

An Analysis of the Effect of Vitamin C on Tooth Growth in Guinea Pigs

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Synopsis

Our analysis examines whether dose (0.5 mg/day, 1 mg/day, 2 mg/day) or delivery method (orange juice vs. ascorbic acid) has an effect on the tooth growth in guinea pigs. We examine 6 individual groups of guinea pigs, each with a different combination of dosage and delivery method, and determine if there are any significant differences between these groups. We find that there is fairly strong evidence that increasing dosage changes tooth growth, but that there is less evidence that delivery method has a impact.

Preliminaries

This section prepares the analysis to run. For more information on replicating this analysis, see the appendix.

```
library(ggplot2); library(plyr);  
data(ToothGrowth);  
options(digits=5);
```

Exploratory Analysis

Summary of Data

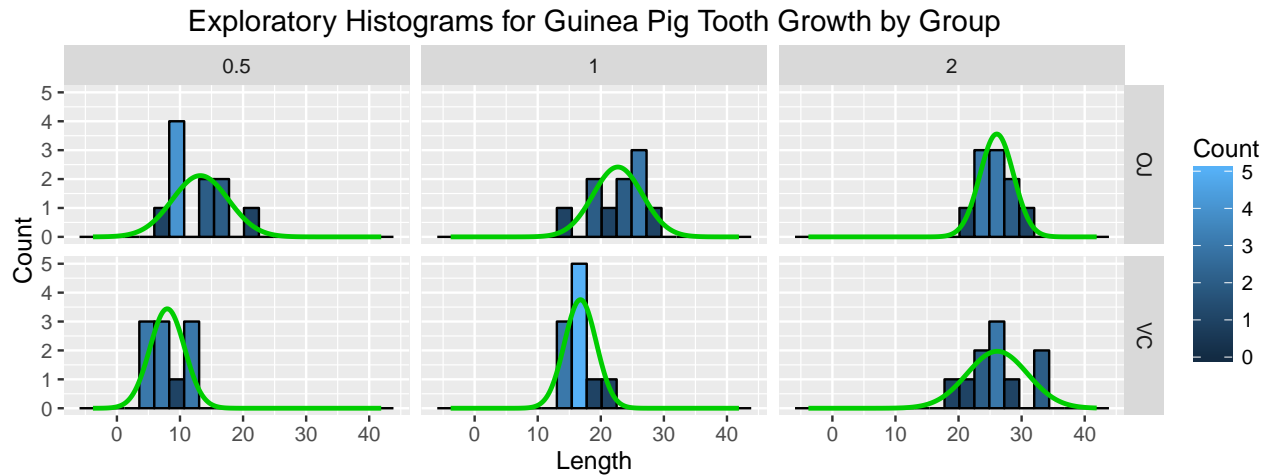
This analysis uses the `ToothGrowth` data set included natively with most R distributions. As described in the R help document, each of 60 guinea pigs was given a dose of 0.5, 1, or 2 mg/day of vitamin C by one of two delivery methods, orange juice (coded OJ) or ascorbic acid (coded VC). The length of their odontoblasts were then measured. The structure of the data set is:

```
str(ToothGrowth)  
  
## 'data.frame':    60 obs. of  3 variables:  
##  $ len : num  4.2 11.5 7.3 5.8 6.4 10 11.2 11.2 5.2 7 ...  
##  $ supp: Factor w/ 2 levels "OJ","VC": 2 2 2 2 2 2 2 2 2 2 ...  
##  $ dose: num  0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 ...
```

Exploratory Graphs

We are interested in seeing if there is a significant difference between the doses, and between the delivery methods. Unfortunately, because of potential correlations between dose and delivery method, we cannot simply compare aggregates, so we will look at all 6 groups individually. Below are histograms showing the distribution of all 60 samples in each of the 6 groups individually, each with a Gaussian curve fit to show how well the sample approximates a normal distribution.

```
# We want to fit normals to the graphs here.
ToothGrowthNorms =
  ddpby( ToothGrowth, .(supp, dose), function(df) {
    lenSeq=seq(min(ToothGrowth$len)-8, max(ToothGrowth$len)+8, length=200 );
    data.frame(len=lenSeq, count=2.37*10*dnorm(lenSeq, mean(df$len), sd(df$len)));
  });
ToothGrowth.histogram =
  ggplot( ToothGrowth, aes( len ) , show.legend = TRUE ) +
  geom_histogram( binwidth=2.37, col = "black", aes( fill = ..count.. ) ) +
  xlab( "Length" ) + ylab("Count") +
  ggtitle( "Exploratory Histograms for Guinea Pig Tooth Growth by Group" ) +
  facet_grid( supp ~ dose ) +
  scale_fill_continuous( name = "Count" ) +
  geom_line(aes(y = count), data=ToothGrowthNorms, color="green3", size=1);
print( ToothGrowth.histogram );
```



It is difficult to tell from these plots if every group is normal. However, since most of them appear to be at least approximately normal, for this analysis, we will assume that they follow the Student's t distribution. This is appropriate both because of the small sample sizes ($n = 10$) and because we do not know the actual population standard deviation.

Hypothesis Testing

We are interested in seeing if there is a difference between individual groups. Our null hypothesis, H_0 , is that there is no difference, and the alternative hypothesis, H_a , is that the dose or delivery method makes a difference. Because of the potential for correlation between doses and delivery methods, we will be looking for significant differences between any two groups, which is $\eta = 15$ hypothesis tests. To control for the number of tests performed, we will use the *Bonferroni correction*. Our $\alpha = 0.05$ for each test individually. With the correction, however, we will be using an effective $\alpha_c = \frac{\alpha}{\eta} = 0.00333$ for each hypothesis test. We will be using a two-sided Student's t test to generate p-values, and we will not treat the variance between groups as equal.

The figure below summarizes the results of the hypothesis testing. Note that p-values that reject the null hypothesis are highlighted in green. (See the Appendix for the full code to generate this figure.)

| Summary of P-Values Calculated Across Groups | | | | | |
|----------------------------------------------|--------------------------|--------------------------|---------------------------|--------------------------|-------------------------|
| Dose/Mthd | 0.5/OJ | 1/OJ | 2/OJ | 0.5/VC | 1/VC |
| 1/OJ | 8.78492×10^{-5} | — | — | — | — |
| 2/OJ | 1.32378×10^{-6} | 0.0392 | — | — | — |
| 0.5/VC | 0.00636 | 3.65521×10^{-8} | 1.36214×10^{-11} | — | — |
| 1/VC | 0.04601 | 0.00104 | 2.36107×10^{-7} | 6.81102×10^{-7} | — |
| 2/VC | 7.19625×10^{-6} | 0.09653 | 0.96385 | 4.68158×10^{-8} | 9.1556×10^{-5} |

Conclusions

There are three sets of p-values that are of clear interest. The first is the increase in dosage in orange juice, and the second is the increase in dosage in ascorbic acid. Examining these p-values, there appears to be a significant effect caused by increasing dosage. The only p-value in these two sets which fails to reject H_0 is between 1/OJ and 2/OJ. In addition, though there may be cross correlations that muddy the data, increase in dosage across delivery methods also appears to generally support the hypothesis that differing doses has a significant impact on tooth growth.

The third set of p-values that interest us are the ones where dosage is held constant, and only the delivery method changes. In this case, there appears to be less evidence of a significant relationship. Only 1/OJ vs. 1/VC rejected H_0 , while 0.5/VC vs. 0.5/OJ and 2/VC vs 2/OJ did not. Still, we conclude that there is likely some effect based on delivery method, though we would probably urge additional testing to be certain.

These conclusions hold only if the underlying samples were normally distributed within their own groups.

Appendix

System Information

This analysis was performed using the hardware and software specified in this section.

```
sessionInfo();

## R version 3.2.4 Revised (2016-03-16 r70336)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 15.10
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
## [1] plyr_1.8.3      ggplot2_2.1.0   rmarkdown_0.9.5
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.4      digest_0.6.9    grid_3.2.4      gtable_0.2.0
##  [5] formatR_1.3      magrittr_1.5     evaluate_0.8.3   scales_0.4.0
##  [9] stringi_1.0-1    reshape2_1.4.1  labeling_0.3     tools_3.2.4
## [13] stringr_1.0.0    munsell_0.4.3   yaml_2.1.13     colorspace_1.2-6
## [17] htmltools_0.3.5  knitr_1.12.3
```

Code to Generate the P-Value Summary Table

```
`r TG = ToothGrowth; TG$group = with(TG, interaction( dose, supp ) );`

\begin{center}
\bgroup
\def\arraystretch{1.5}
\begin{tabular}{|r|c|c|c|c|c|}
\hline
\multicolumn{6}{|c|}{Summary of P-Values Calculated Across Groups} \\
\hline
\hline
Dose/Mthd & 0.5/OJ & 1/OJ & 2/OJ & 0.5/VC & 1/VC \\
\hline
1/OJ
& \cellcolor{green} $`r t.test(TG[TG$group=="0.5.OJ"],"len"),TG[TG$group=="1.OJ"],"len",
alternative="two.sided")$p.value`$
& --- & & --- & & --- & & --- \\
\hline
\end{tabular}
\end{center}
```

```

2/OJ
& \cellcolor{green} $\text{r t.test(TG[TG$group=="0.5.OJ","len"],TG[TG$group=="2.OJ","len"],
alternative="two.sided")$p.value}$
& $\text{r t.test(TG[TG$group=="1.OJ","len"],TG[TG$group=="2.OJ","len"],
alternative="two.sided")$p.value}$
& --- & --- & --- \\
\hline
0.5/VC
& $\text{r t.test(TG[TG$group=="0.5.OJ","len"],TG[TG$group=="0.5.VC","len"],
alternative="two.sided")$p.value}$
& \cellcolor{green} $\text{r t.test(TG[TG$group=="1.OJ","len"],TG[TG$group=="0.5.VC","len"],
alternative="two.sided")$p.value}$
& \cellcolor{green} $\text{r t.test(TG[TG$group=="2.OJ","len"],TG[TG$group=="0.5.VC","len"],
alternative="two.sided")$p.value}$
& --- & --- \\
\hline
1/VC
& $\text{r t.test(TG[TG$group=="0.5.OJ","len"],TG[TG$group=="1.VC","len"],
alternative="two.sided")$p.value}$
& \cellcolor{green} $\text{r t.test(TG[TG$group=="1.OJ","len"],TG[TG$group=="1.VC","len"],
alternative="two.sided")$p.value}$
& \cellcolor{green} $\text{r t.test(TG[TG$group=="2.OJ","len"],TG[TG$group=="1.VC","len"],
alternative="two.sided")$p.value}$
& \cellcolor{green} $\text{r t.test(TG[TG$group=="0.5.VC","len"],TG[TG$group=="1.VC","len"],
alternative="two.sided")$p.value}$
& --- \\
\hline
2/VC
& \cellcolor{green} $\text{r t.test(TG[TG$group=="0.5.OJ","len"],TG[TG$group=="2.VC","len"],
alternative="two.sided")$p.value}$
& $\text{r t.test(TG[TG$group=="1.OJ","len"],TG[TG$group=="2.VC","len"],
alternative="two.sided")$p.value}$
& $\text{r t.test(TG[TG$group=="2.OJ","len"],TG[TG$group=="2.VC","len"],
alternative="two.sided")$p.value}$
& \cellcolor{green} $\text{r t.test(TG[TG$group=="0.5.VC","len"],TG[TG$group=="2.VC","len"],
alternative="two.sided")$p.value}$
& \cellcolor{green} $\text{r t.test(TG[TG$group=="1.VC","len"],TG[TG$group=="2.VC","len"],
alternative="two.sided")$p.value}$ \\
\hline
\end{tabular}
\egroup
\end{center}

```