The effect of vitamin C on tooth growth in guinea pigs

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Introduction

In this assignment we'll use **R** to explore, study, and draw conclusions about the effect of vtamin C on tooth growth in guinea pigs.

The data used is the **ToothGrowth** dataset from R's `datasets` package, and contains observations on odontoblast length in 60 guinea pigs, each of which received one of three dosages of vitamin C (0.5, 1, and 2 mg/day) via one of two delivery methods (orange juice or ascorbic acid).

The data comes from a 1947 study by Crampton, E.W.

Exploratory Data Analysis

The 60 guinea pigs comprising the *Crampton* study were divided into equal groups, such that 30 received vitamin C and 30 received ascorbic acid. Both the vitamin C and ascorbic acid groups were further split into equal groups of ten guinea pigs each, receiving dosages of 0.5, 1, and 2 mg/day respectively via their parent group's delivery method.

To visually explore the sample data from each of the six groups of 10 guinea pigs, we use a boxplot. The boxplot shows quartiles and outliers (indicated by solid dots). Additionally, we've drawn a dashed line connecting the mean of each group.
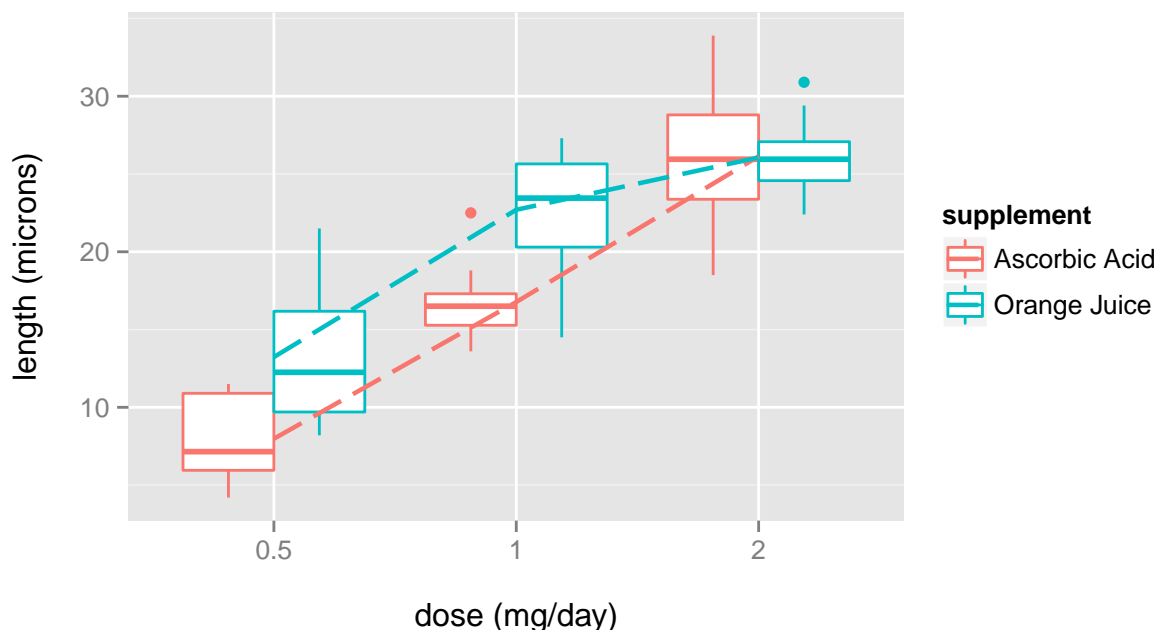
A few things are apparent about the *sample* data; odontoblast length were on average higher in groups with higher dosage, and at `0.5 mg/day` and `1.0 mg/day` dosage groups, supplement means were quite distinct. However, in order to make inferences about *population* means, we turn to hypothesis testing.

Hypothesis testing

We will use hypothesis testing to quantitatively evaluate whether a particular statement about the unknown population means is *likely*. We begin by constructing two hypotheses, the **null hypothesis** and the **alternate hypothesis**. For two groups X and Y with unknown population means μ_X and μ_Y respectively, we construct:

1. **Null hypothesis H0**: $\mu_X - \mu_Y = 0$
2. **Alternate hypothesis Ha**: $\mu_X - \mu_Y \neq 0$

In order to compare X and Y, we must select a suitable two-sample test given the parameters of our experiment, and evaluate the null hypothesis using it.

Assumptions

We make the following basic assumptions:

1. X and Y are i.i.d normal random variables with mean and finite variance.
2. X and Y are independent of each other.
3. $\bar{X}$ and $\bar{Y}$ follow a known distribution.

Because there are 60 distinct guinea pigs in the data (a point once ambiguous in the **R** documentation, but updated as of R 3.2.2), the X and Y populations for no two groups overlap, and we do not have a paired experiment.

For small sample sizes (in our case 10 guinea pigs in each group) it is more robust to assume that the sample means follow a t distribution. For larger samples, the sample mean may instead be treated as normal, owing to the Central Limit Theorem.

The box plot has revealed significant differences in sample variance between the two groups (this is demonstrated explicitly in the Appendix). Because we cannot assume equal variances, we select **Welch's t test** which only requires independence and normality of the populations.

Welch's t test

Welch's t test is a two-sample statistical test evaluating whether the population means of two populations is equal or distinct. It was introduced in the lectures and will be evaluated using the `t.test` function in **R**. A brief summary of test output follows.

Confidence interval

Welch's t test assigns a confidence interval for the difference in population means $\mu_X - \mu_Y$ in terms of known or calculable sample statistics. For a chosen $(1 - \alpha) \cdot 100\%$ confidence level (usually 95%), it is given by:

$$\bar{X} - \bar{Y} \pm t_{\alpha/2, df} \sqrt{\frac{s_X^2}{n_X} + \frac{s_Y^2}{n_Y}}$$

where t is the critical t value (calculable via `qt()`) and the degrees of freedom df is given by:

$$df = \frac{(\frac{s_X^2}{n_X} + \frac{s_Y^2}{n_Y})^2}{\frac{(s_X^2/n_X)^2}{n_X-1} + \frac{(s_Y^2/n_Y)^2}{n_Y-1}}$$

If we chose $\alpha = 0.5$, the resulting 95% confidence interval expresses the idea that 95% of samples will yield a confidence interval that contains the true statistic $\mu_X - \mu_Y$. Therefore, it's unlikely that our observed sample has produced a calculated confidence interval that does *not* include $\mu_X - \mu_Y$, and more simply, we may say there is 95% confidence that the true statistic falls within the interval observed.

The immediate consequences of the 95% confidence interval are:

1. If the interval contains zero, then we cannot reject the **H0** with 95% confidence.

2. If the interval does not contain zero, then we reject **H0** in favor of **Ha** with 95% confidence.

    a. If the interval is strictly positive, then we express confidence in $\mu_X > \mu_Y$.

    b. If the interval is strictly negative, then we express confidence in $\mu_Y > \mu_X$.

The p value

The p value is the result of the statistical test which we select, and quantifies the success or failure of the null hypothesis **H0**. It is the probability that the null hypothesis version of population parameters could have yielded the observed sample statistic.

If the p value is very small, then the evidence against the null hypothesis is considered to be great. In other words, it is considered unlikely to have observed so extreme a result as we have in the sample. Usually, for $p \leq 0.5$, the null hypothesis is rejected, and for $p > 0.5$ the null hypothesis cannot be rejected.

Differences in dosage

Taking X to be the higher dosage group and Y to be the lower dosage group, we evaluate for each supplement:

1. **H0**: $\mu_X - \mu_Y = 0$
2. **Ha**: $\mu_X - \mu_Y \neq 0$

Orange Juice

```
welch.test(length ~ dose,
    data = tg[(dose == 0.5 | dose == 1.0) & (supplement == "Orange Juice")])
```

```
## 95% confidence interval:  -13.4156, -5.52437
## p value:  8.78492e-05
```

```
welch.test(length ~ dose,
    data = tg[(dose == 1.0 | dose == 2.0) & (supplement == "Orange Juice")])
```

```
## 95% confidence interval:  -6.53144, -0.188557
## p value:  0.0391951
```

```
welch.test(length ~ dose,
  data = tg[(dose == 0.5 | dose == 2.0) & (supplement == "Orange Juice")])
```

```
## 95% confidence interval:  -16.3352, -9.32476
## p value:  1.32378e-06
```

There is a clear and strong support for greater odontoblast lengths in populations with higher dosages, in contradiction to the null hypothesis.

Ascorbic Acid

```
welch.test(length ~ dose,
  data = tg[(dose == 0.5 | dose == 1.0) & (supplement == "Ascorbic Acid")])
```

```
## 95% confidence interval:  -11.2657, -6.31429
## p value:  6.81102e-07
```

```
welch.test(length ~ dose,
  data = tg[(dose == 1.0 | dose == 2.0) & (supplement == "Ascorbic Acid")])
```

```
## 95% confidence interval:  -13.0543, -5.68573
## p value:  9.1556e-05
```

```
welch.test(length ~ dose,
  data = tg[(dose == 0.5 | dose == 2.0) & (supplement == "Ascorbic Acid")])
```

```
## 95% confidence interval:  -21.9015, -14.4185
## p value:  4.68158e-08
```

There is a clear and strong support for greater odontoblast lengths in populations with higher dosages, in contradiction to the null hypothesis.

Differences in supplement

Taking X to be the Ascorbic Acid group and Y to be the Orange Juice group, we evaluate for each dosage level:

1. **H0**: $\mu_X - \mu_Y = 0$
2. **Ha**: $\mu_X - \mu_Y \neq 0$

```
welch.test(length ~ supplement, data = tg[dose == 0.5])
```

```
## 95% confidence interval:  -8.78094, -1.71906
## p value:  0.00635861
```

```
welch.test(length ~ supplement, data = tg[dose == 1.0])
```

```
## 95% confidence interval:  -9.05785, -2.80215
## p value:  0.00103838
```

```
welch.test(length ~ supplement, data = tg[dose == 2.0])
```

```
## 95% confidence interval:  -3.63807, 3.79807
## p value:  0.963852
```

At 0.5 and 1.0 mg/day dosage, the low p value suggests that the null hypothesis is considered very unlikely to be true. The 95% confidence interval expresses high confidence that Ascorbic Acid populations have lower odontoblast length than Orange Juice populations.

At 2.0 mg/day dosage, the remarkably high p value $(1 - p \leq 0.05)$ expresses high confidence in the null hypothesis. We must consider it likely for the two population means to be equivalent.

Appendix

```
tg[, var(length), by = .(supplement, dose)]
```

```
##        supplement dose        V1
## 1: Ascorbic Acid  0.5  7.544000
## 2: Ascorbic Acid  1.0  6.326778
## 3: Ascorbic Acid  2.0 23.018222
## 4:  Orange Juice  0.5 19.889000
## 5:  Orange Juice  1.0 15.295556
## 6:  Orange Juice  2.0  7.049333
```