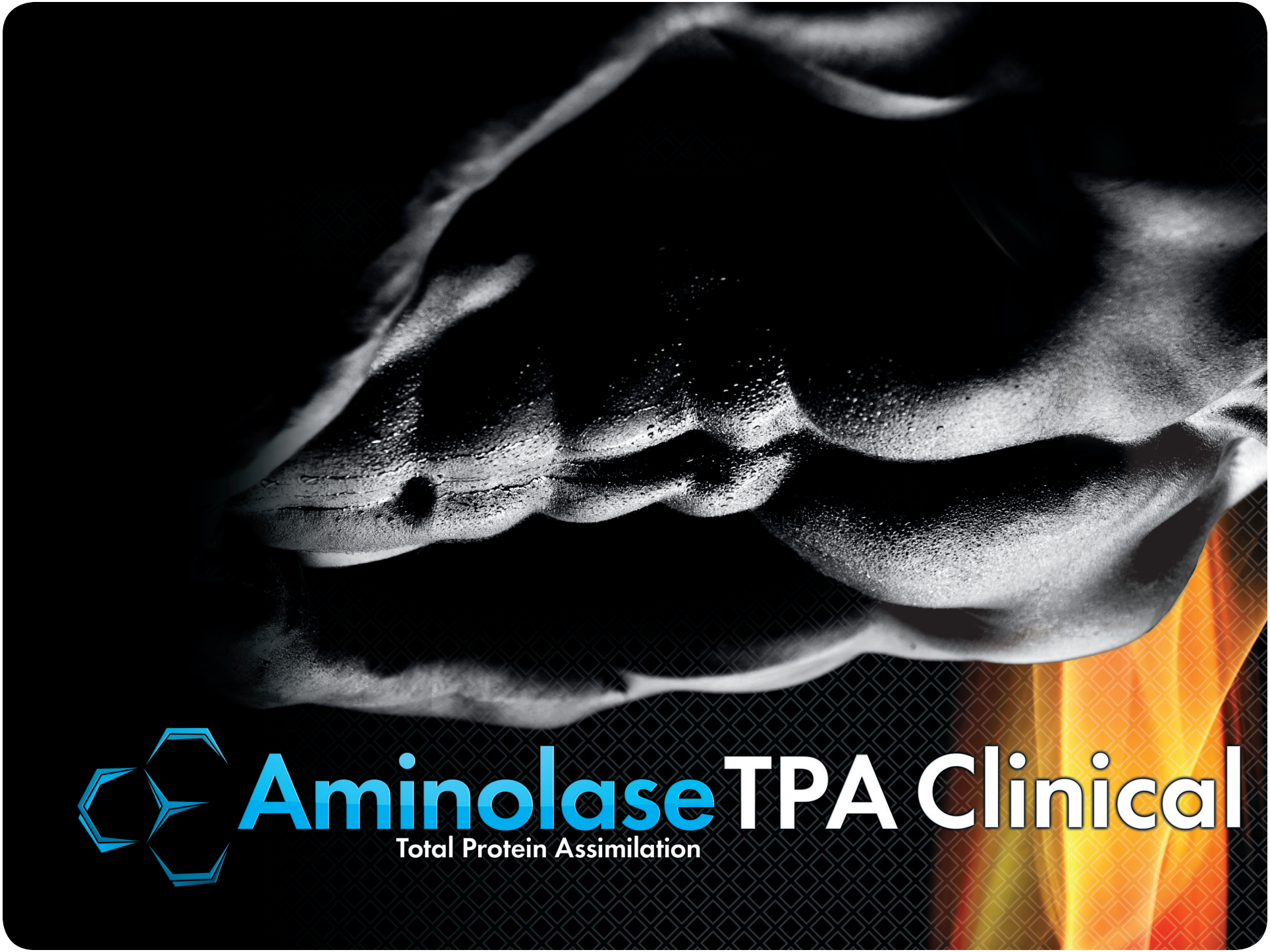

Aminolase TPA Clinical
Total Protein Assimilation

BACKGROUND:

Whey protein is one of the richest sources of all of the 9 essential amino acids which your body is unable to produce. However, whey protein must first be processed into a useable form for building muscle *(peptides and amino acids)* and it must do so in less than two hours to be absorbed. This is the time from the stomach through the small intestine in which digestion/absorption primarily occurs *(Kim SK 522-24)*. Undigested whey will simply be excreted *(wasted amino acids)*.

The use of SDS gel electrophoresis for protease hydrolysis of proteins has been well documented and chosen as the in vitro method to evaluate whey hydrolysis by proteases. A 5 amino acid peptide was created with the help of New England Peptides™ to mark bioavailability mixed with a low molecular weight marker to visualize and determine the size of the peptide fragments created by each protease blend on whey.

The in vitro conditions were designed to mimic the processes that occur in the digestive tract when whey is consumed including; buffering capacity of the drink, pH of the stomach and small intestine, salt conditions, bile salts and transient time. Drinks show a transient time of around 30 minutes in the stomach and less than 2 hours in the small intestine *(Kim SK 522-24)*.

Your body produces proteases of its own including pepsin in the stomach and pancreatic enzymes in the small intestine. Most protein drinks buffer the stomach between 4-6 pH which greatly reduces the acidity needed to unfold/hydrolyze the proteins and the efficiency of pepsin *(Fordtran 645-57;Schaafsma G 1865S-7S)*. This leaves the bile salts *(unfold)* and pancreatic enzymes *(hydrolyze)* as the main means to convert proteins into absorbable peptides and amino acids.

Consuming whey protein without a digestive aid like Aminolase is difficult to break down. This was confirmed by mixing whey with pancreatin using USP small intestinal conditions resulting in very little breack down of the whey *(Figure. 1-B, and Figure 2 grey)*. The pancreatic proteases alone were not enough, further processing of whey by a slight drop in pH and introduction of bile salts resulted in better degradation of whey but still not complete breakdown (Figure 3). However, the Aminolase protease formulation was able to hydrolyze the whey protein with no additional help and in the time needed for absorption in the small intestine *(Figure 1-A and Figure 2 blue)*.

While whey has a high *(1.0)* protein digestibility corrected amino acid score, that score can be misleading. Protein Digestibility Corrected Amino Acid Score *(PDCAAS)* is a method of evaluating the protein quality based on both the amino acid requirements of humans and their ability to digest it. The formula for calculating the PDCAAS percentage is: *(mg of limiting amino acid in 1 g of test protein / mg of same amino acid in 1 g of reference protein)* x fecal true digestibility percentage *(Schaafsma G 1865S-7S)*, which means that the protein can be broken down in the large intestine where very little absorption *(less than 10%)* occurs. Whey also has a high Biological value *(BV)*, which is a measure of the proportion of absorbed protein from a food which becomes incorporated into the proteins of the organism's body. Biological value is determined based on this formula.

$$BV = (Nr / Na) * 100.$$

Where:

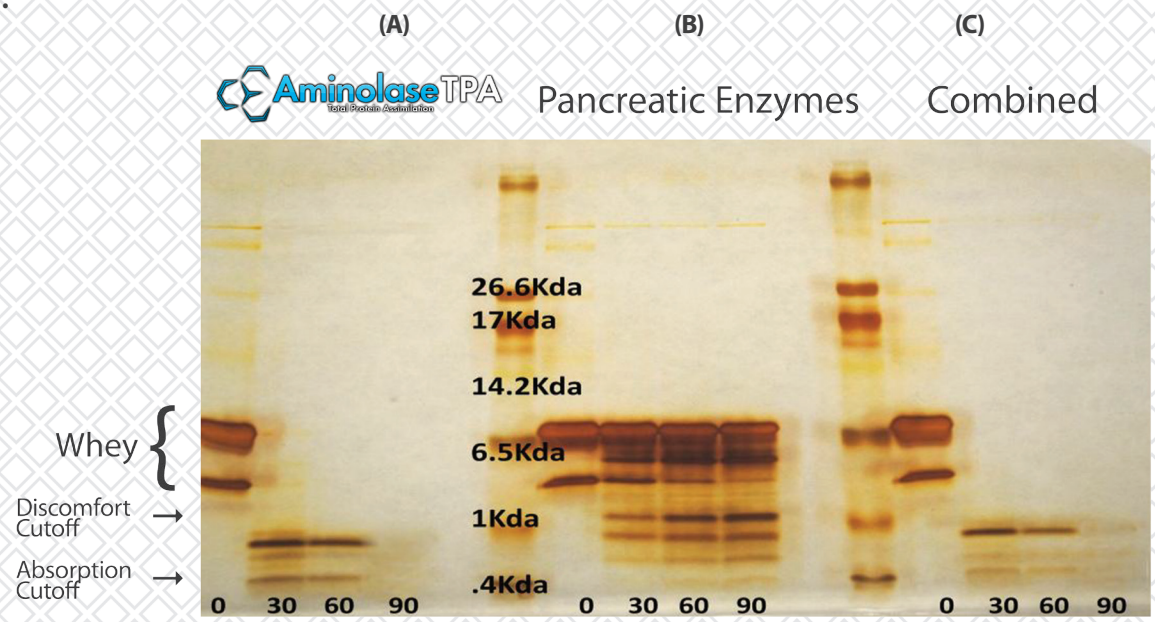
Na = nitrogen absorbed in proteins on the test diet

Nr = nitrogen incorporated into the body on the test diet

However direct measurement of Nr is essentially impossible. While both PDCAA and BV give you a measurement of the nutritional quality of the protein, they have limits to how much of the protein is actually absorbed and used for protein synthesis. The best way to determine if a whey supplement is being processed so your body can use it is by conducting in vitro and in vivo test. The following is an example of the testing of whey protein supplement and whether or not it is processed enough to provide benefit to the user.

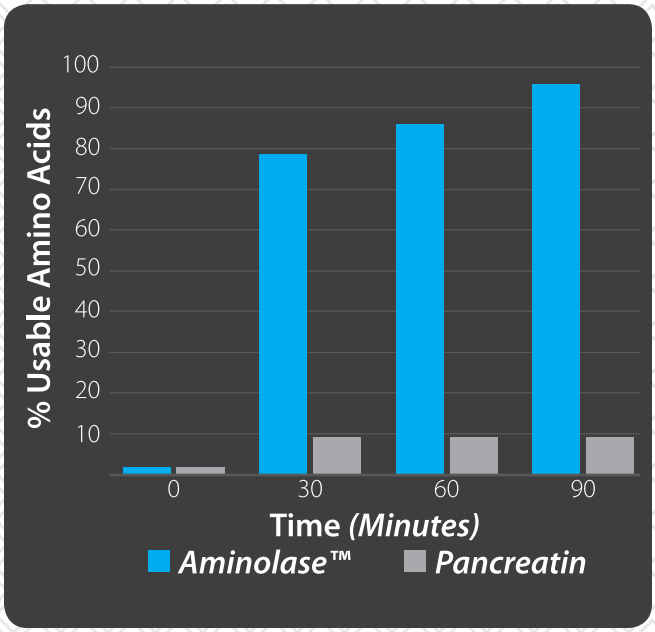
IN VITRO STUDY

Figure 1.



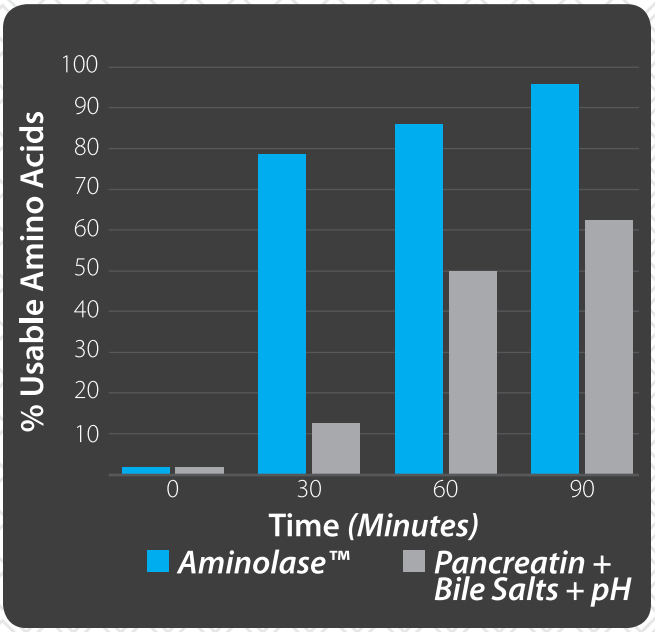
Whey hydrolysis visualized by silver stain gel electrophoresis under USP physiological conditions (37°C, pH6.8)of the **small intestine** *for 90 minutes with either 10mg Aminolase/ g protein or USP pancreatin standard (1%)and a combination of both.*

Figure 2



Whey hydrolysis visualized by silver stain gel electrophoresis densitometry under USP physiological conditions(37°C, pH6.8) of the **small intestine** *for 90 minutes with either 10mg Aminolase/ g protein or USP pancreatin standard (1%).*

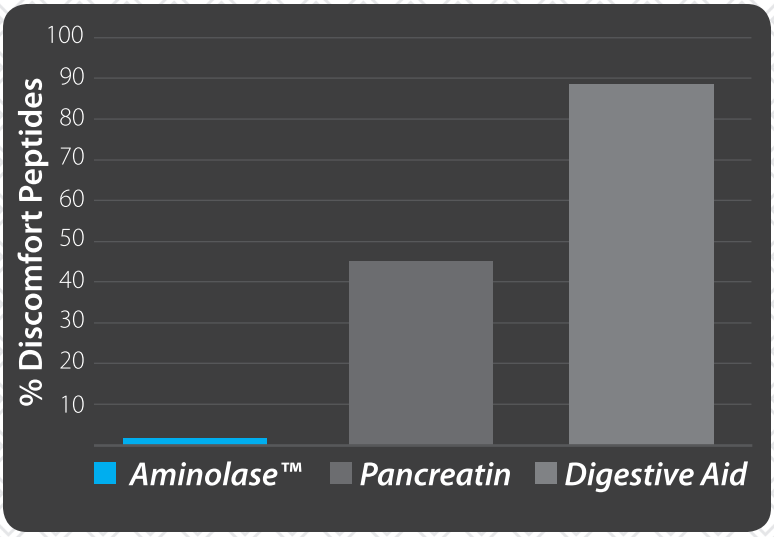
Figure 3.



Whey hydrolysis visualized by silver stain gel electrophoresis densitometry under USP physiological conditions of the **stomach** *(37°C, no pepsin, pH3.0) for 10 minutes and* **small intestine** *(37°C,bile salts, pH6.8) for 90 minutes with either 10mg Aminolase/ g protein or USP pancreatin standard (1%).*

When whey protein is not broken down into the smallest composition, it creates large peptides that can cause discomfort such as bloating, nausea, cramping, pain, etc. When depending solely on the body's own pancreatic enzymes, large peptides can be present for long periods of time in the digestive tract.Under the conditions found in the small intestine in the laboratory, Aminolase™ breaks down these large peptides *(Figure 4)* reducing the potential for discomfort that often comes from consuming large amounts of proteins such as whey. This can be shown in vitro using immunoassays that quantify these peptides down to 1ppm *(Rosendal A 2200-10)*. Whey, hydrolyzed by either Aminolase or Pancreatin and an untreated sports digestive aid alone were examined using this method *(Figure 4)*. The higher the bar, the more "bad" peptides are present.

Figure 4.



Quantifying discomfort peptides in whey after digestion under USP physiological conditions of the stomach (no pepsin, pH3.0) for 10 minutes and small intestine (bile salts, pH6.8) for 90 minutes with either 10mg Aminolase/ g protein or USP pancreatin standard (1%) or digestive aid at their recommended dosage using Veratox kits from Neogen™.

Gel electrophoresis on whey with and without Aminolase™ showed the alpha and beta lacto globulins were being degraded to fragments lower than the 5 amino acid peptide marker, but further analysis was needed to determine if these were di, tri and tetra peptides or individual amino acids. The products of the physiological reactions in the laboratory with whey alone and with Aminolase™ were submitted to Europhins for amino acid analysis. No free amino acids were detectable, indicating that the end products of hydrolysis are in di, tri and tetra peptide form. This observation is important in regards to bioactive peptides produced when whey protein is hydrolyzed the peptides created have been shown to provide benefit to the person *(Yoshikawa 2419-21;Zioudrou 2446-49)*. Many of these bio active peptides have been shown to be in this 2-5 amino acid peptide range *(Antila 215-29) (Table 1)*. Enzymatic digestion of milk proteins represents an important supply of numerous peptides that may have biological activity. The physiological role of these peptides is not yet fully understood. Peptides have been shown to exert beneficial physiological effects. These findings introduce new perspectives in the nutritional and technological evaluation of milk and milk products. These milk peptides may be considered as food additive and perhaps as starting components for some drug developments. It has been shown that some of the biologically active peptides can be released during the in vivo digestion; however more research is needed to fully understand the functional significance of these substances *(Chiba 123-53)*.

Table 1.

Precursor Protein	Fragment	Peptide Sequence	Name	Function
α - lactalbumin	50-53	Tyr-Gly-Leu-Phe	α-lactorphin	opioid agonist, ACE inhibitition
β - lactoglobulin	102-105	Tyr-Leu-Leu-Phe	β lactorphin	non-opioid stimulatory effect on ileum
	142-148	Ala-Leu-Pro-Met-Ile-Arg		ACE-inhibition
	146-149	His-Ile-Arg-Leu	β-lactotensin	ileum contraction

Examples of biologically functional peptides derived from bovine whey proteins

PILOT CLINICAL

Protein must be broken down small enough to pass through the small intestine, absorbed and re-assembled into muscle protein. In order to meet the daily protein intake levels active people require, many turn to protein supplements. These products are usually in the form of protein products rich in essential amino acids (because of cost and concentration) such as whey, soy, and/or egg. Some of these products can have a suggested serving size as high as 50 g of protein, requiring large amounts of processing by the body before they can be absorbed and used for protein synthesis. Protease supplements have been shown to help digest proteins consumed in meals or supplements in the laboratory, the next step is to apply this to human trials. The purpose of this human trial will be to test a protease supplement, Aminolase™, shown in vitro to degrade the essential amino acid rich whey protein, to determine if it significantly increases protein synthesis by increasing the amino acid concentrations in the blood.

PROCEDURES FOR STUDY:

6 healthy, lean, adult, males volunteered for this study. None of the participants were following any particular protein-rich dietary regime, muscle-toning or body building program during the study.

Control Groups

Before the study, all participants reported after an overnight fast for Day 1 of the study. On Day 1, control samples were collected after all participants ingested one 50 g, pre-measured packet of whey protein isolate without Aminolase™. The entire contents of each individual serving packet were emptied into 0.5 L of distilled water, vigorously shaken and consumed. Blood samples were collected at 0 hr *(baseline, immediately prior to ingestion)* 0.5 hr, 1.5 hr, 2.5 hr, 3.5 hr and 4.5 hr. The blood samples were sent to Europhins and tested for amino acids that are essential to the body and those that play a significant role in muscle protein synthesis *(12 totals)*.

Test group

Following 5 days and an over-night fast, the participants returned to the Laboratory for Day 2 of the study. Each participant received 500mg Aminolase™, pre-blended in 50 g whey powder isolate. The entire contents of each individual serving packet contained Aminolase™ was emptied into 0.5 L of distilled water, vigorously shaken and consumed. Blood samples were collected at 0 hr *(baseline, immediately prior to ingestion)* 0.5 hr, 1.5 hr, 2.5 hr, 3.5 hr and 4.5 hr. The blood samples were sent to Europhins and tested for 12 amino acids that are essential to the body and those that play a significant role in muscle protein synthesis. Following the study, the control data from each participant was compared to the data from the corresponding patient in either of the two test groups.

Sample collection

Whole blood samples *(approximately 5 mL)* were collected by a phlebotomist from multiple venous punctures, and transferred to plain Vacutainer ® tubes. Serum was prepared by centrifugation and stored in 200 µl aliquots at -20°C until needed for analyses.

Analytical analyses

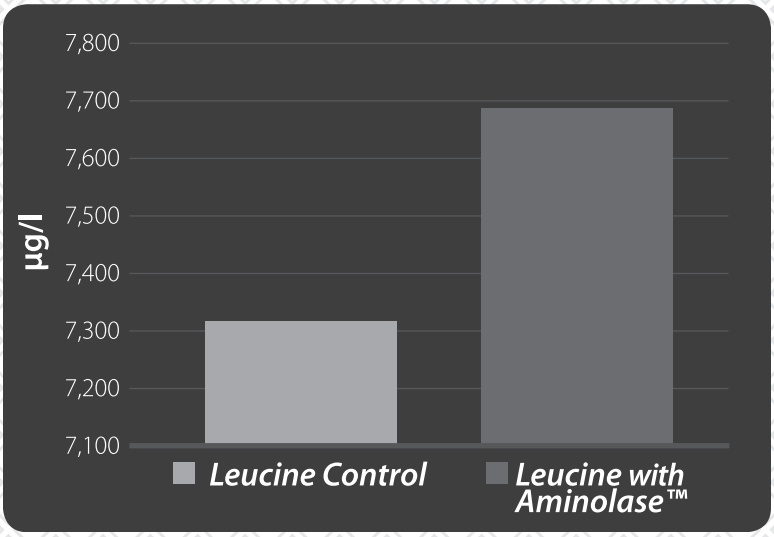
All serum samples were submitted to the laboratory blind to remove any analytical bias. Amino acid analyses consist of quantification of twelve individual serum amino acids for each patient at each time point. Amino acid analyses consisted of quantification of twelve individual serum amino acids for each patient over the 270 minute time period. Analyses were performed on an AA analyzer using ion exchange chromatography and a post column derivatazation with ninhydrin and UV detection.

Results

Amino acid levels *(µg/L)* in the blood at various time points after consumption of 50g of whey protein with 500 mg of Aminolase over a 4.5 hour time period showed a profound increase in these amino acids. The first is the branch chain amino acids *(BCAA)*. BCAA is a combination of essential branched chain amino acids such as leucine, isoleucine, and valine that are of special importance for bodybuilders and athletes because these amino acids are used by the body to build up protein for muscle synthesis, muscle repair, etc *(Eva Blomstrand 269-73)*. Because BCAA's are essential amino acids, not produced by the body, they must be acquired from the diet or from nutritional supplements. BCAA is metabolized in the muscle rather than the liver; consequently the effect of these branched chain amino acids is much quicker and efficient than of any other amino acid. After BCAAs are digested, protein breaks down into individual amino acids that can either be used to build new proteins or used as energy for the body. If the diet is balanced, branched chain amino acids will be used for protein synthesis, essential for endurance athletes and strenuous workouts. BCAA's are also used to reduce fatigue in both anaerobic and endurance sports. Because of its anticatabolic properties and vital role in protein synthesis, leucine is considered to be one of the most critical BCAAs.

Figure 5.

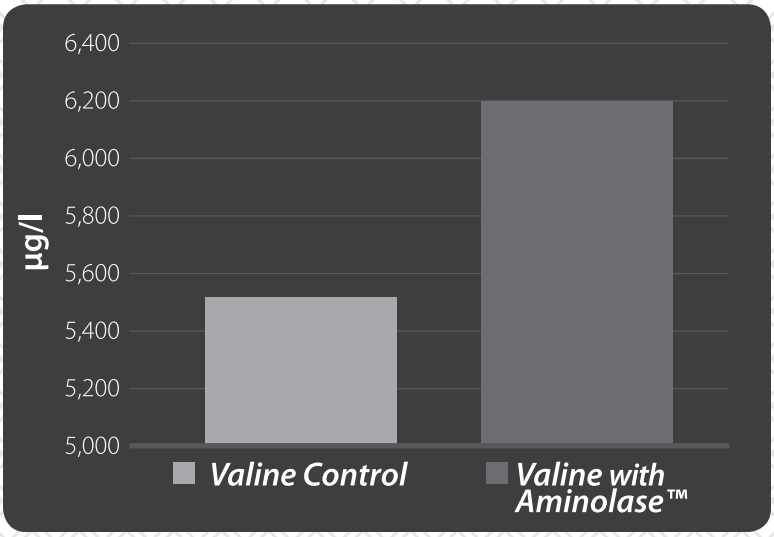
<u>Leucine</u> *(The strongest of the BCAAs is responsible for the regulation of blood-sugar levels, the growth and repair of tissues in skin, bones and of course skeletal muscle. It's a strong potentiator to Human Growth Hormone. It helps in healing wounds, regulating energy and assists in the preventing the breakdown of muscle tissue.)* **increased by 5%**.



Total Leucine levels (µg/L) in the blood after consumption of 50g of whey protein over a 4.5 hour time period.

Figure 6.

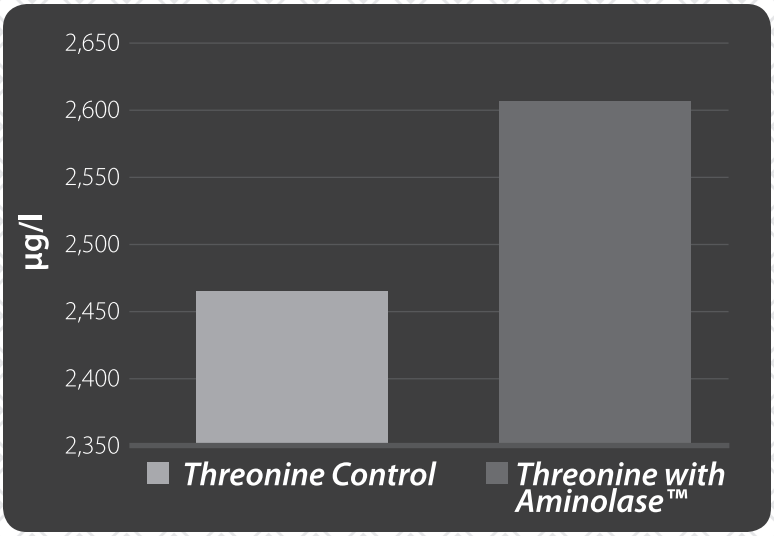
<u>Valine</u> *(Repair and growth of muscle tissue yet again, as commonly attributed to BCAAs. Not processed by the liver; rather actively taken up by muscle. It maintains the nitrogen balance and preserves the use of glucose.)* **increased by 12%**.



Total Valine levels (µg/L) in the blood after consumption of 50g of whey protein over a 4.5 hour time period.

Figure 7.

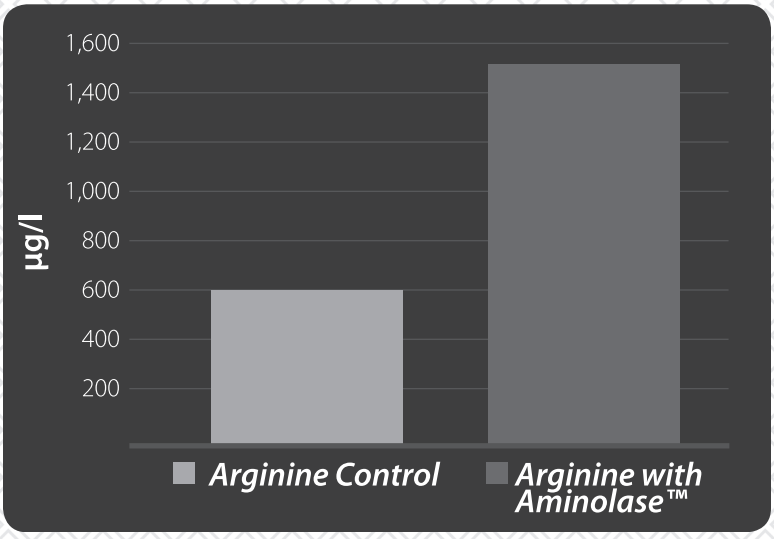
Threonine *(Essential amino acid that is not manufactured within the body, ever. Since its main sources are animal (dairy and meat) this doesn't bode well to vegans. It's found in heart, skeletal muscle and nerve tissue in the central nervous system. Threonine is used to form the body's two most important binding substances, collagen and elastin. Threonine is involved in liver functioning, lipotropic functions (when combined with aspartic acid and methionine) and in the maintenance of the immune system by helping in the production of antibodies and promoting growth and activity of the thymus. But perhaps its most useful property of all is that it allows better absorption of other nutrients, so protein sources containing threonine are more bio-available than others.)* **increased by 6%**.



Total Theronine levels (µg/L) in the blood after consumption of 50g of whey protein over a 4.5 hour time period.

Figure 8.

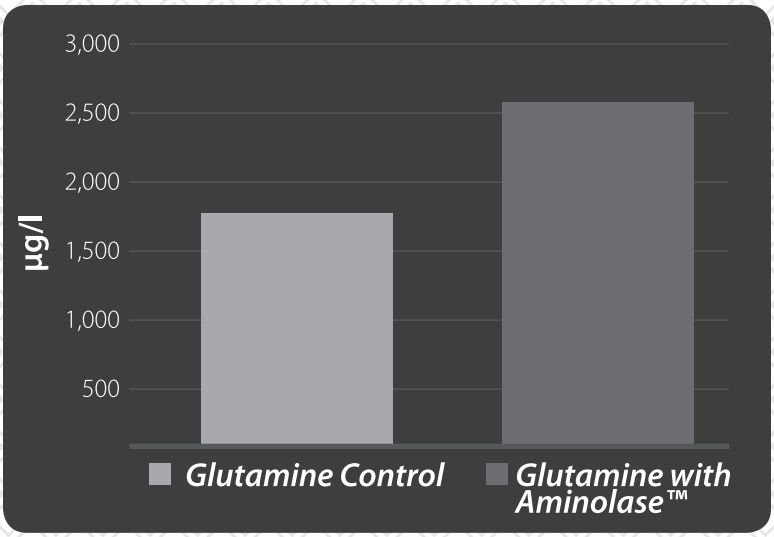
Arginine. *(Arginine has amazing nitrogen retention ability. Nitrogen is one of the key elements in muscle protein synthesis. Some plants can absorb nitrogen, but we mammals have to make do with the stuff we make ourselves. It enhances the immune system, stimulates the size and activity of the Thymus gland which makes it a prime choice for anyone in a condition that is less than optimal for health, such as people recovering from injury and HIV patients. Arginine is also a precursor of very important molecules such as creatine and gamma amino butric acid (GABA, a neurotransmitter in the brain). The hormonal release properties include releasing insulin from the pancreas and a massive stimulator in the manufacture of GH (Growth Hormone) from the anterior pituitary. It increases blood flow. It also improves the health of the liver, skin and connective tissues and may lower cholesterol. But mostly it facilitates muscle mass gain while limiting fat storage, because it keeps fat alive in the system and uses it. It's key in weight control.)* **increased by 150%**. A huge increase, greater than 2.5 times the level obtained from using whey isolate alone.



Total Arginine levels (µg/L) in the blood after consumption of 50g of whey protein over a 4.5 hour time period.

Figure 9.

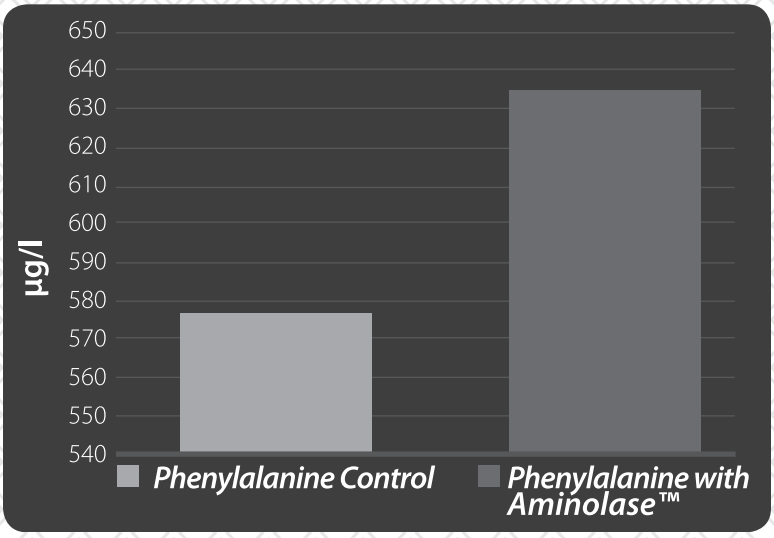
Glutamine *(Glutamine is a non-essential amino acid that is present in the body in large amounts. At some times it forms 60 percent of your total amino acid pool. Because it passes through the blood-brain barrier rather easily it's often called brain-food. In the brain it converts to glutamic acid, which is essential for brain functioning and increase GABA (gamma-amino-butyric-acid, another popular supplemented amino) needed for mental activities. It is used in synthesis of muscle-tissue. It is a nontoxic nitrogen carrier. Most important perhaps is that it balances the acid/alkaline level, so it reduces lactic acid.)* **increased by 45%**.



Total Glutamine levels (µg/L) in the blood after consumption of 50g of whey protein over a 4.5 hour time period.

Figure 10.

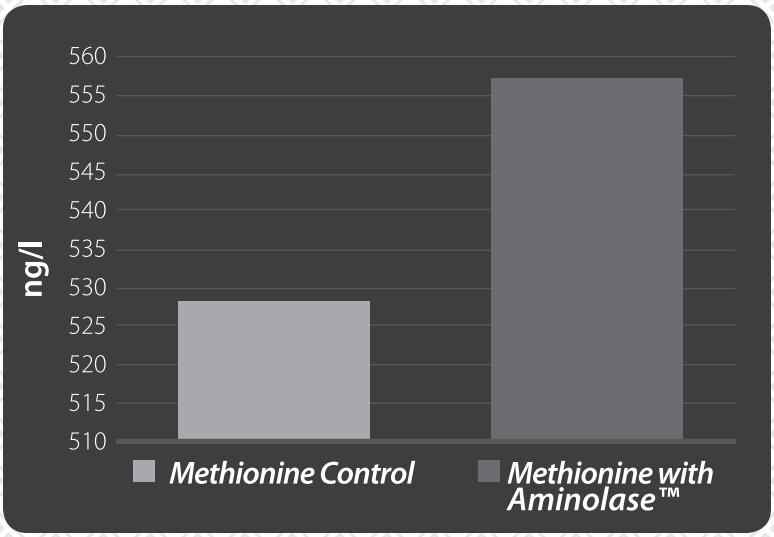
Phenylalanine (The major precursor of tyrosine, enhances learning, memory, mood and alertness. Is a major element in the production of collagen and suppresses appetite.) **increased by 10%**.



Total Phenylalanine levels (µg/L) in the blood after consumption of 50g of whey protein over a 4.5 hour time period.

Figure 11.

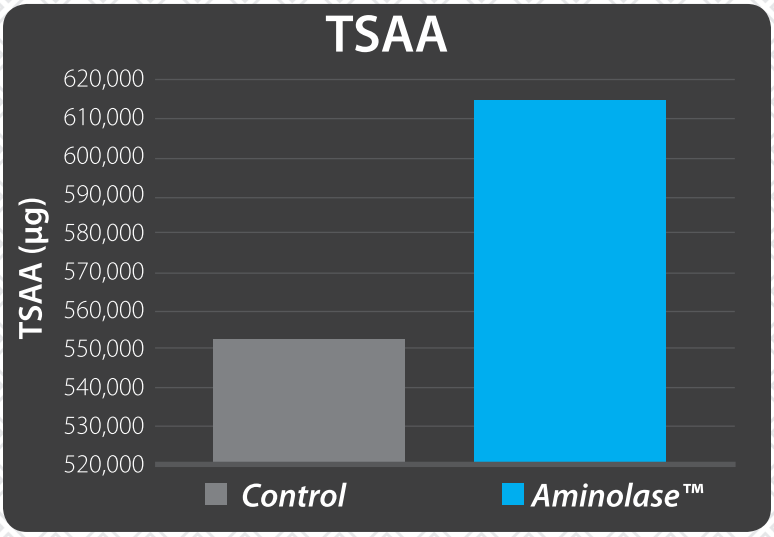
Methionine (Precursor of cystine and creatine, may increase antioxidant levels (glutathione) and reduce blood cholesterol levels and helps remove toxic wastes from the liver and assists in the regeneration of liver and kidney tissue) **increased by 6%.**



Total Methionine levels (µg/L) in the blood after consumption of 50g of whey protein over a 4.5 hour time period.

Figure 12.

Total amino acid concentrations **went up 11%** in the blood over the 270 minutes after ingestion of whey protein isolate with Aminolase when compared to taking whey protein isolate alone.



Total Amino acid levels (µg) in the blood after consumption of 50g of whey protein over a 4.5 hour time period.

Peptide discomfort is caused by the presence of specific sequences of amino acids, called epitopes, in the native protein. Epitopes are areas on the protein surface which are recognized by the immune system and identified as having between 8 – 16 amino acids. It is thought that a discomfort peptide has a minimum of 14 amino acid residues. Trypsin digestion of ß lactoglobulin has shown that there are many epitopes spread over its surface. The molecular weight of a protein also determines whether it will act as a potential discomfort material or not. Potential discomfort material consists of components with the ability to stimulate antibody production and a component with at least two antibody binding sites. The probability that a component will meet these criteria increases above a molecular weight of 3,000 Daltons *(Rosendal A 2200-10)*. In general, the lower the molecular weight of the protein, the lower its discomfort potential. This means a reduction in the likelihood of discomfort effects. Five out of the six participants complained of discomfort when taking 50 grams of whey isolate during the control period, while no one felt any discomfort while taking 50 grams of whey with Aminolase in the test period. These results indicate that Aminolase is able to hydrolyze the peptides in whey that cause discomfort.

Conclusions:

Aminolase helps break down whey protein efficiently and completely so that the body can maximize the absorption of amino acids into the blood stream and reduce the exposure time to whey peptides that can cause discomfort. Consumption of whey with Aminolase significantly raised the level of amino acids in the blood compared with whey isolate alone. Among those amino acids are the branch chain amino acids, which have been shown to play a vital role muscle synthesis and recovery *(Borsheim E648-E657)*.

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