THREE

Products Clinical Study:

Cellular Absorption & Bioavailability

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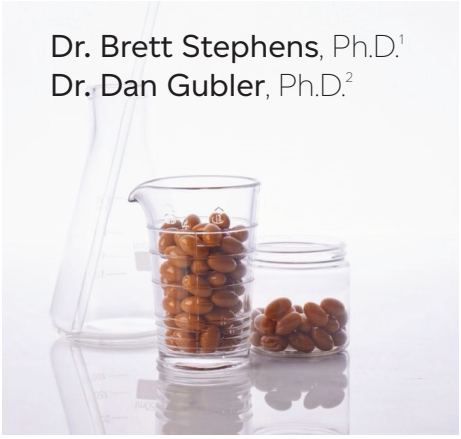


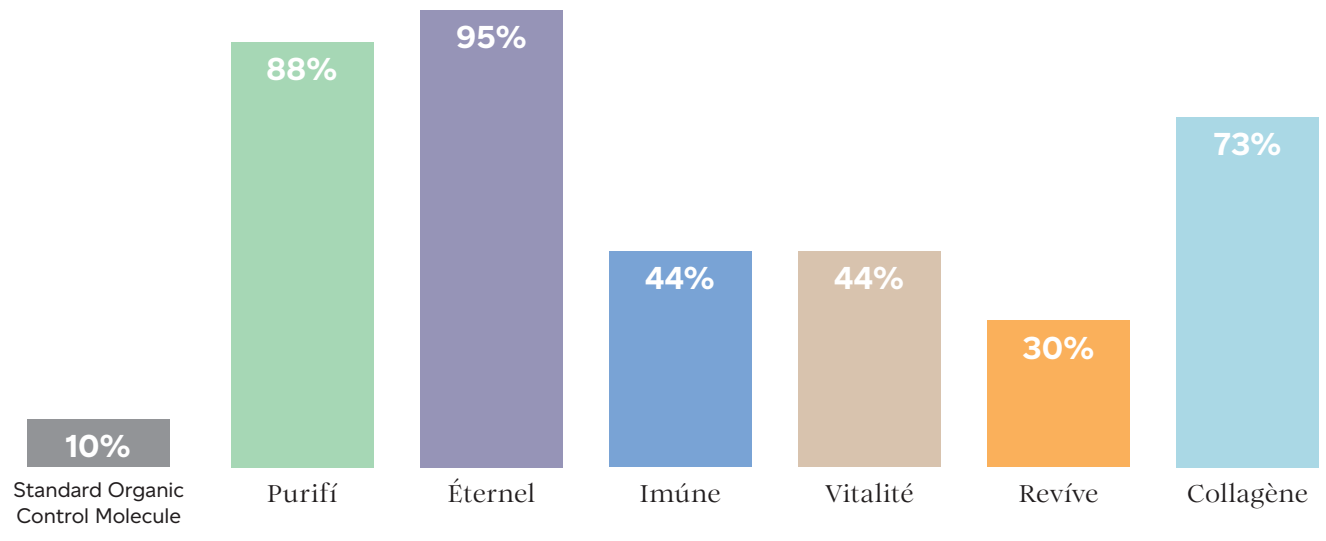
SUMMARY:
The purpose of this clinical study was to determine the cellular absorption and bioavailability of THREE's product line, meaning, at what percentage are the nutrients included in THREE's product line being absorbed by the body?

The results of this study indicate that due to the Cellular Absorption Technologies used in formulation and manufacturing, **THREE's products are between 3 and 9.5 times more absorbable** than the standard organic control molecule used as a baseline in the study.

Increased cellular absorption and bioavailability = increased product efficacy.

Study Snapshot

REMEMBER: Compare the absorption percentage of each product to the **standard organic control molecule**, and **NOT** between products themselves.



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Introduction:
If a nutritional supplement is to improve human health, the bioactive ingredients in the product need to cross the cellular membrane at a high concentration to realize this objective. The problem is this: the bioactive molecules in nutritional supplements are predominantly organic molecules from plants and are hydrophobic (i.e. they are afraid of and are not soluble in water). Because the body is made up of 60% water (the blood is 90% water), these bioactive molecules are poorly absorbed into the cell and have low bioavailability. General absorption and cellular bioavailability of organic molecules is ~10%. THREE has developed and employs many different Cellular Absorption Technologies in its products to increase cellular absorption and bioavailability. The purpose of this clinical study was to determine the cellular absorption and bioavailability of THREE's product line.

Methods:
Cellular absorption and bioavailability was determined by use of a Caco-2 permeability assay using 0.4 µM pore size in a 12-well plate format. Filter was pre-incubated with media for 30 minutes before seeding the cells. 100 µL apical, 600 µL basal. 3X10^5 cells were plated per insert. Media volume was set to 0.5 mL apical and 1.6 mL basal. Media was replaced every 2-3 days until tight junction formation. Products were tested at the concentrations, durations, and then quantified at the absorption ranges shown in **Table 1** below.

Group	Product Tested	Concentration	n=	Duration	abs nm range
1	**Collagène**	4%	3	1hr	275-310
2	**Éternel**	1%	3	1hr	285-320
3	**Revíve**	0.10%	3	1hr	310-325; 400-415
4	**Purifí**	1mg/mL	3	1hr	280-315
5	**Vitalité**	2mg/mL	3	1hr	280-305
6	**Imúne**	0.5mg/mL	3	1hr	285-320
7	**Curcumin**	5µg/mL	2	1hr	415-450

Table 1: Clinical Study Conditions

200 µL aliquot was taken from the basolateral chamber and quantified against a standard curve. Evaluation of permeability was determined by calculation of the apparent permeability coefficient using the following equation:

$$P_{app} = \frac{dQ}{dt} x \frac{1}{A \times C_0}$$

Where dQ/dt = amount of product present in the basal compartment as a function of time (amount/sec) calculated at 1 hr (3600 seconds), A = Area of transwell (1.1 cm2), and C0 = initial concentration of product applied in the apical compartment.

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Results and Discussion:
Predicted cellular absorption and bioavailability percentages of THREE's products are shown in **Figure 1** below. Actual numbers are as follows: Éternel = 95%, Purifí = 88%, Collagène = 73%, Vitalité = 44%, Imúne = 44%, Revíve = 30%, and curcumin (Organic Control) = 10%.

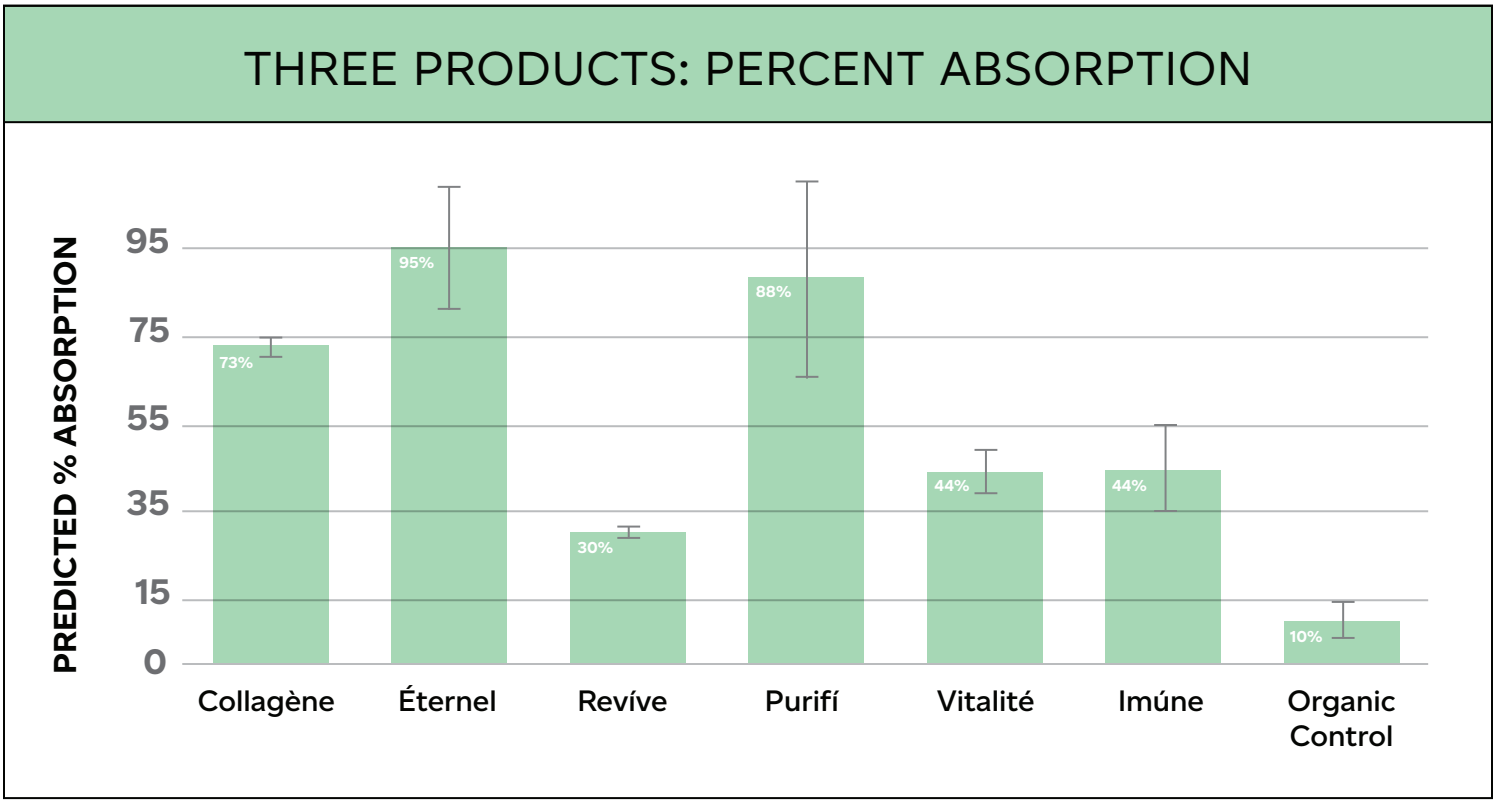

Figure 1: Percent Cellular Absorption and Bioavailabilty of THREE Prodcuts

The correct way to analyze the data in **Figure 1** is to compare the percent absorption of each product to the organic control—and not between products. When looked in this way, Revíve, with a cellular absorption percentage of 30, is 3X more bioavailable than curcumin, which represents a standard organic molecule and served as the control in the experiment. Éternel is 9.5X more bioavailable than curcumin. The significant increases in cellular absorption and bioavailability in THREE's products versus a standard molecule like curcumin is due to the Cellular Absorption Technologies used in THREE's products that help to shuttle the ingredients in the formulas across the cellular membrane and into the cell at a much higher concentration.

Conclusion:
A Caco-2 in-vitro cellular absorption assay was used to calculated percent absorption and bioavailability of THREE's products. The data found that THREE's products, due to the Cellular Absorption Technologies used, are between 3 and 9.5 times more absorbable than a standard organic molecule using no delivery technology. Increased cellular absorption and bioavailability should equate to increased product efficacy.