

# STK4900/9900 - Lecture 4

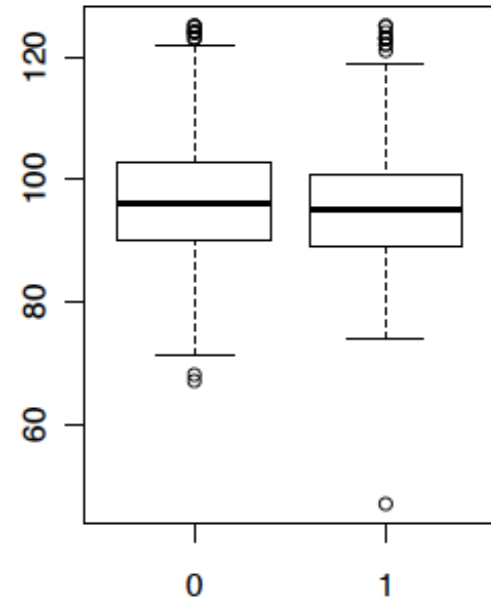
## Program

1. Causal effects
2. Confounding
3. Interaction
4. More on ANOVA
  - Sections 4.1, 4.4, (4.5), 4.6
  - Supplementary material on ANOVA

## Example (cf. practical exercise 10)

How does exercise affect blood glucose level?

Use the HERS data, disregarding women with diabetes



### Simple linear regression:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	97.36	0.282	345.8	< 2e-16
exercise	-1.693	0.438	-3.87	0.00011

Residual standard error: 9.715 on 2030 degrees of freedom

Multiple R-squared: 0.0073, Adjusted R-squared: 0.0068

F-statistic: 14.97 on 1 and 2030 DF, p-value: 0.00011

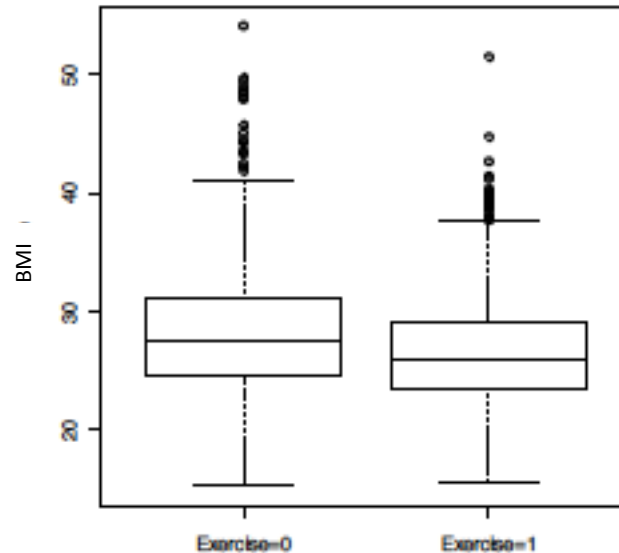
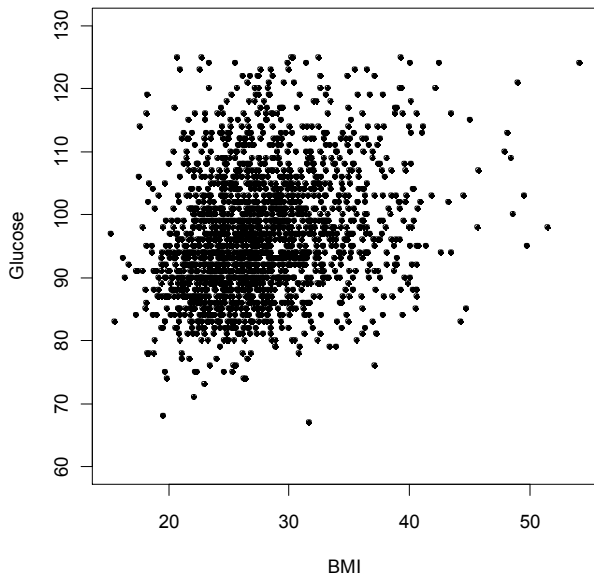
Can we conclude that exercise on average decreases the blood glucose level with 1.7 mg/dL ?

## Problem:

The women who exercise are not a random sample of all women in the cohort (as they would have been in a randomized clinical trial), but differ from the women who don't exercise, e.g. with respect to age, alcohol use, and body mass index (BMI)

Further age, alcohol use, and BMI may influence the glucose level

## Illustration for BMI:



# Confounding

It is possible that the observed significant association between exercise and glucose levels is due to the dependency between exercise and BMI and other covariates, i.e. **not causal**.

In such case we say that the association is spurious and that BMI and the other covariates are **confounding** variables, more precisely we have

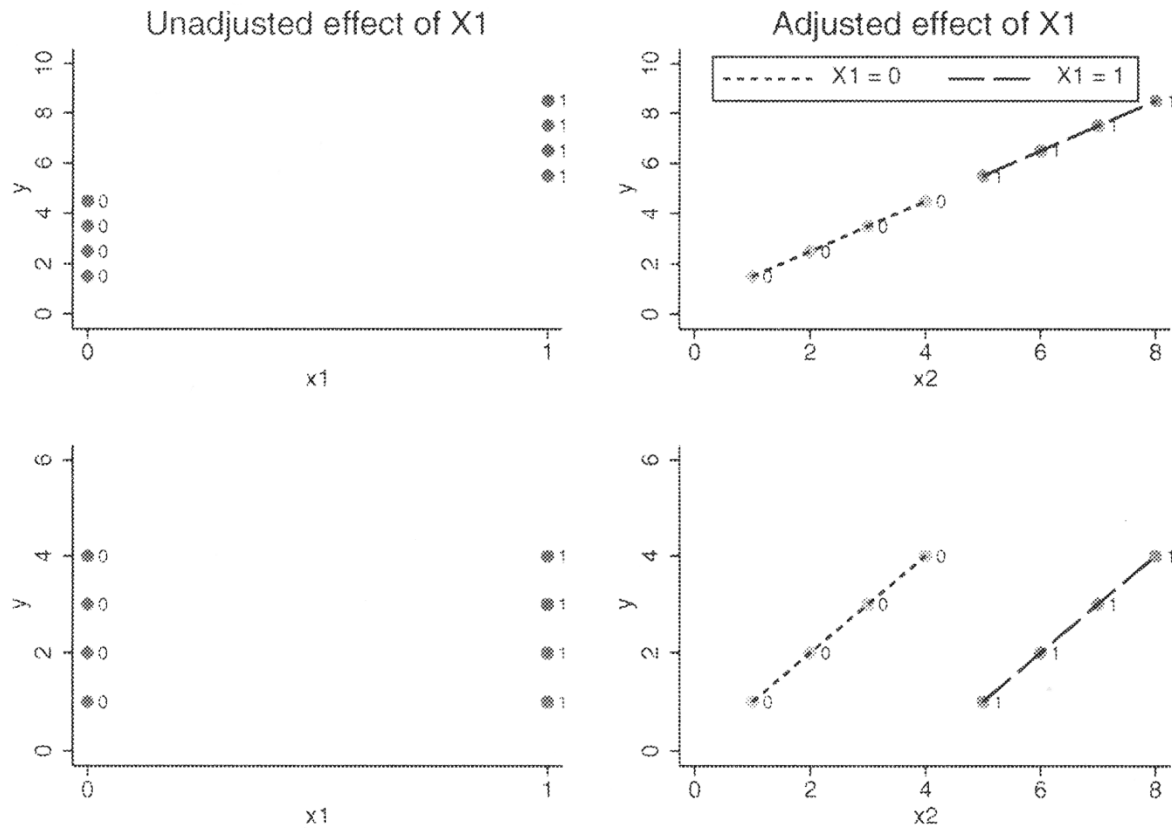
## Conditions for confounding

A covariate  $X_2$  is a confounder for the causal effect of  $X_1$  provided that

- $X_2$  is a causal determinant of the outcome  $Y$   
(or a proxy for such determinants)
- $X_2$  is a causal determinant of  $X_1$   
(or they share a common causal determinant)

# Confounding patterns

Examples of confounding patterns when  $X_2$  is a numerical covariate



Complete  
confounding

Negative  
confounding

Fig. 4.1 in the book

# Control of confounding

Consider the situation where all causal determinants other than  $X_1$  are captured by the binary covariate  $X_2$

Then, given the level of  $X_2$  ( $= 0,1$ ), there is no more confounding and the causal effect of  $X_1$  may be estimated by comparing the means of exposed and unexposed within levels of  $X_2$

In practice this is obtained by fitting the linear model

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \varepsilon_i$$

since here  $\beta_1$  is the effect of one unit's increase in  $X_1$  keeping the value of  $X_2$  constant

In general we may use multiple linear regression to correct for a number of confounders by including them as covariates in the model (assuming that all relevant confounders are recorded in the data)

## Example (contd)

We fit a multiple regression model with blood glucose level as response and exercise, age, alcohol use, and body mass index (BMI) as covariates

### Multiple linear regression:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	78.96	2.592	30.45	<2e-16
exercise	-0.950	0.429	-2.22	0.0267
age	0.064	0.03	2.02	0.0431
drinkany	0.680	0.422	1.61	0.1071
BMI	0.489	0.042	11.77	<2e-16

Residual standard error: 9.389 on 2023 degrees of freedom  
(4 observations deleted due to missingness)

Multiple R-squared: 0.072, Adjusted R-squared: 0.070

F-statistic: 39.22 on 4 and 2023 DF, p-value: < 2.2e-16

We now find that exercise on average decreases the blood glucose level with 1.0 mg/dL

This should be closer to the causal effect of exercise

## In particular:

Suppose that the true model is given by

$$E(Y) = \alpha + \beta_1 x_1 + \beta_2 x_2 \quad (1)$$

but the data are analyzed with a model omitting  $x_2$ , thus as

$$E(Y) = a + bx_1 \quad (2)$$

We then have

$$\hat{b} = \hat{\beta}_1 + \hat{\beta}_2 r_{12} \frac{s_2}{s_1} \quad (3)$$

where  $\hat{b}$  is the least squares estimate of  $b$  under model (2),

$\hat{\beta}_1$  and  $\hat{\beta}_2$  are least squares estimates of model (1),

$r_{12}$  is the Pearson correlation between  $x_1$  and  $x_2$

and the  $s_j$  the empirical standard deviations of  $x_1$  and  $x_2$



## It follows:

When the two covariates are correlated,  $r_{12} \neq 0$ ,  
and when there is a causal effect of  $x_2$  on  $Y$ , so  $\hat{\beta}_2 \neq 0$ ,  
then we estimate different effects of  $x_1$  under model (1) and (2),  
that is:  $\hat{b} \neq \hat{\beta}_1$

However when the two covariates are weakly correlated,  $r_{12} \approx 0$ ,  
or when there is no important causal effect of  $x_2$  on  $Y$ , so  $\hat{\beta}_2 \approx 0$ ,  
then the estimates differ little,  $\hat{b} \approx \hat{\beta}_1$

From equation (3) it follows that inclusion of a new covariate  $x_2$  can  
both make the association between  $x_1$  and  $Y$  weaker as well as  
stronger.

## Example (contd)

We will demonstrate equation (3) on the glucose data. Note that BMI had a strongly significant association with glucose. It turns out that BMI is the essential confounder of the exercise.

However, there were 2 subjects with unknown (missing) BMI. These have to be removed from the data before the comparison

### R code for removing the missing:

```
hers.nob=hers.no[!is.na(hers.no$BMI),]
```

### We then fit models with and without BMI:

```
fit.a=lm(glucose~exercise,data=hers.nob)
```

```
fit.a$coef
```

(Intercept)	exercise
97.370059	-1.701807

```
fit.b=lm(glucose~exercise+BMI,data=hers.nob)
```

```
fit.b$coef
```

(Intercept)	exercise	BMI
83.9422021	-0.9172885	0.4736147

## Example (contd)

We then calculate the correlation between, and the standard deviations of, exercise and BMI

```
r12=cor(hers.nob$exercise,hers.nob$BMI)
```

```
r12
```

```
-0.1587467
```

```
s1=sd(hers.nob$exercise)
```

```
s1
```

```
0.4927197
```

```
s2=sd(hers.nob$BMI)
```

```
s2
```

```
5.141301
```

Finally we demonstrate that equation (3)  $\hat{b} = \hat{\beta}_1 + \hat{\beta}_2 r_{12} \frac{s_2}{s_1}$  holds in the example

```
fit.b$coef[2]+fit.b$coef[3]*r12*s2/s1
```

```
exercise
```

```
-1.701807
```

**The answer is identical to the estimate  $\hat{b}$  for the simple model!**

# Control of confounding

If all confounding variables are recorded and included adequately in a multiple regression model we should then identify the causal effects also in an observational study.

But there is of course no way we can know that all confounders have been identified and measured without error.

We should therefore be cautious about concluding about causal effects from observational studies.

Still we may hope to that we are closer to identifying causality after adjusting (or controlling) for known confounders

## Mediation, Sec. 4.5

Not all measured variables should be adjusted for.

Example: Statin drugs may reduce (bad) cholesterol which in turn may reduce risk of heart attack.

Adjusting for cholesterol measured after taking statins may then hide a causal effect of statins on risk of heart attack.

In this case cholesterol is a mediator, or intermediate variable. It is likely correlated (caused by) statin use and causally related to heart attack. However, since it is on the causal pathway between statin use and heart attack we should not adjust for it.

# Why randomization works

In a study where subjects are randomized to different treatments we can ignore confounding.

This can be deduced from equation (3)  $\hat{b} = \hat{\beta}_1 + \hat{\beta}_2 r_{12} \frac{s_2}{s_1}$

After randomization the treatment  $x_1$  and the confounder  $x_2$  will be (approximately) uncorrelated, thus  $r_{12} \approx 0$  and  $\hat{b} \approx \hat{\beta}_1$ .

**Hence the causal effect is estimated after randomization!**

We don't even need to know the confounding factors

# Interaction for binary covariates

We have considered the situation where two binary predictors  $X_1$  and  $X_2$  have a causal effect on the outcome

We could then estimate the (causal) effects by fitting the linear model

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \varepsilon_i$$

Note that we assume that the effect of  $X_1$  is the same for both levels of  $X_2$  (and vice versa):

$X_1$	$X_2$	$E(y   \mathbf{x})$
0	0	$\beta_0$
1	0	$\beta_0 + \beta_1$
0	1	$\beta_0 + \beta_2$
1	1	$\beta_0 + \beta_1 + \beta_2$

If the effect of  $X_1$  depends on the level of  $X_2$  we have an *interaction*

We may then fit a model of the form

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} + \varepsilon_i$$

The effect for different values of the covariates are then given by:

$X_1$	$X_2$	$X_1 X_2$	$E(y   \mathbf{x})$
0	0	0	$\beta_0$
1	0	0	$\beta_0 + \beta_1$
0	1	0	$\beta_0 + \beta_2$
1	1	1	$\beta_0 + \beta_1 + \beta_2 + \beta_3$



## Example

Use the HERS data to study how low-density lipoprotein cholesterol after one year (LDL1) depends on hormone therapy (HT) and statin use (both binary)

### R commands:

```
ht.fit=lm(LDL1~HT+statins+HT:statins, data=hers)
summary(ht.fit)
```

### R output (edited):

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	145.157	1.326	109.507	< 2e-16
HT	-17.73	1.87	-9.477	< 2e-16
statins	-13.81	2.15	-6.416	1.65e-10
<b>HT:statins</b>	6.24	3.08	2.030	0.0425

(In the model formula **HT:statin** specifies the interaction term "HT\*statin")

The effect of HT seems to be lower among statin users

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	145.157	1.326	109.507	< 2e-16
HT	-17.73	1.87	-9.477	< 2e-16
statins	-13.81	2.15	-6.416	1.65e-10
HT:statins	6.24	3.08	2.030	0.0425

HT reduces LDL cholesterol for non-users of statins by 17.7 mg/dl

For users of statins the estimated reduction is  $17.7 - 6.2 = 11.5$  mg/dl

To obtain the uncertainty, we use the "contrast" library

### R commands:

```
library(contrast)
par1= list(HT=1,statins=1) # specify one set of values of the covariates
par2= list(HT=0,statins=1) # specify another set of values of the covariates
contrast(ht.fit, par1,par2) # compute the difference between the two sets
```

### R output (edited):

Contrast	S.E.	Lower	Upper	t	df	Pr(> t )
-11.48	2.44	-16.27	-6.69	-4.7	2604	0

## Interaction for one binary and one numerical covariate

We now consider the situation where  $X_1$  is a binary predictor and  $X_2$  is numerical

As an illustration we consider the HERS data, and we will see how baseline LDL cholesterol depends on statin use ( $X_1$ ) and BMI ( $X_2$ )

The model

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \varepsilon_i$$

assumes that the effect of BMI is the same for statin users and those who don't use statins

It may be of interest to consider a model where the effect of BMI may differ between statin users and those who don't use statins, i.e. where there is an *interaction*

We then consider the model

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} + \varepsilon_i$$

Note that the model may be written

$$y_i = \begin{cases} \beta_0 + \beta_2 x_{2i} + \varepsilon_i & \text{when } x_{1i} = 0 \\ \beta_0 + \beta_1 + (\beta_2 + \beta_3) x_{2i} + \varepsilon_i & \text{when } x_{1i} = 1 \end{cases}$$

This is a model with different intercepts and different slopes for the numerical covariate depending on the value of the binary covariate

When considering such a model, it is useful to center the numeric covariate (by subtracting its mean) to ease interpretation

In the example, we let  $X_2$  correspond to the centered BMI-values, denoted cBMI

### R commands:

```
hers$cBMI=hers$BMI - mean(hers$BMI[!is.na(hers$BMI)])  
stat.fit=lm(LDL~statins+cBMI+statins:cBMI,data=hers)  
summary(stat.fit)
```

### R output (edited):

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	151.09	0.881	171.58	< 2e-16
statins	-16.72	1.463	-11.43	< 2e-16
cBMI	0.640	0.156	4.09	4.41e-05
statins:cBMI	-0.721	0.269	-2.68	0.0075

## Interaction for two numerical covariates

We finally consider the situation where  $X_1$  and  $X_2$  are both numerical

A model with interaction is then given by

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} + \varepsilon_i$$

For such a model, it is useful to center the covariates.

But even then the interpretation of the estimates is a bit complicated.

# Two-way ANOVA

Consider the situation where the outcome  $y_i$  for an individual depends on two factors, A and B, each with two levels, denoted  $a_1, a_2$  and  $b_1, b_2$

One such example is how LDL cholesterol depends on HT (with levels "placebo" and "hormone therapy") and statin use (with levels "no" and "yes"); cf. slide 16

We may here introduce the covariates:

$$x_{1i} = \begin{cases} 0 & \text{if individ } i \text{ has level } a_1 \text{ for factor A (reference)} \\ 1 & \text{if individ } i \text{ has level } a_2 \text{ for factor A} \end{cases}$$

$$x_{2i} = \begin{cases} 0 & \text{if individ } i \text{ has level } b_1 \text{ for factor B (reference)} \\ 1 & \text{if individ } i \text{ has level } b_2 \text{ for factor B} \end{cases}$$

Then a regression model with interaction takes the form (cf slide 15)

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} + \varepsilon_i$$

If (e.g.) factor B has three levels  $b_1, b_2, b_3$ , we need to introduce two  $x$ 's for this factor (cf slide 26 of Lecture 3):

$$x_{2i} = \begin{cases} 1 & \text{if individ } i \text{ has level } b_2 \text{ for factor B} \\ 0 & \text{otherwise} \end{cases}$$

$$x_{3i} = \begin{cases} 1 & \text{if individ } i \text{ has level } b_3 \text{ for factor B} \\ 0 & \text{otherwise} \end{cases}$$

A model with interaction then takes the form

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \beta_4 x_{1i} x_{2i} + \beta_5 x_{1i} x_{3i} + \varepsilon_i \quad (*)$$

It becomes quite complicated to write the model like this, so it is common to use an alternative formulation



We recapitulate:

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \beta_4 x_{1i} x_{2i} + \beta_5 x_{1i} x_{3i} + \varepsilon_i \quad (*)$$

In order to rewrite model (\*), we denote the outcomes for level  $a_j$  of factor A and level  $b_k$  of factor B by

$$y_{ijk} \quad \text{for } i = 1, \dots, n_{jk}$$

We may then rewrite model (\*) as

$$y_{ijk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk} \quad (**)$$

We have the following relations between the parameters in model (\*) and model (\*\*)

---

(*)	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$	$\beta_4$	$\beta_5$
(**)	$\mu$	$\alpha_2$	$\beta_2$	$\beta_3$	$(\alpha\beta)_{22}$	$(\alpha\beta)_{23}$

---

In model (\*\*) the parameters for the reference levels are 0 :

$$\alpha_1 = \beta_1 = (\alpha\beta)_{11} = (\alpha\beta)_{12} = (\alpha\beta)_{13} = (\alpha\beta)_{21} = 0$$

Note that the model formulation

$$y_{ijk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk} \quad (**)$$

works equally well when factor A has  $J$  levels and factor B has  $K$  levels, while the formulation (\*) would become much more complicated

In Lecture 3 (cf. slide 30), we considered a study of how the extraction rate of a certain polymer depends on temperature and the amount of catalyst used.

We there assumed a linear effect of temperature and the amount of catalyst

We will here consider temperature and catalyst as factors, each with three levels

	0.5%	0.6%	0.7%
50°C	38	45	57
	41	47	59
60°C	44	56	70
	43	57	69
70°C	44	56	70
	47	60	67

## R commands:

```
polymer=read.table("http://www.uio.no/studier/emner/matnat/math/STK4900/v11/polymer.txt",header=T)
polymer$ftemp=factor(polymer$temp)
polymer$fcats=factor(polymer$cat)
fit=lm(rate~ftemp+fcats+ftemp:fcats,data=polymer)
summary(fit)
```

## R output:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	39.5	1.23	32.25	1.30e-10
ftemp60	4.0	1.73	2.31	0.046
ftemp70	6.0	1.73	3.46	0.007
fcats0.6	6.5	1.73	3.75	0.005
fcats0.7	18.5	1.73	10.68	2.06e-06
ftemp60:fcats0.6	6.5	2.45	2.65	0.026
ftemp70:fcats0.6	6.0	2.45	2.45	0.037
ftemp60:fcats0.7	7.5	2.45	3.06	0.014
ftemp70:fcats0.7	4.5	2.45	1.84	0.099

Residual standard error: 1.73 on 9 degrees of freedom

Multiple R-squared: 0.986, Adjusted R-squared: 0.973

F-statistic: 78.78 on 8 and 9 DF, p-value: 2.012e-07

In a planned experiment we can make sure that we have the same number of observations for all the  $J \times K$  combinations of levels of factor A and factor B.

We then have a *balanced* design, and the total sum of squares (TSS) may be uniquely decomposed as a sum of squares for each of the two factors (SSA, SSB), a sum of squares for interaction (SSAB), and a residual sum of squares (RSS):

$$TSS = SSA + SSB + SSAB + RSS$$

To each of these sum of squares there correspond a degree of freedom as given in the ANOVA table on the next slide.

**NB!** If the design is not balanced, the decomposition of the total sum of squares is not unique

The result of a two-way ANOVA may be summarized in the table

Source	df	Sum of squares	Mean sum of squares	F statistics
Factor A	$J - 1$	$SSA$	$SSA / (J - 1)$	$F = \frac{SSA / (J - 1)}{RSS / (n - JK)}$
Factor B	$K - 1$	$SSB$	$SSB / (K - 1)$	$F = \frac{SSB / (K - 1)}{RSS / (n - JK)}$
Interaction	$(J - 1)(K - 1)$	$SSAB$	$SSAB / (J - 1)(K - 1)$	$F = \frac{SSAB / (J - 1)(K - 1)}{RSS / (n - JK)}$
Residual	$n - JK$	$RSS$	$RSS / (n - JK)$	
Total	$n - 1$	$TSS$		

The F-statistics (with their appropriate degrees of freedom) may be used to test the following null hypotheses:

$$H_0 : \text{all } (\alpha\beta)_{jk} = 0 \quad (\text{no interaction})$$

$$H_0 : \text{all } \alpha_j = 0 \quad (\text{no main effect of A})$$

$$H_0 : \text{all } \beta_k = 0 \quad (\text{no main effect of B})$$

For the example:

**R commands:**

```
anova(fit)
```

**R output:**

Analysis of Variance Table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
ftemp	2	332.11	166.06	55.35	8.76e-06
fcats	2	1520.11	760.06	253.35	1.23e-08
ftemp:fcats	4	38.56	9.64	3.213	0.067
Residuals	9	27.00	3.00		

# Higher level ANOVA

Consider for illustration the situation with three factors, A, B, and C.

Data:

$y_{ijkl}$  = observation number  $i$  for level  $a_j$  of factor A,  
level  $b_k$  of factor B, and level  $c_l$  of factor C

Model with interaction:

$$y_{ijkl} = \mu + \alpha_j + \beta_k + \gamma_l + (\alpha\beta)_{jk} + (\alpha\gamma)_{jl} + (\beta\gamma)_{kl} + (\alpha\beta\gamma)_{jkl} + \varepsilon_{ijkl}$$

The result of a three-way ANOVA may be summarized in the table

Source	df *	Sum of squares	Mean sum of squares	F statistics
Factor A		$SSA$	$SSA / df$	$F_A$
Factor B		$SSB$	$SSB / df$	$F_B$
Factor C		$SSC$	$SSC / df$	$F_C$
Interaction AB		$SSAB$	$SSAB / df$	$F_{AB}$
Interaction AC		$SSAC$	$SSAC / df$	$F_{AC}$
Interaction BC		$SSBC$	$SSBC / df$	$F_{BC}$
Interaction ABC		$SSABC$	$SSABC / df$	$F_{ABC}$
Residual		$RSS$	$RSS / df$	
Total	$n - 1$	$TSS$		

\*) can be found on computer output

The decomposition of the total sum of squares is unique if the design is balanced.

Hypothesis testing is similar to two-way ANOVA.