+----------------------
          x6 |   1.0000
             |
          x7 |   0.9466*   1.0000
             |   0.0000
```

Linear combination of random variables

- Rosner6, Eq. 5.12, p. 144:

$$L = \sum_{i=1}^{n} c_i X_i$$

$$Var(L) = \sum_{i=1}^{n} c_i^2 \cdot Var(X_i) + 2 \cdot \sum_{i=1}^{n} \sum_{j=1}^{n} c_i c_j \cdot Cov(X_i, X_j)$$

$$= \sum_{i=1}^{n} c_i^2 \cdot Var(X_i) + 2 \cdot \underbrace{\sum_{i=1}^{n} \sum_{j=1}^{n}}_{i<j} c_i c_j \cdot \sigma_i \sigma_j \cdot Corr(X_i, X_j)$$

If only two random variables X and Y, then $L = c_1 X + c_2 Y$

$$Var(L) = c_1^2 \cdot Var(X) + c_2^2 \cdot Var(Y) + 2 \cdot c_1 \cdot c_2 \cdot Cov(X, Y)$$

Example, ctd..

pnr	x6	x7
301	32	27
302	30	30
304	34	32
306	36	36
307	28	26
308	41	38
309	35	30
310	53	43
311	27	25
312	37	27
313	15	10
314	53	45

Calculate D = x7-x6

pnr	x6	x7	D
301	32	27	-5
302	30	30	0
304	34	32	-2
306	36	36	0
307	28	26	-2
308	41	38	-3
309	35	30	-5
310	53	43	-10
311	27	25	-2
312	37	27	-10
313	15	10	-5
314	53	45	-8

Linear combination of random variables

$$L = c_1 X_1 + c_2 X_2$$

$$Var(L) = c_1^2 \cdot Var(X_1) + c_2^2 \cdot Var(X_2) + 2 \cdot c_1 \cdot c_2 \cdot Cov(X_1, X_2)$$

Example:

Consider the difference $D = X_7 - X_6$

$$= (-1) \cdot X_6 + 1 \cdot X_7$$

$$Var(D) = (-1)^2 \cdot Var(X_6) + 1^2 \cdot Var(X_7) + 2 \cdot (-1) \cdot 1 \cdot Cov(X_6, X_7)$$

$$= Var(X_6) + Var(X_7) - 2 \cdot Cov(X_6, X_7)$$

```
Now insert estimates:
Var(D) = 112.45 + 86.38 -2*93.30 = 12.23
```

… considerably less than either of the original variances!

Linear combination of random variables

pnr	x6	x7	D
301	32	27	-5
302	30	30	0
304	34	32	-2
306	36	36	0
307	28	26	-2
308	41	38	-3
309	35	30	-5
310	53	43	-10
311	27	25	-2
312	37	27	-10
313	15	10	-5
314	53	45	-8

Tasks:

Perform the following tests

- *By hand*
  One sample t-test

- Using your favourite software
  - One sample t-test
  - Two sample t-test
  - Paired samples t-test

Result (descriptive)

```
. summm D, detail

                              D
-------------------------------------------------------------
      Percentiles      Smallest
 1%          -10            -10
 5%          -10            -10
10%          -10             -8       Obs                  12
25%         -6.5             -5       Sum of Wgt.          12

50%           -4                      Mean          -4.333333
                        Largest       Std. Dev.      3.498918
75%           -2             -2
90%            0             -2       Variance       12.24242
95%            0              0       Skewness      -.4674704
99%            0              0       Kurtosis       2.012156
```

One sample t-test

```
. ttest D ==0

One-sample t test
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       D |      12   -4.333333    1.010051    3.498918    -6.55644   -2.110227
------------------------------------------------------------------------------
    mean = mean(D)                                                t =  -4.2902
Ho: mean = 0                                     degrees of freedom =       11

    Ha: mean < 0                 Ha: mean != 0                 Ha: mean > 0
 Pr(T < t) = 0.0006         Pr(|T| > |t|) = 0.0013          Pr(T > t) = 0.9994
```

Example, revisited
now breaking the correlation …

pnr	x6	x7
301	32	27
302	30	30
304	34	32
306	36	36
307	28	26
308	41	38
309	35	30
310	53	43
311	27	25
312	37	27
313	15	10
314	53	45

Shuffle x7 randomly

x7 shuffeled

pnr	x6	x7s
301	32	30
302	30	26
304	34	27
306	36	43
307	28	38
308	41	32
309	35	36
310	53	27
311	27	45
312	37	10
313	15	25
314	53	30

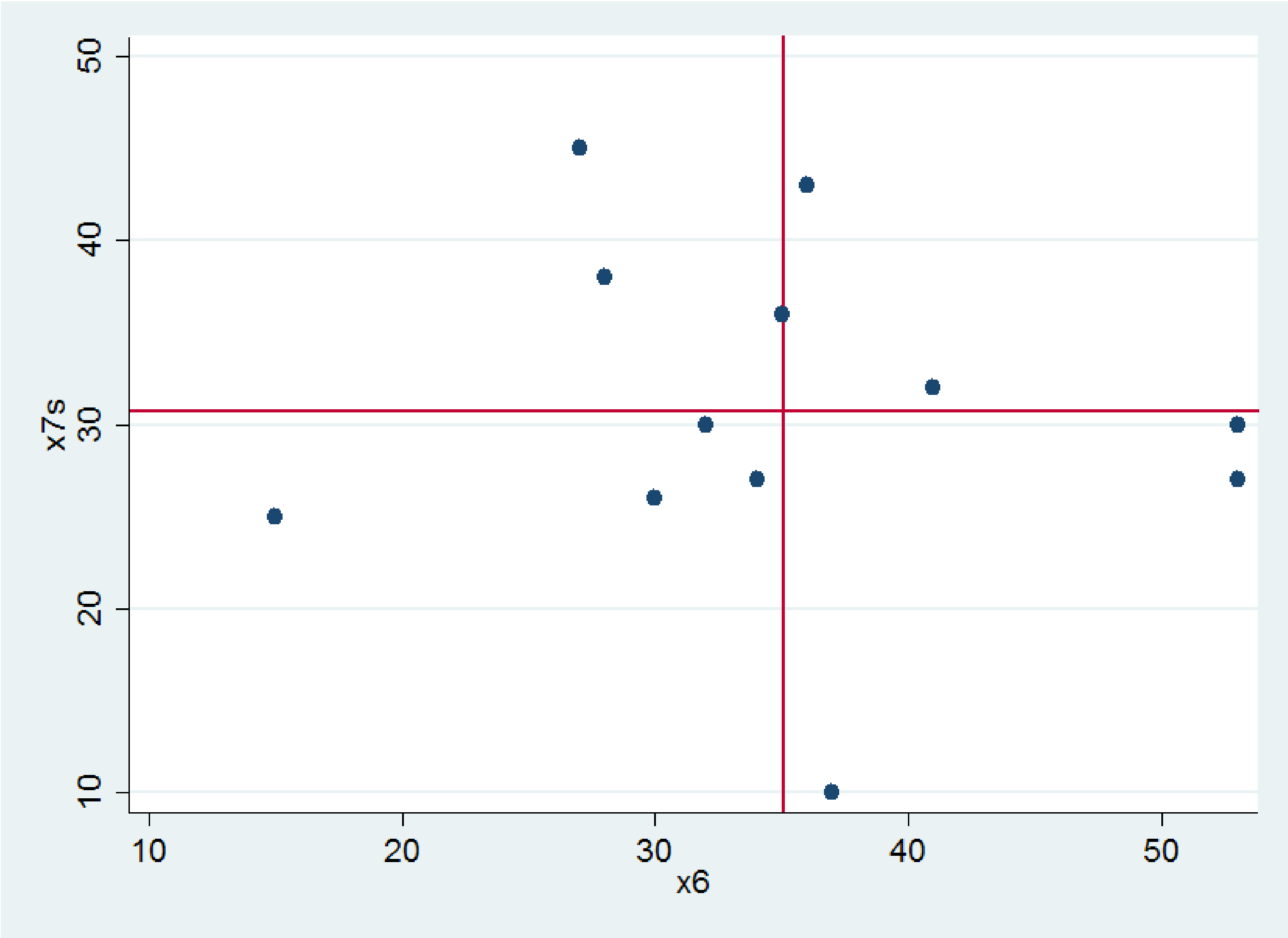
Example, revisited

Re-calculate D

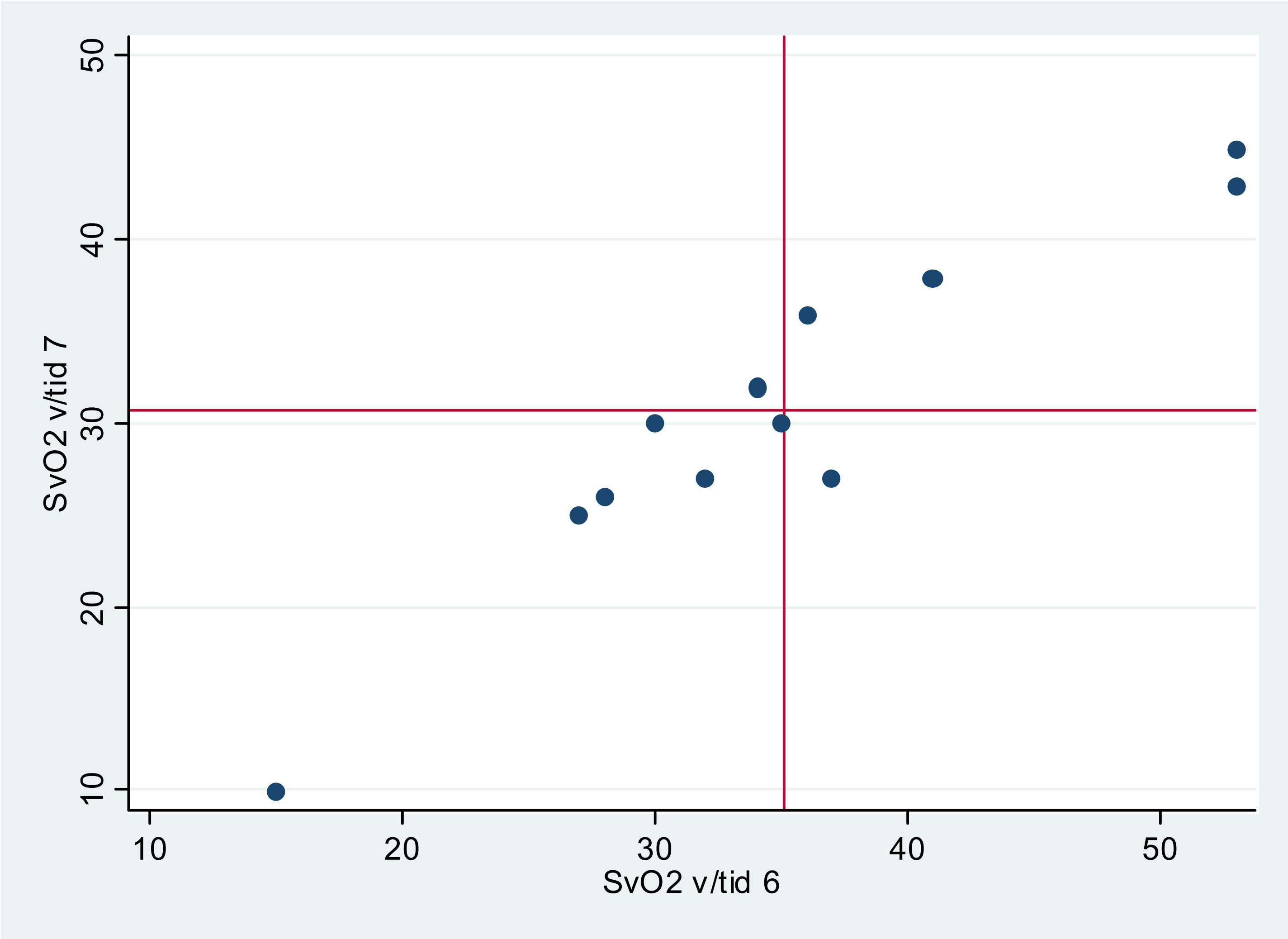
pnr	x6	x7s	D
301	32	30	-2
302	30	26	-4
304	34	27	-7
306	36	43	7
307	28	38	10
308	41	32	-9
309	35	36	1
310	53	27	-26
311	27	45	18
312	37	10	-27
313	15	25	10
314	53	30	-23

- Plot x6 and x7s
- Calculate the following sample statistics, using all observations:
  - mean
  - variance
  - standard deviation
  - covariance
  - correlation coefficient
- Re-calculate the one sample t-test

```
. graph twoway (scatter x7s x6, yline(30.75) xline(35.1))
```



```
. twoway (scatter x7 x6,yline(30.75) xline(35.1))
```



Results…

Kovarians-matrise

```
. cor x6 x7s, cov mean
(obs=12)

    Variable |       Mean    Std. Dev.          Min          Max
-------------+----------------------------------------------------
          x6 |   35.08333     10.6041           15           53
         x7s |      30.75    9.294426           10           45


             |       x6      x7s
-------------+------------------
          x6 |  112.447
         x7s | -11.1591  86.3864
```

Korrelasjons-matrise

```
. pwcorr x6 x7s, star (0.05) sig

             |       x6      x7s
-------------+------------------
          x6 |   1.0000
             |
             |
         x7s |  -0.1132   1.0000
             |   0.7261
             |
```

Result (descriptive)

D

	Percentiles	Smallest		
1%	-27	-27		
5%	-27	-26		
10%	-26	-23	Obs	12
25%	-16	-9	Sum of Wgt.	12
50%	-3		Mean	-4.333333
		Largest	Std. Dev.	14.87116
75%	8.5	7		
90%	10	10	Variance	221.1515
95%	18	10	Skewness	-.2827337
99%	18	18	Kurtosis	1.940189

Again, variance has changed dramatically:

Var(D) = 112.45 + 86.38 +2*11.16 = 221.15

… *considerably more than the sum of the original variances!*

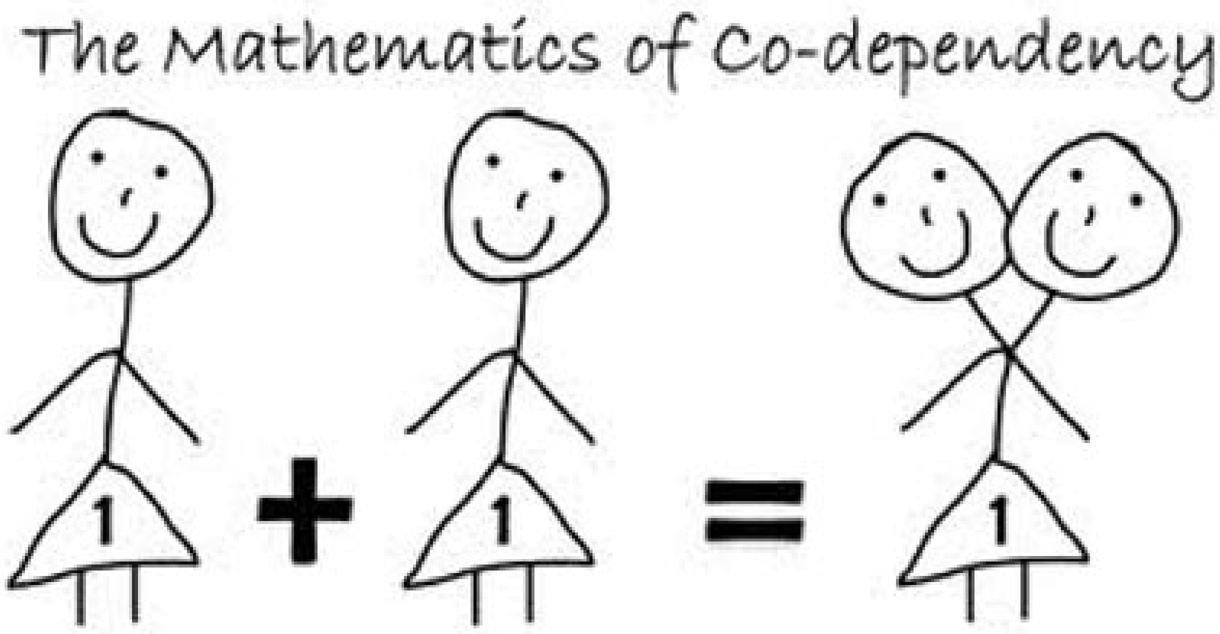
One sample t-test

```
. ttest D ==0 //

One-sample t test
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       D |      12   -4.333333    4.292935    14.87116   -13.78202    5.115353
------------------------------------------------------------------------------
    mean = mean(D)                                                t =  -1.0094
Ho: mean = 0                                     degrees of freedom =       11

    Ha: mean < 0                 Ha: mean != 0                 Ha: mean > 0
 Pr(T < t) = 0.1672         Pr(|T| > |t|) = 0.3345          Pr(T > t) = 0.8328
```

Conclusion



Dependency changes everything!

Review of linear regression

- Rabe-Heskett & Skrondal, chapter 1.
- This material is assumed to be well known from previous courses!
- Points to consider
  - ANOVA
  - Simple linear regression
  - Multiple linear regression
  - Dummy variables
  - Interaction (concept, interpretation)