

“Universal” and “Reliable” Bioavailability Claims: Criteria That May Increase Physician Confidence in Nutritional Supplements

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Abstract

The benefits derived from nutritional supplements are directly related to their bioavailability, yet the dietary supplement industry lacks well-defined standards to ensure adequate bioavailability. Clinical studies of oral bioavailability report data of mean area under the curve (AUC) and standard deviations comparing groups following administration of the active ingredient by oral route over a defined time period. Comparisons primarily focus on statistical descriptions of mean AUC differences between the groups, while often failing to compare or discuss their standard deviations or inter-subject variance. This failure leaves open the question of whether or not an individual in a group is likely to experience the benefits described by the mean-difference comparisons. Further, even if this issue were discussed, it would be difficult to communicate meaning of these inter-subject variances to consumers and/or their physicians.

One way to resolve this problem might be to define “reliable” absorption results as incorporating 84% of the population and “universal” absorption as those incorporating 98% of the population. These indicators can be readily calculated using reported means and standard deviations. Examples are taken from the development of coenzyme Q10 and carotenoid products, demonstrating that some products reported to be “more bioavailable” are in fact too unreliable by these standards, thereby not providing physicians the information needed to make informed decisions.

Introduction

In the pharmaceutical industry, bioavailability is one of the principle pharmacokinetic properties of a drug. The pharmaceutical industry has a concrete definition of bioavailability and uses explicit methods for evaluating the bioavailability of drugs. In addition, bioavailability is a key component in the developing efficacy and safety of any drug. In fact, bioavailability studies are an important part of the information necessary to support an FDA approval.¹ However, while the US dietary supplement industry uses the term “bioavailability” in the marketing of many products, the concept lacks the well-defined standards associated with the pharmaceutical industry. As a result, it is difficult for physicians and consumers to compare the bioavailability claims of different formulations of a supplement. At workshops sponsored by the National Institutes of Health, 8 research priorities were recommended; the following were among them:² Generation of a standardized set of data that would enable consumers and healthcare practitioners to make better educated decisions and choices.

Identification of sources of variability due to formulation factorsImproved consistency of different supplements used in different clinical trialsBioavailability claims for nutritional supplements are too often based on human studies that report statistically significant results between treatments but also report high inter-subject variance within treatment groups. High inter-subject variance refers to the extent to which individual scores within a treatment group typically deviate from the average score within that group. Often expressed as standard deviations, High inter-subject variance in supplement bioavailability studies

may decrease the validity of such studies when applying outcomes to individuals in the general patient population. Physicians therefore may not have the necessary information for making important choices even though the results of the study may suggest otherwise.

This problem is evident in a series of recent bioavailability studies of coenzyme Q₁₀ (CoQ₁₀) and various carotenoids in which serum levels were used as a bioavailability index. In many cases advanced formulations were compared, and while conclusions and claims were drawn from statistically significant differences between treatments, these treatments were neither qualified nor modified to account for high levels of inter-subject variance. This problem is especially acute when the technical results are simplified as a claim that patients can understand. Developing a framework of terms that more easily communicates these technical issues would then be appropriate.

Statistical Significance

Oral bioavailability is defined as the fraction of unchanged drug that reaches the systemic circulation following administration by oral route.³ In the dietary supplement industry, the methodology used most often in clinical trials to show “improved bioavailability” of a particular formulation is the measurement of serum concentration of the substance after ingestion. Trial participants are placed in 2 or more treatment groups. One group is supplemented with Formulation X and one with Formulation Y. Serum levels of the bioactive compound are measured at baseline before ingestion and then at various time intervals after ingestion. The results are reported based on statistically

significant differences between the mean scores in different treatment groups. If the differences are statistically significant and “large,” then the product claim is that it is “X times more bioavailable.” If the difference is modest, then the claim may be simply “more bioavailable.” Some studies circumvent the statistics altogether by singling out the one data point of the best absorber and promote it as “up to X times more bioavailable.” While these claims are mostly based on statistically significant differences between treatment groups, they can be misleading as the average of all the data may include subjects who were poor absorbers as well as those who were super-absorbers, as will be observed in the following analyses.

The problem with the validity of these kinds of claims revolves around the degree of inter-subject variance, relative to the size of the treatment groups. Statisticians have known for decades that inferential significance tests comparing average scores of one treatment group to the next require that certain assumptions be met as to the inter-subject variance.⁴ One of these assumptions, known as the *homogeneity of variance assumption*, assumes that the variance associated with scores within 2 or more groups are approximately equal. In other words, it is assumed that the extent to which individual scores deviate from the mean within each treatment condition is fairly similar. Another assumption is that the scores within each group are distributed normally around this mean score.

Statisticians typically recommend a minimal cell size of 20 participants per condition in order to assume normality and heterogeneity of variance (though this is not always sufficient). However, the monetary and time constraints associated with clinical trials often prohibit developers from using an appropriate number of participants. Despite the existence of non-parametric tests robust to the violations of these assumptions, clinical researchers continue to report simple multiple group comparisons (such as the *t* test and Analysis of Variance) that may be extremely misleading given the variance between subjects in a given group.

In the CoQ₁₀ market for example, product formulations are usually compared to the crystalline form of CoQ₁₀ because it is hydrophobic and poorly absorbed. Consider 2 hypothetical products “New” and “Crystalline” in Figure 1. Their means and standard deviations are presented in Table 1.

FIGURE 1. Hypothetical Comparison of Uptake of CoQ₁₀ between a New Formulation and Crystalline CoQ₁₀

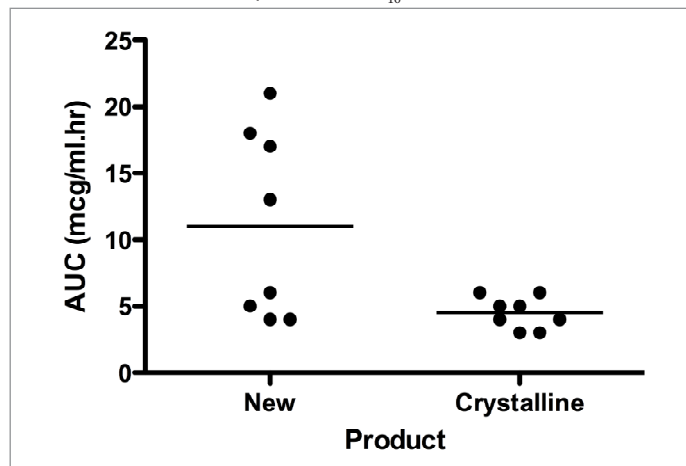


TABLE 1. Hypothetical Comparison of Uptake of CoQ₁₀ between New and Crystalline form of CoQ₁₀ [AUC 0-24hr (mcg/ml*hr)]

	New	Crystalline
Mean AUC	11.00	4.50
Std. Deviation	7.05	1.19

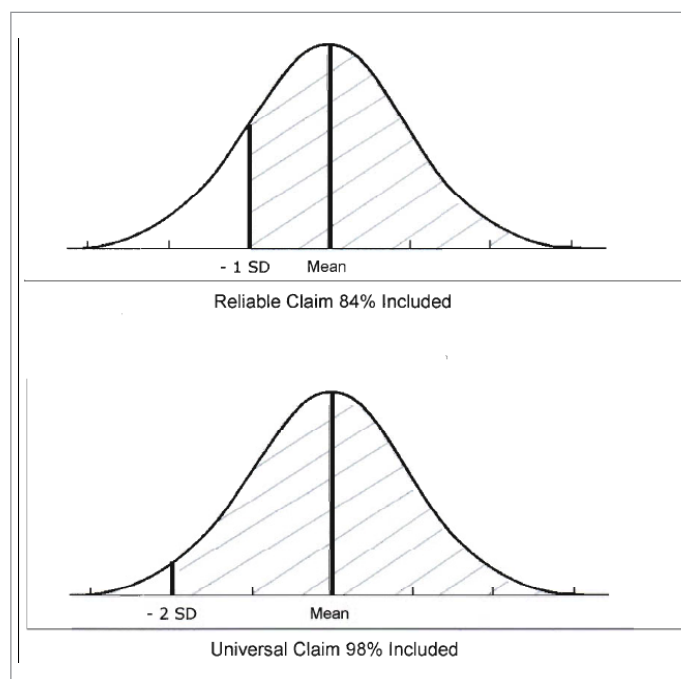
This hypothetical example can demonstrate why statistical significance alone does not constitute “improved bioavailability.” In this simulation, the New product is almost 2.5 times more bioavailable than the Crystalline form (significance at $P < 0.02$ using a *t* test to compare means), yet only 50% of the New product subjects had absorbance values no better than the Crystalline as the scatter plot describes (Figure 1). The manufacturer of the New formulation may then make the following claim: “New CoQ₁₀ product is 2.5 times more bioavailable than crystalline CoQ₁₀.” However, the consumer or physician does not know into which 50% an individual may fall—certainly the claim of 2.5 times more bioavailability is misleading because the inter-subject variance is high. The bioavailability claim based on the difference between group means overlooks the fact that the data indicates that only 50% of the customers may benefit from the product. This problem might interfere with the physician’s goal of developing a wellness regimen for the patient.

A Viable Alternative: Reliable and Universal Claims

For purposes of discussion, the term “reliable” bioavailability is defined as a bioavailability claim that captures 84% of the population and “universal” bioavailability to describe a bioavailability claim that captures 98% of the population (Figure 2). Determining whether the claims derived from a bioavailability study are “reliable” or “universal” requires a straightforward calculation of an “inclusion” value. The “inclusion” value is the lowest absorbance value that still includes 84% (all values above the low SD^a) or 98% (all values above 2 SD below the mean^b) of the results from all subjects. In the hypothetical comparison between the New and Crystalline product, the inclusion value for the New formulation’s “reliable” bioavailability claim would be 11.00 minus 7.05 or 3.95. This means 84% of the subjects would have an absorbance value of 3.95 or greater, which is actually less than or equal to the

- Calculation: 68% of the population is between 1 SD below and 1 SD above the mean. This means that 32% of the population is outside these limits, with 16% below the low SD and 16% above the upper SD. Therefore 84% of the population will be above the lower SD $68\% + 16\% = 84\%$.
- The same reasoning applies to 2 SD where about 98% of the population is above the lower 2 SD from the mean.

FIGURE 2. Reliable and Universal Claims in Normal Distribution



mean absorbance value of the Crystalline group of 4.50. To simplify the discussion, the percent difference between the mean and the inclusion value could be calculated. In this case the mean would have to be reduced by 64% to make a “reliable” claim. It would not be possible to make a “universal” claim, as the difference between the mean and 2 standard deviations below the mean places the inclusion value at less than zero. These distortions are a result of small sample size studies and violations of normality and homogeneity of variance within and between the groups. The key point is that this reliable-universal framework is developed to assist in deciding if a claim is attractive and how to improve communications with physicians and consumers such that they can make educated choices as to the benefits of a formulation for them directly. Finally, and ideally product comparison should be made from direct comparison in the same study. However this framework can help in sorting out claims and counter-claims made in different studies. Inclusion values and percent differences between their means can be calculated from published studies and comparisons made. It should be noted that the reliable-universal framework is not dissimilar to the construction of confidence intervals, which statisticians have long offered as one potential solution for dealing with small samples or violations of statistical assumptions. Basically, in light of the problems associated with large standard deviations, statisticians often determine a level of confidence (e.g., 95%) and create a range calculated by taking the mean plus or minus (in this case) the standard deviation multiplied by 1.96. A claim can now be made that researchers are 95% confident that the population mean falls within a certain range, and effectively rewards distributions that approach normality by more tightly esti-

imating the population mean. In our suggested technique of establishing criteria for “reliable” or “universal” bioavailability, we are simply taking this logic and modifying it slightly to include all scores that exceed 1 (reliable) or 2 (universal) standard deviations below the mean.

Additionally, the FDA criteria for determining bioequivalence require similar assumptions about underlying inter-subject variance confidence intervals and the resulting precautions. The basic difference between the 2 tests is that bioequivalence tests if 2 means are equal, while *t* tests and Analysis of Variance test to see if they are different. However in both test types, inter-subject variance is critical in determining if individual subjects will experience consistent benefits from a generic as compared to a brand name pharmaceutical, for example.

“Reliable” and “Universal” Bioavailability: CoQ₁₀

A series of human studies to be published in the *Journal of Integrative Medicine* compared the bioavailability of a CoQ₁₀-β-cyclodextrin inclusion complex (CoQ₁₀ complex) to an advanced solubilized formulation and the crystalline form of CoQ₁₀.⁵ In one of the studies, 5 subjects participated in a 24-hour crossover design with a single dose of 180 mg of CoQ₁₀. The results indicate that the bioavailability of the CoQ₁₀ complex was significantly better than the crystalline form by a factor of 3.7 (Figure 3).

For each product the percent difference between the mean and the “reliable” or “universal” inclusion value was calculated. Table 2 reports the inclusion values and the associated percentages resulting from the bioavailability of CoQ₁₀ complex, a commercially available oil-solubilized CoQ₁₀ soft-gel formulation containing a proprietary absorption enhancer, and a commercially available crystalline CoQ₁₀ hard gelatin capsule.

These calculations demonstrate that a small inter-subject variance produces corresponding inclusion values and percentage decreases from the mean absorbance values that are quite acceptable. In the case of the CoQ₁₀ complex, a “reliable” bioavailability claim can be made because 84% of all subjects have absorbance values no greater than 17% less than the mean. A “universal” bioavailability claim can also be made because 98% of the subjects have absorbance values no greater than 40% less than the mean. On the other hand, while the solubilized CoQ₁₀ formulation could try to claim higher bioavailability compared to crystalline CoQ₁₀, the claim would be misleading because of the high inter-subject variation in the bioavailability. This product cannot claim “reliable” bioavailability when 84% of all subjects have absorbance values up to 91% below the mean, because some subjects had absorbance values as low as or lower than the crystalline CoQ₁₀. The inter-subject variation is so large that a “universal” bioavailability claim is not even possible. This series of studies also compared CoQ₁₀ complex to the advanced solubilized formulation using 60 mg dose

FIGURE 3. Comparison of Uptake of CoQ₁₀ by Individual Subjects After a Single Oral Dose of 180mg CoQ₁₀

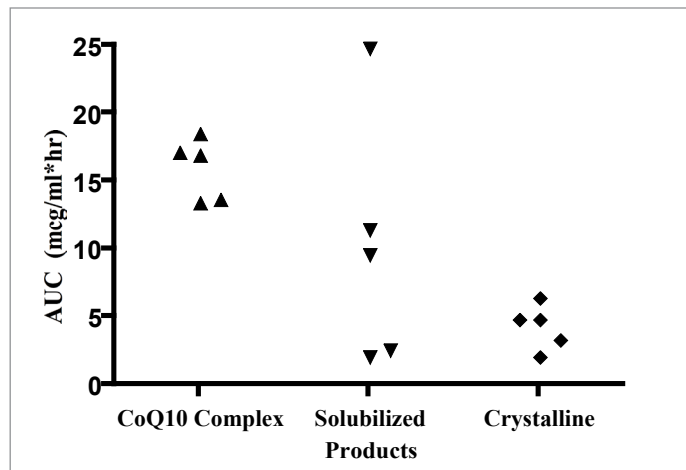


TABLE 2. A Comparison of Uptake of CoQ₁₀ from a Single oral Dose of 180 mg [AUC 0-24hr (mcg/ml*h)] (Adapted from Madhavi and Kagan³)

	Mean AUC	SD	Reliable Inclusion Value	% Decrease from Mean for Reliable Claim	Universal Inclusion Value	% Decrease from Mean for Universal Claim
CoQ ₁₀ complex	15.98	2.27	13.71	17%	11.44	40%
Solubilized CoQ ₁₀	10.13	9.21	0.92	91%	N/A	N/A
Crystalline CoQ ₁₀	4.32	1.65	2.67	62%	N/A	N/A

TABLE 3. Absorption data from other Advanced CoQ₁₀ Products (Adapted from Ullmann, et al.⁴)

Formulation Type	Mean AUC (0-14hr)	SD	% Decrease from Mean for Reliable Claim	% Decrease from Mean for Universal Claim
Microencapsulated	4.61	1.18	26%	52%
Solubilized CoQ ₁₀ “A”	4.98	2.36	47%	94%
Solubilized CoQ ₁₀ “B”	3.56	3.17	89%	N/A

over 24 hours and then a 3-week accumulation. Similar results were found relative to “reliable” and “universal” bioavailability claims. The CoQ₁₀ complex product demonstrated universal improvement in bioavailability while the larger inter-subject variance penalized the solubilized product.

A Review of Other Advanced CoQ₁₀ Formulations

In a review of published studies on other advanced CoQ₁₀ formulations, the inclusion value and percent decreases from the mean have been calculated (see Table 3, adapted from Ullmann, et. al.).⁶ In the study, the microencapsulated formulation could make reasonable “reliable” and “universal” bioavailability claims, but solubilized CoQ₁₀ “A” would have difficulty making an attractive “universal” claim due to its larger inter-subject variance. The solubilized CoQ₁₀ “B” product’s standard deviation was extremely large, indicating a high inter-subject variance, and the bioavailability of solubilized CoQ₁₀ “B” is not any higher than the crystalline product.

Interestingly, the literature describes 4 other products that demonstrate relatively small standard deviations and therefore could claim “reliable” and “universal” bioavailability. However, the increase in bioavailability of these products above the bioavailability of crystalline CoQ₁₀ ranged from 11% to 120%, much more modest than the multiple-fold increase reported by other formulations.^{7,8,9,10}

Another example of the utility of the “universal” bioavailability indicator can be found in a recent study using the reduced form of CoQ₁₀ (Ubiquinol). Interest in Ubiquinol was stimulated by a 4-week accumulation study measuring CoQ₁₀ plasma levels at baseline, 1-, 2-, 3-, and 4-week intervals.¹¹ The study incorporated Ubiquinol into an advanced solubilized formulation and then, unlike other studies, split the 90 mg dose into twice-per-day 45 mg doses. The results were impressive, yet when the “universal” bioavailability concept was applied they seem less so. In comparison, CoQ₁₀ complex was administered once per day in a 60 mg dose for 4 weeks.⁵ Table 4 shows the estimated serum levels after correcting for differences in dosing.

At week 4, Ubiquinol shows a higher mean serum level than CoQ₁₀ complex. However the standard deviation (inter-subject variance) for ubiquinol had increased approximately fivefold while the standard deviation for the CoQ₁₀ complex had increased about threefold. These relative increases affect the “universal” bioavailability such that the CoQ₁₀ complex serum levels are slightly *higher* than those of Ubiquinol. Converting to a ratio of universal value over baseline, a 23% advantage emerges for the CoQ₁₀ complex. Stated otherwise, using the statistical criterion of “universal” bioavailability, the CoQ₁₀ complex appears to have the most consistent improvement in bioavailability compared to Ubiquinol.

“Reliable” and “Universal” Claims: Carotenoids

The bioavailability of carotenoids and associated formulations have not been studied as much as CoQ₁₀ formulations, but illustrative examples do exist. Bowen, et. al., compared the bioavailability of free lutein to its esterified form.¹² The authors reported that the esterified form attained a 61.6% greater AUC than the free lutein (*P* = 0.03). While attention was paid to the study limitations, such as the differences in formulation of the esterified and free forms, the authors concluded that “the lutein diester formulation was more bioavailable than the free lutein form for most individuals.”

A closer look at inter-subject variance and the inclusion values shows that both the free lutein and esterified formulations would have difficulty making a “reliable” claim and it would not be possible to make a “universal” claim (Table 5). The inclusion value for a reliable claim indicates that while the diester may be more bioavailable, as represented by the mean absorbance values, neither carotenoid is reliably bioavailable.

Unusual in bioavailability studies, the authors presented individual absorbance data, which showed that the results were dependent on a few “super” absorbers as compared to many other subjects who absorbed little of either the esterified form or free lutein. Thus it appears the authors’ conclusions about the enhanced bioavailability of the esterified carotenoids were likely derived without reference to the large inter-subject variance.

TABLE 4. A Comparison of the Serum CoQ₁₀ Levels after Four Weeks Supplementation

Formulation Type	Baseline mcg/ml	Standard Deviation Baseline	Week 4 mcg/ml	Standard Deviation Wk 4	Universal Value : 2 SD below mean	Ratio of Universal Value / Baseline
CoQ ₁₀ complex*	0.480	0.11	1.400	0.34	0.72	1.50
Ubiquinol**	0.570	0.18	1.870	0.59	0.69	1.22

* Adapted from Madhavi and Kagan³

**Adapted from Hosoe et al.⁹

TABLE 5. A Comparison of Uptake of Lutein (Adapted from Bowen et al.¹⁰)

	Mean AUC (nmol*h/l)	SD	Reliable Inclusion Value	% Decrease from Mean for Reliable Claim	Universal Inclusion Value	% Decrease from Mean for Universal Claim
Lutein diester	37.0	35.8	1.2	97%	N/A	N/A
Lutein	22.9	19.0	3.9	83%	N/A	N/A

TABLE 6. A Comparison of Uptake of Astaxanthin from Formulations (Adapted from Odeberg et al.¹¹)

	Mean AUC (mcg*h/l)	SD	Reliable Inclusion Value	% Decrease from Mean for Reliable Claim	Universal Inclusion Value	% Decrease from Mean for Universal Claim
Control	1347	501	846	37%	345	74%
Formulation A	2216	547	1669	25%	1122	49%
Formulation B	4960	1504	3456	30%	1952	61%
Formulation C	2580	850	1730	33%	880	66%

In a study of astaxanthin formulations containing algal meal, 3 lipid formulations were compared to a control (algal meal, dextrin): long-chain triglyceride (palm oil) and polysorbate 80 (formulation A); glycerol mono- and dioleate and polysorbate 80 (formulation B); and glycerol mono- and dioleate, polysorbate 80, and sorbitan monooleate (formulation C).¹³ The authors conclude that “all showed enhanced bioavailability” when compared to the control, with formulation B having the largest gain. Applying the inclusion calculations it appears that formulation B can make the best “reliable” and “universal” claims as well, indicating that it is available at a reasonable level for “poor” absorbers (Table 6).

Conclusion

The dietary supplement industry can greatly benefit by improving the standards of reporting the bioavailability of poorly absorbed supplements. The improved bioavailability claims in clinical studies should include both improvement in the uptake as compared to the control and also the inter-subject variance. Such a measure would not only help in defining an improved product, but it would also help physicians decide whether or not a product is likely to be effective. By introducing new terms such as “reliable” and “universal” uptake, factors relating to inter-subject variance and physician/consumer confidence in a formulation can be more easily communicated.

Disclosure: Daniel Kagan, PhD, and Doddabele Madhavi, PhD, are employed by BioActives, LLC. One of the ingredients reviewed are contained in products sold by BioActives, LLC. Ginny Bank, MS, and Kenneth Lachlan, PhD, are not employed by BioActives, LLC.

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