Response of Guinea Pig Incisor Teeth Odontoblasts to Vitamin C Dose and Delivery Method

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Introduction

Statistical inference on whether variations of guinea pig tooth length were related to the dose and form of vitamin C supplmentation in their diets is reported here.

Data

Data are taken from a monograph by C.I. Bliss[1] but were originally reported by E.W. Crampton.[2] A total of 60 guinea pigs were randomly assigned to treatment groups of 10 that were fed a basal diet and supplemental vitamin C as either orange juice or pure ascorbic acid at one of three dose levels (0.5, 1.0, and 2.0 mg per day). Measurements on the incisor teeth of each guinea pig were made after 42 days of feeding. The response is the length of odontoblast[3] cells observed under a microscope (at 440x magnification).

[1] C.I. Bliss. The Statistics of Bioassay. Academic Press, New York, 1952.

[2] E.W. Crampton. The Growth of the Odontoblasts of the Incisor Tooth as a Criterion of the Vitamin C Intake of the Guinea Pig. *J. Nutrition* **33** 491-504 (1947).

[3] Odontoblasts are columnar cells lying at the interface of a tooth's inner enamel surface with the dental pulp. Their function is associated with the production of dentine.

In the data file there are 60 observations of 3 variables

- `len` odontoblast length, a numeric value
- `supp` vitamin C delivery method, a 2-level factor with values ascorbic acid (`VC`) or orange juice (`OJ`)
- `dose` vitamin C dose in mg per day with values 0.5, 1.0, and 2.0

Exploratory Data Analysis

A basic summary of the `ToothGrowth` data is included in Table 1 and, in Figure 1, a scatterplot shows the variation of odontoblast length with vitamin C dose. In the plot, delivery method is indicated by the color of the points. The mean odontoblast lengths from Table 1 are included next to the relevant data in Figure 1.

supplement	dose	mean	variance
ascorbic acid	0.50	13.23	19.89
	1.00	22.70	15.30
	2.00	26.06	7.05
orange juice	0.50	7.98	7.54
	1.00	16.77	6.33
	2.00	26.14	23.02

Table 1: Summary of `ToothGrowth` data

The summarized data and plot suggest that odontoblast length increases with vitamin C dose. The two delivery methods give different response levels as odontoblasts in guinea pigs that were given orange juice (red points) appear to be longer than those given an equivalent dose of ascorbic acid (blue points). An exception to this might be for the 2.0 mg per day dose, for which the mean odontoblast length is similar for vitamin C delivered as orange juice (26.06) and ascorbic acid (26.14). Related to this, for orange juice the increase in odontoblast length between 1.0 mg per day (22.70) and 2.0 mg per day (26.06) may not be large enough to be significant.

Density curves are included in the right margin of the scatterplot. These approximate the distributions of odontoblast lengths with a moving average of frequency. The implications of the shapes of these curves for assumptions in hypothesis testing will be discussed below.

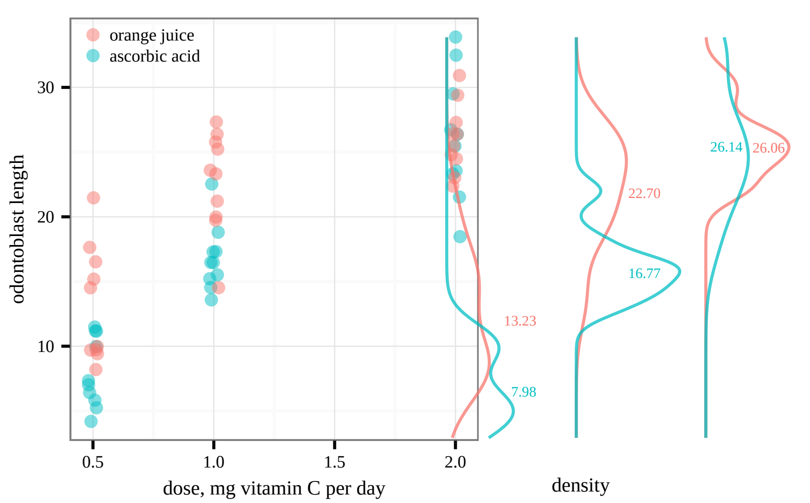

Figure 1: Scatterplot (with jitter added) of guinea pig incisor odontoblast length vs vitamin C dose delivered as either pure ascorbic acid or orange juice. Marginal density plots show the distribution of osteoblast lengths for each treatment.

Hypothesis Tests

Student's t-tests were used to test multiple hypotheses of whether tooth lengths for two treatments were significantly different. For each t-test, it is assumed that the data are sampled from independent and identically distributed populations. The density curves in Figure 1 address the validity of the assumption. Although curves are not exactly those of a normal distribution, they are consistent with what might be observed for relatively small samples ($n = 10$) from a normal distribution. Based on the variances in Table 1 and the width of the density curves in Figure 1, it did not seem appropriate to assume that the populations have equal variance. Testing multiple hypotheses can introduce errors. Using $\alpha = 0.05$ means we expect that in 1 out of every 20 tests, the null hypothesis would incorrectly be rejected (type 1 error). To control this type of error, adjusted α values were used. The adjustment is based on the rank of the calculated p-value. For i^{th} lowest p-value of the 9 tests, α_i is taken as its rank multiplied by 0.5/9 (approximately 0.0056). If p_i is less than α_i then the null hypothesis is rejected. The hypothesis tests with p_i and α_i are reported in Table 2.

	dose		Δ_{mean} 2.5%	50%	97.5%	p	α_i
ascorbic acid	0.5	1.0	-11.27	-8.79	-6.31	0.0000	0.0111
	1.0	2.0	-13.05	-9.37	-5.69	0.0001	0.0278
	2.0	0.5	14.42	18.16	21.90	0.0000	0.0056
orange juice	0.5	1.0	-13.42	-9.47	-5.52	0.0001	0.0222
	1.0	2.0	-6.53	-3.36	-0.19	0.0392	0.0444
	2.0	0.5	9.32	12.83	16.34	0.0000	0.0167
ascorbic acid	0.5	0.5	-8.78	-5.25	-1.72	0.0064	0.0389
vs	1.0	1.0	-9.06	-5.93	-2.80	0.0010	0.0333
orange juice	2.0	2.0	-3.64	0.08	3.80	0.9639	0.0500

Table 2: Hypothesis tests on `ToothGrowth` data set. Different doses (in mg per day) are compared for each delivery method. Then the two delivery methods are compared for each dose. Unadjusted p values and an α value adjusted by the p value rank are reported for each test.

Conclusions

Based on the assumptions described in the *Hypothesis Tests* section and using corrections for testing multiple hypotheses, the following conclusions can be made. The null hypotheses should be rejected for all tests apart from comparing 2 mg/day ascorbic acid to 2 mg/day orange juice. This means that

(i) Odontoblasts lengths differ significantly between any pair of doses as ascorbic acid, with the length increasing as dose increases

(ii) Odontoblasts lengths differ significantly between any pair of doses orange juice, with the length increasing as dose increases

(iii) Odontoblasts lengths differ significantly between ascorbic acid and orange juice at 0.5 mg/day, with the length being greater when delivery is by orange juice

(iv) Odontoblasts lengths differ significantly between ascorbic acid and orange juice at 1.0 mg/day, with the length being greater when delivery is by orange juice

Appendix

```r
library(ggplot2)
library(ggthemes)
library(ggExtra)
library(grid)
library(gridExtra)
library(htmlTable)

# names(meansTooth) <- oldNames

jitterTooth <- ggplot(data = ToothGrowth, aes(x = dose,
    y = len, color = factor(supp, labels = c("orange juice",
        "ascorbic acid")))) + theme_bw() + theme(text = element_text(size = 7,
    family = "serif"), legend.title = element_blank(),
    legend.key = element_blank(), legend.key.height = unit(0.5,
        "line"), legend.key.width = unit(0.5,
        "line"), legend.background = element_blank(),
    legend.position = c(0.175, 0.95)) + geom_jitter(shape = 19,
    alpha = 0.5, position = position_jitter(width = 0.05)) +
    labs(x = "dose, mg vitamin C per day", y = "odontoblast length")

densityTooth <- ggplot() + facet_grid(~dose) +
    theme(text = element_text(size = 7, family = "serif"),
        legend.position = "none", panel.background = element_blank(),
        panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        axis.text = element_blank(), axis.ticks = element_blank(),
        axis.title.x = element_text(hjust = 0.35),
        axis.title.y = element_blank(), strip.text.x = element_blank(),
        strip.background = element_blank(), plot.margin = unit(c(0.4,
            0, 0.5, -1.5), "lines")) + geom_line(data = ToothGrowth,
    aes(x = len, color = factor(supp)), alpha = 0.75,
    stat = "density") + geom_text(data = edaTooth,
    aes(x = mLen, y = c(0.17, 0.17, 0.16, 0.16,
        0.15, 0.07), label = format(mLen, digits = 4),
        color = factor(supp)), size = 1.75, family = "serif",
    hjust = 1, vjust = 1) + coord_flip()

grid.arrange(jitterTooth, densityTooth, ncol = 2,
    widths = c(4, 2.5))

dfH <- data.frame(supp1 = c(rep("VC", 3), rep("OJ",
    3), rep("VC", 3)), dose1 = rep(c(0.5, 1, 2),
    3), supp2 = c(rep("VC", 3), rep("OJ", 6)),
    dose2 = c(rep(c(1, 2, 0.5), 2), 0.5, 1, 2))
```

```
for (i in 1:9) {
    outTests <- t.test(ToothGrowth[ToothGrowth$supp ==
        dfH[i, 1] & ToothGrowth$dose == dfH[i,
        2], 1], ToothGrowth[ToothGrowth$supp ==
        dfH[i, 3] & ToothGrowth$dose == dfH[i,
        4], 1], paired = FALSE, var.equal = FALSE)
    dfH$diffM[i] <- outTests$estimate[1] - outTests$estimate[2]
    dfH$lowCI[i] <- outTests$conf.int[1]
    dfH$upCI[i] <- outTests$conf.int[2]
    dfH$p[i] <- outTests$p.value
}
dfH$rankP <- rank(dfH$p)
dfH$iAlpha <- dfH$rankP * 0.05/9
dfH <- cbind(dfH, c("ascorbic acid", "", "", "orange juice",
    "", "", "ascorbic acid", "vs", "orange juice"))
names(dfH) <- c("supp1", " ", "supp2", "  ", "$\\Delta_{mean}$",
    "   ", "    ", "     ", "rank", "      ",
    "")


xH <- xtable(dfH[, c(11, 2, 4, 6, 5, 7:8, 10)],
    digits = c(0, 0, 1, 1, 2, 2, 2, 4, 4), align = c(rep("l",
        2), rep("r", 5), rep("c", 2)), caption = "Hypothesis tests on \\texttt{ToothGrowth} data set. Diff
addtorow <- list()
addtorow$pos <- list(0)
addtorow$command <- "&  \\multicolumn{2}{c}{dose} &{2.5\\%} &{50\\%} &{97.5\\%} &{$p$}&{$\\alpha_i$}\\\\\n

print(xH, include.rownames = FALSE, comment = FALSE,
    add.to.row = addtorow, sanitize.text.function = function(x) {
        x
    })
```