MONKEY ASSIMILATE
STUDY 1

PROCEDURES FOR STUDY
Six 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 50g, pre-measured packet of whey protein isolate without Monkey Assimilate (ProHydrolase™). 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 total).

TEST GROUP
Following five days and an overnight fast, the participants returned to the laboratory for Day 2 of the study. Each participant received 500mg Monkey Assimilate (ProHydrolase™), pre-blended in 50g whey powder isolate. The entire contents of each individual serving packet containing Monkey Assimilate (ProHydrolase™) 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 twelve 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.

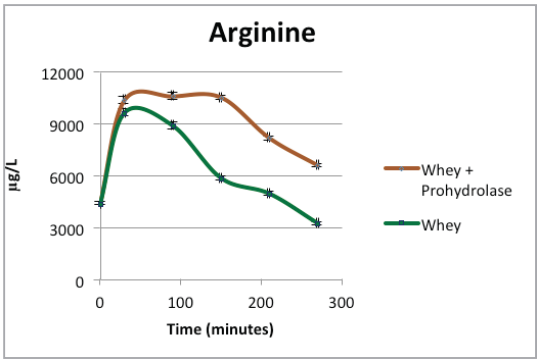
ANALYTICAL ANALYSES
All serum samples were submitted to the laboratory blind to remove any analytical bias. Amino acid analyses consist of quantification of twelve individual serum amino acids for each patient at each time point. Amino acid analyses consisted of quantification of twelve individual serum 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 increased greatly from 0 hr and the first time period taken (0.5 hr) and were fluid on return to baseline between control and test group (Figure 1). The shape of the curve and the quantity of leucine in the blood correlates well with leucine blood levels when taking whey hydrolysates (Tang JE 987-92). This is not a surprise since whey hydrolysate is protease treated to very small peptides, and then dried vs. whey protein that is hydrolyzed by Monkey Assimilate (ProHydrolase) at the point of consumption. This required the amino acid quantity to be calculated from area under the curve (AUC) over the entire time period and compared (270 minutes) instead of individual time points. Quantification was done versus reference standard mixtures and control mixtures of known quantities of all twelve amino acids (Sigma, St. Louis, MO). Amino acid levels are reported in µg/L. Total Serum Amino Acids (TSAA) levels were reported as the total sum of all twelve amino acids. The percent AUC was reported as the amount each amino acid contributed to the total. AUC was calculated using the KaleidaGraph software (Synergy Software, Reading, PA). Most of the samples were bell shape curves, with the lower Y limit set at the time 0 value. Results are expressed as means ± SEM.

FIGURE 5.

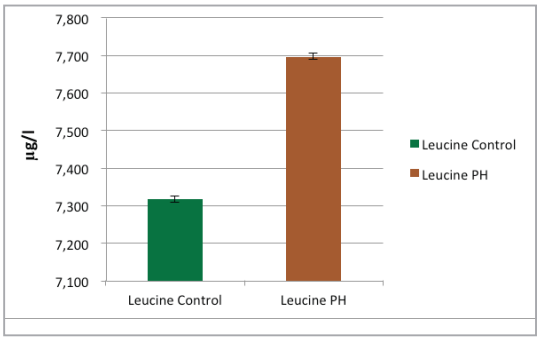
Monkey Assimilate (ProHydrolase™) had a profound increase in these amino acids. The first is the branch chain amino acids (BCAA). BCAA is a combination of essential branched chain amino acids such as leucine, isoleucine, and valine that are of special importance for bodybuilders and athletes because these amino acids are used by the body to build up protein for muscle synthesis, muscle repair, etc (Eva Blomstrand 269-73). Because BCAAs 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. BCAAs 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.



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

FIGURE 6.

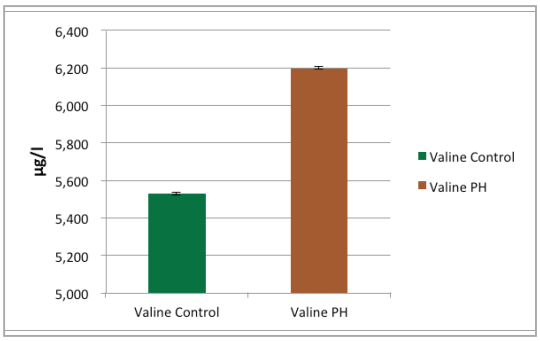
Leucine is the strongest of the BCAAs and is responsible for the regulation of blood-sugar levels, the growth and repair of tissues in skin, bones and of course skeletal muscle. It's a strong potentiator to Human Growth Hormone. It helps in healing wounds, regulating energy and assists in the preventing the breakdown of muscle tissue. Levels 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 7.

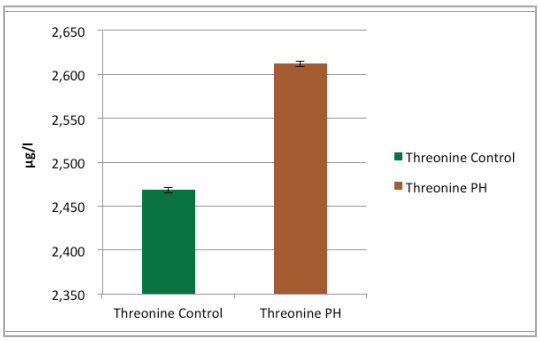
Valine contributes to repair and growth of muscle tissue, as commonly attributed to BCAAs. It is not processed by the liver; rather actively taken up by muscle. It maintains the nitrogen balance and preserves the use of glucose. Levels increased by 11% .



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

FIGURE 8.

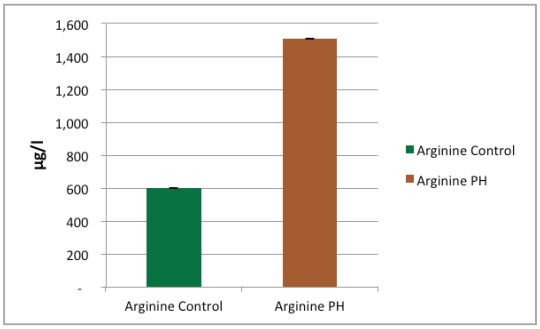
Threonine is an essential amino acid that is never manufactured within the body. Since its main sources are animal (dairy and meat) this doesn't bode well to vegans. It's found in heart, skeletal muscle and nerve tissue in the central nervous system. Threonine is used to form the body's two most important 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. Perhaps its most useful property is that it allows better absorption of other nutrients; therefore protein sources containing threonine are more bioavailable than others. Levels increased by 6%.



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

FIGURE 9.

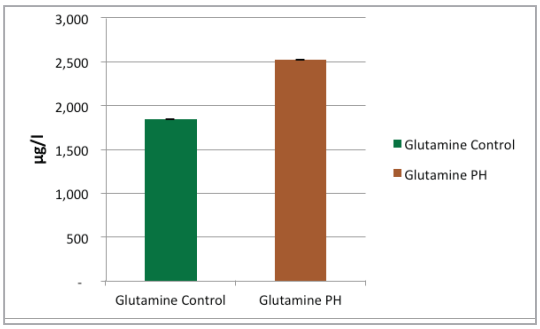
Arginine has amazing nitrogen retention ability. Nitrogen is one of the key elements in muscle protein synthesis. Some plants can absorb nitrogen, but mammals have to make do with what we make ourselves. Arginine enhances the immune system and stimulates the size and activity of the Thymus gland, which makes it a prime choice for anyone in less than optimal 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. Levels increased by 60%, more 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 10.

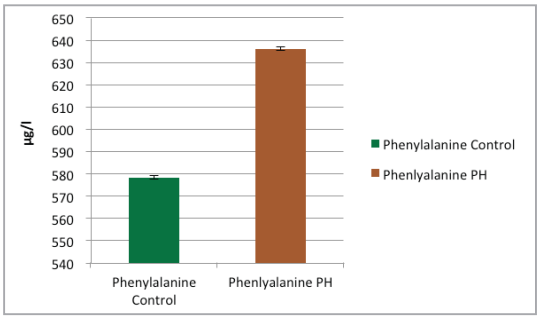
Glutamine is a non-essential amino acid that is present in the body in large amounts. At some times it forms 60 percent of the total amino acid pool. Because it passes through the blood-brain barrier rather easily, it's often called "brain-food". In the brain it converts to glutamic acid, which is essential 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.Perhaps most importantly, it balances the acid/alkaline level, so it reduces lactic acid. Levels increased by 28%.



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

FIGURE 11.

Phenylalanine, the major precursor of tyrosine, enhances learning, memory, mood and alertness. Is a major element in the production of collagen and suppresses appetite. Levels increased by 9%.



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

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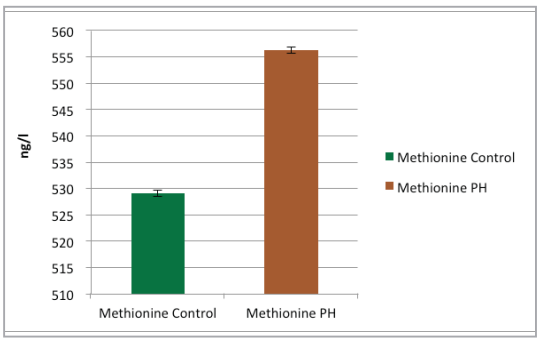
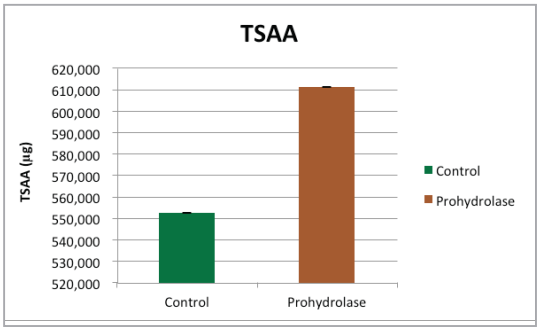
FIGURE 12.

Methionine is the precursor of cystine and creatine, and may increase antioxidant levels (glutathione) and reduce blood cholesterol levels. It also helps remove toxic wastes from the liver and assists in the regeneration of liver and kidney tissue. Levels 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 13.
Total amino acid concentrations increased 60mg in the blood over the 270 minutes after ingestion of whey protein isolate with Monkey Assimilate (ProHydrolase™) when compared to taking whey protein isolate alone. This correlates well with published results showing amino acids increases when taking whey hydrolysate vs. whey protein unprocessed.



Total Amino acid levels(µg/L) 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 β-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 potentially 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 Da ltons(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 when taking 50 grams of whey with Monkey Assimilate (ProHydrolase™) in the test period. These results indicate that Monkey Assimilate (ProHydrolase™) is able to hydrolyze the peptides in whey that cause discomfort, consistent with the Veratox in vitro results in the laboratory.

CONCLUSIONS

The amount of amino acids found in the blood with whey and the increase with whey/ Monkey Assimilate (ProHydrolase™) is consistent with the study and benefits of whey hydrolysate (Koopman 106-15). Monkey Assimilate (ProHydrolase™) 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 Monkey Assimilate (ProHydrolase™) 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). Based on the success of this clinical study, preparation for an advanced clinical to further investigate the benefits of Monkey Assimilate (ProHydrolase™)/WHEY is underway.