BME 3005

Biostatistics

Lecture 4: *Analysis of Variance*

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Introduction

- So far, we have learned how to summarize the data
  – Mean, variance, SD, median, percentiles
  – Standard error of the mean to estimate the precision with which a sample mean estimates the population mean
- Now testing scientific hypotheses – These are called *tests of significance*
  – They yield a ‘p’ value
  – $P<0.05$ is considered significant

Summary of Statistical Methods (table at the cover)

Summary of Some Statistical Methods to Test Hypotheses

Scale of measurement	Type of experiment: Two treatment groups consisting of different individuals	Three or more treatment groups consisting of different individuals	Before and after a single treatment in the same individuals	Multiple treatments in the same individuals	Association between two variables
Interval (and drawn from normally distributed populations*)	Unpaired *t* test (Chapter 4)	Analysis of variance (Chapter 3)	Paired *t* test (Chapter 9)	Repeated-measures analysis of variance (Chapter 9)	Linear regression, Pearson product-moment correlation, or Bland-Altman analysis (Chapter 8)
Nominal	Chi-square analysis-of-contingency table (Chapter 5)	Chi-square analysis-of-contingency table (Chapter 5)	McNemar's test (Chapter 9)	Cochrane Q†	Relative rank or odds ratio (Chapter 5)
Ordinal‡	Mann-Whitney rank-sum test (Chapter 10)	Kruskal-Wallis statistic (Chapter 10)	Wilcoxon signed-rank test (Chapter 10)	Friedman statistic (Chapter 10)	Spearman rank correlation (Chapter 8)
Survival time	Log-rank test or Gehan's test (Chapter 11)				

*If the assumption of normally distributed populations is not met, rank the observations and use the methods for data measured on an ordinal scale.
†Not covered in this text.
‡Or interval data that are not necessarily normally distributed.

Problem

- Null hypothesis: "Diet has no effect on the mean cardiac output of people living in a small town."

Samples

- 4 groups each of 7 people from a town of 200 people
  - Control group: continue eating normally
  - Second group: eat only spaghetti
  - Third group : eat only steak
  - Fourth group: Eat only fruits and nuts.
- All participants give informed consent..
- After 1 month, each person has a cardiac catheter inserted and the cardiac output is measured.

Control Group

- Hypothesis: all treatments have the same effect.
- The study includes a control group.
  - Experiments generally should.
- Our hypothesis: diet has no effect on cardiac output.

Figure 3.1 -- all the cardiac outputs

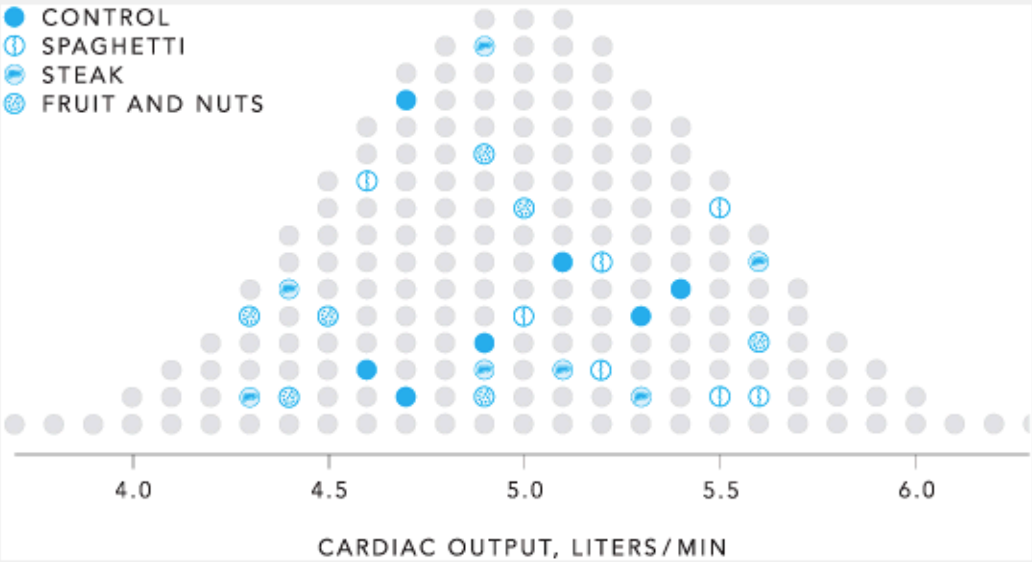


Figure 3–1 The values of cardiac output associated with all 200 members of the population of a small town. Since diet does not affect cardiac output, the four groups of seven people each selected at random to participate in our experiment (control, spaghetti, steak, fruit and nuts) simply represent four random samples drawn from a single population.

In fact: the hypothesis is true. The distribution is normal.
So there is no link between cardiac output and the diet.

Fig 3.2

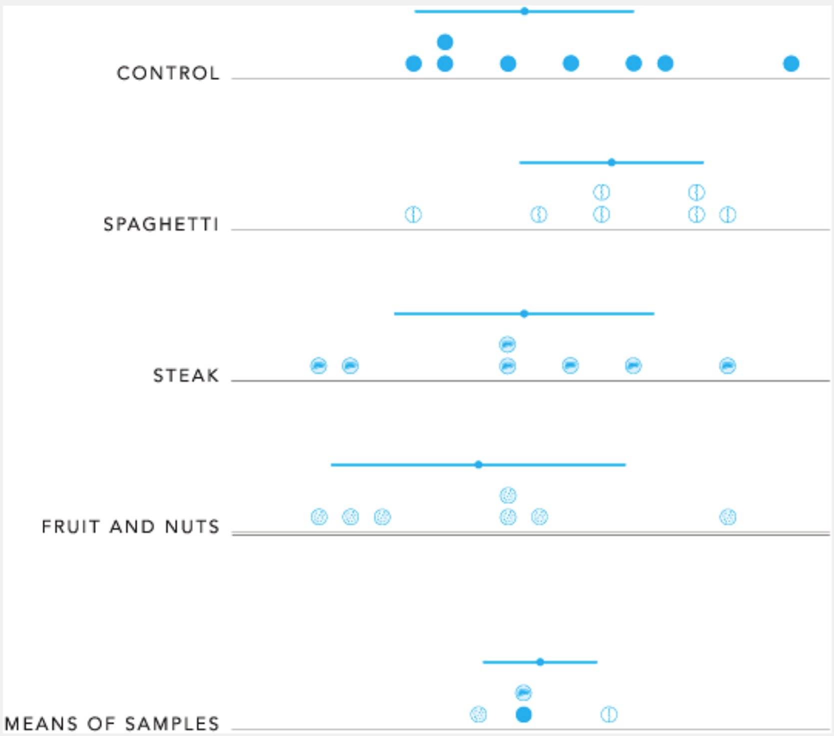


Figure 3–2 An investigator cannot observe the entire population but only the four samples selected at random for treatment. This figure shows the same four groups of individuals as in Fig. 3–1 with their means and standard deviations as they would appear to the investigator. The question facing the investigator is: Are the observed differences due to the different diets or simply random variation? The figure also shows the collection of sample means together with their standard deviation, which is an estimate of the standard error of the mean.

- The investigator cannot observe the whole population.
  So, he is left with the four samples selected at random.
- The main question is: Are the observed differences due to the dietary differences or simply random variation?
- The data looks like there is no difference.

No Effect

- We assume that diet has no effect on cardiac output.
- Since we assume diet does not matter, we then assume that the four experimental groups of seven people each are four random samples of size 7 drawn from a single population of 200 people.

Random Sampling

- Since the samples are drawn at random from a population with some variance, we expect the samples to have different means and std’s

- But if our null hypothesis is correct, the observed difference are simply due to random sampling.

Figure 3.3

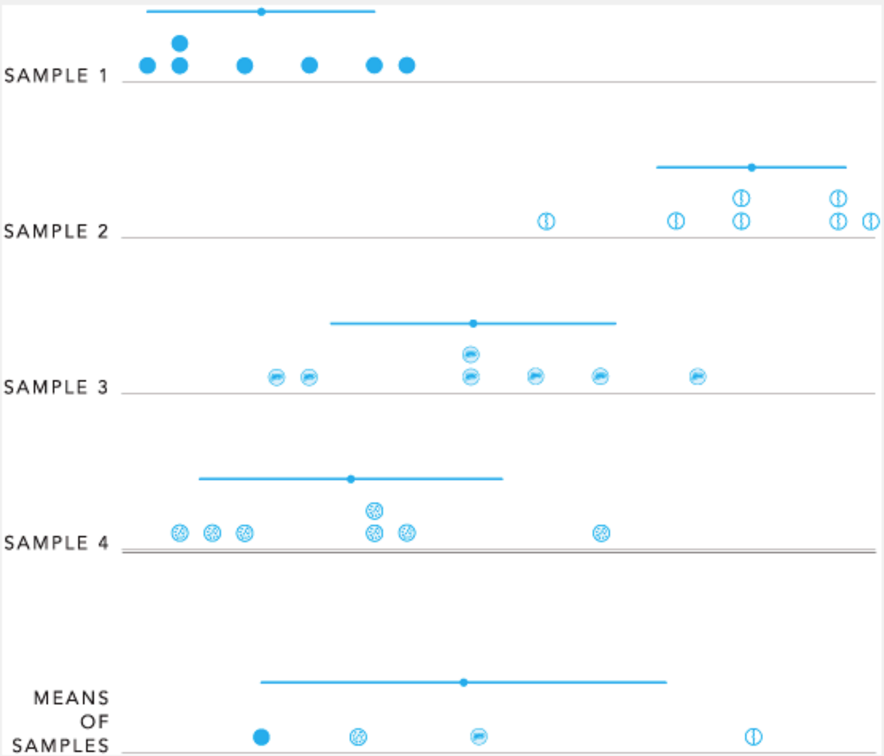


Figure 3–3 The four samples shown are identical to those in Fig. 3–2 except that the variability in the mean values has been increased substantially. The samples now appear to differ from each other because the variability between the sample means is larger than one would expect from the variability within each sample. Compare the relative variability in mean values with the variability within the sample groups with that seen in Fig. 3–2.

Another sample

Looks like there is a difference.

All the sample means differ from each other

Figure 3.4

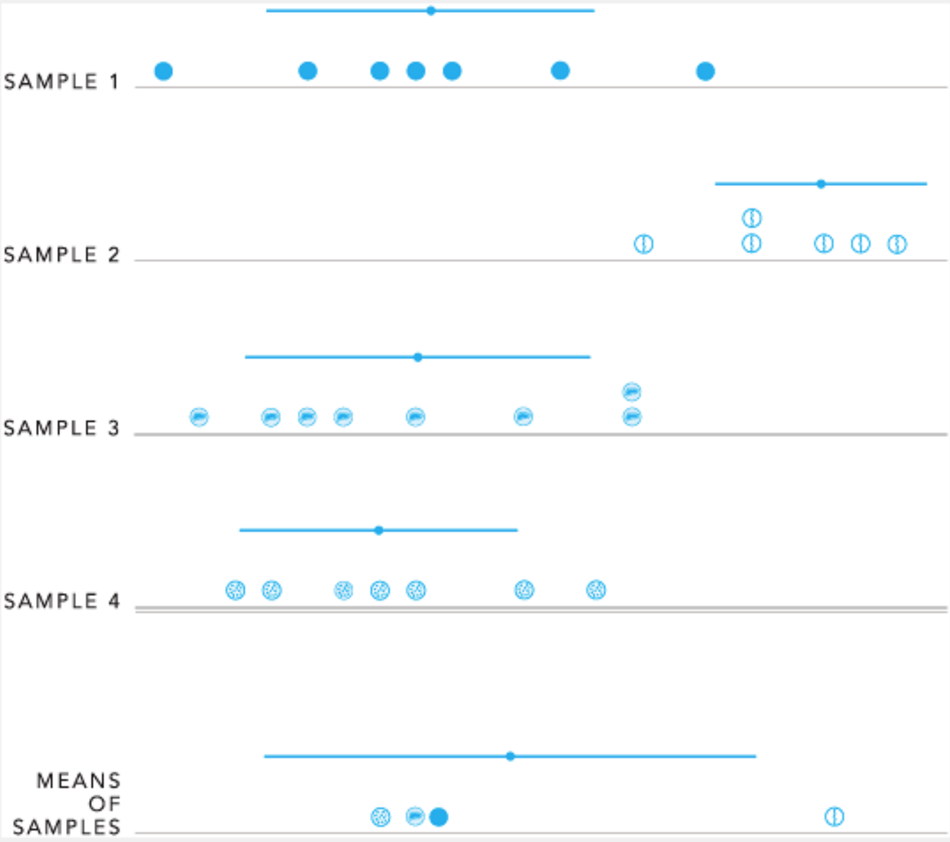


Figure 3–4 When the mean of even one of the samples (sample 2) differs substantially from the other samples, the variability computed from within the means is substantially larger than one would expect from examining the variability within the groups.

Another sample

Looks like there is difference.

One sample mean differs
From all others

The notion of difference

- **Why does one think that**
  - Fig 3.2: There is no difference between different diets in terms of their effect on cardiac output
  - Fig 3.3 & 3.4: There is a difference between different diets in terms of their effect on cardiac output
- The variability (std) within each sample is approximately same
  - Fig 3.2: the variability in the mean value of the samples is consistent with the variability one observes within the individual samples
  - Fig 3.3 & 3.4: the variability among sample means is much larger than one expect from the variability within each sample.

Parametric Statistical Methods

- Chap 2: Two population parameters, mean and std completely describe a normally distributed population.

- Therefore, we will use our raw data to compute these parameters and then base our analysis on their values rather than the raw data itself.

- Since the procedures we will now develop are based on these parameters: ‘parametric statistical methods’

Nonparametric statistical methods

- They don’t use normally distributed assumption
- Data can be any distribution
- They use, ranks, percentiles, frequencies etc
- Parametric methods generally provide more information about the treatment studied and are more likely to detect a real treatment effect when the underlying population is normally distributed.

How to test for differences between groups: Analysis of variance

- Hypothesis: On the average, different treatments all affect some variable identically.

- Null hypothesis: the hypothesis of “NO EFFECT”
  - There is no difference between the treatments..

- The resulting test can be generalized to analyze data obtained in experiments involving any number of treatments..

Estimating the population variance

- 2 different ways

    1)

    – The std or variance computed from each sample is an estimate of the std or variance of the entire population.

    – Since each estimate of population variance is computed from within each sample group, the estimates will not be affected by any differences in the mean values of different groups.

    2)

    – Means of each sample will be used to determine a second estimate of the population variance.

    – In this case, the differences between the means will affect the resulting estimate of the population variance.

Main Idea

- If all the samples were drawn from the same population (diet has no effect on cardiac output)
    - Two different ways of estimating population variance should yield approximately the same answer.
    - The samples were drawn from a single population.
- If all the samples were not drawn from the same population (diet has effect on cardiac output)
    - Reject the null hypothesis
    - Conclude that at least one of the samples was drawn from a different population.

First Way of Estimating the Population Variance

When the hypothesis that diet has no effect is true, every sample will give as good an estimate of the population variance.

So simply take the *average of four estimates of variance within the treatment groups.*

$$s^2_{\text{wit}} = {}^1\!/\!_4\,(s^2_{\text{con}} + s^2_{\text{spa}} + s^2_{\text{st}} + s^2_{\text{f}})$$

Population variance= within-groups variance= any variance of a group = s_{wit}^2

Second way of estimating the population variance

- Estimate the population variance from the means of the samples
- Hypothesis: all samples were drawn from a single population
  - Std (four sample means) will approximate Standard Error of the Mean (SEM)
- Remember: SEM was?(the Std of Sample means)
  - SEM ($\sigma_{\overline{X}}$) is related to the population std (σ) and sample size (n) as,

$$\sigma_{\overline{X}} = \frac{\sigma}{\sqrt{n}}.$$

  - True population variance σ^2 is related to the sample size as,

$$\sigma^2 = n\sigma_{\overline{X}}^2$$

Second way to estimate population variance

- We will use the variability between sample means to estimate SEM and call it $s_{\overline{X}}^2$ and use that to estimate the population variance.

$$s_{\text{bet}}^2 = n s_{\overline{X}}^2$$

- s_{bet}^2 is *between-groups variance*.
- =The estimate of the population variance computed from between the sample means.

F

- If the hypothesis that all the samples were drawn from a single population is true, then the within group or between group or the real population variances should be almost EQUAL.

$$F = \frac{\text{population variance estimated from sample means}}{\text{population variance estimated as average of sample variances}}$$

$$F = \frac{s^2_{\text{bet}}}{s^2_{\text{wit}}}$$

- F should be about 1.

Rule

- *If F is a big number, the variability between the sample means is larger than expected from the variability within the samples, so reject the hypothesis that all samples were drawn from the same population.*

- F for Fig 3.2 is almost 1, Fig 3.3 is 3.68, Fig3.4 is 4.86. So for Fig 3.2, continue accepting the null hypothesis, and reject it for Fig 3.3, Fig 3.4.

Figure 3.5

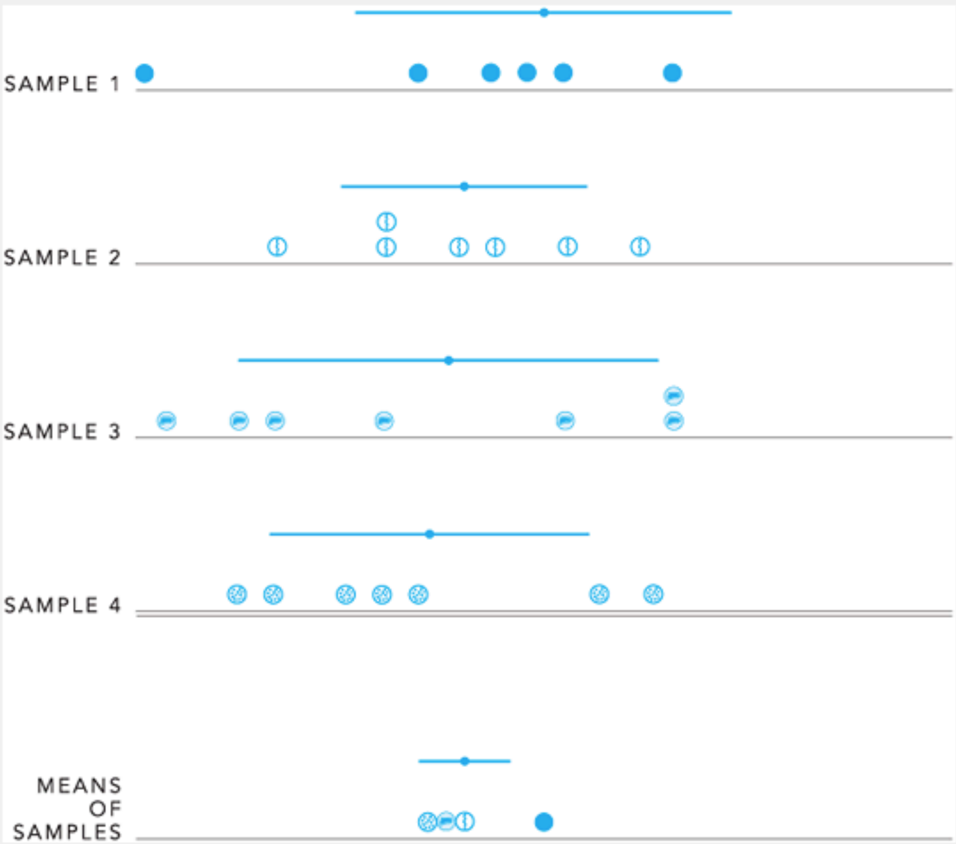


Figure 3–5 Four samples of seven members each drawn from the population shown in Fig. 3–1. Note that the variability in sample means is consistent with the variability within each of the samples, $F = 0.5$.

$F = 0.5$

Another set of 4 samples of 7 people out of town of 200 people.

We would get a slightly different F each time due to random variation even if diet had no effect on cardiac output.

Figure 3.6

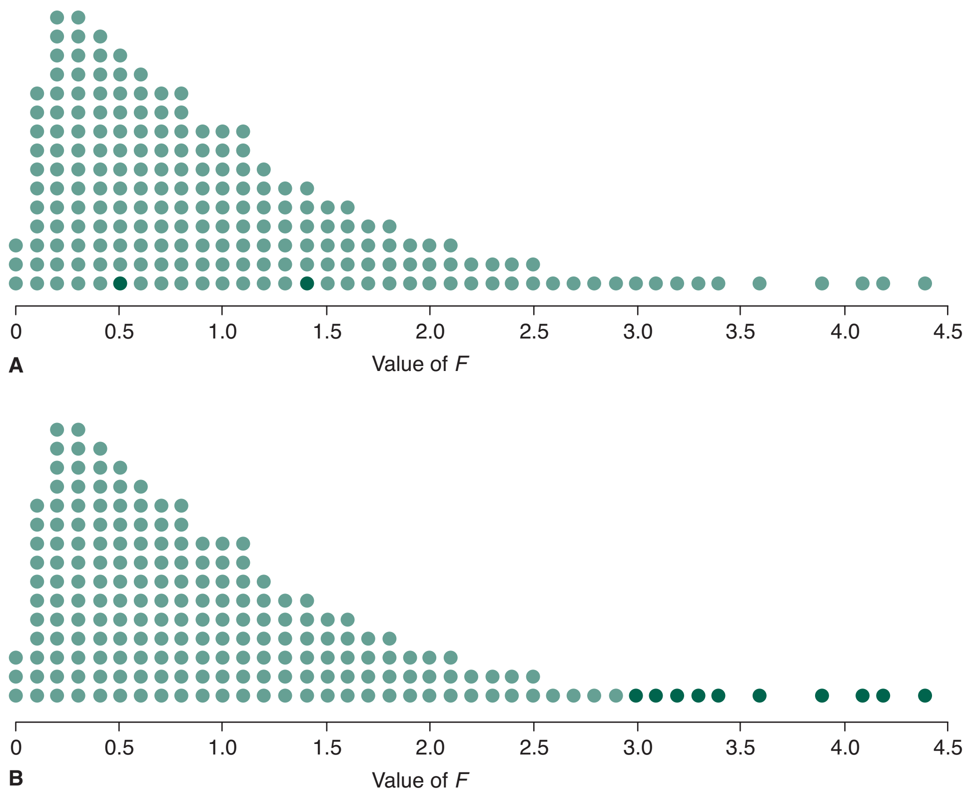


FIGURE 3-6. (A) Values of *F* computed from 200 experiments involving four samples, each of size 7, drawn from the population in Figure 3-1. **(B)** We expect *F* to exceed 3.0 only 5% of the time when all samples were, in fact, drawn from a single population. *(continued)*

F values calculated from 200 different samples

A. Dark circles represent the F's calculated form samples of Fig 3.2 and Fig 3.5

Exact shape of F distribution depends on how many samples were drawn, the size of each sample, and the distribution of the population from where the samples were drawn.

Figure 3.6 (cont)

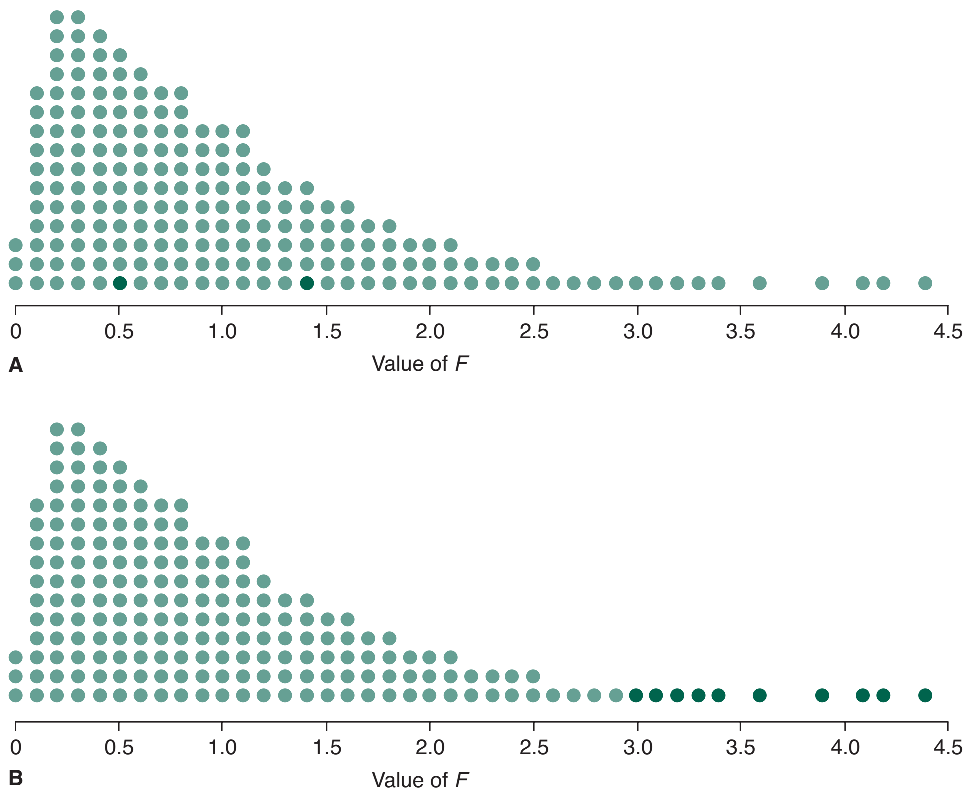


FIGURE 3-6. (A) Values of *F* computed from 200 experiments involving four samples, each of size 7, drawn from the population in Figure 3-1. **(B)** We expect *F* to exceed 3.0 only 5% of the time when all samples were, in fact, drawn from a single population. *(continued)*

Most of the F values are around 1 (between 0 and 2)
Some of them are much larger (dark circles part B of the figure) but they are unlikely.
Only 5% of 200 experiments (10 Experiments) produced F>3.

“BIG” F

Since F exceeded 3 only 10 out of 200 times *when all samples were drawn from the same population, we might decide F is big when it exceeds 3.0 and reject the hypothesis that all the samples were drawn from the same population.*

p<0.05 ??

- We accept the risk of erroneously rejecting the null hypothesis 5 percent of the time because F will be 3.0 or greater about 5% of the time, even when the treatment does not alter mean response.
- When we obtain such a “big” F, we reject the hypothesis that all the means are same and report P<0.05.
- This means that “there is a less than a 5% chance of getting a value of F as big or bigger than the computed value if the original hypothesis (null hypothesis) is true (diet did not affect cardiac output)”.

Figure 3.6 (c&d)

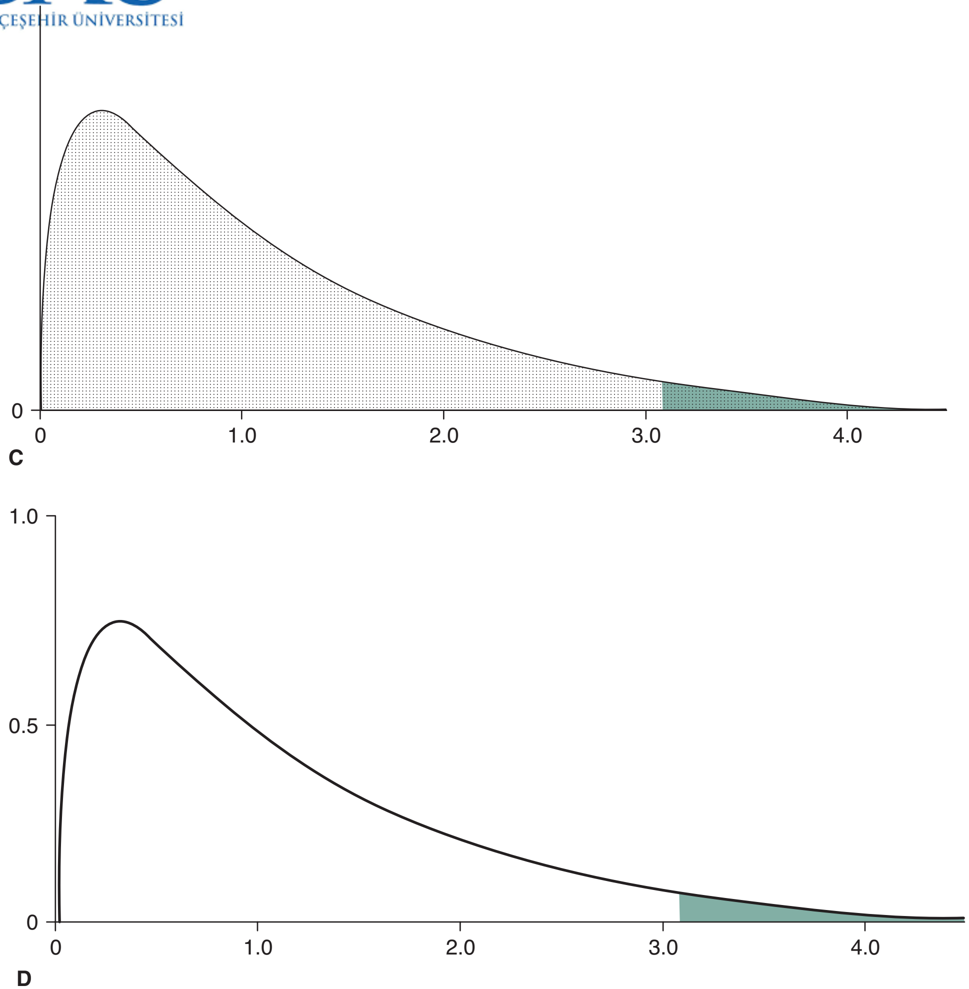


FIGURE 3-6. *(Continued)* **(C)** Results of computing the *F* ratio for all possible samples drawn from the original population. The 5% of most extreme *F* values are shown darker than the rest. **(D)** The *F* distribution one would obtain when sampling an infinite population. In this case, the cutoff value for considering *F* to be "big" is that value of *F* that subtends the upper 5% of the total area under the curve.

All 1042 experiments
Dark sand -- 5% highest F

Areas under the curve are analogous to the fractions of total number of circles.

Cutoff value that corresponds to $p<0.05$ and $p<0.01$ are 3.01 and 4.72

Table 3.1 (critical values of F corresponding to p<0.05 and p<0.01)

v_d \ v_n	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	∞
1	161	200	216	225	230	234	237	239	241	242	243	244	245	246	248	249	250	251	252	253	253	254	254	254
	4052	**4999**	**5403**	**5625**	**5764**	**5859**	**5928**	**5981**	**6022**	**6056**	**6082**	**6106**	**6142**	**6169**	**6208**	**6234**	**6261**	**6286**	**6302**	**6323**	**6334**	**6352**	**6361**	**6366**
2	18.51	19.00	19.16	19.25	19.30	19.33	19.36	19.37	19.38	19.39	19.40	19.41	19.42	19.43	19.44	19.45	19.46	19.47	19.47	19.48	19.49	19.49	19.50	19.50
	98.49	**99.00**	**99.17**	**99.25**	**99.30**	**99.33**	**99.36**	**99.37**	**99.39**	**99.40**	**99.41**	**99.42**	**99.43**	**99.44**	**99.45**	**99.46**	**99.47**	**99.48**	**99.48**	**99.49**	**99.49**	**99.49**	**99.50**	**99.50**
3	10.13	9.55	9.28	9.12	9.01	8.94	8.88	8.84	8.81	8.78	8.76	8.74	8.71	8.69	8.66	8.64	8.62	8.60	8.58	8.57	8.56	8.54	8.54	8.53
	34.12	**30.82**	**29.46**	**28.71**	**28.24**	**27.91**	**27.67**	**27.49**	**27.34**	**27.23**	**27.13**	**27.05**	**26.92**	**26.83**	**26.69**	**26.60**	**26.50**	**26.41**	**26.35**	**26.27**	**26.23**	**26.18**	**26.14**	**26.12**
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.93	5.91	5.87	5.84	5.80	5.77	5.74	5.71	5.70	5.68	5.66	5.65	5.64	5.63
	21.20	**18.00**	**16.69**	**15.98**	**15.52**	**15.21**	**14.98**	**14.80**	**14.66**	**14.54**	**14.45**	**14.37**	**14.24**	**14.15**	**14.02**	**13.93**	**13.83**	**13.74**	**13.69**	**13.61**	**13.57**	**13.52**	**13.48**	**13.46**
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.78	4.74	4.70	4.68	4.64	4.60	4.56	4.53	4.50	4.46	4.44	4.42	4.40	4.38	4.37	4.36
	16.26	**13.27**	**12.06**	**11.39**	**10.97**	**10.67**	**10.45**	**10.29**	**10.15**	**10.05**	**9.96**	**9.89**	**9.77**	**9.68**	**9.55**	**9.47**	**9.38**	**9.29**	**9.24**	**9.17**	**9.13**	**9.07**	**9.04**	**9.02**
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.03	4.00	3.96	3.92	3.87	3.84	3.81	3.77	3.75	3.72	3.71	3.69	3.68	3.67
	13.74	**10.92**	**9.78**	**9.15**	**8.75**	**8.47**	**8.26**	**8.10**	**7.98**	**7.87**	**7.79**	**7.72**	**7.60**	**7.52**	**7.39**	**7.31**	**7.23**	**7.14**	**7.09**	**7.02**	**6.99**	**6.94**	**6.90**	**6.88**
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.63	3.60	3.57	3.52	3.49	3.44	3.41	3.38	3.34	3.32	3.29	3.28	3.25	3.24	3.23
	12.25	**9.55**	**8.45**	**7.85**	**7.46**	**7.19**	**7.00**	**6.84**	**6.71**	**6.62**	**6.54**	**6.47**	**6.35**	**6.27**	**6.15**	**6.07**	**5.98**	**5.90**	**5.85**	**5.78**	**5.75**	**5.70**	**5.67**	**5.65**
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.34	3.31	3.28	3.23	3.20	3.15	3.12	3.08	3.05	3.03	3.00	2.98	2.96	2.94	2.93
	11.26	**8.65**	**7.59**	**7.01**	**6.63**	**6.37**	**6.19**	**6.03**	**5.91**	**5.82**	**5.74**	**5.67**	**5.56**	**5.48**	**5.36**	**5.28**	**5.20**	**5.11**	**5.06**	**5.00**	**4.96**	**4.91**	**4.88**	**4.86**
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.13	3.10	3.07	3.02	2.98	2.93	2.90	2.86	2.82	2.80	2.77	2.76	2.73	2.72	2.71
	10.56	**8.02**	**6.99**	**6.42**	**6.06**	**5.80**	**5.62**	**5.47**	**5.35**	**5.26**	**5.18**	**5.11**	**5.00**	**4.92**	**4.80**	**4.73**	**4.64**	**4.56**	**4.51**	**4.45**	**4.41**	**4.36**	**4.33**	**4.31**
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.97	2.94	2.91	2.86	2.82	2.77	2.74	2.70	2.67	2.64	2.61	2.59	2.56	2.55	2.54
	10.04	**7.56**	**6.55**	**5.99**	**5.64**	**5.39**	**5.21**	**5.06**	**4.95**	**4.85**	**4.78**	**4.71**	**4.60**	**4.52**	**4.41**	**4.33**	**4.25**	**4.17**	**4.12**	**4.05**	**4.01**	**3.96**	**3.93**	**3.91**
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.86	2.82	2.79	2.74	2.70	2.65	2.61	2.57	2.53	2.50	2.47	2.45	2.42	2.41	2.40
	9.65	**7.20**	**6.22**	**5.67**	**5.32**	**5.07**	**4.88**	**4.74**	**4.63**	**4.54**	**4.46**	**4.40**	**4.29**	**4.21**	**4.10**	**4.02**	**3.94**	**3.86**	**3.80**	**3.74**	**3.70**	**3.66**	**3.62**	**3.60**
12	4.75	3.88	3.49	3.26	3.11	3.00	2.92	2.85	2.80	2.76	2.72	2.69	2.64	2.60	2.54	2.50	2.46	2.42	2.40	2.36	2.35	2.32	2.31	2.30
	9.33	**6.93**	**5.95**	**5.41**	**5.06**	**4.82**	**4.65**	**4.50**	**4.39**	**4.30**	**4.22**	**4.16**	**4.05**	**3.98**	**3.86**	**3.78**	**3.70**	**3.61**	**3.56**	**3.49**	**3.46**	**3.41**	**3.38**	**3.36**
13	4.67	3.80	3.41	3.18	3.02	2.92	2.84	2.77	2.72	2.67	2.63	2.60	2.55	2.51	2.46	2.42	2.38	2.34	2.32	2.28	2.26	2.24	2.22	2.21
	9.07	**6.70**	**5.74**	**5.20**	**4.86**	**4.62**	**4.44**	**4.30**	**4.19**	**4.10**	**4.02**	**3.96**	**3.85**	**3.78**	**3.67**	**3.59**	**3.51**	**3.42**	**3.37**	**3.30**	**3.27**	**3.21**	**3.18**	**3.16**
14	4.60	3.74	3.34	3.11	2.96	2.85	2.77	2.70	2.65	2.60	2.56	2.53	2.48	2.44	2.39	2.35	2.31	2.27	2.24	2.21	2.19	2.16	2.14	2.13
	8.86	**6.51**	**5.56**	**5.03**	**4.69**	**4.46**	**4.28**	**4.14**	**4.03**	**3.94**	**3.86**	**3.80**	**3.70**	**3.62**	**3.51**	**3.43**	**3.34**	**3.26**	**3.21**	**3.14**	**3.11**	**3.06**	**3.02**	**3.00**
15	4.54	3.68	3.29	3.06	2.90	2.79	2.70	2.64	2.59	2.55	2.51	2.48	2.43	2.39	2.33	2.29	2.25	2.21	2.18	2.15	2.12	2.10	2.08	2.07
	8.68	**6.36**	**5.42**	**4.89**	**4.56**	**4.32**	**4.14**	**4.00**	**3.89**	**3.80**	**3.73**	**3.67**	**3.56**	**3.48**	**3.36**	**3.29**	**3.20**	**3.12**	**3.07**	**3.00**	**2.97**	**2.92**	**2.89**	**2.87**
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.45	2.42	2.37	2.33	2.28	2.24	2.20	2.16	2.13	2.09	2.07	2.04	2.02	2.01
	8.53	**6.23**	**5.29**	**4.77**	**4.44**	**4.20**	**4.03**	**3.89**	**3.78**	**3.69**	**3.61**	**3.55**	**3.45**	**3.37**	**3.25**	**3.18**	**3.10**	**3.01**	**2.96**	**2.98**	**2.86**	**2.80**	**2.77**	**2.75**
17	4.45	3.59	3.20	2.96	2.81	2.70	2.62	2.55	2.50	2.45	2.41	2.38	2.33	2.29	2.23	2.19	2.15	2.11	2.08	2.04	2.02	1.99	1.97	1.96
	8.40	**6.11**	**5.18**	**4.67**	**4.34**	**4.10**	**3.93**	**3.79**	**3.68**	**3.59**	**3.52**	**3.45**	**3.35**	**3.27**	**3.16**	**3.08**	**3.00**	**2.92**	**2.86**	**2.79**	**2.76**	**2.70**	**2.67**	**2.65**
18	4.41	3.55	3.16	2.93	2.77	3.66	2.58	2.51	2.46	2.41	2.37	2.34	2.29	2.25	2.19	2.15	2.11	2.07	2.04	2.00	1.98	1.95	1.93	1.92
	8.28	**6.01**	**5.09**	**4.58**	**4.25**	**4.01**	**3.85**	**3.71**	**3.60**	**3.51**	**3.44**	**3.37**	**3.27**	**3.19**	**3.07**	**3.00**	**2.91**	**2.83**	**2.78**	**2.71**	**2.68**	**2.62**	**2.59**	**2.57**
19	4.38	3.52	3.13	2.90	2.74	2.63	2.55	2.48	2.43	2.38	2.34	2.31	2.26	2.21	2.15	2.11	2.07	2.02	2.00	1.96	1.94	1.91	1.90	1.88
	8.18	**5.93**	**5.01**	**4.50**	**4.17**	**3.94**	**3.77**	**3.63**	**3.52**	**3.43**	**3.36**	**3.30**	**3.19**	**3.12**	**3.00**	**2.92**	**2.84**	**2.76**	**2.70**	**2.63**	**2.60**	**2.54**	**2.51**	**2.49**

v_n = degrees of freedom for numerator; v_d = degrees of freedom for denominator.

Table 3.1 (critical values of F corresponding to p<0.05 and p<0.01)

TABLE 3-1. Critical Values of *F* Corresponding to *P* < .05 (Lightface) and *P* < .01 (Boldface) (Continued)

	v_n																							
v_d	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	∞
20	4.35	3.49	3.10	2.87	2.71	2.60	2.52	2.45	2.40	2.35	2.31	2.28	2.23	2.18	2.12	2.08	2.04	1.99	1.96	1.92	1.90	1.87	1.85	1.84
	8.10	**5.85**	**4.94**	**4.43**	**4.10**	**3.87**	**3.71**	**3.56**	**3.45**	**3.37**	**3.30**	**3.23**	**3.13**	**3.05**	**2.94**	**2.86**	**2.77**	**2.69**	**2.63**	**2.56**	**2.53**	**2.47**	**2.44**	**2.42**
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.28	2.25	2.20	2.15	2.09	2.05	2.00	1.96	1.93	1.89	1.87	1.84	1.82	1.81
	8.02	**5.78**	**4.87**	**4.37**	**4.04**	**3.81**	**3.65**	**3.51**	**3.40**	**3.31**	**3.24**	**3.17**	**3.07**	**2.99**	**2.88**	**2.80**	**2.72**	**2.63**	**2.58**	**2.51**	**2.47**	**2.42**	**2.38**	**2.36**
22	4.30	3.44	3.05	2.82	2.66	2.55	2.47	2.40	2.35	2.30	2.26	2.23	2.18	2.13	2.07	2.03	1.98	1.93	1.91	1.87	1.84	1.81	1.80	1.78
	7.94	**5.72**	**4.82**	**4.31**	**3.99**	**3.76**	**3.59**	**3.45**	**3.35**	**3.26**	**3.18**	**3.12**	**3.02**	**2.94**	**2.83**	**2.75**	**2.67**	**2.58**	**2.53**	**2.46**	**2.42**	**2.37**	**2.33**	**2.31**
23	4.28	3.42	3.03	2.80	2.64	2.53	2.45	2.38	2.32	2.28	2.24	2.20	2.14	2.10	2.04	2.00	1.96	1.91	1.88	1.84	1.82	1.79	1.77	1.76
	7.88	**5.66**	**4.76**	**4.26**	**3.94**	**3.71**	**3.54**	**3.41**	**3.30**	**3.21**	**3.14**	**3.07**	**2.97**	**2.89**	**2.78**	**2.70**	**2.62**	**2.53**	**2.48**	**2.41**	**2.37**	**2.32**	**2.28**	**2.26**
24	4.26	3.40	3.01	2.78	2.62	2.51	2.43	2.36	2.30	2.26	2.22	2.18	2.13	2.09	2.02	1.98	1.94	1.89	1.86	1.82	1.80	1.76	1.74	1.73
	7.82	**5.61**	**4.72**	**4.22**	**3.90**	**3.67**	**3.50**	**3.36**	**3.25**	**3.17**	**3.09**	**3.03**	**2.93**	**2.85**	**2.74**	**2.66**	**2.58**	**2.49**	**2.44**	**2.36**	**2.33**	**2.27**	**2.23**	**2.31**
25	4.24	3.38	2.99	2.76	2.60	2.49	2.41	2.34	2.28	2.24	2.20	2.16	2.11	2.06	2.00	1.96	1.92	1.87	1.84	1.80	1.77	1.74	1.72	1.71
	7.77	**5.57**	**4.68**	**4.18**	**3.86**	**3.63**	**3.46**	**3.32**	**3.21**	**3.13**	**3.05**	**2.99**	**2.89**	**2.81**	**2.70**	**2.62**	**2.54**	**2.45**	**2.40**	**2.32**	**2.29**	**2.23**	**2.19**	**2.17**
26	4.22	3.37	2.98	2.74	2.59	2.47	2.39	2.32	2.27	2.22	2.18	2.15	2.10	2.05	1.99	1.95	1.90	1.85	1.82	1.78	1.76	1.72	1.70	1.69
	7.72	**5.53**	**4.64**	**4.14**	**3.82**	**3.59**	**3.42**	**3.29**	**3.17**	**3.09**	**3.02**	**2.96**	**2.86**	**2.77**	**2.66**	**2.58**	**2.50**	**2.41**	**2.36**	**2.28**	**2.25**	**2.19**	**2.15**	**2.13**
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2.30	2.25	2.20	2.16	2.13	2.08	2.03	1.97	1.93	1.88	1.84	1.80	1.76	1.74	1.71	1.68	1.67
	7.68	**5.49**	**4.60**	**4.11**	**3.79**	**3.56**	**3.39**	**3.26**	**3.14**	**3.06**	**2.98**	**2.93**	**2.83**	**2.74**	**2.63**	**2.55**	**2.47**	**2.38**	**2.33**	**2.25**	**2.21**	**2.16**	**2.12**	**2.10**
28	4.20	3.34	2.95	2.71	2.56	2.44	2.36	2.29	2.24	2.19	2.15	2.12	2.06	2.02	1.96	1.91	1.87	1.81	1.78	1.75	1.72	1.69	1.67	1.65
	7.64	**5.45**	**4.57**	**4.07**	**3.76**	**3.53**	**3.36**	**3.23**	**3.11**	**3.03**	**2.95**	**2.90**	**2.80**	**2.71**	**2.60**	**2.52**	**2.44**	**2.35**	**2.30**	**2.22**	**2.18**	**2.13**	**2.09**	**2.06**
29	4.18	3.33	2.93	2.70	2.54	2.43	2.35	2.28	2.22	2.18	2.14	2.10	2.05	2.00	1.94	1.90	1.85	1.80	1.77	1.73	1.71	1.68	1.65	1.64
	7.60	**5.42**	**4.54**	**4.04**	**3.73**	**3.50**	**3.33**	**3.20**	**3.08**	**3.00**	**2.92**	**2.87**	**2.77**	**2.68**	**2.57**	**2.49**	**2.41**	**2.32**	**2.27**	**2.19**	**2.15**	**2.10**	**2.06**	**2.03**
30	4.17	3.32	2.92	2.69	2.53	2.42	2.34	2.27	2.21	2.16	2.12	2.09	2.04	1.99	1.93	1.89	1.84	1.79	1.76	1.72	1.69	1.66	1.64	1.62
	7.56	**5.39**	**4.51**	**4.02**	**3.70**	**3.47**	**3.30**	**3.17**	**3.06**	**2.98**	**2.90**	**2.84**	**2.74**	**2.66**	**2.55**	**2.47**	**2.38**	**2.29**	**2.24**	**2.16**	**2.13**	**2.07**	**2.03**	**2.01**
32	4.15	3.30	2.90	2.67	2.51	2.40	2.32	2.25	2.19	2.14	2.10	2.07	2.02	1.97	1.91	1.86	1.82	1.76	1.74	1.69	1.67	1.64	1.61	1.59
	7.50	**5.34**	**4.46**	**3.97**	**3.66**	**3.42**	**3.25**	**3.12**	**3.01**	**2.94**	**2.86**	**2.80**	**2.70**	**2.62**	**2.51**	**2.42**	**2.34**	**2.25**	**2.20**	**2.12**	**2.08**	**2.02**	**1.98**	**1.96**
34	4.13	3.28	2.88	2.65	2.49	2.38	2.30	2.23	2.17	2.12	2.08	2.05	2.00	1.95	1.89	1.84	1.80	1.74	1.71	1.67	1.64	1.61	1.59	1.57
	7.44	**5.29**	**4.42**	**3.93**	**3.61**	**3.38**	**3.21**	**3.08**	**2.97**	**2.89**	**2.82**	**2.76**	**2.66**	**2.58**	**2.47**	**2.38**	**2.30**	**2.21**	**2.15**	**2.08**	**2.04**	**1.98**	**1.94**	**1.91**
36	4.11	3.26	2.86	2.63	2.48	2.36	2.28	2.21	2.15	2.10	2.06	2.03	1.98	1.93	1.87	1.82	1.78	1.72	1.69	1.65	1.62	1.59	1.56	1.55
	7.39	**5.25**	**4.38**	**3.89**	**3.58**	**3.35**	**3.18**	**3.04**	**2.94**	**2.86**	**2.78**	**2.72**	**2.62**	**2.54**	**2.43**	**2.35**	**2.26**	**2.17**	**2.12**	**2.04**	**2.00**	**1.94**	**1.90**	**1.87**

Table 3.1 (critical values of F corresponding to p<0.05 and p<0.01)

38	4.10	3.25	2.85	2.62	2.46	2.35	2.26	2.19	2.14	2.09	2.05	2.02	1.96	1.92	1.85	1.80	1.76	1.71	1.67	1.63	1.60	1.57	1.54	1.53
	7.35	**5.21**	**4.34**	**3.86**	**3.54**	**3.32**	**3.15**	**3.02**	**2.91**	**2.82**	**2.75**	**2.69**	**2.59**	**2.51**	**2.40**	**2.32**	**2.22**	**2.14**	**2.08**	**2.00**	**1.97**	**1.90**	**1.86**	**1.84**
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.07	2.04	2.00	1.95	1.90	1.84	1.79	1.74	1.69	1.66	1.61	1.59	1.55	1.53	1.51
	7.31	**5.18**	**4.31**	**3.83**	**3.51**	**3.29**	**3.12**	**2.99**	**2.88**	**2.80**	**2.73**	**2.66**	**2.56**	**2.49**	**2.37**	**2.29**	**2.20**	**2.11**	**2.05**	**1.97**	**1.94**	**1.88**	**1.84**	**1.81**
42	4.07	3.22	2.83	2.59	2.44	2.32	2.24	2.17	2.11	2.06	2.02	1.99	1.94	1.89	1.82	1.78	1.73	1.68	1.64	1.60	1.57	1.54	1.51	1.49
	7.27	**5.15**	**4.29**	**3.80**	**3.49**	**3.26**	**3.10**	**2.96**	**2.86**	**2.77**	**2.70**	**2.64**	**2.54**	**2.46**	**2.35**	**2.26**	**2.17**	**2.08**	**2.02**	**1.94**	**1.91**	**1.85**	**1.80**	**1.78**
44	4.06	3.21	2.82	2.58	2.43	2.31	2.23	2.16	2.10	2.05	2.01	1.98	1.92	1.88	1.81	1.76	1.72	1.66	1.63	1.58	1.56	1.52	1.50	1.48
	7.24	**5.12**	**4.26**	**3.78**	**3.46**	**3.24**	**3.07**	**2.94**	**2.84**	**2.75**	**2.68**	**2.62**	**2.52**	**2.44**	**2.32**	**2.24**	**2.15**	**2.06**	**2.00**	**1.92**	**1.88**	**1.82**	**1.78**	**1.75**
46	4.05	3.20	2.81	2.57	2.42	2.30	2.22	2.14	2.09	2.04	2.00	1.97	1.91	1.87	1.80	1.75	1.71	1.65	1.62	1.57	1.54	1.51	1.48	1.46
	7.21	**5.10**	**4.24**	**3.76**	**3.44**	**3.22**	**3.05**	**2.92**	**2.82**	**2.73**	**2.66**	**2.60**	**2.50**	**2.42**	**2.30**	**2.22**	**2.13**	**2.04**	**1.98**	**1.90**	**1.86**	**1.80**	**1.76**	**1.72**
48	4.04	3.19	2.80	2.56	2.41	2.30	2.21	2.14	2.08	2.03	1.99	1.96	1.90	1.86	1.79	1.74	1.70	1.64	1.61	I.56	1.53	1.50	1.47	1.45
	7.19	**5.08**	**4.22**	**3.74**	**3.42**	**3.20**	**3.04**	**2.90**	**2.80**	**2.71**	**2.64**	**2.58**	**2.48**	**2.40**	**2.28**	**2.20**	**2.11**	**2.02**	**1.96**	**1.88**	**1.84**	**1.78**	**1.73**	**1.70**
50	4.03	3.18	2.79	2.56	2.40	2.29	2.20	2.13	2.07	2.02	1.98	1.95	1.90	1.85	1.78	1.74	1.69	1.63	1.60	1.55	1.52	1.48	1.46	1.44
	7.17	**5.06**	**4.20**	**3.72**	**3.41**	**3.18**	**3.02**	**2.88**	**2.78**	**2.70**	**2.62**	**2.56**	**2.46**	**2.39**	**2.26**	**2.18**	**2.10**	**2.00**	**1.94**	**1.86**	**1.82**	**1.76**	**1.71**	**1.68**
60	4.00	3.15	2.76	2.52	2.37	2.25	2.17	2.10	2.04	1.99	1.95	1.92	1.86	1.81	1.75	1.70	1.65	1.59	1.56	1.50	1.48	1.44	1.41	1.39
	7.08	**4.98**	**4.13**	**3.65**	**3.34**	**3.12**	**2.95**	**2.82**	**2.72**	**2.63**	**2.56**	**2.50**	**2.40**	**2.32**	**2.20**	**2.12**	**2.03**	**1.93**	**1.87**	**1.79**	**1.74**	**1.68**	**1.63**	**1.60**
70	3.98	3.13	2.74	2.50	2.35	2.23	2.14	2.07	2.01	1.97	1.93	1.89	1.84	1.79	1.72	1.67	1.62	1.56	1.53	1.47	1.45	1.40	1.37	1.35
	7.01	**4.92**	**4.08**	**3.60**	**3.29**	**3.07**	**2.91**	**2.77**	**2.67**	**2.59**	**2.51**	**2.45**	**2.35**	**2.28**	**2.15**	**2.07**	**1.98**	**1.88**	**1.82**	**1.74**	**1.69**	**1.62**	**1.56**	**1.53**
80	3.96	3.11	2.72	2.48	2.33	2.21	2.12	2.05	1.99	1.95	1.91	1.88	1.82	1.77	1.70	1.65	1.60	1.54	1.51	1.45	1.42	1.38	1.35	1.32
	6.96	**4.88**	**4.04**	**3.56**	**3.25**	**3.04**	**2.87**	**2.74**	**2.64**	**2.55**	**2.48**	**2.41**	**2.32**	**2.24**	**2.11**	**2.03**	**1.94**	**1.84**	**1.78**	**1.70**	**1.65**	**1.57**	**1.52**	**1.49**
100	3.94	3.09	2.70	2.46	2.30	2.19	2.10	2.03	1.97	1.92	1.88	1.85	1.79	1.75	1.68	1.63	1.57	1.51	1.48	1.42	1.39	1.34	1.30	1.28
	6.90	**4.82**	**3.98**	**3.51**	**3.20**	**2.99**	**2.82**	**2.69**	**2.59**	**2.51**	**2.43**	**2.36**	**2.26**	**2.19**	**2.06**	**1.98**	**1.89**	**1.79**	**1.73**	**1.64**	**1.59**	**1.51**	**1.46**	**1.43**
120	3.92	3.07	2.68	2.45	2.29	2.18	2.09	2.02	1.96	1.91	1.87	1.84	1.78	1.73	1.66	1.61	1.56	1.50	1.46	1.39	1.37	1.32	1.28	1.25
	6.85	**4.79**	**3.95**	**3.48**	**3.17**	**2.96**	**2.79**	**2.66**	**2.56**	**2.47**	**2.40**	**2.34**	**2.23**	**2.15**	**2.03**	**1.95**	**1.86**	**1.76**	**1.70**	**1.61**	**1.56**	**1.48**	**1.42**	**1.38**
∞	3.84	2.99	2.60	2.37	2.21	2.09	2.01	1.94	1.88	1.83	1.79	1.75	1.69	1.64	1.57	1.52	1.46	1.40	1.35	1.28	1.24	1.17	1.11	1.00
	6.63	**4.60**	**3.78**	**3.32**	**3.02**	**2.80**	**2.64**	**2.51**	**2.41**	**2.32**	**2.24**	**2.18**	**2.07**	**1.99**	**1.87**	**1.79**	**1.69**	**1.59**	**1.52**	**1.41**	**1.36**	**1.25**	**1.15**	**1.00**

v_n = degrees of freedom for numerator; v_d = degrees of freedom for denominator.

Degrees of Freedom

- The degrees of freedom simply represent the way number of samples and sample size enter the mathematical formulas used to construct all statistical tables.

- v_n is the numerator degrees of freedom
- v_d is the denominator degrees of freedom

- Between group degrees of freedom (numerator degrees of freedom because between group variance is in the numerator of F) is number of samples -1, v_n=m-1 (4 groups, 4-1=3)

- The within groups degrees of freedom (denominator) number of samples times 1 less than the size of each sample, v_d =m(n-1) (4 groups, size 7 each, 4(7-1)=24)

How to read F table?

Table 3-1 Critical Values of *F* Corresponding to *P* < .05 (Lightface) and *P* < .01 (**Boldface**)

	ν_n																							
ν_d	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	∞
24	4.26	3.40	3.01	2.78	2.62	2.51	2.43	2.36	2.30	2.26	2.22	2.18	2.13	2.09	2.02	1.98	1.94	1.89	1.86	1.82	1.80	1.76	1.74	1.73
	7.82	**5.61**	**4.72**	**4.22**	**3.90**	**3.67**	**3.50**	**3.36**	**3.25**	**3.17**	**3.09**	**3.03**	**2.93**	**2.85**	**2.74**	**2.66**	**2.58**	**2.49**	**2.44**	**2.36**	**2.33**	**2.27**	**2.23**	**2.21**

Find v_n and v_d and lookup the corresponding significance level.

For p<0.05, look up the value on the top, for p<0.01 lookup the value on the bottom.

Calculate your F value, then compare it against the cutoff values of p<0.05 and p<0.01.

Restrictions

- Mathematicians assumed four things about the underlying population that must be satisfied for the tables to be applicable to the data:
  - Each sample must be independent of other samples.
  - Each sample must be randomly selected from the population being studied.
  - The populations from which the samples were drawn must be "normally distributed".
  - The variances of each population must be equal, even when the means are different, i.e. when the treatment has an effect.
- ANOVA as discussed up to now requires each sample contain the same number of members.

Example 1. Glucose Levels in Children of Parents with Diabetes

- To investigate whether abnormalities in carbohydrate metabolism could be identified in non-diabetic young adults whose parents had a history of type 2 diabetes

- Children with history of type 2 diabetes in their parents --- *cases*

- Age matched children with no history of diabetes in their family --- *controls*

Observational Studies

- This is an *observational study.*
  – *Obtain data by simply observing events without controlling them (like giving a drug etc.)*

- Observational studies are prone to 2 problems:
  – The groups may vary in ways the investigators don't notice or choose to ignore, and these factors rather than the treatment itself may be the reason of differences --- *confounding factors*

  – Observational studies are prone to bias in patient recall, investigator assessment and selection of the treatment or control group.

Figure 3.7


CONTROL
n = 25
X̄ = 82.2
s = 2.49
70
75
80
85
90
95
10
DIABETIC PARENT
n = 25
X̄ = 86.1
s = 2.09
70
75
80
85
90
95
10
FASTING GLUCOSE LEVELS, mg/dL

Children selection criteria

- Parental history of diabetes was verified by a physician through medical records to exclude possible type I diabetics.
- No child had parents who were both diabetics.
- The cases and control groups had similar ages (15.3±4.5 SD and 15.1±5.7 SD).
- Control offspring were matched according to age of parents from families with no history of diabetes in parents, grandparents, uncles, or aunts.

Null hypothesis

- Null hypothesis: Fasting glucose level do not differ in children of parents with type 2 diabetes compared to children with parents that did not have a history of type 2 diabetes.
- Question: How likely are the differences between the two samples of children to be due to random sampling rather than a difference based on parental history of diabetes?

Method

$$s^2_{wit} = \tfrac{1}{2}(s^2_{diabetes} + s^2_{control}) = \tfrac{1}{2}(2.09^2 + 2.49^2) = 5.28\ (\text{mg/dL})$$

$$\bar{X} = \tfrac{1}{2}(\bar{X}_{diabetes} + \bar{X}_{control}) = \tfrac{1}{2}(86.1 + 82.2) = 84.2\ \text{mg/dL}$$

$$s_{\bar{X}} = \sqrt{\frac{(\bar{X}_{diabetes} - \bar{X})^2 + (\bar{X}_{control} - \bar{X})^2}{m - 1}} = \sqrt{\frac{(86.1 - 84.2)^2 + (82.2 - 84.2)^2}{2 - 1}} = 2.76\ \text{mg/dL}$$

$$s^2_{bet} = ns^2_{\bar{X}} = 25(2.76^2) = 190.13\ (\text{mg/dL})^2$$

Method (cont)

$$F = \frac{s^2_{\text{bet}}}{s^2_{\text{wit}}} = \frac{190.13}{5.28} = 36.01$$

$\nu_n = 2-1 = 1$

$\nu_d = 2(25-1) = 48$

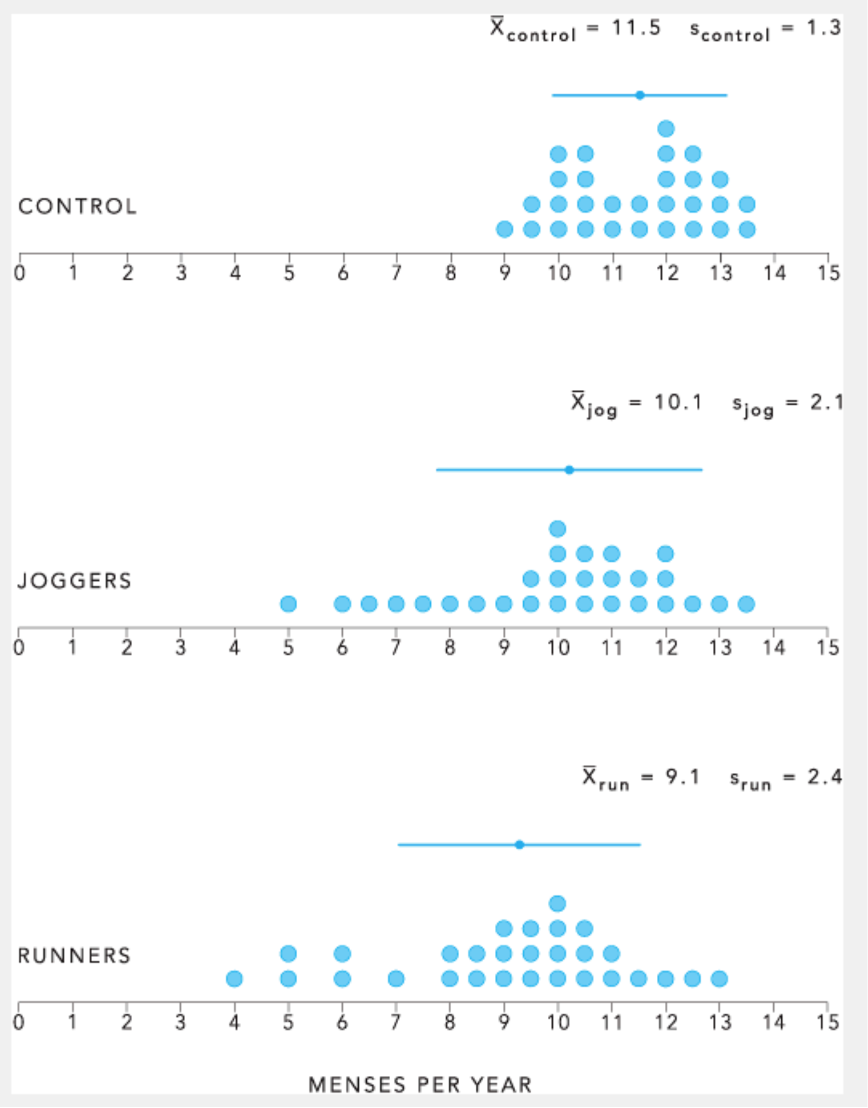
$F > 7.19 \rightarrow p < 0.01$

F associated with our observations is big and we reject the null hypothesis.

So, the fasting glucose levels are higher in the children of diabetics than non-diabetics.

ν_d	1
36	4.11 7.39
38	4.10 7.35
40	4.08 7.31
42	4.07 7.27
44	4.06 7.24
46	4.05 7.21
48	4.04 7.19

Figure 3.9



The number of menstrual cycles in women who are sedentary, joggers or runners

3 groups 26 women in each

Method

$$s^2_{\text{wit}} = \tfrac{1}{3}(s^2_{\text{con}} + s^2_{\text{jog}} + s^2_{\text{run}})$$
$$= \tfrac{1}{3}(1.3^2 + 2.1^2 + 2.4^2) = 3.95\ (\text{menses/year})^2$$

$$\bar{X} = \tfrac{1}{3}(\bar{X}_{\text{con}} + \bar{X}_{\text{jog}} + \bar{X}_{\text{run}})$$
$$= \tfrac{1}{3}(11.5 + 10.1 + 9.1) = 10.2\ \text{menses/year}$$

$$s_{\bar{X}} = \sqrt{\frac{(\bar{X}_{\text{con}} - \bar{X})^2 + (\bar{X}_{\text{jog}} - \bar{X})^2 + (\bar{X}_{\text{run}} - \bar{X})^2}{m-1}}$$
$$= \sqrt{\frac{(11.5 - 10.2)^2 + (10.1 - 10.2)^2 + (9.1 - 10.2)^2}{3-1}}$$
$$= 1.2\ \text{menses/year}$$

$$s^2_{\text{bet}} = ns^2_{\bar{X}} = 26(1.2^2) = 37.44\ (\text{menses/year})^2$$

$$F = \frac{s^2_{\text{bet}}}{s^2_{\text{wit}}} = \frac{37.44}{3.95} = 9.48$$

Method (cont)

- $\nu_n = m-1=3-1=2$
- $\nu_d = m(n-1) = 3(26-1) = 75$

ν_d	1	2
36	4.11 7.39	3.26 5.25
38	4.10 7.35	3.25 5.21
40	4.08 7.31	3.23 5.18
42	4.07 7.27	3.22 5.15
44	4.06 7.24	3.21 5.12
46	4.05 7.21	3.20 5.10
48	4.04 7.19	3.19 5.08
50	4.03 7.17	3.18 5.06
60	4.00 7.08	3.15 4.98
70	3.98 7.01	3.13 4.92
80	3.96 6.96	3.11 4.88

F will exceed 4.90 only 1 percent of the time when all groups are drawn from a single population.

So, we conclude that jogging or running has an effect on the frequency of menstruation.

Summary

- We have developed a statistical method to test the significant differences among three or more different groups of individuals.
- The groups must not overlap (different individuals in each group).
- The group sizes should be same.
- The data should be drawn from normally distributed populations.
- How about which of the groups differed from the others? -- Chapter 4 -- the unpaired t-test

Problem 3.4, Primer of Biostatistics, Glantz (6th Edition)

3-4 If heart muscle is briefly deprived of oxygen—a condition known as ischemia—the muscle stops contracting and, if the ischemia is long enough or severe enough, the muscle dies. When the muscle dies, the person has a myocardial infarction (heart attack). Surprisingly, when the heart muscle is subjected to a brief period of ischemia before a major ischemic episode, the muscle is more able to survive the major ischemic episode. This phenomenon is known as ischemic preconditioning. This protective effect of ischemic preconditioning is known to involve activation of adenosine A1 receptors, which stimulate protein kinase C (PKC), a protein involved in many cellular processes including proliferation, migration, secretion, and cell death. Akihito Tsuchida and colleagues ("α_1-Adrenergic Agonist Precondition Rabbit Ischemic Myocardium Independent of Adenosine by Direct Activation of Protein Kinase C," *Circ. Res.*, 75: 576–585, 1994) hypothesized that α_1-adrenergic receptors might have an independent rule in this process. To address this question, Tsuchida and colleagues subjected isolated rabbit hearts to a brief 5-min ischemia or exposed the hearts to a variety of adenosine and α_1-adrenergic agonists and antagonists. In any case, following a 10-min recovery period, the heart was subject to ischemia for 30 min and the size of the resulting infarct measured. The control group was only subjected to 30 min of ischemia. If each group included 7 rabbit hearts, is there evidence that pretreatment with ischemia or a pharmacological agent affected infarct size, measured as the volume of heart muscle that dies?

	Infarct size, cm^3	
Group	Mean	SEM
Control	0.233	0.024
Ischemic preconditioning (PC)	0.069	0.015
α_1-Adrenergic receptor agonist (Phenylephrine)	0.065	0.008
Adenosine receptor antagonist (8-p-[sulfophenyl] theophylline)	0.240	0.033
α_1-Adrenergic receptor antagonist (Phenoxybenzamine)	0.180	0.033
Protein kinase C inhibitor (Polymyxin B)	0.184	0.038

BAHÇEŞEHİR ÜNİVERSİTESİ

Problem 3.6, Primer of Biostatistics, Glantz (6th Edition)

3-6 Burnout is a term that loosely describes a condition of fatigue, frustration, and anger manifested as a lack of enthusiasm for and feeling of entrapment in one's job. This situation can arise when treating people who have serious diseases. In recent years, AIDS has joined the list of diseases that may have a negative impact on professionals serving people suffering from this disease. To investigate whether there were differences in burnout associated with caring for people who have AIDS compared with other people who have serious diseases, J. López-Castillo and coworkers ("Emotional distress and occupational burnout in health care professionals serving HIV-infected patients: A comparison with oncology and internal medicine services," *Psychother. Psychosom.* 68: 348–356, 1999) administered the Maslach Burnout Inventory questionnaire to health professionals working in four clinical units: infectious disease, hemophilia, oncology, and internal medicine in Spain. (Ninety percent of the people in the infectious disease and 60 percent of the people in the hemophilia unit were HIV-positive.) Are there differences in burnout scores between health professionals working in these different units?

	Infectious Disease	Hemophilia	Oncology	Internal Medicine
Mean	46.1	35.0	44.4	47.9
Standard deviation	16.1	11.1	15.6	18.2
Sample size	25	25	25	25