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Making Data Manageable

Fold changes, ratios of means and not the mean ratio

OFTEN IN TESTING groups or treatments, you need to know what has changed and how it has changed during the test or experiment. In comparing an outcome of two conditions, *X* and *Y*, you can look at the difference in the outcomes in several ways. The simplest is the difference (*X* - *Y*) of the outcomes.

This difference, however, is difficult to compare across multiple repeated measures because it can be biased by differences in technique, baseline values or controls. One way to overcome this is to look at the change between *X* and *Y* as a ratio or percentage, or the fold change. A widely accepted calculation in biology, and in particular in microarray analyses[1] and gene expression, it is also useful in assessing the magnitude of changes over time in other research areas.

A fold change is the ratio of the final to the initial value or, when comparing two treatments, the ratio of the outcome of treatment *X* to the outcome of treatment *Y*. By definition, an increase from 100 customers per day to 200 customers per day would be a relative increase of 100% ((200 - 100)/100), or a two-fold change (200/100). Fold change =

$$\frac{\text{Final value or outcome in condition } X}{\text{Initial value or outcome in condition } Y.}$$

The outcome used for fold change is usually expressed as a summary statistic (mean or median) for a group of experiments. There continues to be debate about what to do when the ratio is between 0 and 1, meaning there's a decrease.

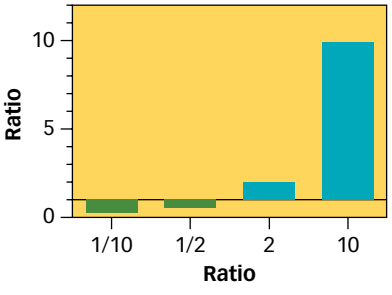
Now, as it's relatively standard in biology, these values are given as the negative reciprocal of the ratio.[2] A change from 100 to 25, for example, would be a fold change of 0.25, while a change from 25 to 100 would be a fold change of 4. Instead of 0.25, this is often expressed as a fold change of -4 (or -1*(1.0/0.25)).

An advantage of the fold change, aside from its simple calculation, is the ease of interpretability because it is unitless and maintains its relative difference when transformed. Graphing the fold change also is made easier by transforming the data into log base 2.

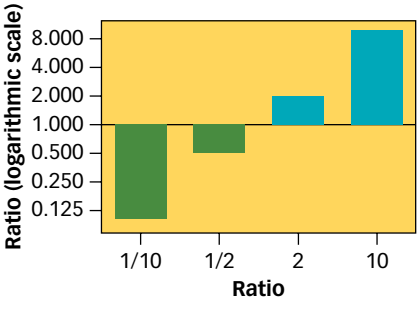
As illustrated using GraphPad (www.graphpad.com), simply plotting the untransformed calculated fold change will not show the equivalence (in loss vs. gain) between a ratio of 0.1 and a ratio of 10, while the log base 2 transformed graph maintains the equivalence. See Figures 1 and 2. Another way to preserve the ratio equivalence is to use the negative reciprocal (in which (-1*(1/0.1)) = -10) in charts.

One shortcoming in using a single fold change as an outcome is that it does not incorporate any measures of variability that might be needed for statistical comparisons. Also, being a relative term, there are no strict guidelines for importance for the magnitude of the change. Its importance is determined specifically by its interpretation in the analysis.

Untransformed fold change / FIGURE 1



Log base 2 transformed fold change / FIGURE 2



Ratio of means vs. mean of ratios

The fold change is a ratio of two means or medians and is not identical to the mean of a group of ratios of values within or between experiments. These measures can have very different interpretations. The fold change is based on averaging the individual numerator and denomina-

tor and calculating the fold change, while the mean ratio would be the average of a group of individual ratios. Both are useful in analyses.

Suppose you are repeating an experiment looking at customer levels after two promotional periods, T_A and T_B, five times. There are two possible interpretations of the results you might make:

1. If you are interested in the overall fold change for the customers and treating the five experiments as replicates and averaging the results for each treatment, then compute the ratio:

Fold change = (Average(T_{A1}, …T_{A5})) / (Average(T_{B1}, …T_{B5}))

2. If you are interested in the fold change for each individual experiment and average (and variability) over all of these experiments, you would compute the mean of the ratios:

Mean of ratios = Σ [(T_{A1}/ T_{B1}) + … + (T_{A5}/ T_{B5})] /5.

The results may be quite different in each case. For example, suppose the results of your experiments are as follows: T_A = [50, 25, 20, 25, 100] and T_B = [10, 25, 20, 10, 20]. Your resulting ratios would be:

Fold change = ((50 + 25 + 20 + 25 + 100)/5)/(10 + 25 + 20 + 10 + 20)/5) = 44/17 = 2.6 average fold change over all experiments, and

Mean of ratios = (50/10 + 25/25 + 20/20 + 25/10 + 100/20)/5 = (5 + 1 + 1 + 2.5 + 5)/5 = 14.5/5 = 2.9 = the mean of the five-fold changes for the experiment.

The mean of ratios is larger and could lead to misinterpretation of the effectiveness of promotion A if used instead of the fold change ratio of means because it is greatly influenced by the final unusual day of 100 customers data point for promotion A.

Testing for differences

Analyzing experiments for significant fold change requires determination of the fold change that is important experimentally, as well as a statistical test to examine the significant difference.

Mark R. Dalman and his coauthors suggested a tiered approach to testing fold change that incorporates the fold change cut-off and adjustment for any multiple comparisons in the statistical test if more than two fold changes are being compared.[3]

In practice, the t-statistic or one-way analysis of variance is used for comparing the fold changes, although nonparametric approaches to these data also can be used as well as a comparison of the proportion of fold changes above a cut-off value.

Greater uses of fold change

While highly used in genomic microarray and proteomic analyses, the fold change is important in many other fields. Fold changes are great when comparing the effect of multiple conditions to one another. In general, all the conditions are calculated against the same baseline for comparison to the untreated or calculated against their own initial value for comparisons over time.

When comparing over time, the null or untreated condition fold change can be defined as one and all other conditions are calculated and corrected to this ratio. This correction is useful when even the null condition does contain change over time. An example of fold change comparisons includes comparing the tumor shrinkage effects of multiple new drugs to the standard of care.

Fold changes also can be used to compare statistics, such as comparing the regression coefficients between two conditions. Additionally, fold change may be used in sample size power calculations. Fold change is a very powerful analysis tool because it can greatly simplify a lot of data into manageable and easy-to-compare numbers. QP

Fold change is a **very powerful analysis tool** because it can **greatly simplify a lot of data** into manageable and easy-to-compare numbers.

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