



## ProHydrolase® Clinical Phase II Study

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## ProHydrolase® Clinical—Phase II Study

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# ProHydrolase® Clinical—Phase II Study

## Introduction

Whey protein supplementation is a common practice to promote muscle building and muscle recovery for people with an active lifestyle (body-builders, fitness enthusiasts), as well as others who experience muscle wasting due to disease or treatment of disease. Whey protein supplementation has been shown to increase muscle protein synthesis and reduce muscle protein degradation.

When whey protein is digested, it's broken down in the digestive tract into small peptides, and eventually into amino acids that will be absorbed in the intestines. To be effective, whey protein must be broken down into a smaller particle size within approximately 90 minutes of consumption; otherwise, the protein goes undigested and excreted from the body. During digestion, the larger peptides composed of more than seven amino acids may trigger an immune response, causing discomfort and even inflammation in the gut.

A proprietary digestive enzyme formulation has been developed to be consumed in conjunction with a whey protein supplement in order to provide some pre-digestion of the protein, in order to take full advantage of the availability of the essential amino acids for building muscle and improving muscle recovery. The pre-digestion will also ensure that smaller, non-immunogenic peptides will be formed, therefore reducing the potential for discomfort that is often associated with protein consumption.

## Purpose of the Study

The purpose of this study is to determine if the multi-enzyme formulation (ProHydrolase®) combined with whey protein results in improved digestion and absorption of amino acids along with reduction of the immunogenic responses associated with whey protein consumption, compared to whey protein alone.

## Procedure

Whey protein supplements were formulated with and without ProHydrolase, tested and distributed by a third party to 20 test subjects with the following common characteristics:

- Ages 19-35 years
- Normal body weight (BMI = 18.5-25)
- No known food allergies or intolerance
- Non-weight training subjects who agree not to begin a new exercise program during the course of the study

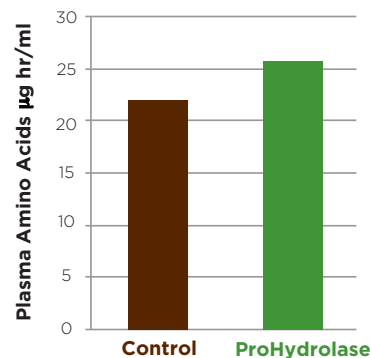
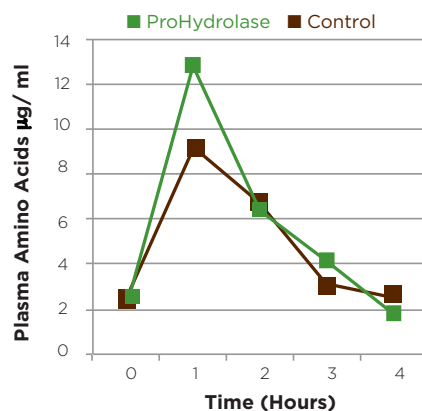
Two trials were completed by each test subject, one using whey protein alone and one with the addition of ProHydrolase. Each trial occurred at the end of a nine-day period in which subjects consumed a specific diet of approximately 2200 kcal; 40% carbohydrate, 25% protein, 35% fat. Prior to each of the nine pretrial days, subjects were given a specific meal. On the day of each trial, subjects were tested following a 12 hour fast. A catheter was placed in the arm or hand vein and seven 5 ml blood samples were drawn at time 0 (before consumption), 1, 2, 3, and 4 hours following consumption of the supplement. Blood samples were used to measure levels of amino acids, C-reactive protein, and insulin.



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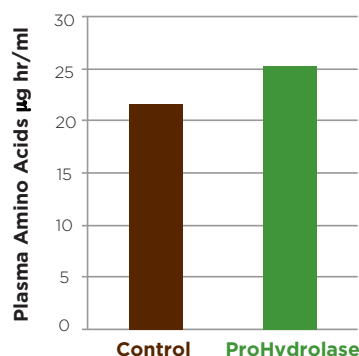
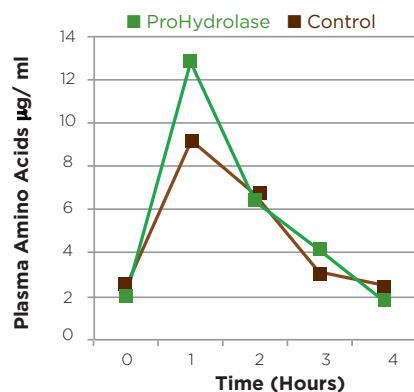
## Amino Acids Analysis - Results

Individual Time Points      Area Under the Curve  
Leucine



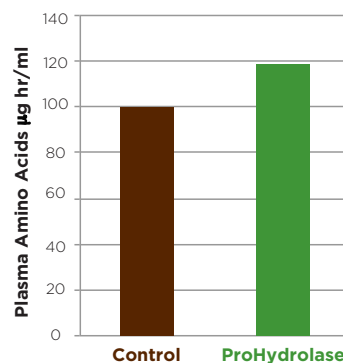
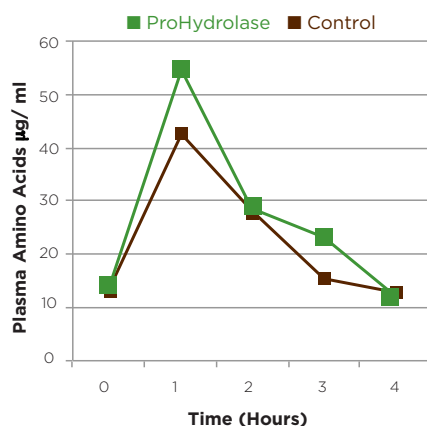
**Leucine** is the strongest of the branched chain amino acids (BCAAs), and is responsible for the regulation of blood-sugar levels, the growth and repair of tissues in skin, bones and skeletal muscle. It's a strong potentiator to Human Growth Hormone. It helps in healing wounds, regulating energy and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

Isoleucine



**Isoleucine** is a BCAA that promotes muscle recovery after physical exercise. Alone, it's needed for the formation of hemoglobin as well as assisting with regulation of blood sugar levels and energy levels. It's also involved in blood-clot formation.

Valine



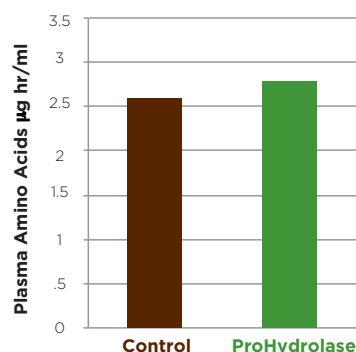
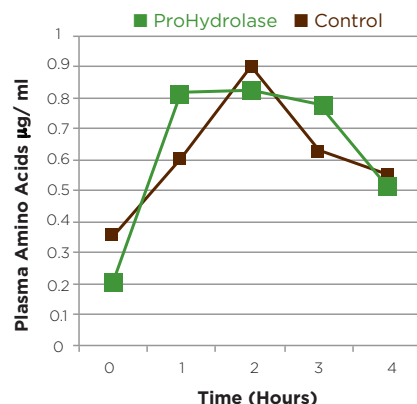
**Valine** assists in the repair and growth of muscle tissue, as commonly attributed to BCAAs. It's not processed by the liver; rather actively taken up by muscle. It maintains the nitrogen balance and preserves the use of glucose.



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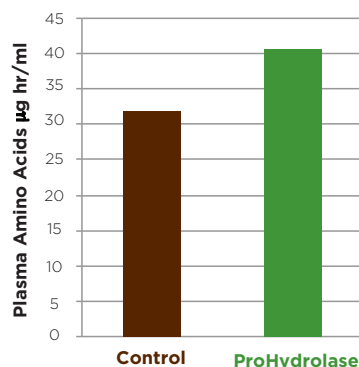
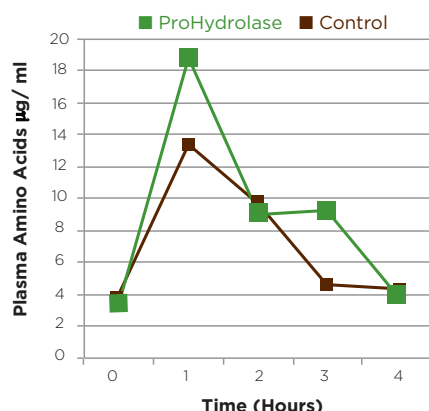
## Amino Acids Analysis - Results

Individual Time Points      Area Under the Curve  
Aspartate



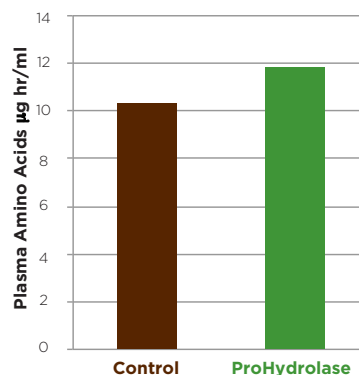
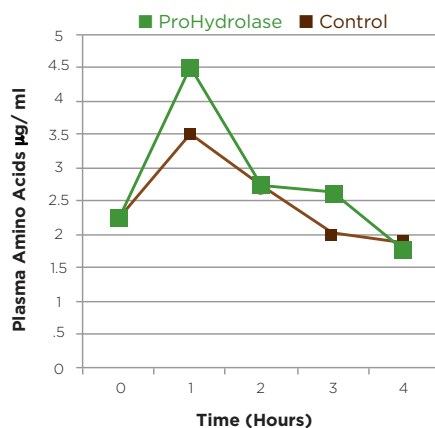
**Aspartate** is used to increase absorption of the minerals with which they combine, and to enhance athletic performance. It's needed for stamina, brain and neural health and assists the liver by removing excess ammonia and other toxins from the bloodstream. It is also very important in the functioning of RNA, DNA, as well as the production of immunoglobulin and antibody synthesis.

Glutamate



**Glutamate** is the most common excitatory (stimulating) neurotransmitter in the central nervous system. More than 50% of the amino acid composition of the brain is derived from glutamic acid and its derivatives, providing fuel for the brain. Glutamic acid acts as carrier for potassium across the brain/blood barrier. Glutamic acid is instrumental in the metabolism of other amino acids as well as sugars and fats.

Cysteine



**Cysteine** is required in the manufacture of the amino acid taurine and is a component of the antioxidant glutathione. It helps remove harmful toxins from the body and protect the brain and liver from damage from alcohol, drugs etc. Research has shown that it may help in strengthening the protective lining of the stomach as well as intestines, which may help prevent damage caused by aspirin and similar drugs. Cysteine is also critical to the metabolism of a number of essential biochemicals including coenzyme A, heparin, biotin, lipoic acid, and glutathione.

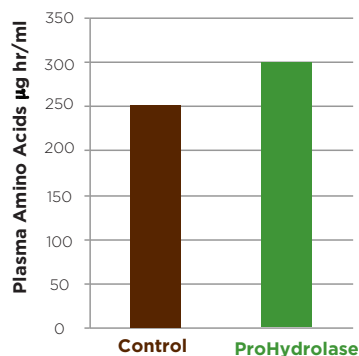
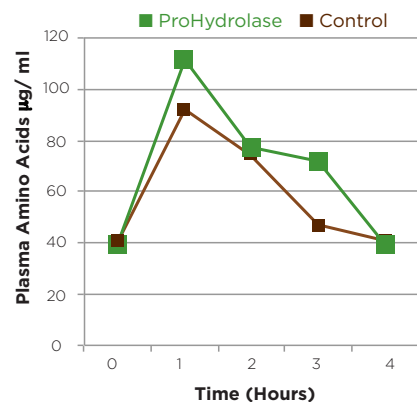


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## Amino Acids Analysis – Results

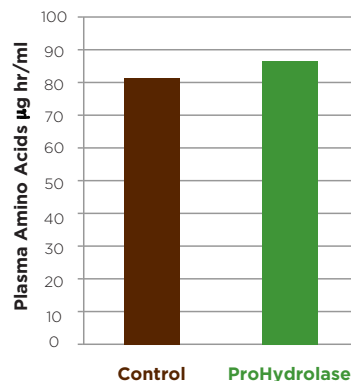
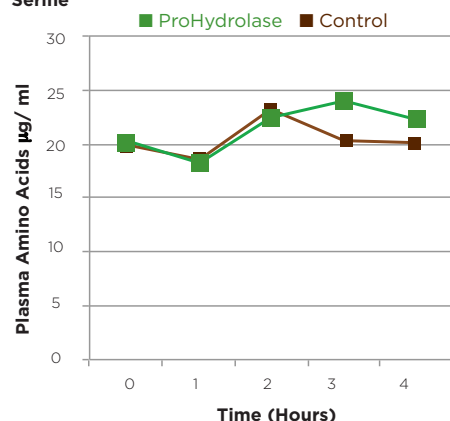
Individual Time Points      Area Under the Curve

Lysine



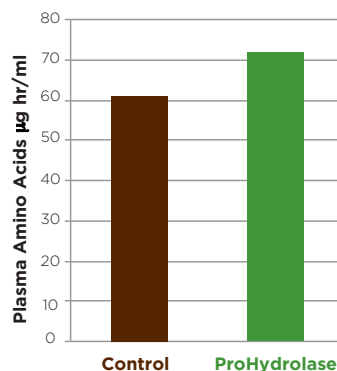
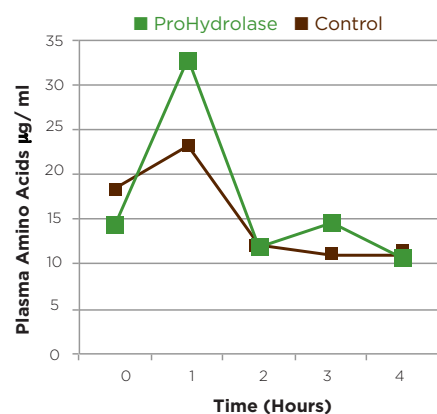
**Lysine** is an essential amino acid, important for proper growth. It plays an essential role in the production of carnitine, a nutrient responsible for converting fatty acids into energy and helping to lower cholesterol. Lysine helps the body absorb calcium, and it plays an important role in the formation of collagen, a substance important for bones and connective tissues including skin, tendon, and cartilage.

Serine



**Serine** helps produce immunoglobulins and antibodies for a strong immune system, and also aids in the absorption of creatine, a substance made from amino acids that helps build and maintain all the muscles in the body, including the heart.

Histidine

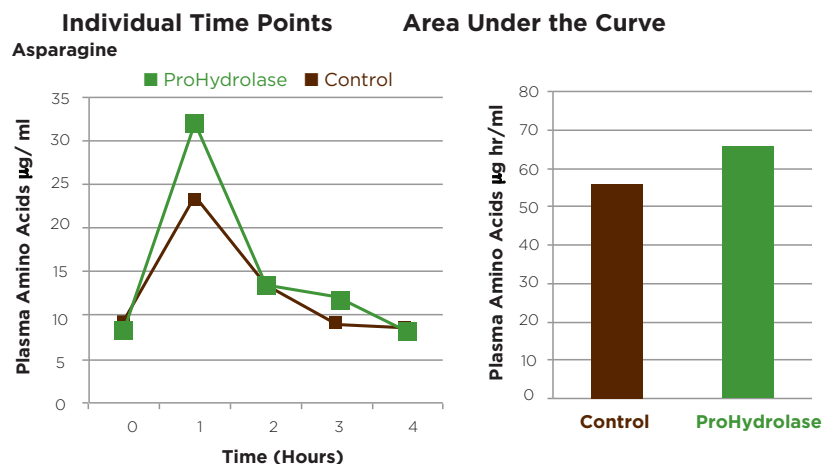


**Histidine** is needed for growth and for the repair of tissue, as well as the maintenance of the myelin sheaths that act as a protector for nerve cells. It's required for the manufacture of both red and white blood cells, and helps to protect the body from damage caused by radiation and in removing heavy metals from the body.

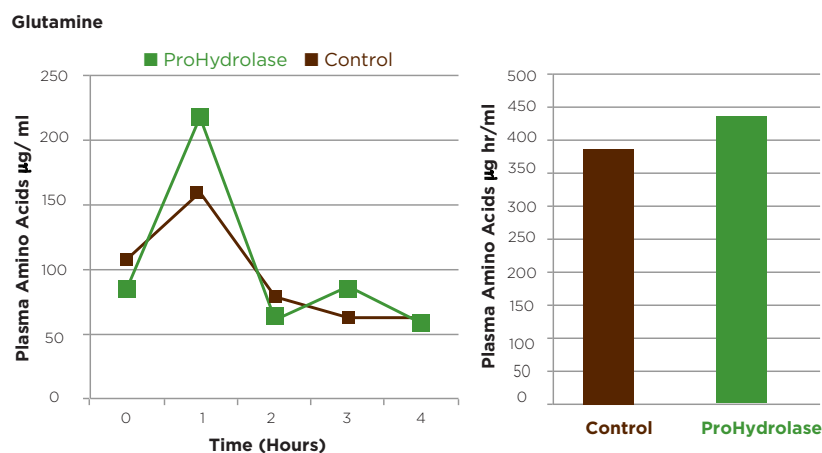


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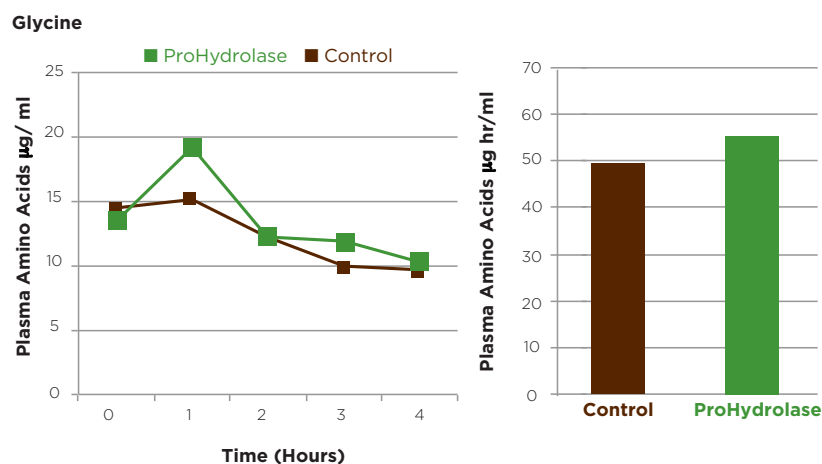
## Amino Acids Analysis - Results



**Asparagine** is vital in nerve and brain health and an important building block for proteins and muscle mass. It's an important aspect of metabolism, because it aids in the removal of toxic ammonia from the body.



**Glutamine** is produced in the muscles and distributed by the blood to the organs that need it. Glutamine may help gut function, the immune system, and other essential processes in the body, especially in times of stress. It is also important for providing “fuel” (nitrogen and carbon) to many different cells in the body. Glutamine is needed to make other chemicals in the body such as other amino acids and glucose (sugar).



**Glycine** functions as a neurotransmitter and a precursor to metabolic intermediates such as purines, which are components of DNA. Glycine increases GH production, removing unwanted substances from the body (i.e. lactic acid), enhancing energy levels and calming the brain.



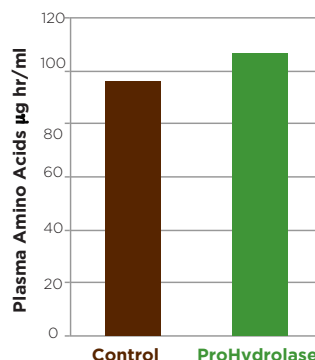
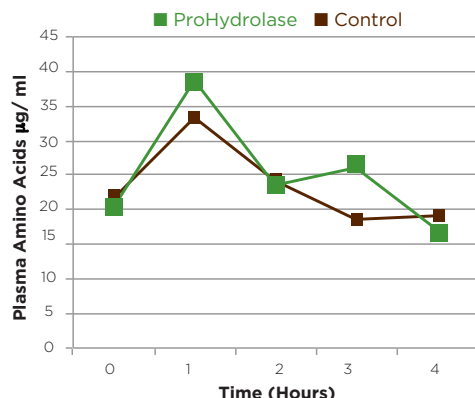
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## Amino Acids Analysis – Results

### Individual Time Points

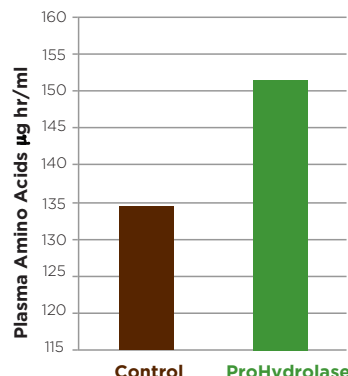
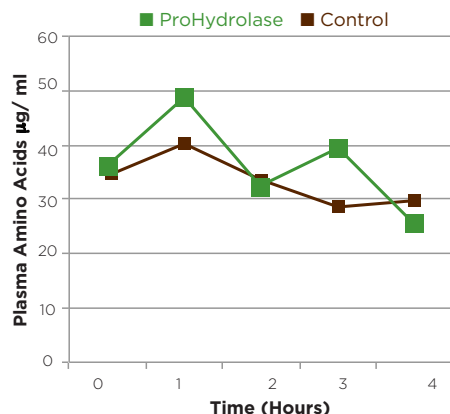
### Area Under the Curve

#### Threonine



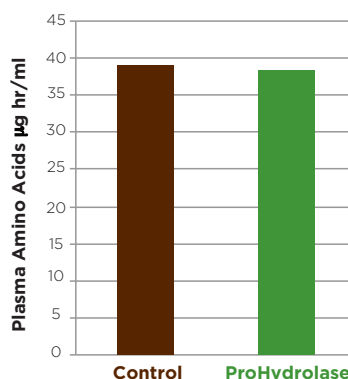
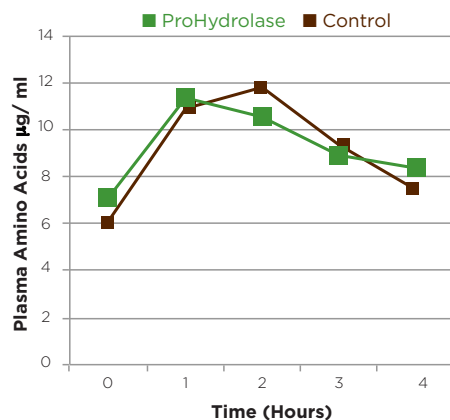
**Threonine** is an essential amino acid that is never manufactured within the body. Its main sources are animal (dairy and meat), which presents a challenge to those on a vegan diet. It's found in heart, skeletal muscle and nerve tissue in the central nervous system. Threonine is used to form the body's two most important binding substances, collagen and elastin. It's involved in liver function, 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. Arguably its most useful property, it allows better absorption of other nutrients, making protein sources containing threonine more bio-available than others.

#### Alanine



**Alanine** is an amino acid that helps the body convert the simple sugar glucose into energy and eliminate excess toxins from the liver. Alanine has been shown to help protect cells from being damaged during intense aerobic activity, when the body cannibalizes muscle protein to help produce energy.

#### Tyrosine



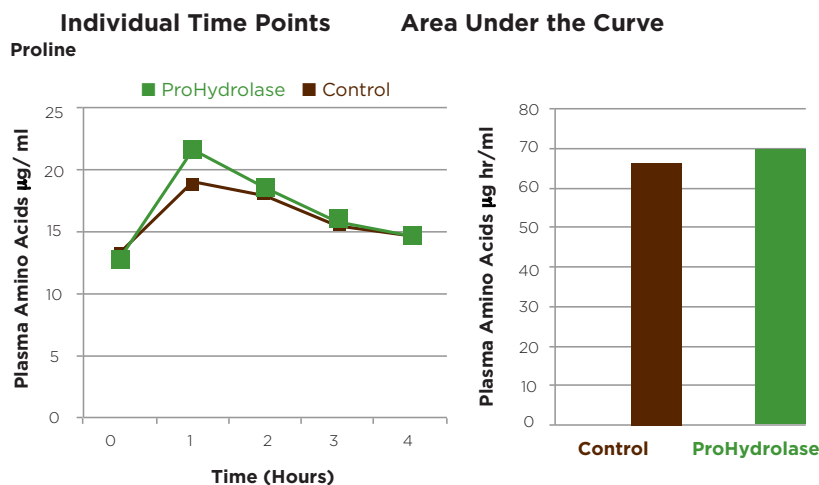
**Tyrosine** initiates and propels the development of neurotransmitters and hormones that work to keep the mind sharp. It's also the precursor of several neurotransmitters, including L-dopa, dopamine, norepinephrine, and epinephrine.





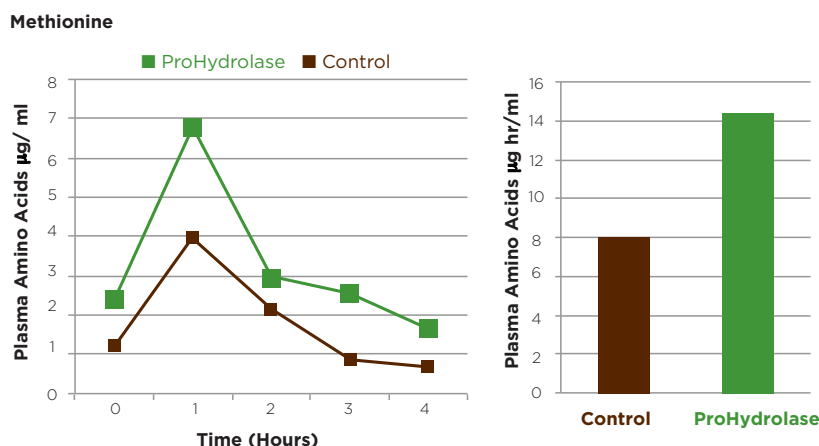
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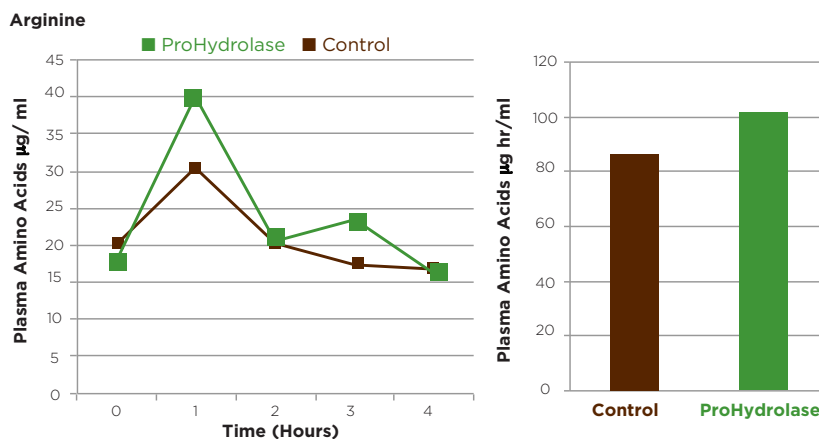


**Proline** is needed for the production of collagen and cartilage. It keeps muscles and joints flexible and helps reduce sagging and wrinkling that accompany UV exposure and normal aging of the skin.

**Methionine** is an essential sulfur amino acid, which must be obtained from food or supplement sources. L-methionine contributes to the synthesis of S-adenosyl-L-methionine (S-AdoMet), which may elevate mood and support joint health, and L-cysteine, which is a component of glutathione, an important antioxidant molecule in the body. Methionine is also a transporter of the antioxidant mineral, selenium.



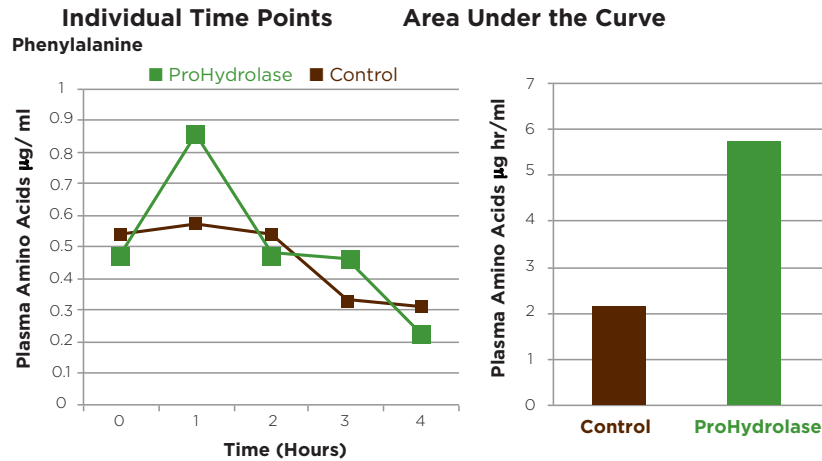
**Arginine** has remarkable nitrogen retention ability. Nitrogen is one of the key elements in muscle protein synthesis. It enhances the immune system and stimulates the size and activity of the thymus gland, making it a prime choice for anyone in less than optimal health, such as those recovering from injury or HIV patients. Arginine is also a precursor of very important molecules such as creatine and gamma amino butyric 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. Primarily, 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.



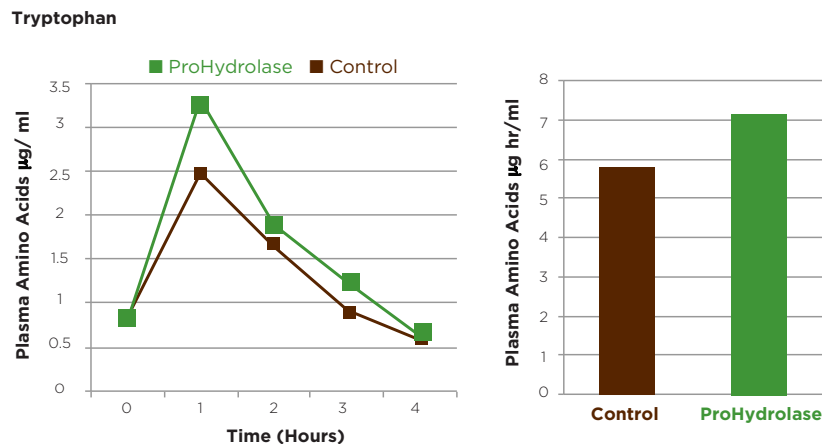


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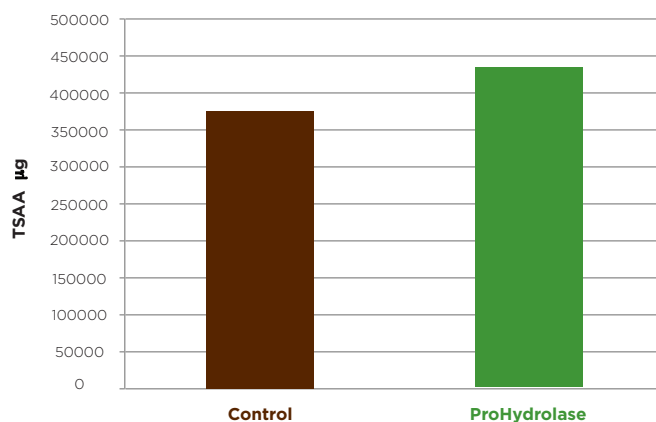


**Phenylalanine** is the essential building block of new cells that help to restore damaged cells. It helps to build up tissues in muscles.



**Tryptophan** is an essential amino acid that helps support mood, relaxation, and restful sleep. It plays a part in the synthesis of both melatonin and serotonin, hormones involved with mood and stress response. It also supports immune functions, as the body's precursor to the kynurenes that regulate immunity.

### Total Serum Amino Acids



Total amino acid concentrations increased 55mg in the blood over the 270 minutes after ingestion of whey protein isolate with ProHydrolase when compared to taking whey protein isolate alone. This is a 20% increase in amino acids when whey isolate is combined with ProHydrolase vs. consuming whey isolate alone. This correlates with published results showing the increase of amino acids when taking whey hydrolysate vs. whey protein unprocessed.



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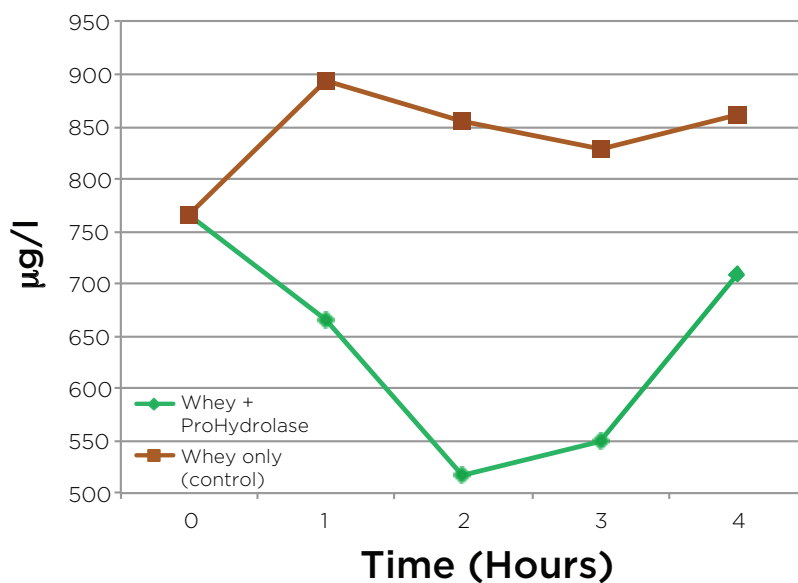
### Amino Acids Analysis

Nine of the 20 amino acids the body uses are essential and must be obtained through diet and/or supplementation. However, all amino acids may become essential if the body is unable to produce them or produce them fast enough to keep up with protein synthesis. People with low-protein diets or eating disorders, liver disease, diabetes, or genetic conditions that cause Urea Cycle Disorders (UCDs), may need to take amino acid supplements to avoid a deficiency. Physically active people may need additional amino acids due to the high rate of protein synthesis that occurs when building and repairing muscle and tissue during and after workouts. The most common amino acid supplementation is protein in the form of whey, egg, soy, etc. These proteins contain a high concentration of amino acids, however they're bound together in a peptide complex that must be broken down in order to be utilized. ProHydrolase provides the enzymes needed to break down these bound amino acids so they can be fully utilized by the body for muscle building and recovery.

### Bioactive Peptides

ProHydrolase is a unique blend of proteases that quickly and effectively degrades proteins such as whey to small peptides. These peptides can be used for both their amino acid content, as well as bioactive peptides. Bioactive peptides have been defined as “specific protein fragments that have a positive impact on body functions and conditions”. Specifically, the peptides present in milk have been shown to have intestinal anti-inflammatory activities.

Levels of C-reactive protein indicate general levels of inflammation in the body, and can be determined by a blood test.



**Figure 1:** CRP levels start at 750µg/l, and increase to approximately 900µg/l after consuming whey.

With the addition of ProHydrolase, levels decrease to 500µg/l, and then gradually increase over the 4 hours to 700µg/l.



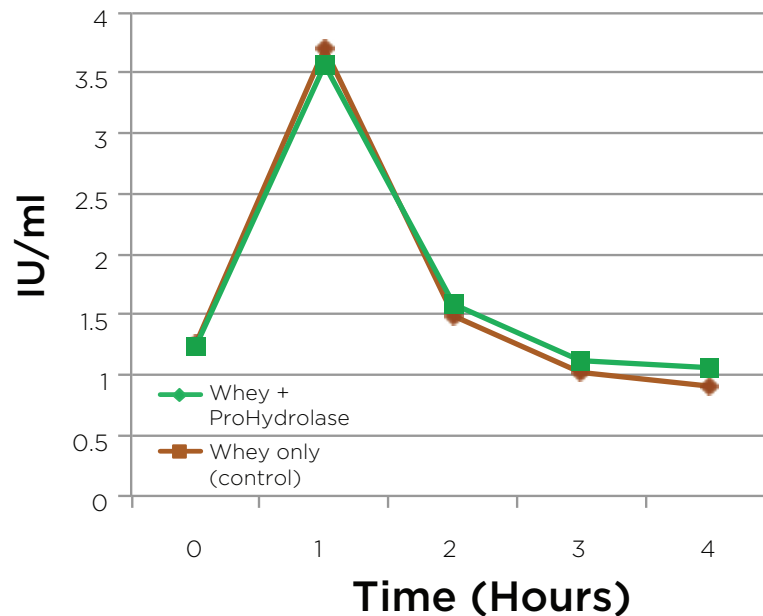
## ProHydrolase® Clinical—Phase II Study

CRP levels were only affected in the test group that consumed whey+ProHydrolase; these levels decreased significantly ( $p<0.039$ ). The control group that consumed only whey experienced no significant change in CRP levels.

### Insulin

In addition to insulin's effect on entry of glucose into cells, it also stimulates the active uptake of amino acids, again contributing to its overall anabolic effect. When insulin levels are low, as in the fasting state, the balance is pushed toward intracellular protein degradation. ProHydrolase produces di, tri and tetra peptides but no single amino acids. Di and tri peptides use proton driven active transporters to pass from the intestine to the blood, which may or may not be insulin driven.

To test whether insulin levels change with the consumption of whey protein plus Prohydrolase, blood samples were tested for insulin after consuming whey with and without ProHydrolase. Insulin levels spike in both cases but no additional insulin increase/decrease is detected versus the control, indicating the new peptides created by ProHydrolase do not influence insulin levels.



**Figure 2.** Insulin levels increased both when consuming whey isolate alone and with ProHydrolase. There was no difference in insulin levels between the two groups during the four hours.



## ProHydrolase® Clinical—Phase II Study

### Conclusion

In summary, this clinical study has demonstrated the benefits of adding the protease blend ProHydrolase to whey protein isolate. When whey protein isolate is consumed along with ProHydrolase, amino acid concentrations in the blood increase by 20%, compared to consuming whey protein alone. In addition, C-reactive protein levels decrease as a result of the bioactive peptides created by the hydrolyzed protein, indicating a reduction of inflammation in the body. ProHydrolase has been determined to have no effect on insulin levels.



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