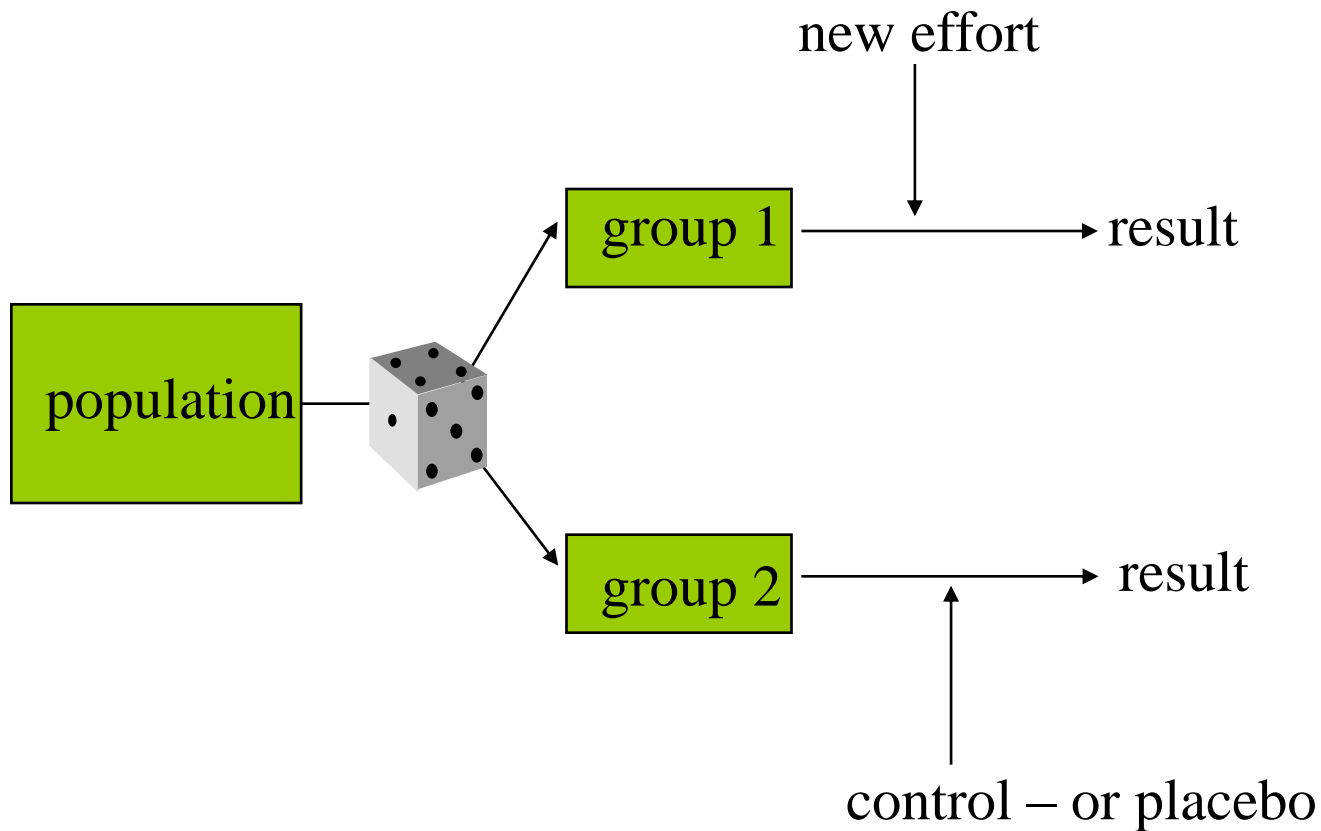


# Design of experiments

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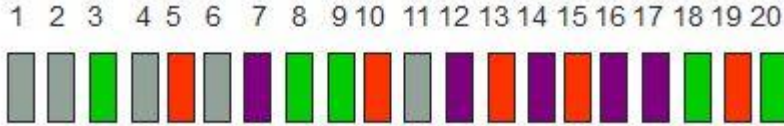
# Randomized controlled trial (RCT)



**Blinded** – the patients do not know which group they belong to

**Double blinded** – neither the patients nor the researchers know which group the patients belong to

## The completely randomised design:



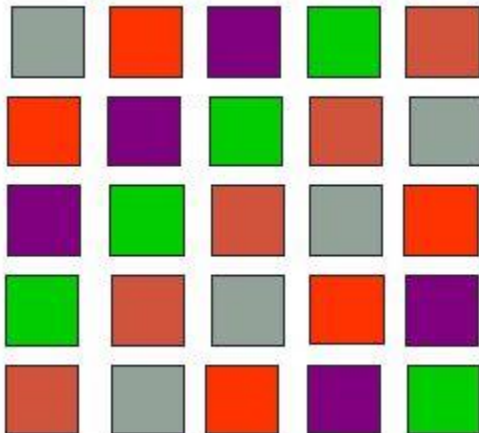
- each experimental unit is assigned to a treatment strictly at random without taking account of any individual characteristics
- demands relatively homogeneous experimental units

## The randomised block design:



- used to control a source of random variation which might otherwise obscure the effect of a treatment
- it is assumed that differences between treatments *are* of interest while differences between blocks, which are random effects are of *no* interest

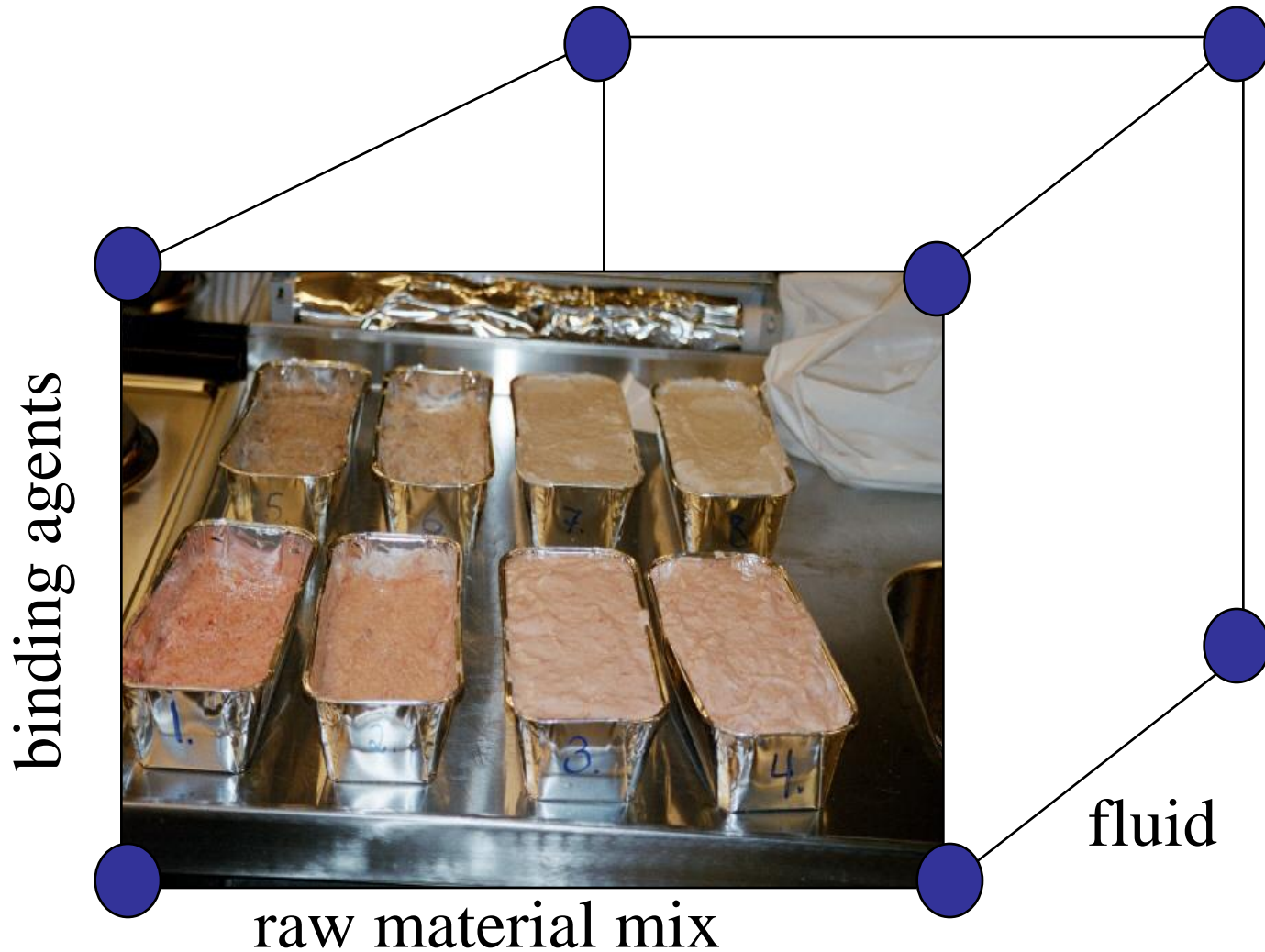
## The Latin square design:



- treatments (colours) are squared (e.g. 5 x 5 here)
- rows might be working days of the week
- columns might be time of the day
- randomise whole rows and then whole columns
- best suited for exp. with 4 – 7 treatments

*Factorial design:*

## Experimental design: fish pudding



# Coded set-up and the taste response (Y1):

---

TESTS:	Potato starch	Milk	-1=Saith +1=Salmon	TASTE (Y1)
Test 1	-1	-1	1	8,3
Test 2	1	-1	1	6,3
Test 3	-1	1	1	3,8
Test 4	1	1	1	5,5
Test 5	-1	-1	-1	4
Test 6	1	-1	-1	2,8
Test 7	-1	1	-1	2,3
Test 8	1	1	-1	5,5

---

## Preparation from the recipes



## Process



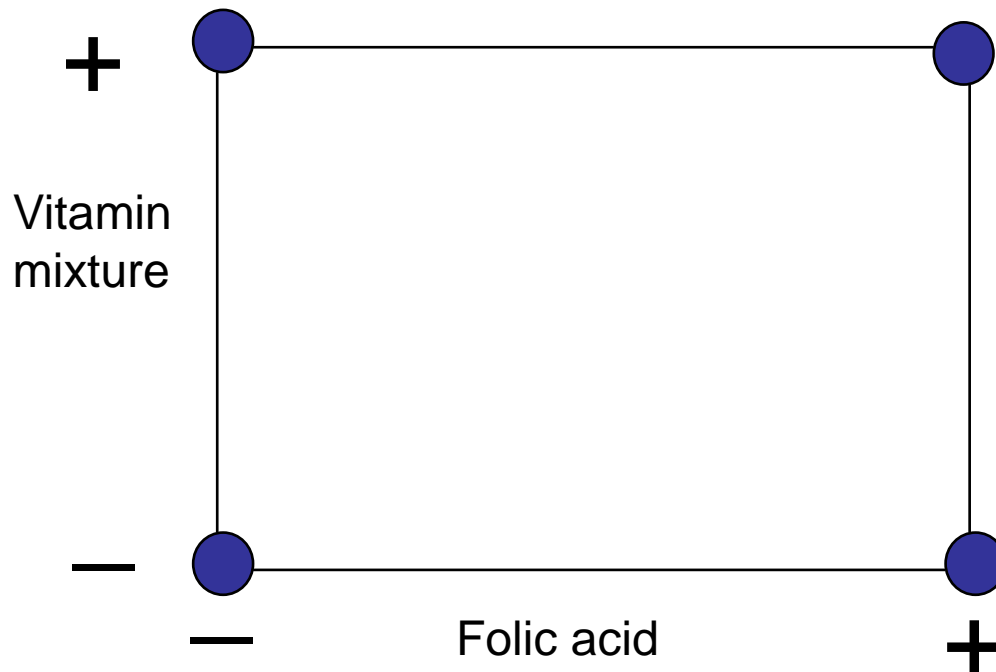
Modelling:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + \dots + b_1 b_2 X_1 X_2 + \dots + \text{error}$$

$$\text{Taste} = 4,8 + 0,2 \text{ potato starch} - 0,5 \text{ milk} + 1,2 \text{ salmon}$$

# Prevention of neural tube defects in the fetus by vitamins

A randomised double-blind prevention trial based on factorial design was conducted at 33 centres in seven countries to determine whether supplementation of folic acid or a mixture of seven other vitamins around the time of conception can prevent neural tube defects.



A total of 1817 women at high risk were allocated at random to one of the four groups

# Results

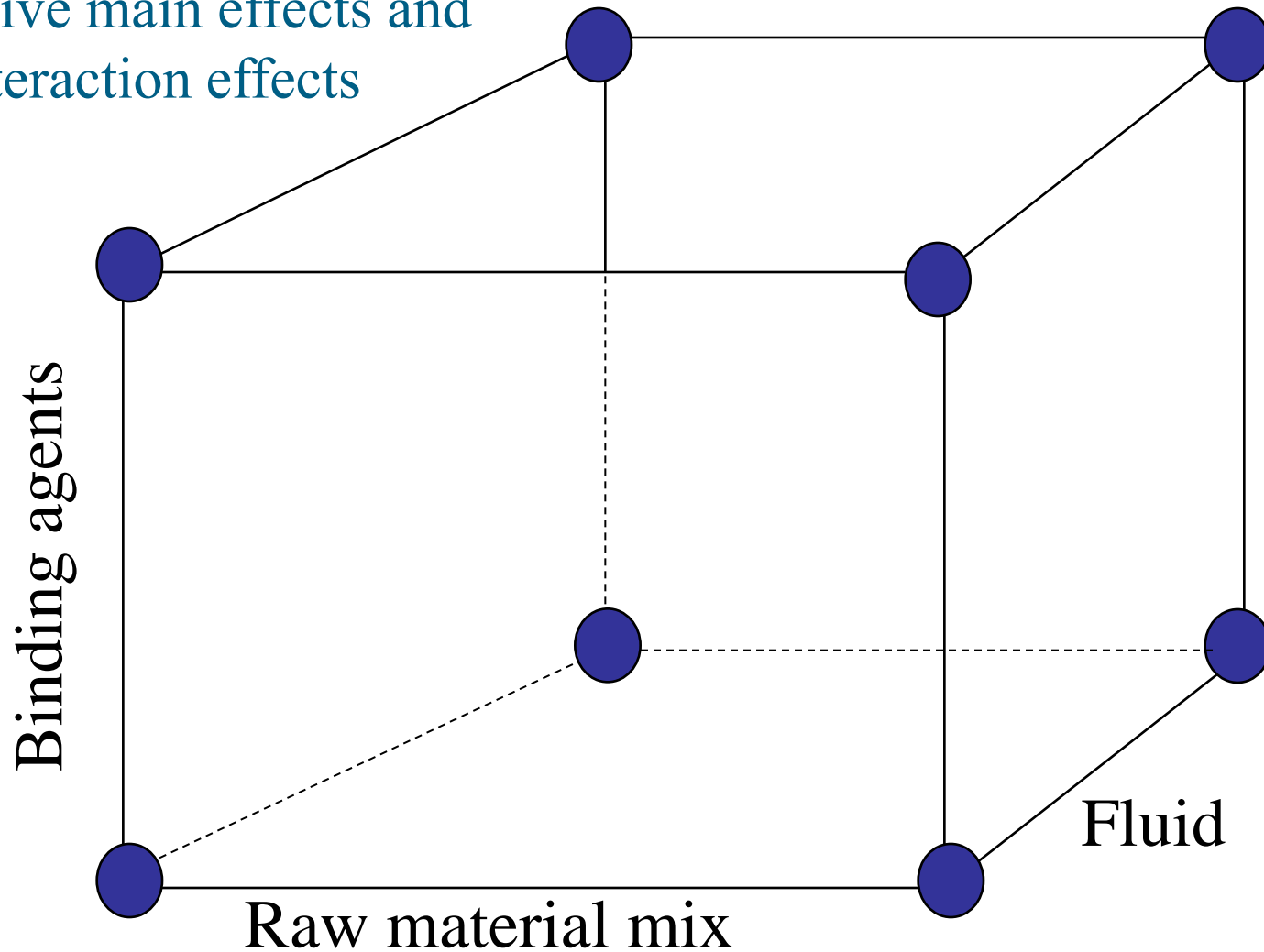
Randomisation group			All women	
			NTD/all	Relative risk: folic acid vs non-folic acid (95% CI)
	Folic acid	Other vitamins		
A	+	-	2/298	} 6/593 (1.0%) } } 21/602 (3.5%) }
B	+	+	4/295	
C	-	-	13/300	
D	-	+	8/302	

- Women given folic acid showed a 1,0 % risk for NTD in fetus
- Women in the other groups showed a 3,5% risk for NTD in fetus
- Women in the vitamin mix group showed no sign.diff. from other groups
- Folic acid supplementation before pregnancy is recommended



# Full factorial design

-Give main effects and Interaction effects



# Effects of changes in fat, fish and fibre intakes on death and myocardial reinfarction

A randomised controlled trial with a factorial design ( $2^3$ ) was done to examine the effects of dietary intervention in the secondary prevention of Infarction (MI).

2033 men under 70 years, admitted to 21 hospitals, and who had recovered from MI were allocated to receive or not to receive advice on each of the three dietary factors.

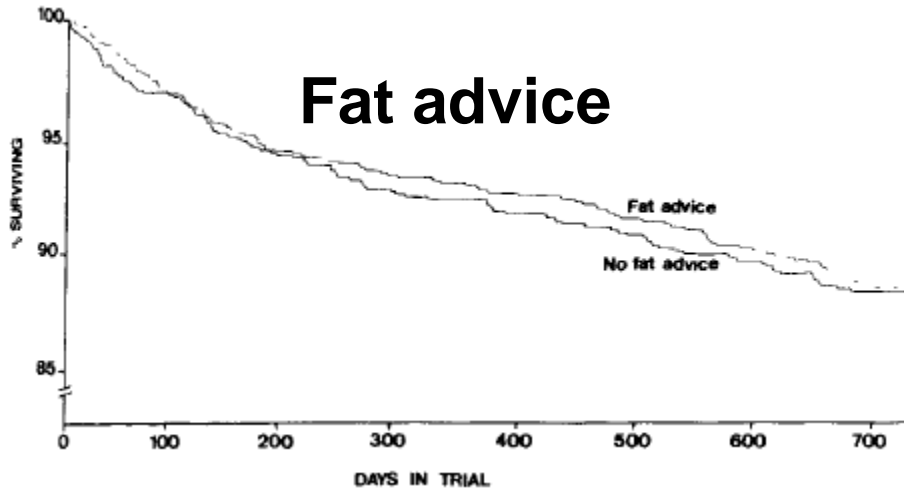
	Dietary intake	Dietary intake
	At 6 months	At 2 years
<i>% fat energy</i>		
Fat advice	32.1 (6.0, 937)*	32.3 (5.9, 869)
No fat advice	35.3 (5.9, 942)	35.0 (5.8, 876)
<i>P/S ratio</i>		
Fat advice	0.78 (0.30, 937)	0.78 (0.32, 869)
No fat advice	0.40 (0.23, 942)	0.44 (0.25, 876)
<i>EPA (g per week)</i>		
Fish advice	2.3 (1.3, 947)	2.4 (1.4, 883)
No fish advice	0.7 (0.7, 932)	0.6 (0.7, 862)
<i>Cereal fibre (g per day)</i>		
Fibre advice	19 (8, 926)	17 (8, 849)
No fibre advice	9 (5, 953)	9 (5, 896)

\*Mean (SD, no of subjects).

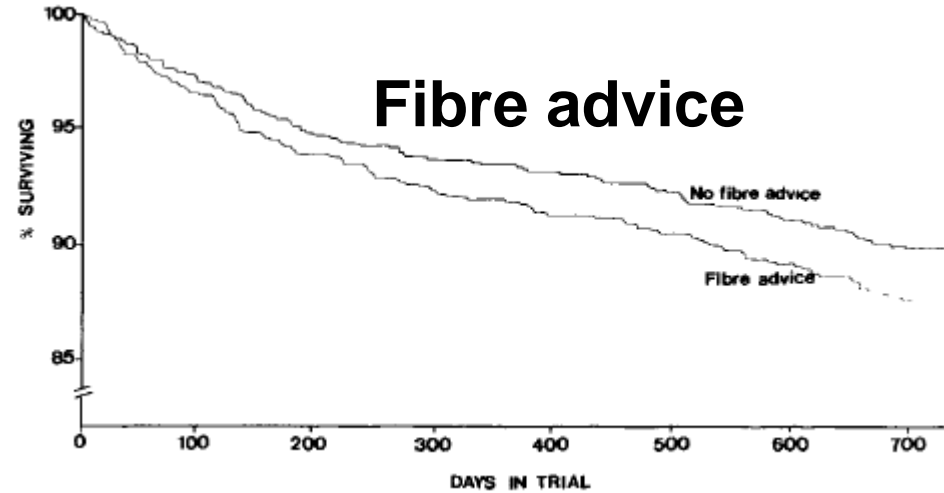
P/S = polyunsaturated / saturated ratio of fatty acids intake

# Survival

## Fat advice

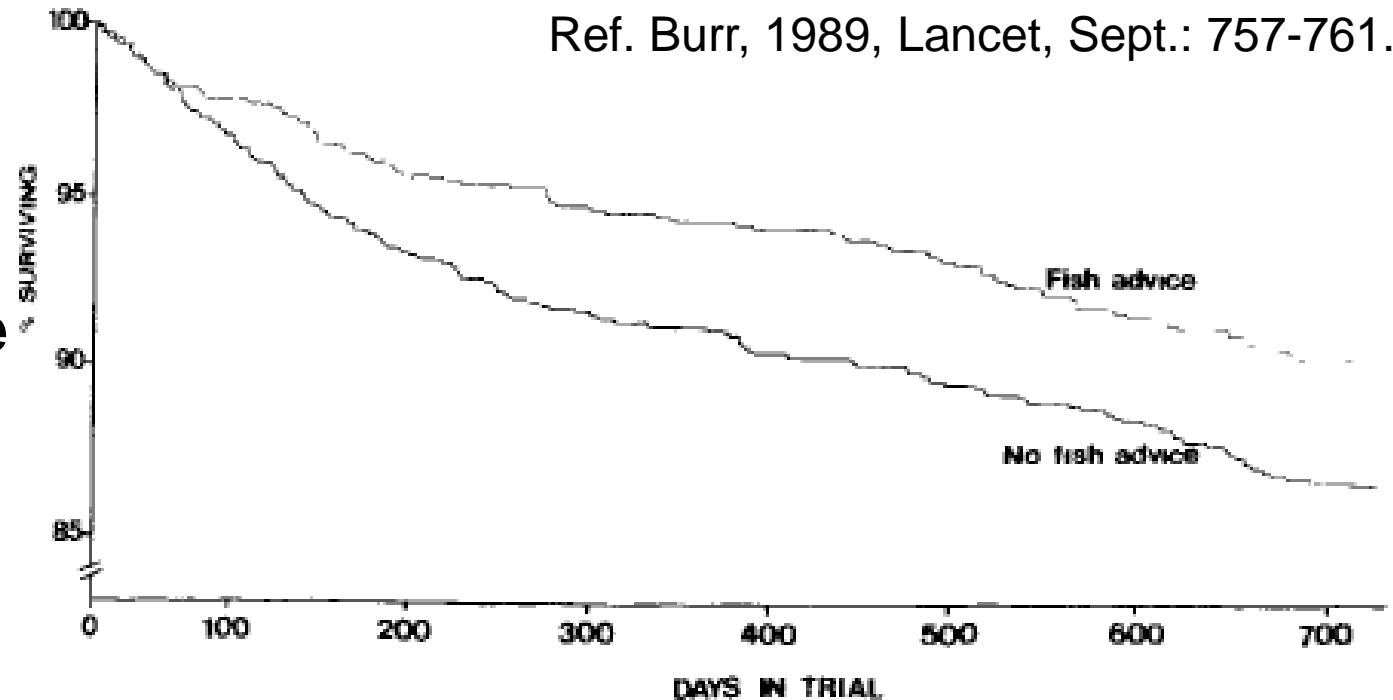


## Fibre advice



Ref. Burr, 1989, Lancet, Sept.: 757-761.

## Fish advice



# Results

Diet group	All deaths
Fat advice	111 (10.9%)
No fat advice	113 (11.1%)
Fish advice	94 (9.3%)*
No fish advice	130 (12.8%)
Fibre advice	123 (12.1%)
No fibre advice	101 (9.9%)

The subjects advised to eat fatty fish had a 28 % reduction in 2 year all-cause mortality compared with those not so advised.



# Example: Fish

## Multivariate evaluation of feed for Atlantic halibut

Ragnar Nortvedt <sup>a</sup>, Stig Tuene <sup>b</sup>

Table 1

Experimental design. The order of the experimental units was randomized. The first three variables (vars.) were varied according to  $2^3$  FD, whereas the other four vars. show the actual diet composition (in % of dry matter). Var. No. 2 is a qualitative description of floating feed (+1), sinking feed (-1) or a mix of the two types (0). Tank Nos. 1, 2, 5 and 7 represent the replicated center points in the design

Tank No.	Design variables			Diet composition (%)			
	1: (%) dry matter	2: floating vs. sinking	3: pellet size (mm)	4: carbo-hydrate	5: ash	6: protein	7: fat
9	95.0	+1	3.0	10.2	13.7	53.6	22.5
4	96.1	+1	8.0	8.9	14.2	53.3	23.6
10	95.6	-1	3.5	7.6	11.8	58.7	21.9
3	94.3	-1	7.0	8.2	11.3	53.4	27.1
11	32.2	+1	4.0	11.3	14.0	53.4	21.3
6	36.3	+1	9.0	8.8	13.0	55.3	22.9
12	32.3	-1	3.5	10.0	13.6	55.6	20.8
8	32.3	-1	9.0	10.9	12.6	55.1	21.4
1	63.6	0	5.0	10.8	12.2	54.1	22.9
2	63.6	0	5.0	10.8	12.2	54.1	22.9
5	63.6	0	5.0	10.8	12.2	54.1	22.9
7	63.6	0	5.0	10.8	12.2	54.1	22.9

# Results

Table 4

Explained variance in PLS response models of the gross feed conversion efficiency (GFCE), protein efficiency ratio (PER), protein productive value (PPV), fat efficiency ratio (FER), fat productive value (FPV) and neutralized fat productive value (FPV<sub>n</sub>) in the periods  $P_1$  (2.07–7.08) and  $P_2$  (10.08–5.09) and in the total experimental period (Tot: 12.06–5.09.93). The models were based upon the designed variables and their cross terms. The weighted regression coefficients of the significant variables are shown according to:  $Y = \beta_0 + \beta_1 \times \text{dry matter} + \beta_2 \times \text{floating feed} + \beta_3 \times \text{pellet size} + \beta_4 \times (\text{dry matter})^2 + \beta_5 \times (\text{dry} \times \text{floating}) + \beta_6 \times (\text{dry} \times \text{pellet size}) + \beta_7 \times (\text{floating})^2 + \beta_8 \times (\text{floating} \times \text{size}) + \beta_9 \times (\text{pellet size})^2$

Response	Variance (%)	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$	$\beta_4$	$\beta_5$	$\beta_6$	$\beta_7$	$\beta_8$	$\beta_9$
GFCE <sub>P1</sub>	21.6	0.946	0.001	–	0.019	–	–	$3.2 \times 10^{-4}$	–	–	0.001
GFCE <sub>P2</sub>	72.2	1.014	–	0.018	0.018	–	$1.9 \times 10^{-4}$	$2.0 \times 10^{-4}$	–	0.003	0.001
GFCE <sub>tot</sub>	51.5	0.856	–	–	0.023	–	–	$1.5 \times 10^{-4}$	0.054	–	0.002
PER <sub>tot</sub>	52.7	1.571	–	–	0.042	–	–	$2.8 \times 10^{-4}$	0.086	–	0.003
PPV <sub>tot</sub>	36.0	0.166	$7.1 \times 10^{-4}$	0.014	0.005	$5.0 \times 10^{-6}$	$1.7 \times 10^{-4}$	$1.0 \times 10^{-4}$	–	0.002	$4.2 \times 10^{-4}$
FER <sub>tot</sub>	44.7	4.413	–0.003	–	0.086	$-1.5 \times 10^{-5}$	–	–	0.274	–	0.008
FPV <sub>tot</sub>	83.5	–0.287	$1.5 \times 10^{-3}$	–	–	$4.3 \times 10^{-6}$	–	$2.5 \times 10^{-4}$	0.056	–	–
FPV <sub>n</sub>	58.3	3.449	–0.005	–	–	$-4.1 \times 10^{-5}$	–	$-7.5 \times 10^{-4}$	–	–	–

# A few conclusions:

- The systematic variation in the designed parameters influenced fat retention stronger than growth rate over the total experimental period
- Pellet size (var. No. 3) and the combined effect from pellet size and high dry matter content (var. Nos. 1x3) were the most significant positive parameters upon GFCE and protein utilization
- High dry matter content (var. No. 1) and the combined effect from pellet size and high dry matter content (var. Nos. 1x3) were most important to fat utilization
- Future fish feeds should consequently maximize the parameters above, within biological reasonable limits, to achieve the best utilization of the feed

## Example mice:

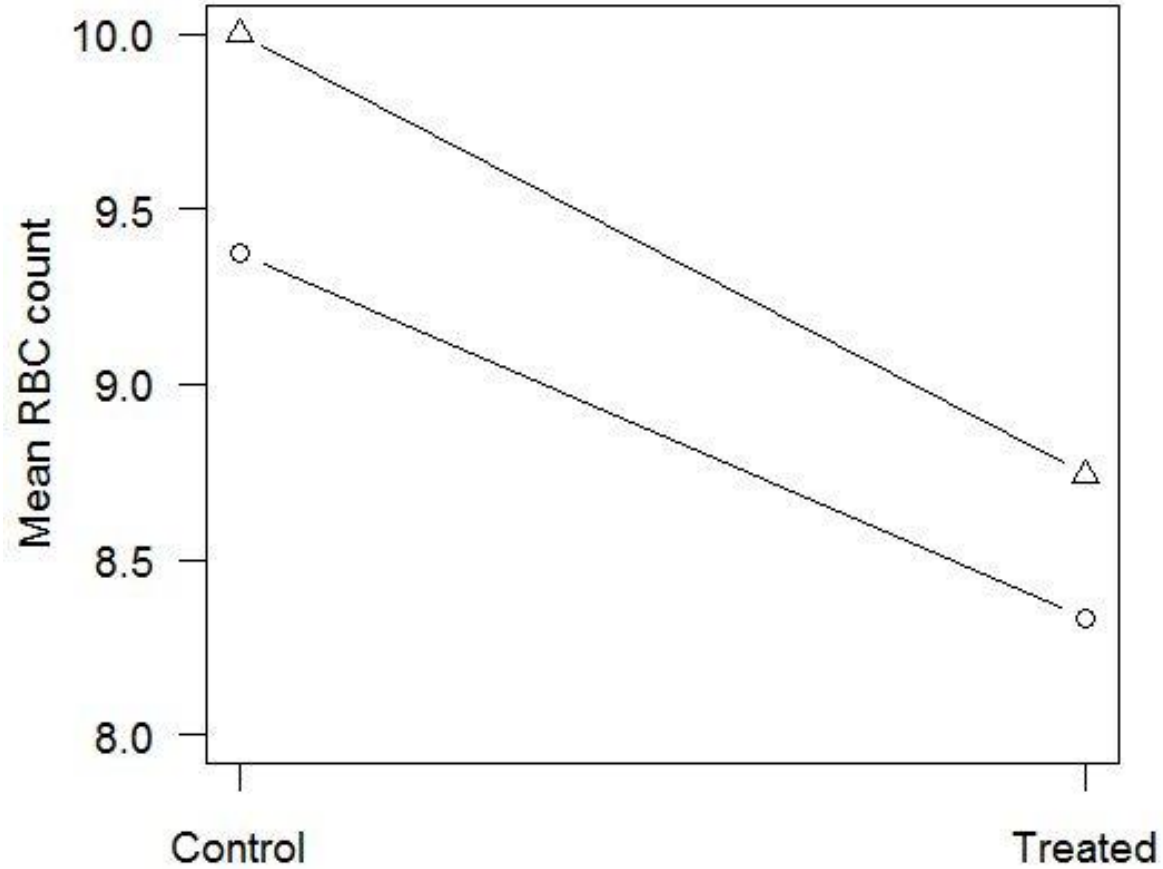
Strain	Red Blood Cell counts		Strain means
	Control	Treated	
BALB/c	10.10	8.95	
	10.08	8.45	
	9.73	8.68	
	10.09	8.89	9.37
C57BL	9.60	8.82	
	9.56	8.24	
	9.14	8.18	
	9.20	8.10	8.86
Treatment Mean	9.69	8.54	

In this study mice of two strains (BALB/c and C57BL) were dosed with with chloramphenicol at 2000mg/kg. This is a 2(strains) x 2(dose levels) factorial design.

We want to know:

- does treatment have an effect on RBC counts (results above)
- do strains differ in RBC counts
- do strains differ in their response to chloramphenicol (the interaction).



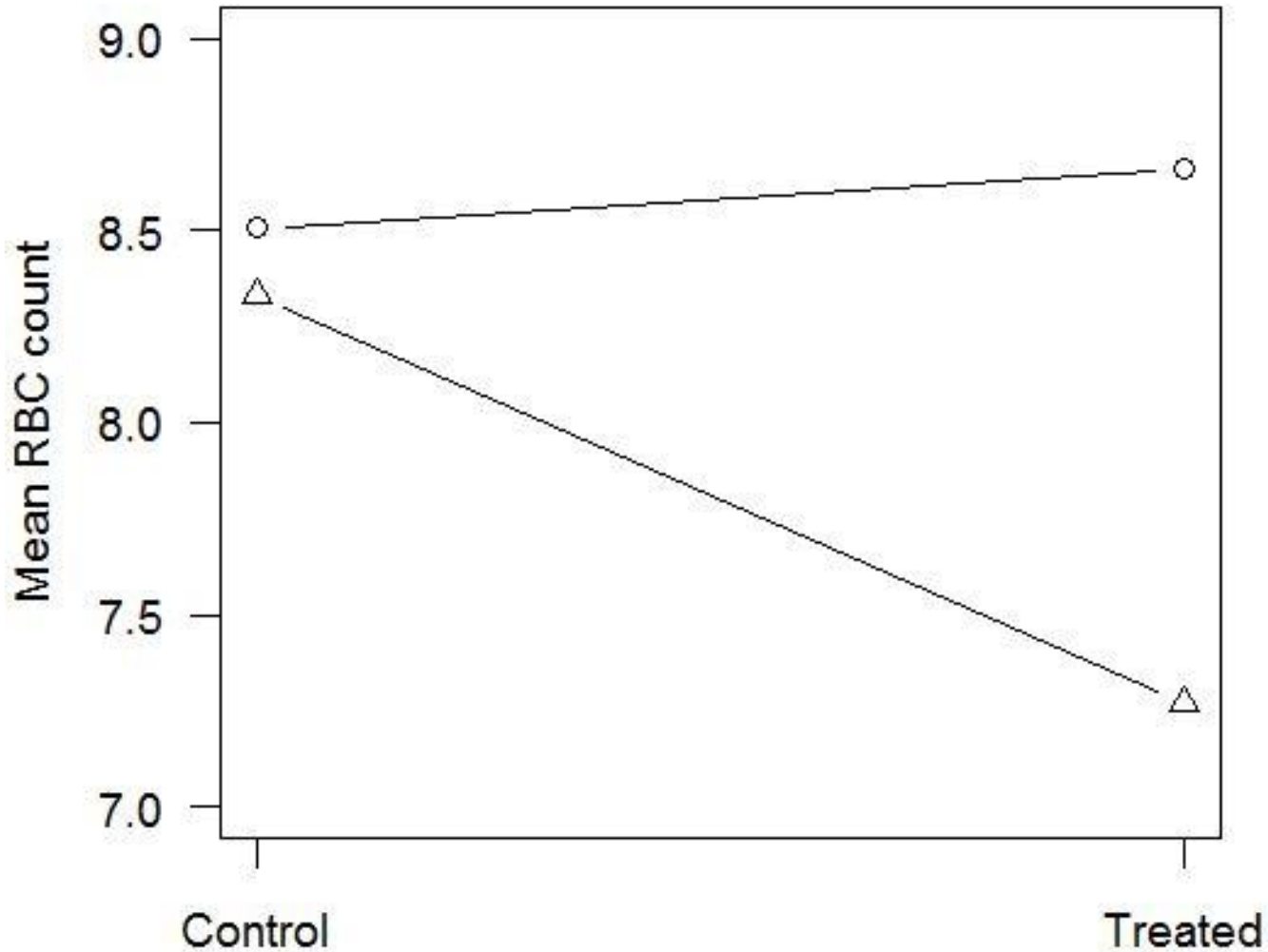


No interaction effect

# Another mice example with different strains:

Red Blood Cell counts

Strain	Control	Treated	Strain means
C3H	7.85	7.81	7.80
	8.77	7.21	
	8.48	6.96	
	8.22	7.10	
CD-1	9.01	9.18	8.58
	7.76	8.31	
	8.42	8.47	
	8.83	8.67	
Treatment means	8.42	7.96	



Interaction effect! This means that results based on a single strain cannot be generalised.

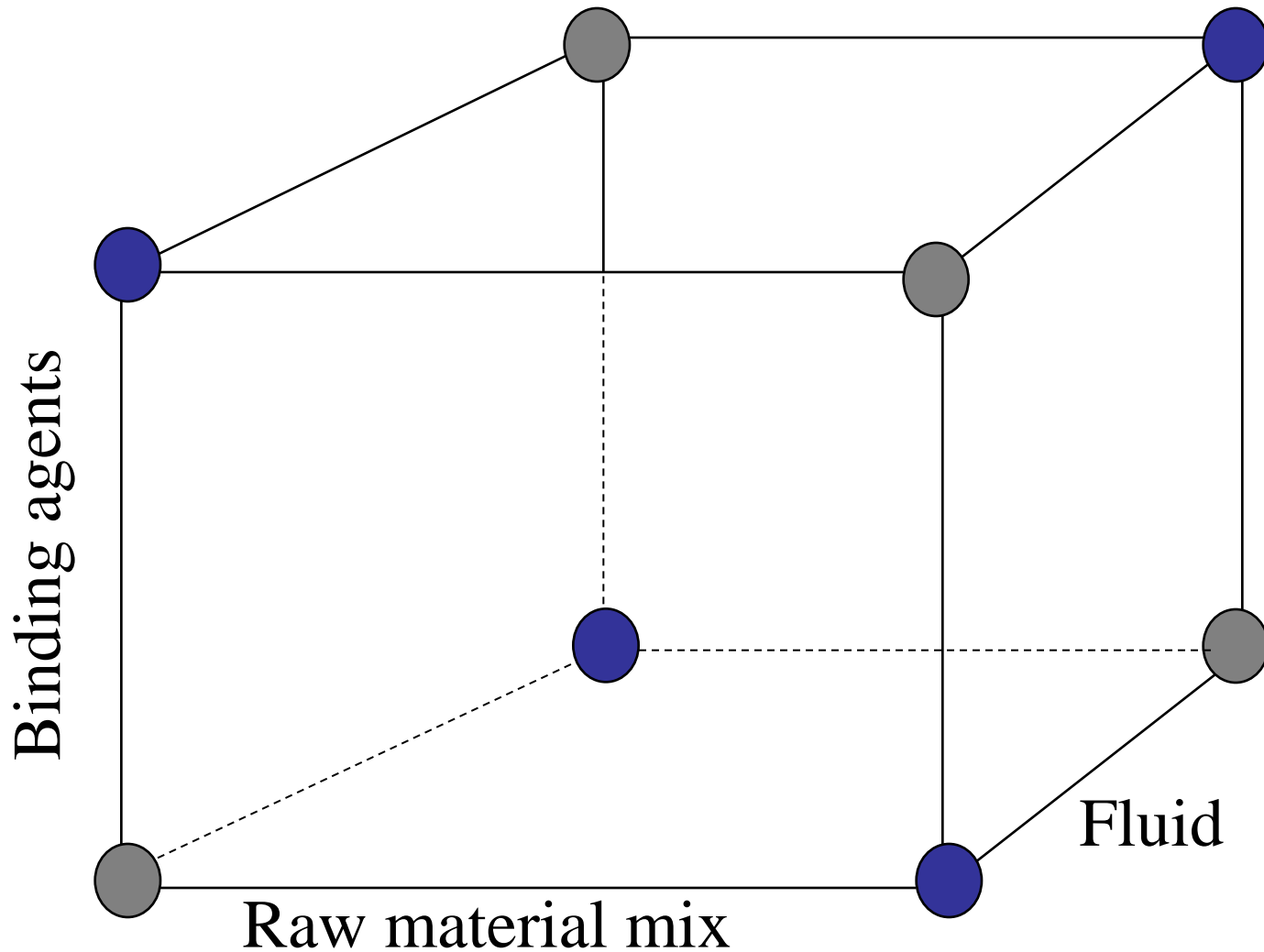
See also: Richter CA, Birnbaum LS, Farabollini F et al. In vivo effects of bisphenol A in laboratory rodent studies. *Reprod Toxicol* 2007;24:199-224.

# Test your calculations:

What is the calculated interaction effect between Factor 2 (milk) and Factor 3 (salmon) in the initial Fish pudding example?

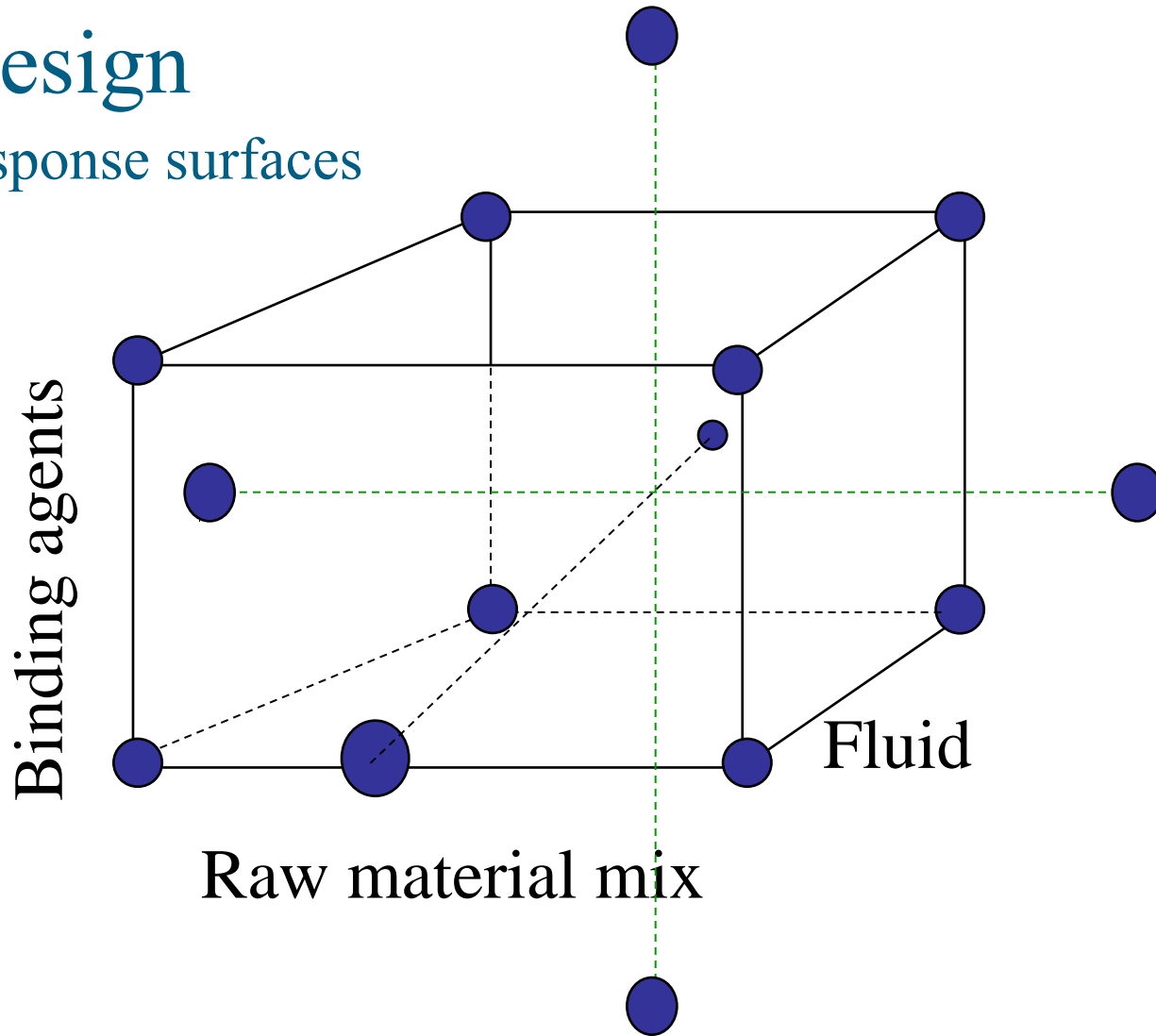
# Reduced factorial design

- Give main effects, more cost effective in the initial test phase

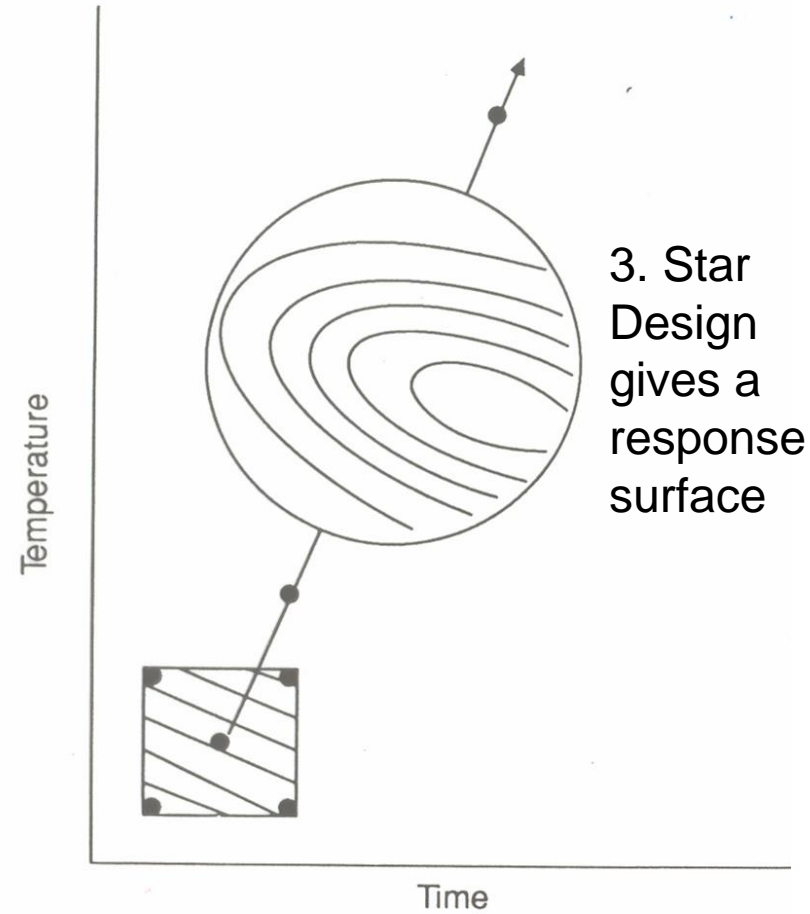
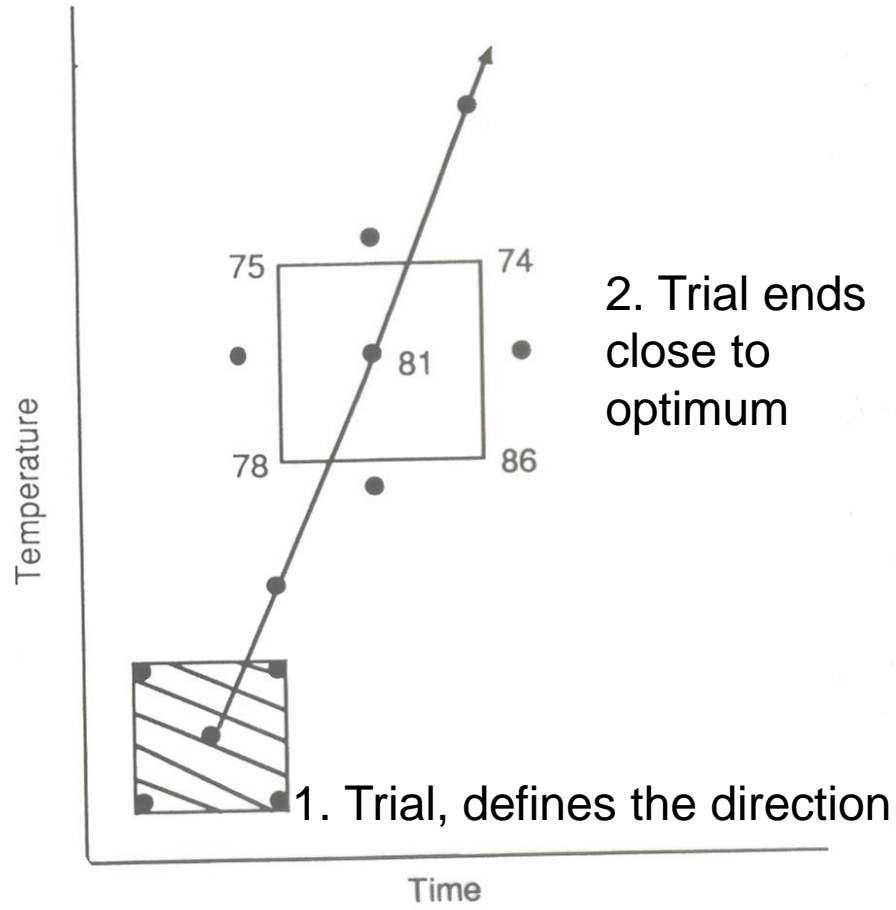


# Star design

- Give response surfaces



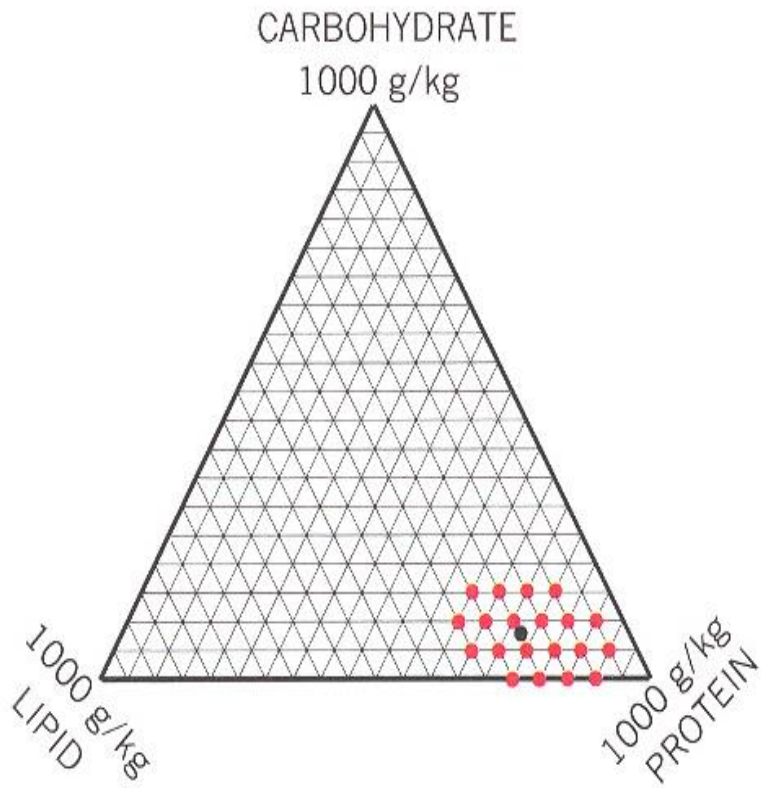
# Strategic approach towards optimum, here with two variables:



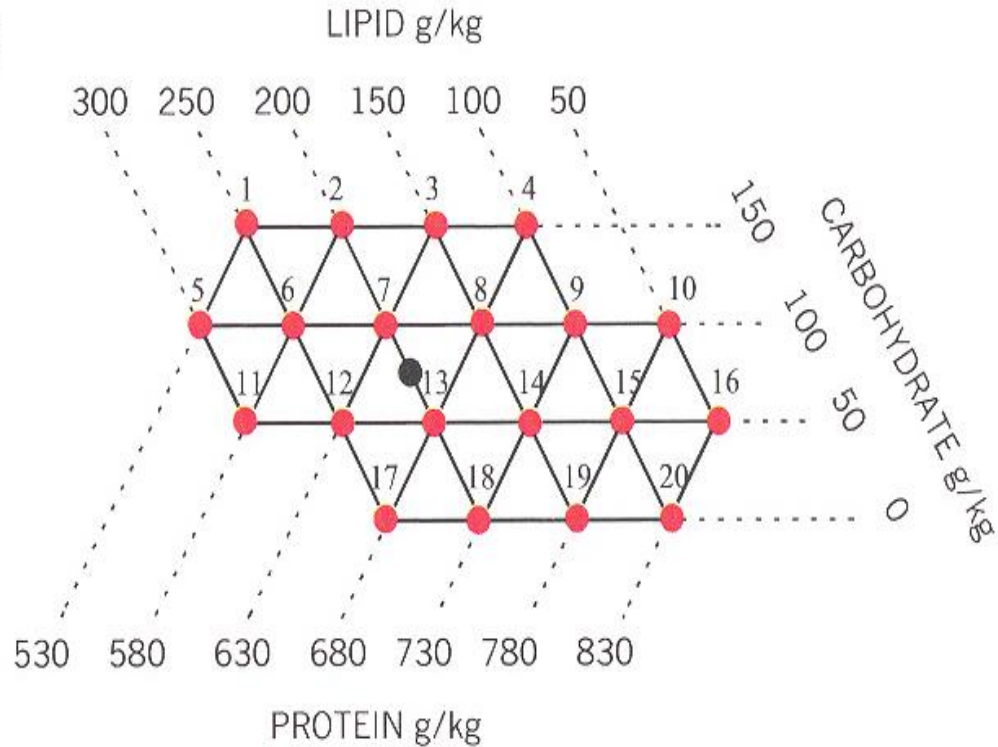
# Mixture design

- Applied when the combination opportunities are 'closed'

a)

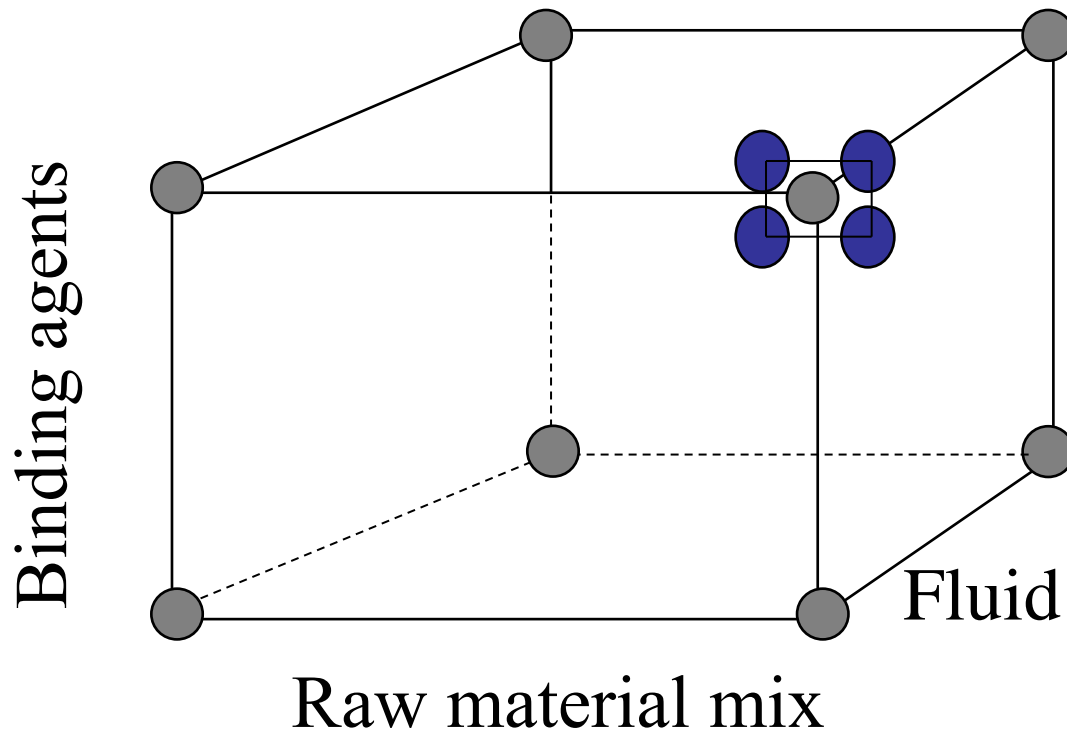


b)





Check the variability around optimum!



# Robustness in product testing

## Taguchi design

Design variable			Variables representing the customers'					
Raw mix	fluid	Bind. agent	handling of the product				Average	
			Temp.	-1	-1	1	1	response
			Tid	-1	1	-1	1	Yw
-1	-1	-1		Y1	Y9	Y17	Y25	5,6
1	-1	-1		Y2	Y10	Y18	Y26	7
-1	1	-1		Y3	Y11	Y19	Y27	7,1
1	1	-1		Y4	Y12	Y20	Y28	8
-1	-1	1		Y5	Y13	Y21	Y29	4
1	-1	1		Y6	Y14	Y22	Y30	2,3
-1	1	1		Y7	Y15	Y23	Y31	4
1	1	1		Y8	Y16	Y24	Y32	7

# Experimental strategy towards optimum (approx. 20% of costs in each stage)

- 1<sup>st</sup> stage: Reduced FD, several relevant variables and wide variation
- 2<sup>nd</sup> stage: Focus on the most important variables and limited variation. Apply FD or a randomized block design
- 3<sup>rd</sup> stage: Apply a star design or a mixture design (D-optimal) near optimum to get response surfaces
- 4<sup>th</sup> stage: Test variability near optimum
- 5<sup>th</sup> stage: Test robustness by Taguchi design