Recall our study of the effects of obesity and a new drug on the cholesterol levels of 40 subjects. Ten of the 20 non-obese individuals were given a placebo while the other ten were given an experimental new drug. The 20 obese individuals were also equally assigned to the placebo and treatment groups. After 6 months, the cholesterol levels of individuals in each group were measured. Run an AxB ANOVA on the following data (to simplify calculations, only the  $W_1D_2$  group of cholesterol levels has changed):

	D <sub>1</sub> (placebo)	D <sub>2</sub> (drug)	
W₁ (non-obese)	85.08 92.68   90.60 89.86   76.85 77.63   90.24 107.21   91.38 90.24   Mean = 89.2 StDev = 8.5	92.11 85.14 107.13 87.59 91.20 79.83 82.05 103.61 101.25 99.96 Mean = 93.0 StDev = 9.5	Overall Non-Obese 20 subjects Avg. Cholesterol = 91.1 St. Deviation = 9.0
W₂ (obese)	103.13 95.17 116.50 100.63 102.87 115.14 101.10 100.16 109.19 94.27 Mean = 103.8 StDev = 7.6	96.97 105.78 85.51 94.67 90.53 94.39 87.55 106.52 108.24 86.86 Mean = 95.7 StDev = 8.5	Overall Obese 20 subjects Avg. Cholesterol = 99.8 St. Deviation = 8.9
	Overall Placebo 20 subjects Avg. Cholesterol = 96.5 St. Deviation = 10.8	Overall Drug 20 subjects Avg. Cholesterol = 94.3 St. Deviation = 8.9	Overall Study 40 subjects Avg. Cholesterol = 95.4 St. Deviation = 9.9

1) What assumptions must be met before running an AxB ANOVA? Are these assumptions met?

2) Graph the group means, using separate lines for the obese and non-obese groups. Does it appear as though obesity has a significant impact on cholesterol (a significant row effect)? Does it look as though there is a significant column effect (the drug impacts cholesterol)? Does it look as though we have a significant interaction between the drug and obesity treatments?



3) Write out the model for an AxB ANOVA. Select a couple cholesterol measurements and calculate the parameters in the model. What impact does being in the placebo, drug, obese, or non-obese groups have? What is the value of the interaction parameter for each group?

## $x_{ijk} = \mu + \alpha_j + \beta_k + \alpha \beta_{jk} + \varepsilon_{ijk}$

Given	Overall Mean	Alpha	Beta	Interaction	Error
x <sub>ijk</sub>	μ	$\alpha_{j} = (\mu_{Aj} - \mu)$	$\boldsymbol{\beta}_k = (\boldsymbol{\mu}_{Bk} - \boldsymbol{\mu})$	$\alpha\beta_{jk} = (\mu_{AjBk} - \mu_{Aj} - \mu_{Bk} + \mu)$	

4) Let's run the computations on this AxB ANOVA. The SS values have been filled in (*verify these when you have time*). Fill-in the missing degrees of freedom and calculate the MS values. Finally, calculate the three MSRs.

Source	Sums of Squares	Degrees of freedom	Mean Square	Mean Square Ratio
Drug (A)	46.343			
Obesity (B)	752.816			
Interaction (AB)	355.245			
Error	2638.774			
Total	3793.178			

5) Remember that the total variance in an AxB ANOVA is partitioned into four components. Make a diagram of this partitioning (write each SS value next to its place in the diagram). Explain what all of this means.

6) In this type of analysis, our first step is to always check for significant interaction. Find the critical F-value to compare to your MSinteraction. Is the interaction significant? What does this mean? 7) We now know that the efficacy of the drug depends on whether or not an individual is obese. Our original intent was to determine the overall efficacy of the drug. What additional analyses could we conduct to determine the drug's efficacy?

Once we find significant interaction, we should look at the *simple effects* of our variable of interest. To do this, we split our study into two groups (If variable A is important, we split the study into groups B1 and B2; if variable B is important, we split the study into groups A1 and A2).

8) Our variable of interest is "drug," so we need to split our study into two groups: obese and non-obese. The following two tables demonstrate this "split." What kind of analysis can we run on each of these tables?

	D <sub>1</sub> (placebo)	D <sub>2</sub> (drug)	
W₁ (non-obese)	n=10 Mean = 89.2 StDev = 8.5	n=10 Mean = 93.0 StDev = 9.5	Overall Non-Obese 20 subjects Avg. Cholesterol = 91.1 St. Deviation = 9.0

	D <sub>1</sub> (placebo)	D <sub>2</sub> (drug)	
W <sub>2</sub> (obese)	n=10 Mean = 103.8 StDev = 7.6	n=10 Mean = 95.7 StDev = 8.5	Overall Obese 20 subjects Avg. Cholesterol = 99.8 St. Deviation = 8.9

9) If we're now only comparing 2 group means, we can run a standard t-test. We would use the overall MSE as our best estimate of the pooled variance. If we're comparing 2+ group means, we can conduct a one-way ANOVA on our split data. In this ANOVA, we would use the MSE from the original analysis. Use the formulas for this type of analysis to verify the following calculations. Use the summary tables to draw conclusions from your study.

$$t_{df_{E}} = \frac{\bar{X}_{1} - \bar{X}_{2}}{\sqrt{MS_{E}}\sqrt{\frac{1}{n_{1}} + \frac{1}{n_{2}}}}$$

Source	Sums of Squares	Degrees of freedom	Mean Square	Mean Square Ratio
SSA Treatment/Factor (Between)	$SS_A = \sum n_a \left(\overline{X}_a - M\right)^2$	a-1	$\frac{SS_A}{df_A}$	
	SSE from ANOVA	df <sub>E</sub> from ANOVA		
SSE Error (Within)	or $\sum_{n=1}^{n} (1 - 1) r^2$	or	$\frac{SS_E}{df_E}$	
	$\sum_{i=1}^{n} (n_a - 1)s_a$	N-a		

## 10) Let's run through the calculations for our example:

	D <sub>1</sub> (placebo)	D <sub>2</sub> (drug)	
W₁ (non-obese)	<b>85.08</b> 92.68 90.60 89.86 76.85 77.63 90.24 107.21 91.39 90.24 Mean = 89.2	92.11 85.14 107.13 87.59 91.20 79.83 82.05 103.61 101.25 99.96 Mean = 93.0	<u>Overall Non-Obese</u> 20 subjects Avg. Cholesterol = 91.1 St. Deviation = 9.0
	StDev = 8.5	StDev = 9.5	
W <sub>2</sub> (obese)	103.13 95.17 116.50 100.63 102.87 115.14 101.10 100.16 109.20 94.27 Mean = 103.8	96.97 105.78 85.51 94.67 90.53 94.39 87.55 106.52 108.24 86.86 Mean = 95.7	Overall Obese 20 subjects Avg. Cholesterol = 99.8 St. Deviation = 8.9
	Overall Placebo	Overall Drug	Overall Study
	20 subjects	20 subjects	40 subjects
	Avg. Cholesterol = 96.5 St. Deviation = 10.8	Avg. Cholesterol = 94.3 St. Deviation = 8.9	Avg. Cholesterol = 95.4 St. Deviation = 9.9

Source	Sums of Squares	Degrees of freedom	Mean Square	Mean Square Ratio
Drug (A)	46.343	1	46.343	0.632
Obesity (B)	752.816	1	752.816	10.27
Interaction (AB)	355.245	1	355.245	4.85
Error	2638.774	36	73.299	
Total	3793.178	39		

Non-Obese Analysis

$$t_{36} = \frac{(103.8 - 95.7)}{\sqrt{73.3}\sqrt{\frac{1}{10} + \frac{1}{10}}} = \frac{8.1}{3.829} = 2.11 \sim t_{36}^{\alpha = 0.05}(1.688)$$

If we can assume equal variances, we can use SSE and MSE from the AxB ANOVA:

$$SS_A = 10(103.8 - 99.8)^2 + 10(95.7 - 99.8)^2 = 328.1$$

Source	Sums of Squares	Degrees of freedom	Mean Square	Mean Square Ratio
Drug	328.1	1	328.1	4.476
Error	2638.774	36	73.3	
Total	N/A	N/A		

If we cannot assume equal variances, we can calculate SSE with our data:

$$SS_E = (10 - 1)(7.6^2) + (10 - 1)(8.5^2) = 1170.09$$

Source	Sums of Squares	Degrees of freedom	Mean Square	Mean Square Ratio
Drug	328.1	1	328.1	5.05
Error	1170.09	18	65.0	
Total	N/A	19		

11) Write out a paragraph explaining the results of this study.