

## Effects of L-glutamine on the cellular protein synthesis *in vitro.* Glutazorb<sup>®</sup> stabilized glutamine-vs-L-Glutamine Standard

Test Performed by

Medical University-Sofia, Faculty of Pharmacy Department of Pharmacology, Pharmacotherapy & Toxicology Lab of Experimental Chemotherapy and Molecular Pharmacology Sofia, Bulgaria

Project Coordinator & Senior Researcher Asst. Prof. Georgi Tsvetanov Momekav, MPharm, PhD

> Report Date February 10, 2010

L-glutamine is the most abundant free alpha-amino acid both in the body's tissues and in the circulation. Although traditionally considered as non-essential because it can be readily synthesized in the liver, in more recent studies a solid body of evidence unambiguously indicates that glutamine can become essential under circumstances, such as debilitating illness or injury, severe stress, and excessive exertion, whereby significant glutamine depletion has taken place. Glutamine is a precursor to glucose, some amino-acid neurotransmitters and many peptides, proteins, and nucleotides, and functions as an energy substrate for most cells. Glutamine plays a central role for the normal function of the nervous system, immunity, skeletal muscle, and the alimentary. In the immune system, glutamine is used as an energy source fuel by fibroblasts, lymphocytes, and macrophages, and is also used for nucleotide synthesis. Skeletal muscle is the primary storage site for glutamine, and also the primary depot of glutamine for other tissues. The GI tract utilizes glutamine as a fuel source, and uses more glutamine than any other area of the body.

It has been well established that persisting, exhaustive exercise causes a significant decline in plasma glutamine levels, and moreover, the signs of overtraining have been also associated with glutamine depletion. On this basis, it has been proposed that a lot of conditions associated with such depletion could be at least partly corrected with supplemental glutamine. Some of the proposed benefits of glutamine supplementation for athletes include augmented immune function, anabolic effects upon protein and glycogen synthesis and inhibition of protein catabolism.

While the anabolic and the other beneficial effects of glutamine supplementation in athletes are well-established, there is a lot of controversy regarding the efficacy of glutamine in some controlled studies, which to a great extent is due to the quality of the products tested.

In order to comparatively evaluate the properties of a stabilized glutamine formula (Glutazorb<sup>®</sup> with a standard, high quality glutamine [Sigma Chemical Co.]), we sought to determine the effect of the stabilization processing upon the metabolic effects in vitro. To address this issue, established monolayer cultures of muscle cells (RD) were subcultured in a deficient growth medium containing only 2.5% fetal calf serum (instead of 10% which is mandatory for the optimal cell growth and maintenance in vitro) and without an addition of I-glutamine (essential component of all human and animal cell line growth media). Thereafter, cells were exposed to either culture medium (controls) or 0.5 mmol conventional glutamine or stabilized glutamine (Glutazorb<sup>®</sup>). At different exposure periods (48h or 72 h), the cells were detached from the cell culture flasks via trypsinization, washed thrice in PBS to remove residual protein from the culture medium, and counted. Thereafter, the protein content in each sample was determined using the Lowry method. The results were calculated as mg protein/106 cells and expressed as percentage of the untreated control (set as 100%).

As evidenced from the results obtained, both glutamine formulations (conventional and stabilized) caused a significant time-dependent increase in the protein content of FCS-deprived muscle cells. In all experiments however, the stabilized glutamine formula (Glutazorb<sup>®</sup>) caused more pronounced augmentation of the protein anabolism as compared to the non-stabilized product (Table 1; Figure 1).

The achieved results unambiguously indicate that the presented processed formulation is characterized with superior effects upon protein synthesis in vitro, as compared to a conventional glutamine product, under the chosen experimental conditions.

## Appendix

Experimental Data for the Effects of Glutamine on Cellular Protein Synthesis

**Table 1.** Effects of glutamine supplementation on the protein content of human muscle cells cultured in FCS-deficient medium.

	Protein Content (% of untreated control, set as 100%)	
Exposure Period (h)	Conventional Glutamine	Stabilized Processed Glutamine Glutazorb <sup>®</sup>
48	122* ± 7	136** ± 5
72	131* ± 11	143** ± 7

\* Statistically significant (p<0.05) vs. the untreated control.

\*\*Statistically significant (p<0.05) vs. the equivalent concentration of conventional glutamine (Student's t-test).

**Figure 1.** Effects of Glutamine – conventional (grey columns) or processed (Glutazorb<sup>®</sup>) (black columns) on the protein content in cells RD cells cultured in FCS-deficient medium after 48 or 72 h incubation. Each column represents the arithmetic mean  $\pm$  sd of four (4) independent experiments.

