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MEMORANDUM

02-20-08

Team AAETF,

As promised, here is the second of four Kre-Alkalyn studies that were performed in the last two years.

This particular study is rather interesting because it helps to show what happens once Kre-Alkalyn is introduced into a cell. This study also used an advanced technology called a "cell-line study". It allows researchers to see exactly what is happening inside a cell (in vitro) without using live participants.

To give you some perspective, this is also the same methodology, and facility - Greenberg Medical Center in Sofia, that many pharmaceutical companies use to test new drugs before they go to market. (In fact, our study was sandwiched in-between a cell line study for Pfizer and one for Bristol-Myers.)

Attached to this memo is the actual second study, *"Effects Of Creatine Supplementation On Cellular Protein Metabolism In Vitro "Kre-Alkalyn -vs- Creatine Monohydrate"*.

Once again, please forward this information to your clients and contacts right away. Thanks again for your efforts. I'll be seeing most of you at the Arnold next week (please remember to bring your business cards with you).

All The Best,

Brian Andrews
President, All American EFX

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Effects Of Creatine Supplementation On Cellular Protein Metabolism In Vitro “Kre-Alkalyn -vs- Creatine Monohydrate”

January 31st, 2008

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Objectives: The anabolic properties of creatine supplementation are well established, and we sought to determine the effects the stabilization process (used to manufacture Kre-Alkalyn) might have upon these metabolic effects.

Methods: To address this issue, established monolayer cultures of muscle cells (RD) or chondrocytes (SW1353) were subcultured in a deficient medium containing only 2.5 % fetal calf serum, instead of 10% which is mandatory for the optimal cell growth and maintenance in vitro. Thereafter, cells were exposed to either culture medium (controls) or 0.5 mmol conventional creatine (Creatine Monohydrate) or buffered creatine (Kre-Alkalyn). At different exposure periods (12, 24 or 48 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 method of Lowry. The results were calculated as mg protein/ 10^6 cells and expressed as percentage of the untreated control (set as 100%).

Results: Evident from the results obtained, both creatine formulations (conventional creatine and stabilized Kre-Alkalyn) caused a significant time dependent increase in the protein content of either muscle or cartilage cells. In all experiments, however, the Kre-Alkalyn formulation caused a more pronounced augmentation of the protein anabolism, as compared to the non-stabilized counterpart (Creatine Monohydrate) (Table 4.1.; Figure 4.1.).

Appendix 4.

Experimental data for the effects of creatine supplementation on cellular metabolism.

Table 4.1. Effects of creatine supplementation on the protein synthesis of human muscle or cartilage cells cultured in FCS-deficient medium.

Exposure period (h)	Protein content (% of untr. control)	
	Conventional creatine	Kre-Alkalyn
RD muscle cells		
12	109* ± 4	111* ± 1
24	112* ± 3	121*# ± 4
48	117* ± 2	124*# ± 2
SW1353 cartilage cells		
12	105* ± 4	110*# ± 3
24	111* ± 4	117*# ± 3
48	115* ± 3	122*# ± 4

* Statistically significant ($p < 0.05$) vs. the untreated control; # Statistically significant ($p < 0.05$) vs. the equivalent concentration of conventional creatine (Student's t-test).

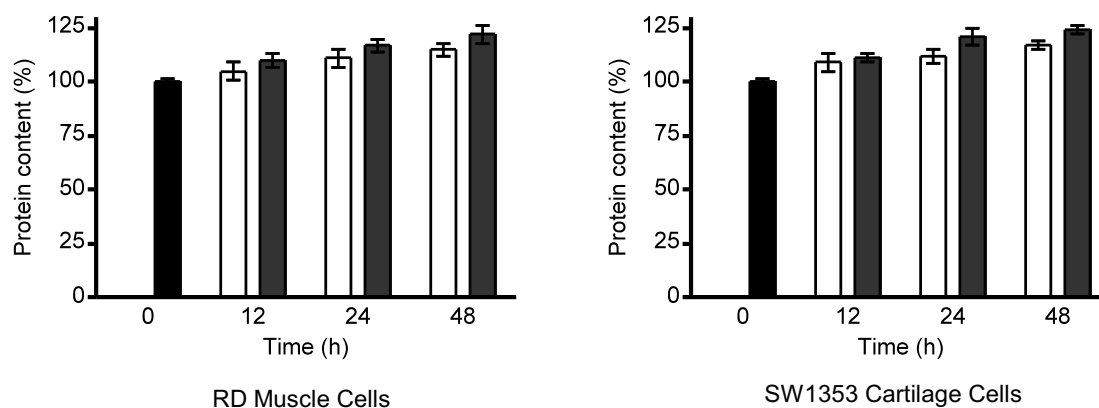


Figure 4.1. Effects of creatine – conventional (white columns) or stabilized Kre-Alkalyn (grey columns) on the protein content in cells (upper plot) or RD cells (lower plot), cultured in FCS-deficient medium after a 12, 24 or 48 h incubation period. Each column represents the arithmetic mean ± sd of 4 independent experiments.

Conclusion: Kre-Alkalyn proved to exhibit a greater anabolic effect compared to regular creatine. Thus, considering the equivalent controlled conditions of the experiment, the observed greater anabolic effects of Kre-Alkalyn could be ascribed solely to the superior stability afforded by the processing manipulations used to manufacture this compound.