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ENHANCED PHYSICAL PERFORMANCE THROUGH USE OF ENRICHED AMINO ACID SUPPLEMENTS

Dudrick, S; Watterson, S; Feste, A; Drummond R

Achieving peak physical performance has been a goal of athletes for self-improvement as well as for competitive purposes. The most effective means for improving performance involves prolonged systematic exercise training. Increases in power and endurance of over 50% have been achieved in previously sedentary individuals through conditioning and adherence to optimal nutritional diets. Another potential means for enhancing performance involves taking supplements or “ergogenic” aids. It has been estimated that these supplemental aids do not usually result in more than a 7% improvement in performance, especially when given to untrained individuals. Serious, well-trained athletes, however, usually maintain their fitness at near their maximum tolerance limits. Why such competitors would want to utilize supplements for any potential benefits these aids might provide to further improve physical performance is, therefore, understandable.

A wide variety of commercial supplements are available, but no well designed experiments have been performed to prove that they provide significant benefit to elite athletes. The following study was undertaken to evaluate a popular protein supplement by monitoring power output generated by peak conditioned athletes using a knee extension/flexion exercise machine in a randomized controlled crossover model. Plasma and urine amino acid concentrations were determined and based on amino acid disappearance profiles (reflecting utilization), an enriched protein supplement was formulated that resulted in significantly improved power and endurance for these athletes.

METHODS

Preliminary

An exercise protocol was established involving alternating knee extensions and flexions using the OMITRON^R apparatus. Participants were asked to use maximum effort with each motion. The resistance was adjusted on the apparatus to setting #8 or #9 (consistent per athlete per testings) to allow a complete range of motion so that multiple repetitions could be performed. VO₂ max was 60%-70%, indicating exercise of moderate intensity. The set number of repetitions followed by a rest period constituted on cycle. Multiple cycles were repeated for the exercise testing. Power output was recorded for each extension and flexion. The apparatus was calibrated prior to each use.

The basic supplement was taken prior to the exercise program. Blood was drawn for plasma amino acid profiles before the supplementation and 30 minutes afterwards. Repeat profiles were obtained after the exercise testing. The amino acid levels that fell considerably during exercise were then added to the basic supplement to constitute the “enriched supplement”. The “enriched supplement” was then tested and effect on exercise performance (power generation) was compared with the effect produced by the basic supplement. The basic supplement used in this study is described in Table 1.

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Table 1: AMINO BLEND

Amino Blend is an enzymatically-digested milk protein consisting primarily of free amino acids and small chain peptides. This product is an excellent source of amino acids for use in tablet or capsule formulations.

Typical Analysis:

Total Nitrogen	13.2%
Amino Nitrogen	5.2%
Moisture	3.6%
Ash	4.9%
pH (2% solution)	6.9

Total Amino Acid Assay (percent):

Lysine	13.4
Histidine	2.7
Arginine	3.5
Aspartic Acid	6.7
Threonine	3.7
Serine	4.8
Glutamic Acid	20.8
Proline	9.9
Glycine	1.9
Alanine	3.1
Cystine	0.8
Valine	6.5
Methionine	2.9
Isoleucine	5.3
Leucine	9.3
Thyrosine	1.4
Phenylalanine	2.0
Tryptophan	1.2

Microbiological:

Standard Plate Count	Less than 5,000/gram
Salmonella	Negative
E. Coli	Negative

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Subjects

To avoid a conditioning effect, the study utilized seven adult males who were all in peak physical condition, achieved mainly by weightlifting. All were accustomed to using daily protein supplements.

Diet

The athletes' usual self-selected dietary practices obtained by daily dietary recall are described in Table 2. The average daily intake was 30.2 kcal/kg and 1.62 gm/kg of protein. None of the athletes were taking anabolic steroids during the study.

Table 2: SELF-SELECTED DIETARY PRACTICES

AA No	Name	Age	Weight (lbs)	Weight (Kg)	Daily Intake-Energy (Kcal)	Daily Intake-Energy (Kcal/Kg)	Daily Intake-Protein (gm)	Daily Intake-Protein (gm/Kg)
30	FW	37	240	105.1	3800	34.8	162	1.48
32	ME	32	230	104.5	3540	33.9	283	2.71
33	BA	32	204	92.7	1153	12.4	86	0.93
34	GR	28	190	86.4	2906	33.6	116	1.34
35	MR	28	192	87.3	2116	24.2	79	0.90
36	JD	29	207	94.1	3367	35.0	202	2.15
37	SJ	27	200	90.0	3335	36.7	167	1.84

Four days before testing, the athletes stopped taking their regular daily supplement and began taking a randomly assigned supplement as they continued their daily workouts. The supplement used at this stage was administered in the form of capsules to disguise taste. During the three days prior to testing, the athletes took 20 capsules (15 grams) per day. During the 24 hour period prior to testing, the athletes did not take any supplements, consume alcohol or exercise. On the day of testing, the athletes did not eat for the four hours immediately prior to testing but took 30 grams of supplement powder dissolved in 300 cc of aspartame-flavored Koolaid^R 30 minutes prior to testing. The athletes then remained sedentary for 30 minutes prior to exercising. During testing, the athletes were allowed to sip water ad libidum to prevent dehydration.

Exercise Testing

“Warm” Up: The athletes began the test protocol with a warm up involving 20 minutes of pedaling on a stationary bicycle set at a fixed speed (setting #3) and tension for each testing session.

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Phase I: The athletes began extension followed by flexion in a forceful but non-exhaustive manner. After 7 consecutive extensions and flexions (one cycle), the athletes rested for 45 seconds. This routine was repeated until 35 cycles were completed.

Interval: The athletes were kept warm on a stationary bicycle for 10 minutes set at the same parameters of speed and tension as used during warm up.

Phase II: The athletes then resumed extension and flexion with 10 repetitions constituting one cycle. After a 45 second rest, the cycles were repeated consecutively until exhaustion. Phase II was terminated after the athlete completed the last full cycle of 10 repetitions.

Laboratory Testing

Blood: After the athletes arrived for the day of testing, a 24-hour urine collection was completed (see below) and a blood specimen was taken ($t=1$). The supplement was ingested and following a rest of 30 minutes, the next blood specimen was taken ($t=2$). Following completion of the exercise testing, a third blood specimen was taken ($t=3$). These blood specimens were assayed for amino acid concentrations.

Urine: For the 24 hour period preceding the testing, all urine was collected to assay for amino acid profiles. Using the concentration of each amino acid and the known urine volume, an hourly output of each amino acid was determined. Following completion of the exercise testing, a specimen was again taken for urine produced during the three hours at the beginning of the exercise testing period. Amino acid concentrations were determined and based on the known urine volume from the three hour period collected, the hourly output for each amino acid was obtained.

Repeat Testing with Alternate Supplement

After the above sequence was completed, each athlete resumed his usual exercise and dietary pattern. The above sequence was repeated the following week using the alternate supplement based on the prior random assignment with each athlete thus serving as his own control. A paired t-test comparing the two supplements in each athlete served as the basis for data analyses for power generated and for the biochemical test results. All athletes were well informed of the study protocol beforehand and gave written informed consent before the studies were conducted.

Amino Acid Analysis

The Pico Tag^R Physiological Amino Acid Analysis System was comprised of a Digital Professional 350 Computer, a Digital LA50 Printer, a System Interface Module, two Model 510 Pumps, a Wisp Model 710B Autosampler, a Temperature Control Module and a Model 440 Absorbance Detector.

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Samples were prepared for ultrafiltration by diluting one hundred ul of plasma 1:1 with methionine sulfoxide (0.2 um/l in 0.1 m/L HCL) internal standard solution. After mixing, 200 ul of the diluted plasma was placed into an ultrafiltration device directly onto the PLGC membrane. The samples were spun in a fixed angel centrifuge for 15 min at ambient temperature and 4300 rpms. After centrifugation, 25 ul aliquots of the ultrafiltrate were placed into Pico Tag reaction vials. Urine samples were not ultrafiltered; they were diluted 1:1 with methionine sulfoxide internal standard solution. The 25 ul aliquots were then dried, redried, and subsequently derivatized with phenylisothiocyanate (26).

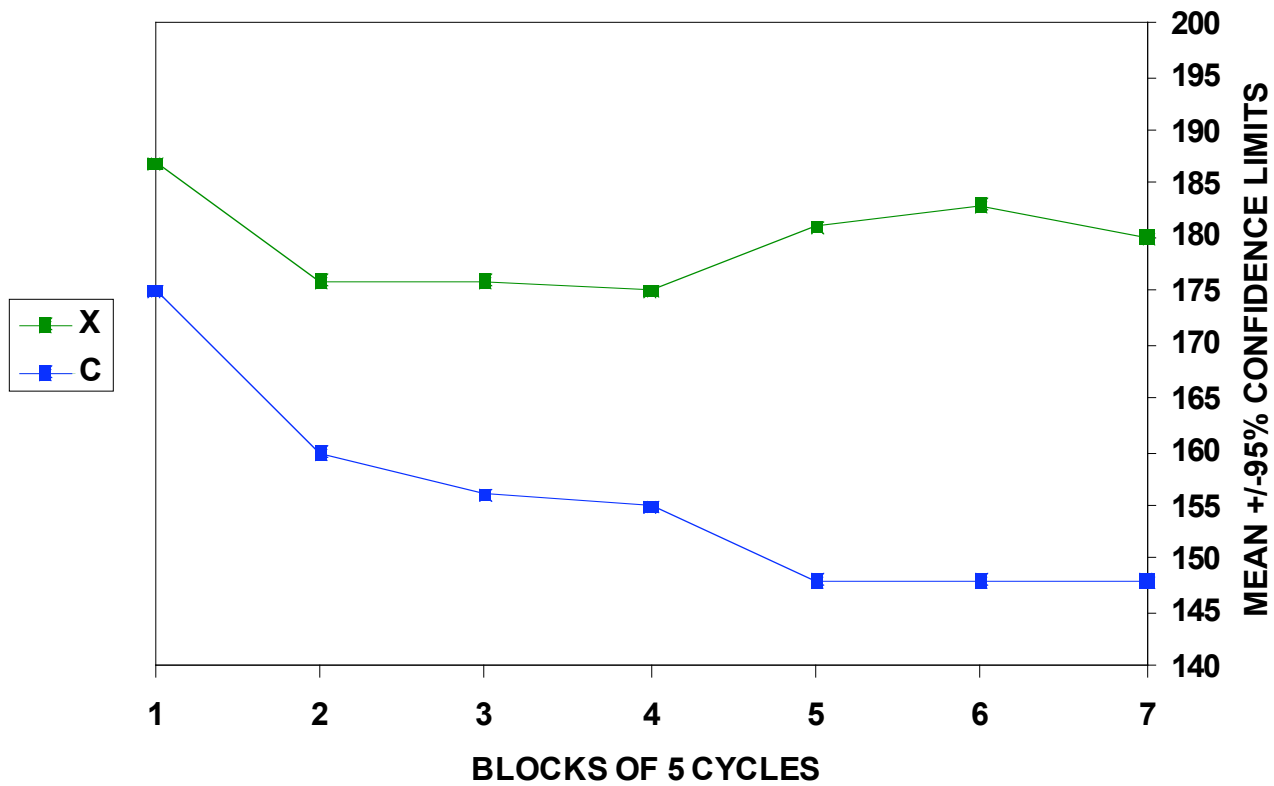
The Phenylisothiocarbamyl (PTC) amino acid derivatives were separated on C18 Reverse Phase Pico Tag Free Amino Acid Analysis Column (3.9mm X 30cm). The column temperature was 46 degrees C., and the separation was accomplished by employing a binary gradient at flow rate of 1.0 ml/min (26). The chromatographic system was controlled by WatersTM software. The PTC amino acid derivatives were detected by their absorbance at 254 nm. The method of internal standard analysis using peak height for quantitation was used. The separation required 68 min and an additional 20 min was required for equilibration of the column with Pico Tag Eluent 1. Volumes of 10 ul and 20 ul were injected for standards and plasma/urine samples respectively. Both volumes contained 500 ppm of each internal standard.

RESULTS

Power Output

Phase I: To identify trends in the data (35 cycles), each five succeeding cycles was grouped together in blocks, yielding 7 sequential blocked periods. The average power for flexions and extensions in the blocks yielded for the particular supplement a mean plus standard error of the mean (see figure 1). Each athlete served as his own control using two different supplements. Paired comparisons for the flexion and extension in each repetition then served as the basis for a paired t-test between respective blocks.

Figure 1: ATHLETES DATA: POWER, n=490 REPETITIONS/BLOCK



The enriched supplement resulted in higher power in each block. The increased power achieved was sustained throughout this phase of testing (p 0.05). The non-enriched supplement resulted in less power per block, and the power output steadily tapered off over time, demonstrating decreased endurance.

Phase II: Because of different numbers of cycles achieved by the different athletes prior to exhaustion, a paired t-test for each athlete using the two different supplements was then performed on the basis of total power generated as well as the number of cycles completed (see Table 3). For each athlete, use of the enriched supplement resulted in significantly (p<0.05) more total power (a 91% increase compared with the basic supplement). Endurance was also prolonged with the enriched supplement as 6 of 7 subjects completed more cycles (for an average increase of 69%). For each supplement, the average power generated per cycle was calculated for all athletes as a group. As time (cycles) elapsed, successive averages reflected the power of those athletes continuing to exercise. Graphs of these averages (figures 2, 2A) display the greater power and endurance achieved with the enriched supplement during phase II.

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Table 3: TOTAL POWER AND TOTAL CYCLES

<u>Athlete</u>	<u>C Power</u>	<u>X Power</u>	<u>C Cycles</u>	<u>X Cycles</u>
30	23635	78464	7	25
32	19596	34910	17	20
33	49949	106474	20	35
34	91288	129531	25	34
35	34698	131275	10	35
36	31472	31838	12	11
37	66508	96073	26	35
Total	317148	608565	117	195

NOTE: C=control formula, X=experimental formula

PAIRED SAMPLES

<u>Variable</u>	<u>C Cycles</u>	<u>X Cycles</u>
Mean	16.714	27.857
Standard Deviation	7.387	9.529
Paired Observations	7	

t-statistics	-3.307	Hypothesis
Degrees of Freedom	6	Ho: $\mu_1 = \mu_2$
Significance	0.016	Ha: $\mu_1 \neq \mu_2$

<u>Variable</u>	<u>C Power</u>	<u>X Power</u>
Mean	45369.286	86938.000
Standard Deviation	25731.655	40901.141
Paired Observations	7	

t-statistics	-3.466	Hypothesis
Degrees of Freedom	6	Ho: $\mu_1 = \mu_2$
Significance	0.013	Ha: $\mu_1 \neq \mu_2$

Figure 2: ATHLETE STUDY: PHASE II

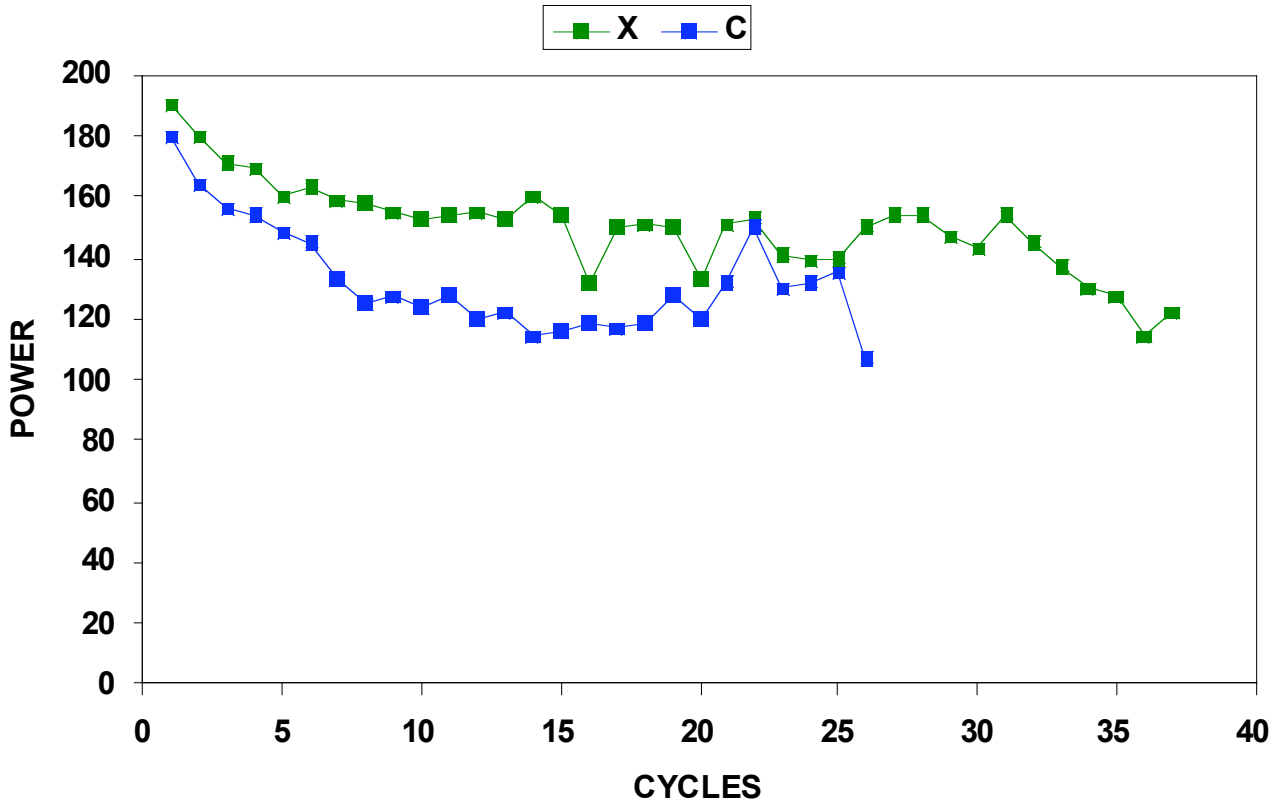
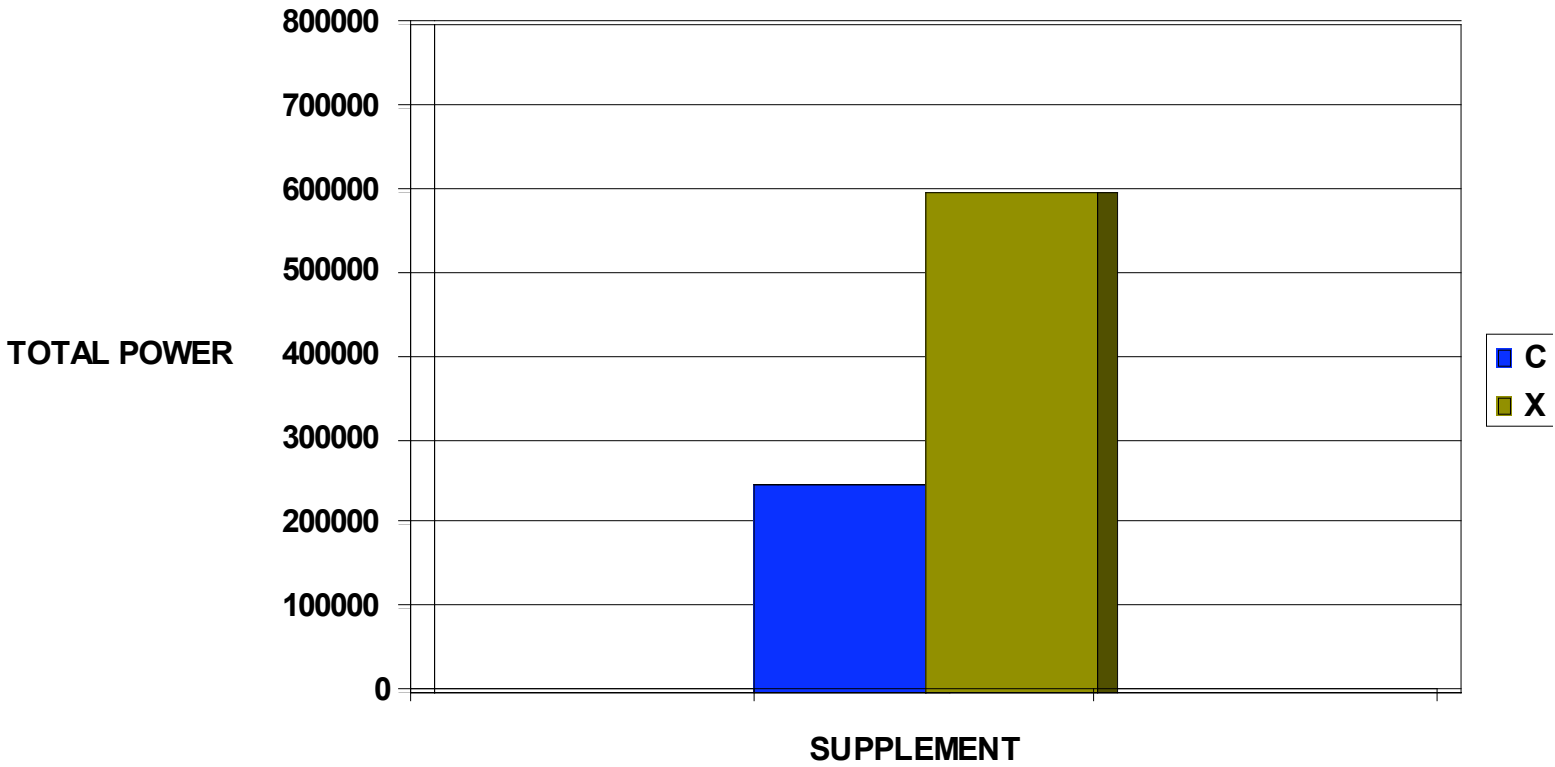


Figure 2A: STUDY II: PHASE II, TOTAL POWER



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Amino Acid Profiles

Prior to administration of supplement (t=1):

Plasma: Amino acid profiles were similar for the two supplements except that the enriched formulation had more phenylalanine.

Urine: Hourly outputs were also identical except that the enriched supplement resulted in less serine.

After administration of supplement but prior to exercise testing (t=2):

Plasma: Administration of both supplements followed by the ½ hour of absorption, however, caused significant ($p < 0.05$) alterations in the profiles for each supplement reflecting uptake of amino acids. Figure 3 for the basic non-enriched supplement and Figure 4 for the enriched formulation showed significant ($p < 0.05$) increases in most amino acids except for phosphoserine which declined.

After exercise testing (t=3)

Plasma: Most of the amino acid elevations noted prior to testing were then reversed except for alanine which remained high for both supplements (see figure 3 and 4).

Urine: For both supplements the urine produced during exercise showed similar amino acid profiles.

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Figure 3: PLASMA: C MEAN

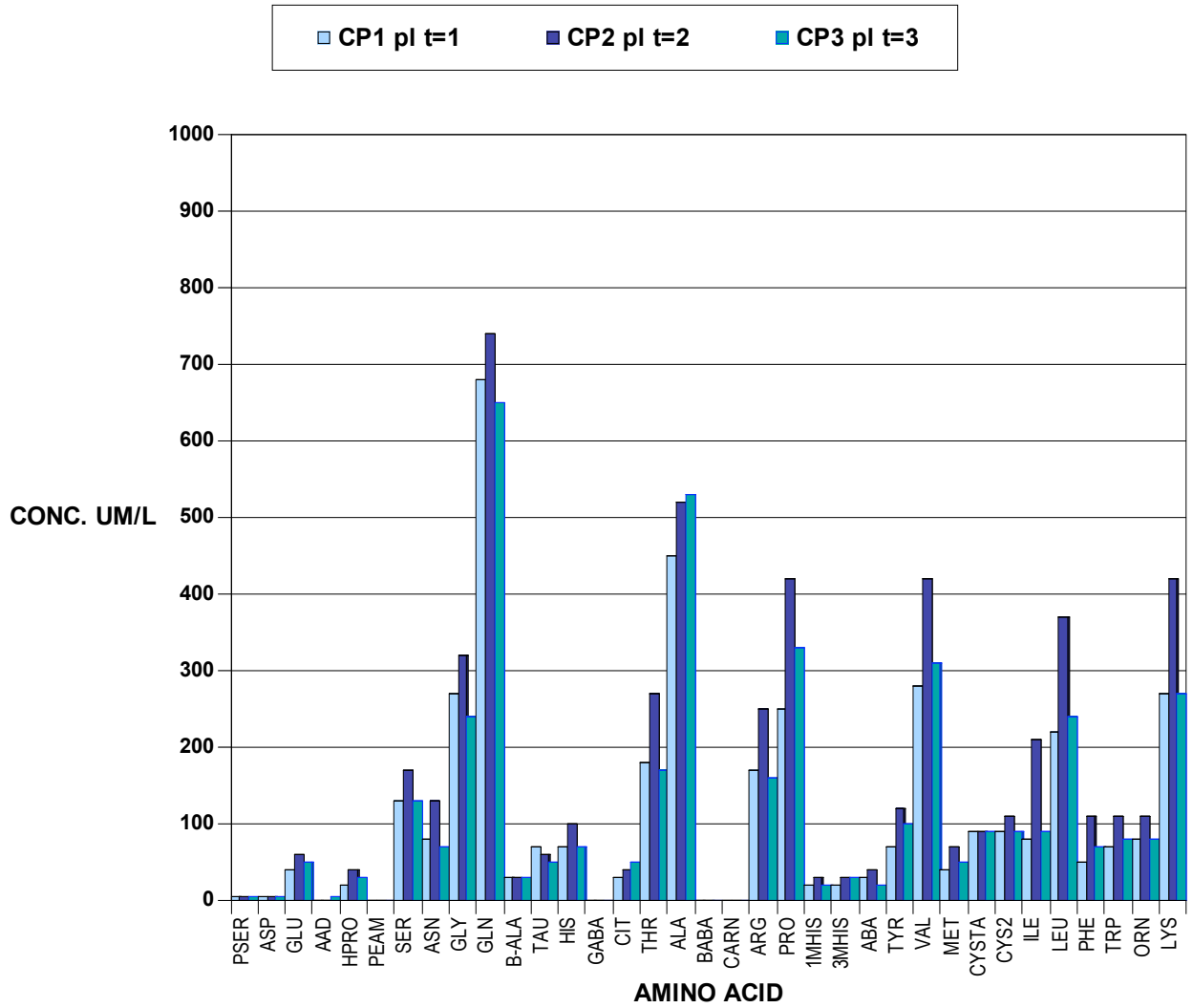
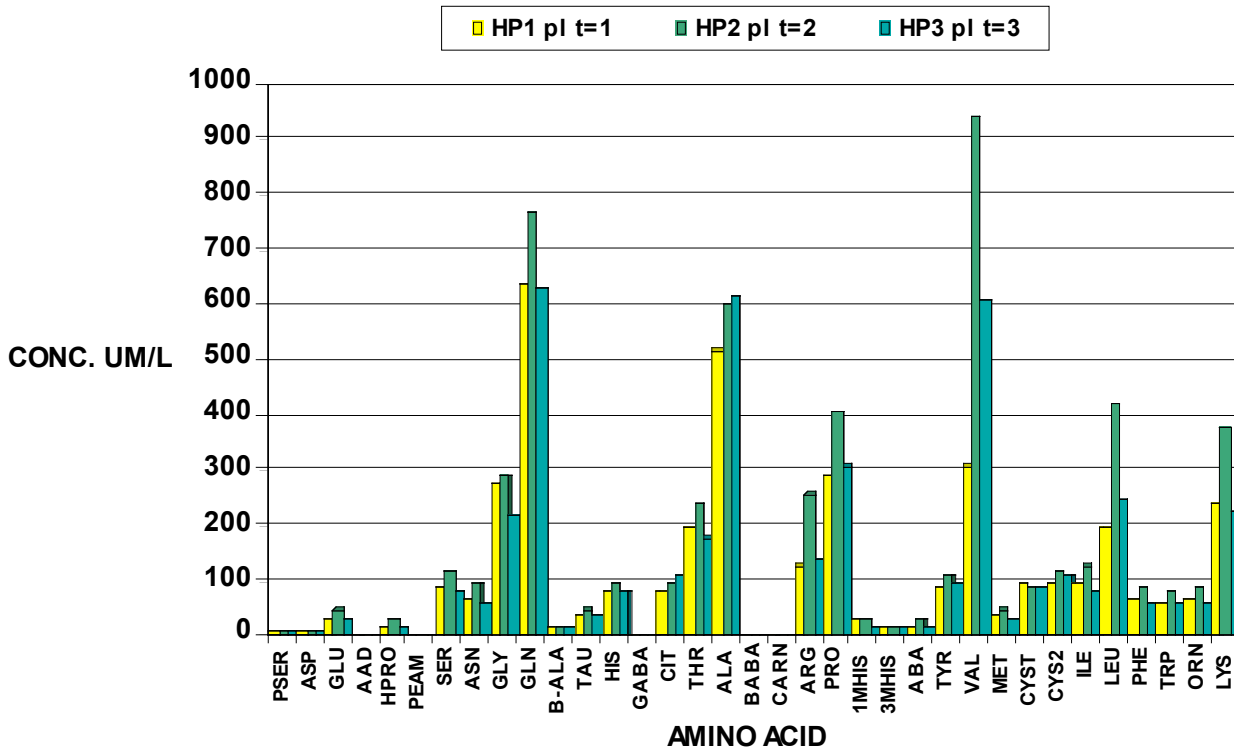


Figure 4: PLASMA: X MEAN



DISCUSSION

The two phases of this experimental model suggest that improved power and endurance are possible with a specifically enriched supplement. When tested for a set number of knee extension and flexions (Phase I), the athletes taking the enriched formulation always generated more net power during each block for testing. Furthermore, they were able to sustain a consistent and high level of power. When the same athletes were not taking the enriched supplement, they displayed a steady decline in power generation demonstrating less endurance. During the second phase of the testing when the athletes exercised to the point of exhaustion, the power generation was consistently higher with the enriched formula and their endurance was significantly prolonged before exhaustion forced them to stop. With each athlete serving as his own control, it appears that the enriched protein supplement provided a significant improvement in energy generation when it was taken for three days prior to testing as well as one-half hour before beginning the warm up and exercise testing. The concept of protein as an energy source is not new. What is novel is the documentation of a specific blend of amino acids serving as a performance enhancer.

During ordinary physical exercise, the usual endogenous sources of energy are known to be primarily carbohydrate and to a lesser extent fat with protein considered to provide no

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more than 5 to 10% of the energy expended. The energy sources, however, are also known to vary with body composition and the type and duration of exercise. Short duration sprinters depend primarily on muscle glycogen stores relying on a burst of anaerobic metabolism. Moderate to heavy intensity exercise of more prolonged duration relies mainly on fat stored although protein catabolism is also known to make a contribution. Protein is normally considered to provide a structural basis for tissues but not to serve as a significant energy store.

Certain earlier studies have suggested that protein intake above the Recommended Daily Allowance (RDA) for sedentary individuals provided no exercise improvement. Rasch, et al. (11), investigated the effect of administering commercial protein supplement tablets on muscle hypertrophy and strength but found no beneficial effects. Consolazio, et al. (4), gave exercising men 1.4 or 2.8 grams of protein per kilogram per day. The higher protein intake led to increased muscle mass but did not improve work performance. Muscle bulk and power increases have been hypothesized to be mediated via growth hormone; however, increasing growth hormone concentrations orally (usually with arginine and ornithine supplements which are thought to stimulate growth hormone release) has not produced any documented major increase in muscle bulk or power. Butterfield (3) and Wolfe (12) have pointed out that studies recommending increased daily protein intake for physically active individuals frequently suffer from flaws in experimental design or methodology fueling a major controversy in Exercise Physiology pertaining to whether physically active individuals do in fact require increased amounts of daily protein intake. Meanwhile the National Research Council has recommended for the general population a minimum protein intake of 0.8 grams per kilogram body weight per day. These lines of evidence suggest that with increased exercise, whole body protein use and turnover are augmented (9). Studies in rodents and man have demonstrated a net breakdown of protein during exercise, freeing amino acids that can be utilized for oxidation and gluconeogenesis. This mobilization is the result of decreased protein synthesis in liver and muscle, plus increased protein degradation in liver and also possibly in muscle (7). While it is not known whether exercise increases oxidation and utilization of all amino acids, increased catabolism of branched chain amino acids (BCAA) has been documented (2). Experimental data suggest that exercise increases branched chain ketoacid dehydrogenase activity in skeletal muscle, and that this is the limiting enzyme in the metabolic pathway of branched chain amino acid oxidation. Therefore, as exercise frees amino acids from skeletal muscle and liver, BCAA metabolism is enhanced resulting in the generation of branched chain ketoacids as well as providing a major source of amino groups for de novo alanine synthesis. Alanine is also available for hepatic gluconeogenesis to help prevent hypoglycemia during exercise. Exercising athletes who experienced declines in blood sugar developed earlier onset of fatigue. Similar exercise-induced increases in carnitine palmitoyl transferase (10) would enable utilization of these branched chain ketoacid carbon structures as energy sources within mitochondria. It has also been documented that glutamine undergoes enhanced oxidation during exercise as a contribution to amino acid sources for energy generation (8).

To investigate supplements of specific amino acids our experiments were designed to utilize exquisitely trained athletes who had been steadily taking a self-selected dietary

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regimen consisting of 30.2 kcal/kg/day as well as 1.6 gm/kg of protein per day. This dietary program appears sufficient to provide the daily energy and protein needs of these athletes. A conditioning phase for the athletes on this testing regimen was obviated by utilizing dedicated elite weight lifters in peak condition who were tested on a knee extension/flexion exercise machine that is integral to their routine training program. Moreover, these athletes had also been taking commercially available protein supplements regularly as part of their daily workout regimen which increased their dietary protein intake by 23%. Prior to the exercise testing, the warm up helped the athletes to get their “second wind” and pass through the “circulatory phase” so that during testing they were relying primarily on carbohydrate as well as protein metabolism (1). The experimental protocol then involved studies of sequential phases of exercise performance; Phase I dealing with a moderate intensity exercise for a set period followed by Phase II with a slightly heavier intensity of exercise continued until exhaustion. The randomly selected supplement (either basic or enriched form) was given sequentially over two weeks and performance was evaluated for differences in power generation. The question thus arises how the amino acids used to enrich the basic supplement may be contributing to the improved power and endurance that was observed (e.g., served as and extra fuel source). It is known that for physically active individuals increased dietary protein intake together with sufficient caloric intake maintains energy as well as nitrogen equilibrium or positive balance and can contribute to increased tissue stores such as muscle mass. Ultimately, a plateau is reached in terms of weight and tissue accretion as muscle, fat and carbohydrate. During vigorous exercise associated with increased metabolism of specific amino acids, energy sources must necessarily be recruited from existing tissue stores. Skeletal muscle and the liver do not preferentially store in reserve and then release those specific amino acids that are utilized in increase amounts with exercise (e.g., branched chain amino acids, glutamine and possibly arginine and lysine among others). Otherwise, plasma levels of these specific amino acids would not decrease with vigorous exercise as was consistently observed. Those amino acids metabolized in increase amounts during vigorous are obviously present endogenously in only limited quantities for energy generation and work with power output restricted by substrate availability as well as obligatory muscle catabolism. The hypotheses to be tested in this experiment was that by providing an exogenous source (oral supplement) for the rate-limiting endogenous amino acids, performance might be significantly enhanced as a result of augmenting the amino acid pool highly utilized but otherwise maintained in limited supply. Normally, one would expect athletes to utilize very little protein during exercise and to consume only a small meal before commencement of physical activity. Moreover, as blood flow is shunted away from the viscera during exercise, one would expect diminished absorption of dietary amino acids. This experiment has demonstrated, however, that with a liquid oral supplement taken shortly (1/2 hour) prior to moderate exercise, amino acids can in fact be absorbed efficiently via the GI tract and appear in the blood stream in significant quantities to have a measurable effect. Furthermore, it appears that this means of supplementing fuel sources of athletes can provide readily available energy substrates resulting in significantly more strength, power and endurance than had been available through regular conditioning and routine dietary means alone.

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Exhibits:

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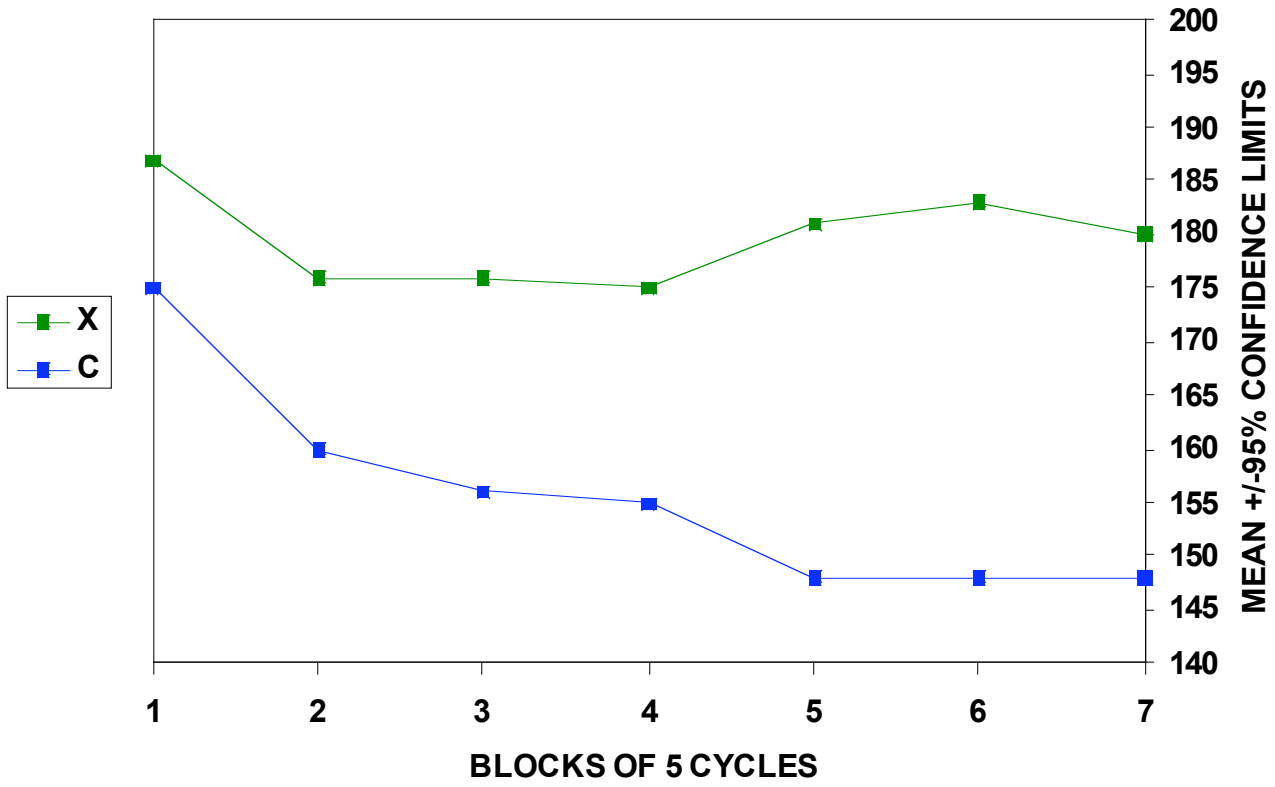


Figure 2: ATHLETE STUDY: PHASE II

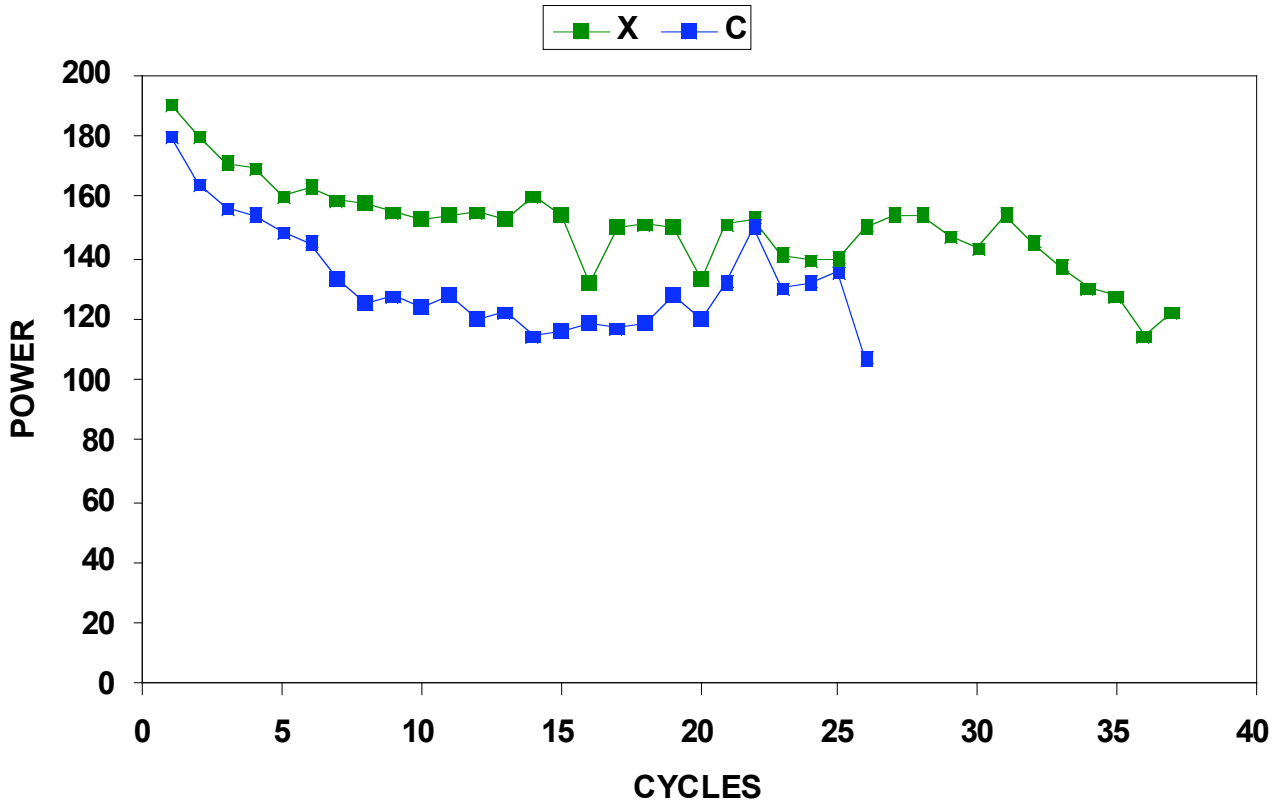
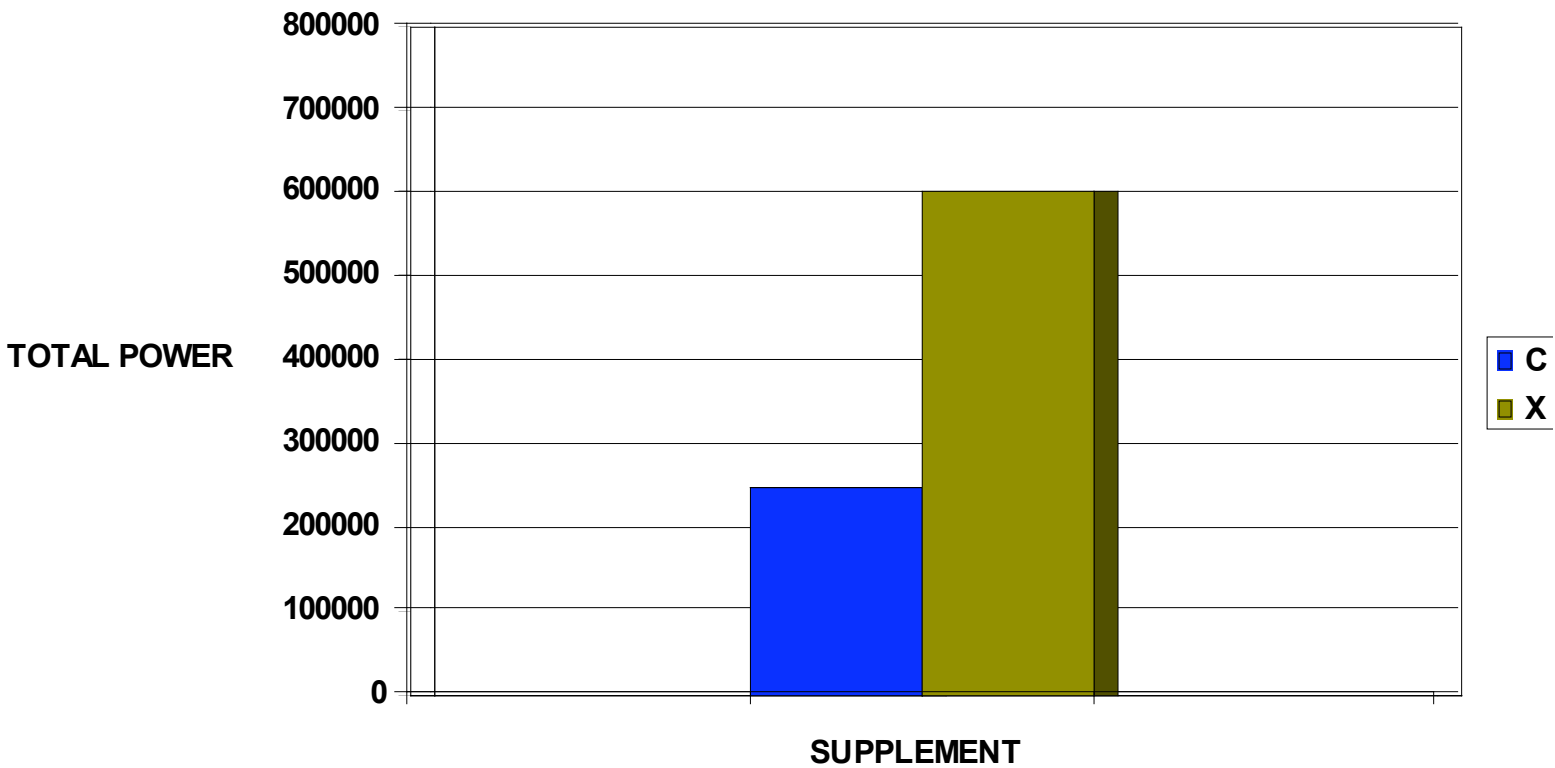


Figure 2A: STUDY II: PHASE II, TOTAL POWER



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Figure 3: PLASMA: C MEAN

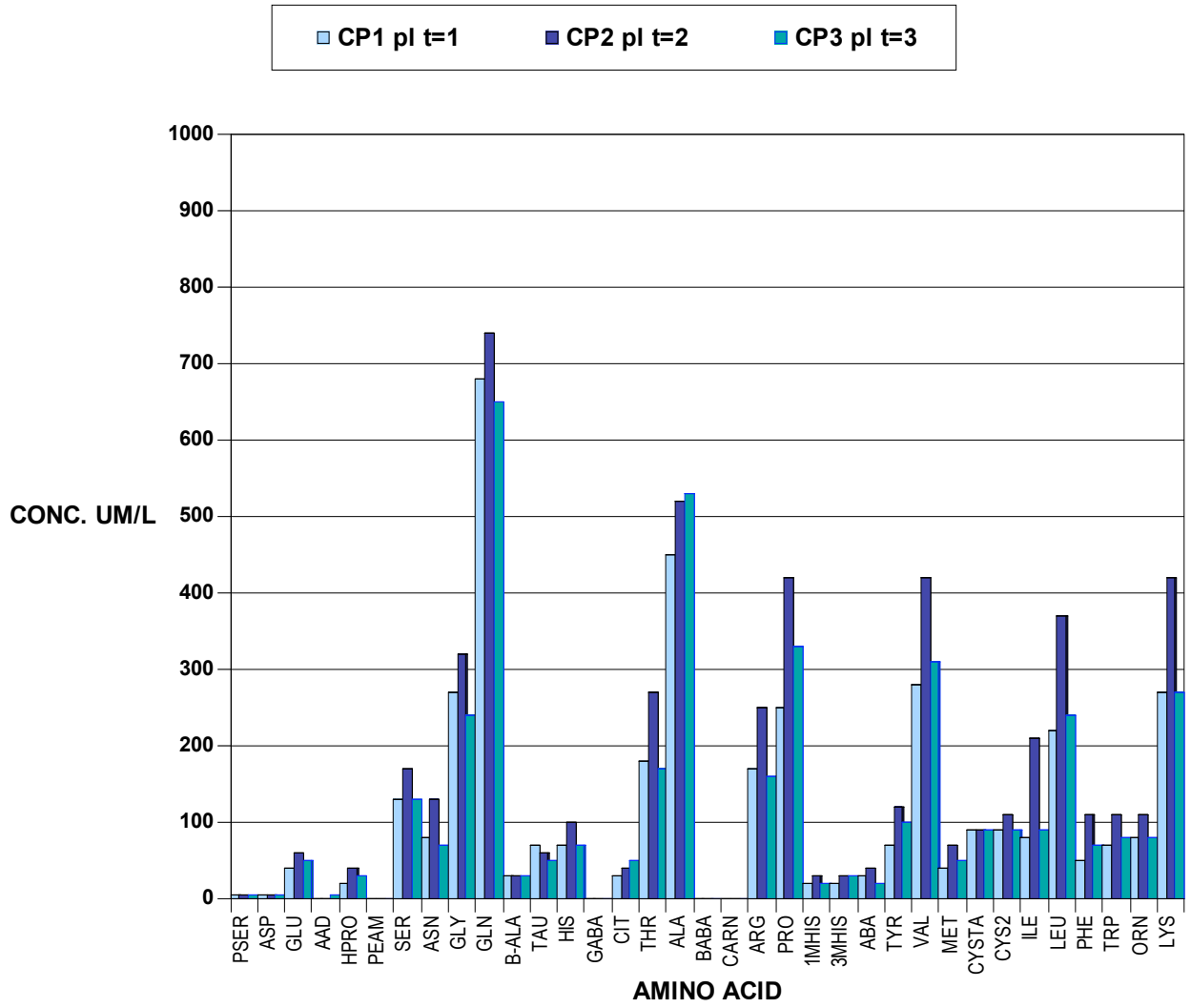


Figure 4: PLASMA: X MEAN

