AstraZyme®

VEHICLE OF ENERGY

Enzymology Research Center
Catalysts, Inc.

A VEHICLE OF ENERGY

Enzymology Research Center Catalysts, Inc.

The information contained in this publication was written to explain how AstraZyme®, a proprietary and clinically proven combination of enzymes and herbs, takes the digestive and absorption of protein to its highest level.

The statements found within this publication have not been evaluated by the FDA. Any products mentioned are not intended to diagnose, treat, cure or prevent any disease.

The information in this publication is for educational purposes only. If you have any specific questions as to how this information pertains to your health, consult with a qualified healthcare professional.

First Printing July 2016

Enzymology Research Center Catalysts, Inc.
215 Main Street
Miltona, MN 56354
Office: (218) 943-7904

AstraZyme®

A VEHICLE OF ENERGY

TABLE OF CONTENTS

Houston…. we have a problem! 5

Preparing for a successful launch 8

Pre-launch: Enzymes 9

Stage 1: Enzymes 11

Stage 2: Enzymes + AstraGin® 13

Stage 3: Enzymes + AstraGin® + Trace Minerals 15

AstraZyme®: Mission Accomplished! 17

Benefits of Divine Digestion 33

AstraLog 34

HOUSTON... WE HAVE A PROBLEM!

We have been temporarily issued a magnificently designed machine, our human body, which may be likened to a rocket. The rocket's physical form has been created to support its function. Likewise, the body is designed to support our function over a lifetime.

In the lifespan of the rocket, it will blast off, accelerate through time and space, prevail over atmospheric resistance to reach its destination, deliver its payload, and accomplish its mission. Our journey on earth represents a similar voyage: we are born; we accelerate through time and distance; we overcome untold obstacles to achieve our destiny or mission.

In order to be successful, rockets require a dynamic fuel source like a kerosene and oxygen combination. For humans, our fuel is food and consists of three essential parts: proteins, fats and carbohydrates.

Proteins play the most significant role in terms of overall importance and function. Consider that 17% of our body consists of protein; the percentage is second only to water at 60%. Proteins are vital to our body because they

1. Energize metabolism
2. Balance coagulation (when the blood clots normally)
3. Provide structural support
4. Protect us with antibodies in our immune system
5. Perform fluid balance
6. Regulate hormones
7. Transport materials in and out of cells
8. Enable muscle contraction
9. Comprise the physical structure of all enzymes

Amino acids are the building blocks of protein, aiding in metabolism at the cellular level. They are required for our bodies to function and the essential ones (meaning the body cannot produce these on its own) must be taken in every day as food. If we do not take in and fully assimilate proteins every day, our bodies will suffer. In fact, the vast majority of diseases and disorders including premature aging are the end result of incompletely digested proteins.

The main challenge with proteins, whether from plant or animal sources, is when the body is unable to completely break them down into individual amino acids. There are a number of factors that contribute to this dilemma such as low gastric hydrochloric acid (HCl or stomach acid) levels, consuming too much protein as well as the loss of digestive enzymes through the course of aging.

Returning to our rocket analogy, what if an unrefined (instead of the required highly-refined) kerosene was put into the fuel tank? It would lead to very poor performance and increase the risk for a potential melt down of the rocket itself. The same thing is true for the human body. If we do not put high-octane fuel (completely digested proteins) into our tanks we can expect to experience a variety of performance issues and eventually a potential health crisis.

Here is a brief list of undesirable risks associated with poor protein digestion.

1. Stomach and intestinal discomfort including indigestion, gas, bloating, acid reflux, etc.
2. The formation of excess mucus when eating any of the eight (8) . major hard to digest proteins:
    a. Milk
    b. Eggs
    c. Fish
    d. Shellfish
    e. Tree nuts
    f. Peanuts
    g. Wheat
    h. Soybeans

3. Excessive stress on the liver and kidneys.
4. Calcium deficiencies eventually lead to low levels found in bones.
5. Calcium deficiency that irritates the nervous system contributing to nervousness and irritability.
6. Immune system is forced to clean excess undigested protein out of the blood stream.
7. Excess undigested protein lowers oxygen levels in the blood stream which may eventually trigger various forms of disease.
8. The body may have to take some of the enzymes from the defense system for digestion, which lead to their not being available to fight off major illnesses or diseases, let alone the common cold.
9. Loss or lack of energy. Poor fuel equals poor performance.

Statistically, close to 30% of Americans experience digestive issues every year that result in:

- over 20 million hospital visits
- doctors writing millions of antacid prescriptions without considering the dangers of taking excess aluminum
- the spending of over $2 billion on Over The Counter (OTC) digestive symptom relievers
- more than $140 billion in total costs for digestive disorders

PREPARING FOR A SUCCESSFUL LAUNCH

Is there a safe and effective solution that will completely digest and fully utilize all of the proteins (from plant or animal sources) that we ingest?

Yes, in fact there is only one substance on the entire planet that can fulfill this formidable task—they are called enzymes.

Is there something available that has no side effects, no toxicity levels or contraindications?

Yes, enzymes have more research and studies completed than any other nutritional supplement.

Is there a solution that is earth-friendly, biodegradable and renewable?

Yes, enzymes are grown through a fermentation process that is completely renewable and fully biodegradable.

Is there something that has a long history and proven track record?

Enzymes were first discovered in 1897 and have had a flawless track record for over 119 years.

Finally, if this solution does exist is it affordable and cost effective?

Enzymes have become incredibly cost effective and affordable such that the average person can take them three times a day for less than $1.50.

Now that you know there is a safe and effective solution let's take a closer look into the wonderful world of enzymes.

PRE-LAUNCH: ENZYMES

Enzymes are among the most wonderful and amazing substances on the planet. Many scientists have come to the conclusion that the moment enzymes appeared is when life began. Enzymes are found in every living entity (plants, animals, humans). In fact, life can be defined as an orderly and integrated succession or cascade of enzymatic reactions.

Every chemical reaction in every living entity is governed by an enzyme. We would not be able to think, breathe or move without them. The theory that life itself not only began with enzymes, but also continues to be methodically controlled by them is of monumental importance. The reason enzymes can perform all of these functions is they are embodied with an unlimited source of energy. Even with advanced technology, researchers cannot fully explain where this energy comes from.

A broad view of the most important functions of enzymes include:

1. **Catalysts**
   Enzymes speed up reactions. A reaction that would normally take 78 million years to occur takes less than 20 milliseconds.The average enzyme catalyzes 100 to 1,000 reactions every second.
2. **Energy Part 1**
   Energy is transformed and stored by enzymes through two major pathways. The first is through the respiratory system and the second is through the citric acid cycle (think ATP or cellular energy).
3. **Energy Part 2**
   Enzymes slowly and gradually release this stored energy so that it doesn't cause spikes or increases in our body temperature.
4. **Energy Part 3**
   Every chemical reaction requires an enormous amount of initial energy to get the ball rolling so to speak. Enzymes reduce this "activation" energy by more than 75% with many reactions happening "spontaneously" (meaning no energy input is required).

5. **Connection**
   Enzymes consist of two parts and act as a bridge between the physical (visible) and non-physical (invisible) worlds. The physical part is demonstrated by a protein molecule, while the non-physical part is represented via electrical energy. We could say that the energy uses the protein as a vehicle to accomplish its work. Is it a coincidence that we could say the exact same thing about the human body? We have a physical protein vehicle that is ultimately directed and guided by an electrical energy source that we cannot see.
6. **Efficient**
   Enzymes are so efficient that it only takes a single tablespoon or 14 grams to completely breakdown an entire dump truck filled with 4,000 pounds of egg whites.
7. **Light**
   Enzymes produce a very unique source of light. It is referred to as "bioluminescence", a cold light because it does not produce any heat. This light is how information is transmitted (at 386,000 miles per second) between the trillions of cells found in the human body.

No matter your age, if you are experiencing a lack of energy it could be that your body is not digesting food as efficiently as possible. Human beings contain the largest number of enzymes at birth. Have you ever wondered why children have seemingly unlimited energy? By the age of 30, we lose 20% of our enzymes and by 60, over 80%. In order to experience an abundance of energy throughout your life, it's important to maintain your enzyme levels and use them wisely.

Please note there is a difference between the energy found in enzymes versus the type of energy found in caffeine, coffee, energy drinks, etc. We could relate enzymatic energy to liquid rocket fuel because it can be adjusted and controlled and will burn for a very long time.

Whereas the superficial energy found in coffee and energy drinks would be more like solid rocket fuel that is uncontrollable and once lit, burns up quickly. In other words hold on and enjoy the ride while it lasts. Of course, repeatedly taking energy short-cuts will eventually lead to some rather serious side effects.

As a positive contrast, enzyme energy from supplementation burns clean so there are no side effects, toxicity levels or contraindications to worry about.

STAGE 1: ENZYMES

Enzymes are very special protein molecules responsible for trillions of bio chemical reactions in the human body. In fact, the body will convert up to 50% of the protein ingested into enzymes. They are the catalysts that initiate and then control every biochemical process in the body. Scientists have identified over 5,000 unique enzymes. Every enzyme is important to the overall health of our bodies and they play a very critical role when it comes to digestion. Because the body obtains energy from eating, the only avenue the human race has to accessing the energy contained in food is through enzymes.

Here is a review of some specific functions of digestive enzymes.

1. They break down proteins (very long chains of amino acids) into single amino acids, so your body can use them to create more enzymes as well as to repair and rebuild your muscles, tissues and organs which in turn increases energy.

2. They are able to break down fats (mainly triglycerides) into single fatty acids so your body can store them or utilize them as an energy source.

3. They are able to break down carbohydrates commonly called sugars (oligosacharides or polysaccharides) into single sugars termed monosaccharides so your body can use them for energy.

4. They are able to break down all of these food types in a very short time as they pass through your digestive tract which increases energy.

5. They take stress off the pancreas so it does not have to produce as many enzymes which also increases energy.

6. They relieve stress from the liver and kidneys because the food is already broken down into its smallest pieces which increases energy.

7. They also allow your immune system to rest from having to clear the constant flow of partially digested food that finds its way into the blood stream called “circulating immune complexes” which increases energy.

8. All food passes through the digestive tract more quickly and efficiently, so issues like indigestion, gas, bloating and discomfort are reduced, which increases energy.

9. They support normal mucus levels in the body.

10. Did we mention they increase energy levels?

It is interesting to note that the only source of energy known to break down food is still an enigma or mystery to scientists. Enzymes provide us energy through “sharing” their own energy. There is no shortage of energy in the world of enzymes, so our only mission is to tap into them.

STAGE 2: ENZYMES + ASTRAGIN®

We are now going to narrow our focus to the digestion of proteins and introduce AstraGin®. There are two main aspects to the full utilization of any protein: breakdown and absorption. In order to utilize the food ingested, complete breakdown of the long chains of amino acids into smaller peptides and single amino acids must occur.

The second essential mechanism of digestion is absorption. For the molecules to be of value, efficient absorption of the amino acids and smaller peptides must also take place. Unless addressed by diet and supplementation, here is where digestive issues may become systemic issues.

Break down and absorption are separate processes and optimum health requires both. As we age, the body becomes deficient in its own enzyme production and supplementation becomes necessary. Enzyme supplements complete the first part of the equation by digesting or breaking down the proteins relatively quickly, within 30-60 minutes. They also perform their work in a very wide pH range (from 2-10) which means they are active throughout the entire digestive tract.

For the absorption process, combining enzymes with AstraGin® makes all the difference. AstraGin® is a proprietary combination of extracts from Astragalus membranaceus and Panax notoginseng. This unique combination allows the uptake of small peptides and amino acids on a cellular level. Numerous clinical studies have been done both in-vitro (in a test tube) as well as several in-vivo (in a living being).

Studies conducted over the past 14 years continue to prove the efficacy of this amazing combination. Specifically, they document dramatic increases in the absorption rates of the following substances:

- L-Arginine by 66%
- L-Citruline by 45%

- L-Tryptophan by 53%
- Glucose by 55%
- Folate by 50%
- Glucosamine by 23%
- Agmatine by 36%
- B-alanine by 26%
- Creatine by 33%
- Omega-7 fatty acid by 39%
- Peptides by 40%
- Curcumin by 92%

As you will note from the study data, (starting on page 19) the relationship between enzymes and AstraGin® is synergistic; together the two produce exceptional results.

Regarding digestion, enzyme supplementation is essential to health and choosing an effective product makes the difference. A progression of digestion could be summarized like this.

1. Without enzymes, "You are what you eat."
   Your body could still be processing today what you ate yesterday or even the day before.
2. With enzymes, "You are what you digest."
   Your body may break down the food, but may not be able to fully absorb it.
3. With enzymes and AstraGin® , "You are what you absorb."
   Finally, this one-two punch delivers the "knock out blow" to the age-old absorption question.

This winning combination is representative of the two companies that discovered and developed it: NuLiv Science and Enzymology Research Center Catalysts. NuLiv Science is a global supplier and developer of natural ingredients for the nutraceutical industry. NuLiv Science specializes in the research and development of proprietary and clinically proven nutraceutical ingredients through a unique platform of integrated biologics. *www.nulivscience.com*

Enzymology Research Center Catalysts is a global supplier of enzymes (microbial, bacterial, plant and animal). *www.erccatalysts.com*

STAGE 3: ENZYMES + ASTRAGIN® + MINERALS

The third and final stage of successfully launching a rocket requires that both fuels types be blended together (as in enzymes and AstraGin®) and then ignited with a spark. The spark that ignites our powerful combination product is a unique blend of trace minerals. It takes just a spark to ignite two potent fuels and that is also true when it comes to initiating the fire of enzymes and AstraGin®.

Each enzyme depends on a specific mineral to reach its full catalytic effect. An example of this is zinc, which is responsible for aiding over 300 different enzymes in performing more effectively. It has been said that minerals are the enzymes for enzymes!

After considerable research, a trace mineral complex consisting of 72 individual minerals demonstrated the most potent results. This trace mineral complex also mirrors the same microscopic geometric pattern as those of the enzymes, tetrahedrons or four-sided pyramids. Found within these miniature pyramids are all of the trace minerals. The pyramid acts as a vehicle or delivery system for the minerals.

Once the payload of minerals has been delivered to the cell, this unique tetrahedron complex also aids in the uptake of various waste and by-products. These microscopic structures act like sponges that will soak them up and then safely pull them out of the body.

The unique aspect regarding our particular type and source of mineral complex is that it enjoys a GRAS (generally recognized as safe) status in the supplement industry and is used in medical and pharmaceutical applications. Because of the purity and potency of the minerals only very small doses are required.

The results achieved by the trio of enzymes, AstraGin® and trace minerals are astounding and have been documented in a landmark clinical study. The name of this unique combination is AstraZyme®. “Astra” which relates to the energy source of the enzyme as well as a reference to AstraGin®; “Zyme” which corresponds to the physical or vehicle portion of the enzyme. The word AstraZyme® literally means “energy vehicle” or “vehicle of energy”.

ASTRAZYME®: MISSION ACCOMPLISHED!

AstraZyme® enhances digestion by successfully addressing both aspects of protein:

1. **its complete breakdown**
2. **its full absorption**

AstraZyme® is:

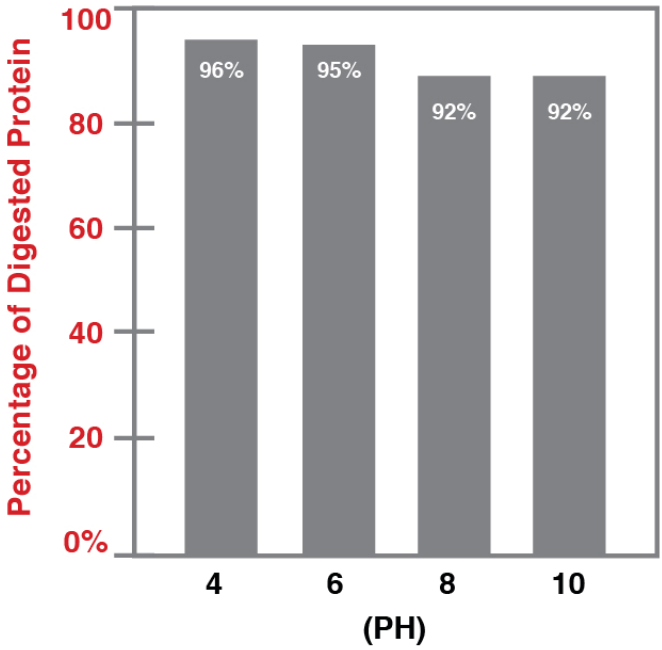
- Non-GMO
- Non-dairy
- Non-soy
- Gluten-free
- Allergen-free
- Vegan

Please review the following four charts and complete study in more detail so you can discover just how effective it is.

AstraZyme®

CHART 1

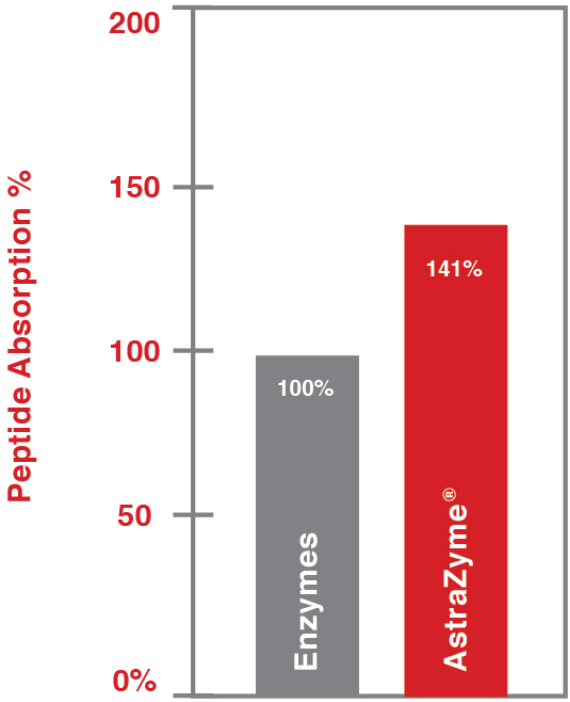
Complete digestion of proteins into peptides and amino acids within 30-60 minutes in a pH range from 4.0 – 10.0.



Percentage of digested protein in 30-60 minutes in various PH ranges.

CHART 2

Quantity of peptides absorbed increased by 41%

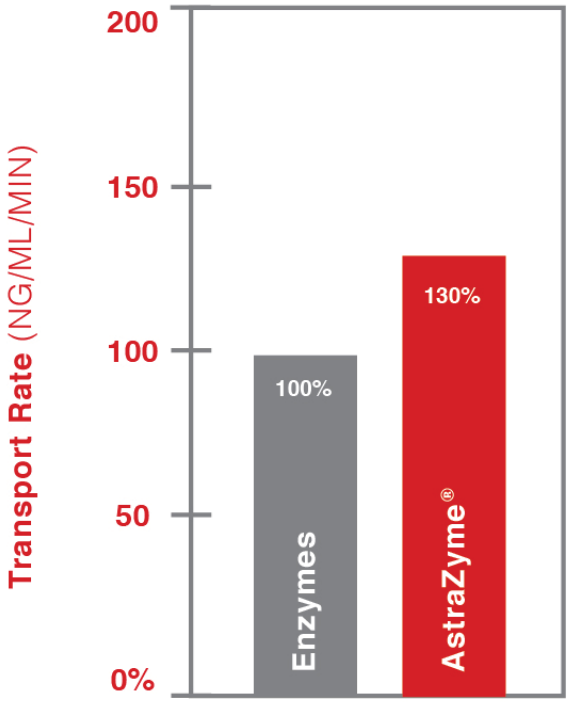


Total quantity of Peptides absorbed by CACO-2 cells in 45 minutes.

AstraZyme®

CHART 3

Rate of absorption of peptides increased by more than 30%

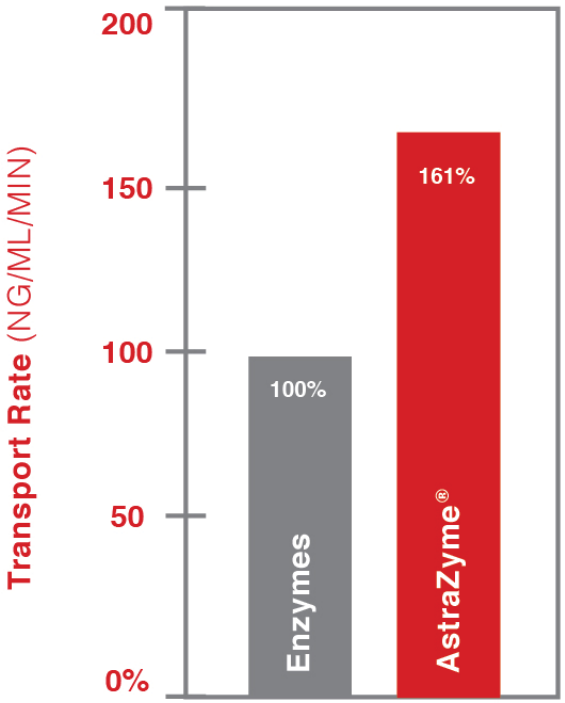


Absorption rate of Peptides in 45 minutes.

AstraZyme®

CHART 4

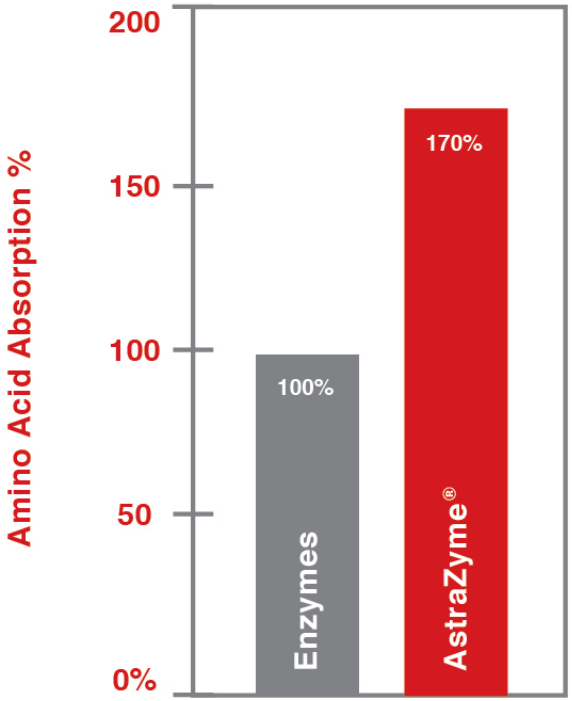
Rate of absorption of amino acids increased by more than 61%



Absorption rate of amino acids in 15 minutes.

CHART 5

Quantity of amino acids absorbed increased by 70%



Total quantity of Amino Acids absorbed by CACO-2 cells in 45 minutes.

Enzymology Research Center Catalysts, Inc.
215 Main Street, Miltona, MN 56354 U.S.A.
Phone: 1.218.943.7904

AstraZyme® Study

AstraZyme® is a unique combination of proteolytic enzymes with trace minerals (ERCC1™) and extracts of Astragalus membranaceus and Panax notoginseng (AstraGin®).

Part 1- The effect of ERCC1™ (a proprietary ERCC enzyme blend with trace minerals) on the breakdown of protein (bovine hemoglobin) into peptides and amino acids.

Part 2- The effect of AstraGin® on the absorption of peptides and amino acids (derived from ERCC1™) in human small intestinal Caco-2 cells.

Table of Contents

1. Abstract Page 23
2. Summary Pages 24-25
3. Objective Page 25
4. Materials & Methods Pages 25-26
5. Results
   A. Protease Activity Pages 27-29
   B. Peptide Absorption Pages 29-30
   C. Amino Acid Absorption Pages 30-31
6. Discussion Page 31
7. Reference Page 32

1. Abstract

The objectives of the present study are threefold:

1. To evaluate the ability of ERCC1™ on bovine hemoglobin digestion.

In the bovine hemoglobin digestion study, ERCC1™ hydrolyzed >90% of the hemoglobin to peptides and amino acids in 60 minutes over a wide range of pH. This indicates that ERCC1™ works well in human digestive tract. In another time-course digestion test, ERCC1™ hydrolyzed greater than 95% of the hemoglobin to peptides and amino acids in acidic environment within 60 minutes and the percent of unhydrolyzed hemoglobin continually decreased as time passed.

2. To observe the effect of AstraGin® on the absorption of the hemoglobin-derived peptides hydrolyzed by ERCC1™ in human small intestinal Caco-2 cell monolayers.

In the peptide absorption study, no peptides were absorbed in Caco-2 cells when whole

hemoglobin solution was added to the medium without ERCC1™. When the hemoglobin was hydrolyzed by ERCC1™, AstraGin® was able to increase the amount of peptides absorption (AUC) by 41% and 83% in 45 minutes at 1X and 10X strength and the rate of absorption by 30% and 66% in 45 minutes at 1X and 10X strength.

3. To observe the effect of AstraGin® on the absorption of the hemoglobin-derived amino acids hydrolyzed by ERCC1™ in human small intestinal Caco-2 cell monolayers.
In the amino acids absorption study, very low amount of amino acids were absorbed in Caco-2 cells when whole hemoglobin solution was added to the medium without ERCC1™. When the hemoglobin was hydrolyzed by ERCC1™, AstraGin® was able to increase the amount of amino acids (AUC) absorption by 70% and 125% with 1X and 10X AstraGin® and the rate of absorption by 61% and 110% in 15 minutes with 1X and 10X strength.

In summary, the study demonstrates that ERCC1™ was able to hydrolyze 90% or greater amount of the bovine hemoglobin to peptides and amino acids. AstraGin® was shown to increase the amount as well as rate of absorption of the peptides and amino acids.

AstraZyme® is a proprietary and proven combination of proteolytic enzymes and trace minerals (ERCC1™) with extracts of Astragalus membranaceus and Panax notoginseng (AstraGin®).

2. Summary

Table 1. Percent of unhydrolyzed hemoglobin with ERCC1™ at indicated pH buffer

Undigested hemoglobin (%)	pH in buffer			
	4	6	8	10
No ERCC1™	100.00±0.30			
With ERCC1™	3.34±0.22	4.29±0.231	9.76±0.11	9.12±0.40

Table 2. Percent of unhydrolyzed hemoglobin with ERCC1™ at indicated time.

With ERCC1™ at pH4 buffer		Undigested hemoglobin (%)
Time (min)	0	100.00±0.38
	30	12.17±0.10
	60	4.30±0.02
	90	1.87±0.02
	120	2.04±0.02

Table 3. Transport rate of peptides in 45 minutes

AstraGin® doses	ERCC1™	Relative transport rate of peptides in 45 minutes (%)
0X	-	<1
0X	+	100.00 ± 14.24
1X	+	130.38 ± 12.14*,##
10X	+	165.87 ± 12.21*,##

Table 4. Total amount of peptides (AUC) absorbed in Caco-2 cells in 45 minutes

AstraGin® doses	ERCC1™	Relative area under curve of peptide absorption (%)
0X	-	<1
0X	+	100.00±15.82
1X	+	141.04±13.48*
10X	+	183.02±13.57*

Table 5. Transport rate of amino acids in 15 minutes

AstraGin® doses	ERCC1™	Relative transport rate of amino acids in 15 minutes (%)
0X	-	<1
0X	+	100.00 ± 2.85
1X	+	161.38 ± 13.01**.##
10X	+	209.76 ± 20.33**.##

**p<0.01, when compared to ERCC1™ only group
p<0.01, when compared to Blank group (No ERCC1™, no AstraGin® added)

Table 6. Total amount of amino acids absorbed in Caco-2 cells in 15 minutes.

AstraGin® doses	ERCC1™	Relative area under curve of total amino acids absorption (%)
0X	-	<1
0X	+	100.00±3.81
1X	+	170.36±7.25**.##
10X	+	224.95±8.41**.##

3. Objective

AstraGin® has been validated and demonstrated to enhance the cellular absorption of amino acids, vitamins, and glucose in NuLiv Science's ***In vitro*** and ***In vivo*** studies. Details of the studies are presented in the AstraGin™ product dossier.

The purpose of this study is to assess the effectiveness of ERCC1™ on protein digestion (specifically bovine hemoglobin) and AstraGin® on the absorption of hemoglobin-derived peptides and amino acids hydrolyzed by ERCC1™ in human small intestine Caco-2 cells.

AstraZyme® is a proprietary and proven combination of proteolytic enzymes and trace minerals (ERCC1™) with extracts of Astragalus membranaceus and Panax notoginseng (AstraGin®).

4. Materials & Methods

Protease activity assay

Protease activities of the samples were measured by QuantiCleave™ Protease Assay Kit according to the manufacturer's protocol (Pierce, Rockford, IL). Measure absorbances of wells in a plate reader set to 450nm. For each well calculate the change in absorbance at 450nm (ΔA450) by subtracting the A450 of the blank from that of the corresponding substrate well. This ΔA450 is the absorbance generated by the proteolytic activity of the protease. The protease activity was determined by SPECTRA MAX190 (Molecular Device, USA)

Cell Culture

The Caco-2 cell line was obtained from ATCC (Philadelphia, PA, USA). The Caco-2 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Gibco Life Technology), nonessential amino acids, L-glutamine and penicillin/streptomycin. The Caco-2 cells were incubated at 37℃ in a humidified atmosphere containing 5% CO_2. The cells used in the experiments were between passages 10 and 20. Caco-2 cells were subcultured weekly by trypsin and were seeded at a ratio of 1:3 upon reaching 80% confluence. The culture medium was changed every 2–3 days. For the transport experiments, the cells were seeded at a density of $9x10^5$ cells/cm^2 in 6-well filter support inserts with polyethylene membranes (0.4 μm pore size, 24 mm diameter, 4.67 cm^2 growth surface area; Costar, Corning Inc., Corning, NY). The monolayers reached confluence in 3 days after seeding, and the cells were differentiated for at least an additional 14 days prior to the transepithelial transport experiments. The integrity of the Caco-2 cell monolayers and the tight junctions were monitored before every experiment by determining the transepithelial electrical resistance

(TEER) measurements using an epithelial Volt-Ohm Meter (Millicell ERS-2, Millipore, Bedford, MA). Only the Caco-2 monolayers with TEER values higher than 700Ω·cm² were used for the experiments.

Preparation of bovine hemoglobin hydrolysate

Bovine hemoglobin was dissolved at 5% (w/v) in water, and mixed vigorously and filtered through a glass wool filter. Hemoglobin solution was diluted to 4% with 300mM HCl. Immediately before digestion, prepare ERCC1™ in sodium acetate buffer, pH4. 2% hemoglobin solution was digested by incubation with 1% (w/w) ERCC1™ for 2 h at 37℃ in a orbital incubator during the reaction, aliquots of the hemoglobin and ERCC1™ mixture were taken out at various times. Each aliquot was immediately added with ammonium hydroxide to a value of pH 10 and heated in boiling water for 10 min to inactivate the enzyme activity and stop the reaction. The precipitate was removed from the peptic digest by centrifugation (10000 rpm, 4℃, 20 min). The supernatants were lyophilized before further use. Sample mixtures were stored at -30℃.

Tris-Tricine-SDS-PAGE electrophoresis

Tricine-SDS-PAGE was carried to evaluate the peptide profile after ERCC1™ treatment. The Tris-Tricine-SDS-PAGE method was carried out according to Schägger & von jagow (1987), using 19.5% separating gel and 10x10 cm glass plates in the Bio-Rad system. Staining was performed according to a standard procedure.

Transepithelial transport studies

After TEER measurement, the differentiated Caco-2 monolayers were gently rinsed twice with Hank's balanced salt solution (HBSS) and equilibrated for 30 min at 37℃. Then the medium was replaced with fresh HBSS containing the peptides derived from bovine hemoglobin hydrolyzed by ERCC1™. The transwells were incubated at 37℃ for 120 min and the apical and basolateral medium were sampled at the designated time intervals and analyzed by fluoraldehyde (OPA) peptide assay. During and at the end of the experiments, TEER was measured and data were recorded only from experiments in which TEER was higher than 250Ω·cm²

Fluoraldehyde (OPA) peptide assay

Peptide concentration was measured by the method of fluoraldehyde reaction using bovine serum albumin (BSA) as a standard. BSA (2mg/ml) was digested by incubation with 1% (w/w) trypsin for 72 h at 37℃ in a orbital incubator. The undigested protein was removed by the addition of TCA, followed by centrifugation at 10,000g for 20 min, the peptide content of the supernatant was measured. Peptide concentrations of the samples were measured by the fluoraldehyde (OPA) assay kit according to the manufacturer's protocol (Pierce, Rockford, IL). Briefly, prepare samples, blanks and standards in an opaque plate. Add optimal volume of OPA reagent solution to each well and mix well. Measure the fluorescence at excitation 330-390nm and emission at 436-475nm. The peptide concentrations were determined by Polarstar Galaxy (BMG LABTECH, DE).

MALDI-TOF mass spectrometry

The MALDI-TOF MS experiments were performed on microflex (Bruker Daltonics, USA) equipped with an N_2 laser. Prior to the MS analysis, the dried peptide mixtures were dissolved in ddH_2O, and desalted using C18 Zip-Tip pre-packed micro-columns (Millipore, Bedford, MA, USA) previously equilibrated with aqueous 0.1% TFA (v/v) and eluted with 70% acetonitrile (v/v) containing 0.1% TFA (v/v). The HPLC peaks were directly loaded onto a stainless steel plate together with α-cyano-4-hydroxycynnamic acid matrix and air-dried. The mass spectra were acquired in the positive ion reflector mode by accumulating 400 laser pulses. The external mass calibration was performed with mass peptide standards (Sigma). The spectra were analyzed with the FlexAnalysis version 3.4 (Bruker Daltonics, USA).

Amino acid quantitation analysis

Amino acids concentration was measured by using commercial Menagent test kits (BioVision, USA). Briefly, prepare samples, blanks and standards in an opaque plate. Add optimal volume of amino acid reaction mix solution to each well and mix well. Measure the fluorescence at excitation 535 nm and emission at 590nm. The amino acid concentrations were corrected background by subtracting blank samples.

A. Protease Activity

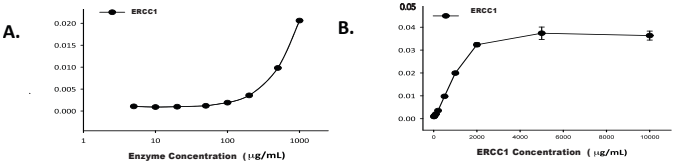


Figure1. ERCC1™ enzymes activity (A) Limit detection of enzymes activity. Enzyme concentrations are plotted using logarithmic scales; (B) Dose effect of enzymes activity.

This ΔA450 is the absorbance generated by the proteolytic activity of ERCC1™. When ΔA450 is higher, their proteolytic activity is higher. The Lower limit of activity assay for ERCC1™ was 50μg/mL. When ERCC1™ concentration <1000 μg/mL, their proteolytic activities proceed at a rate that is dependent of reactant concentration (initial rate of reaction), and when concentration >1000 μg/mL, their proteolytic activities proceed at a rate that is independent of reactant concentration.

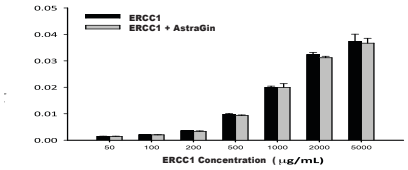


Figure2. Effect of AstraGin® on ERCC1™ activity.

Effect of AstraGin® on ERCC1™ activity：There was no statistical difference between ERCC1™ group and ERCC1™ +AstraGin® group. It also means AstraGin® 's involvement is independent of ERCC1™ activity.

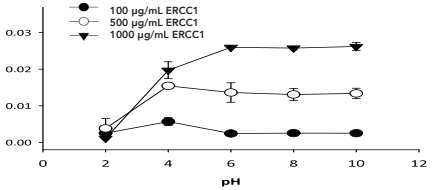


Figure3. Effect of pH on ERCC1™ activity. ERCC1™ activity at various pH conditions.

100-1000μg/mL of ERCC1™ was selected to observe pH effect. Most ERCC1™ concentrations have higher enzyme activity in pH4, except for when 1000 μg/mL used. Effect of pH on ERCC1™ activity is more noticeable in lower concentrations.

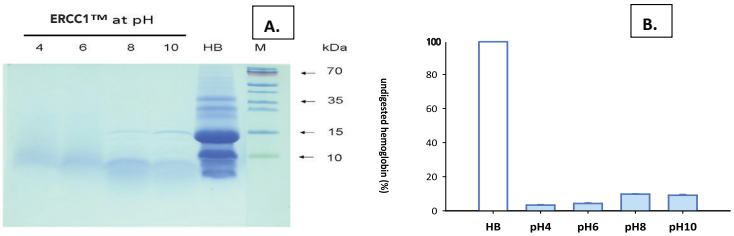


Figure 4. Undigested hemoglobin with ERCC1™ at indicated pH buffer. (A) Tricine-SDS-PAGE of hemoglobin hydrolysates by ERCC1™. 4, pH4; 6, pH6; 8, pH8; 10, pH10; HB, 2% hemoglobin in H_2O; M, pre-stained protein marker; kDa, separated standard protein molecular weight. (B) Quantitative analysis of the results shown in panel.

Table 1. Percent of undigested hemoglobin with ERCC1™ at indicated pH buffer.

Undigested hemoglobin (%)	pH in buffer			
	4	6	8	10
No ERCC1™	100.00±0.30			
With ERCC1™	3.34±0.22**	4.29±0.231**,#	9.76±0.11**,##	9.12±0.40**,##

** p<0.01, when compared to hemoglobin only (No ERCC1™) group.
# p<0.05, when compared to hemoglobin+ ERCC1™ at pH2 group
p<0.01, when compared to hemoglobin+ ERCC1™ at pH2 group

As indicated in Fig.4, after 1 h of incubation at 37℃ of hemoglobin in the presence of 1% (w/w) ERCC1™, the hemoglobin bands almost completely disappeared on the electrophoresis gel, especially at acidic buffer, e.g. pH4. The higher subunits of hemoglobin (>25kDa) were completely undetectable. The smaller smeared bands were mainly produced forms of ERCC1™'s digestion. ERCC1™ adapted well to a wide range of pH, and most high enzymatic activity appeared in acidic environment. Even though there was a 15kDa subunit slightly appeared at pH8 or pH10 buffer when hemoglobin digested by ERCC1™, this didn't change the fact that ERCC1™ had the highly digestive capacity to digest >90% hemoglobin in 60 minutes over wide range of pH.

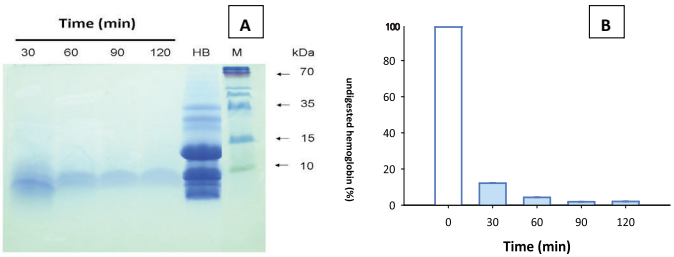


Figure5. Undigested hemoglobin with ERCC1™ at indicated time. (A) Tricine-SDS-PAGE of hemoglobin hydrolysates by ERCC1™. (B) Quantitative analysis of the results shown in panel.

Table 2. Percent of undigested hemoglobin with ERCC1™ at indicated time.

With ERCC1™ at pH4 buffer		Undigested hemoglobin (%)
Time (min)	0	100.00±0.38
	30	12.17±0.10**
	60	4.30±0.02**,##
	90	1.87±0.02**,##,††
	120	2.04±0.02**,##,††

** p<0.01, when compared to hemoglobin only group.
p<0.01, when compared to "hemoglobin digestion by ERCC1™ for 30min" group.
†† p<0.01, when compared to "hemoglobin digestion by ERCC1™ for 60min" group.

As indicated in Fig.5, incubation of hemoglobin in the presence of 1% (w/w) ERCC1™ at pH4 buffer, the hemoglobin digestion appeared partial after 30 min with the apparition of bands with smaller molecular weights. The relative percentage of undigested hemoglobin after 30min of ERCC1™'s action was approximately 10%. By time pass, the amounts of undigested hemoglobin were decreased. The maximum limitation of ERCC1™ was reached after 90 minutes of digestion time. There was no statistical difference between 90 minutes and 120 minutes.

B. Peptide absorption

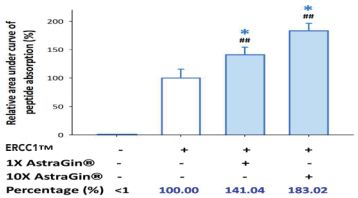


Figure 6. Effect of AstraGin® on the absorption rate of peptides produced from ERCC1™ in 45 minutes.

Table 3. Relative transport rate of peptides in 45 minutes.

AstraGin® doses	ERCC1™	Relative transport rate of peptides in 45 minutes (%)
0X	-	<1
0X	+	100.00 ± 14.24
1X	+	130.38 ± 12.14*,##
10X	+	165.87 ± 12.21*,##

*p<0.05, when compared to ERCC1™ only group.
p<0.01, when compared to Blank group (No ERCC1™, no AstraGin® added)

The differentiated Caco-2 cell monolayers were pretreated with AstraGin® for 24h, and then incubated for 120 min with the peptides derived from bovine hemoglobin hydrolyzed by ERCC1™. During the incubation, the medium from the basolateral compartments were collected at designated time intervals and analyzed by fluoraldehyde (OPA) peptide assay. Our results indicated that AstraGin® increased the initial absorption rate of peptides derived from bovine hemoglobin hydrolyzed by ERCC1™ by 30.39% and 65.87% with 1X and 10X AstraGin® respectively when compared to the ERCC1™ only group. Notably, it was not feasible to observe the peptide transport phenomenon when hemoglobin without ERCC1™'s action. This was due to intact protein too large to across the intestinal membrane. Without enzyme digestion, it was difficult to transport large size of protein into the intestinal cells.

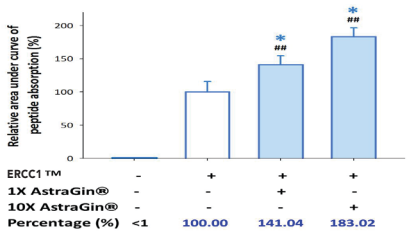


Figure 7. Effect of AstraGin® on the total quantity of peptides derived from bovine hemoglobin hydrolyzed by ERCC1™ in 45 minutes

Table 4. Total amount of peptides absorbed by Caco-2 cells in 45 minutes.

AstraGin® doses	ERCC1™	Relative area under curve of peptide absorption (%)
0X	-	<1
0X	+	100.00±15.82
1X	+	141.04±13.48*
10X	+	183.02±13.57*

*p<0.05, when compared to ERCC1™ only group
p<0.01, when compared to Blank group (No ERCC1™, no AstraGin® added)

When bovine hemoglobin was not hydrolyzed by ERCC1™, the total quantity of peptides absorbed in Caco-2 cells was minimum with or without AstraGin®. When bovine hemoglobin was hydrolyzed by ERCC1™, the total quantity of peptides absorbed in Caco-2 cells was 41% and 83% with 1X and 10X AstraGin® respectively.

C. Amino Acid Absorption

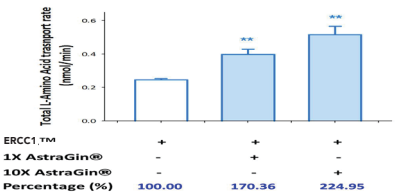


Figure 8. Effect of AstraGin® on the absorption rate of bovine hemoglobin-derived amino acids hydrolyzed by ERCC1™ in 15 minutes.

Table 5. Relative transport rate of amino acids in 15 minutes.

AstraGin® doses	ERCC1™	Relative transport rate of amino acids in 15 minutes (%)
0X	-	<1
0X	+	100.00 ± 2.85
1X	+	161.38 ± 13.01**,##
10X	+	209.76 ± 20.33**,##

**p<0.01, when compared to ERCC1™ only group
p<0.01, when compared to Blank group (No ERCC1™, no AstraGin® added)

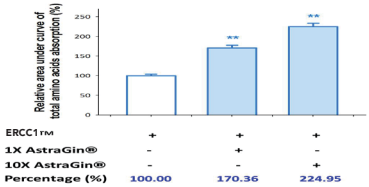


Figure 9. Effect of AstraGin® on the absorption of total amount of bovine hemoglobin-derived amino acids hydrolyzed by ERCC1™ in 15 minutes

Table 6. Total amount of amino acids absorbed in Caco-2 cells in 15 minutes.

AstraGin® doses	ERCC1™	Relative area under curve of total amino acids absorption (%)
0X	-	<1
0X	+	100.00±3.81
1X	+	170.36±7.25**,##
10X	+	224.95±8.41**,##

The differentiated Caco-2 cell monolayers were pretreated with AstraGin® for 24 hours, and then incubated for 120 min with the bovine hemoglobin-derived amino acids hydrolyzed by ERCC1™. During the incubation, the medium from the basolateral compartments were collected at designated time intervals and analyzed by L-amino acid quantitation fluorometric kit. In the amino acids absorption study, very low amount of amino acids were absorbed in Caco-2 cells when whole hemoglobin solution was added to the medium without ERCC1™. After bovine hemoglobin was hydrolyzed by ERCC1™, AstraGin® was able to increase the total amount of amino acids (AUC) absorption by 70% and 125% with 1X and 10X AstraGin® in 45 minutes and the rate of absorption by 61% and 110% with 1X and 10X AstraGin® in 15 minutes.

6. Discussion

In the protease activity assay, the detection limit for ERCC1™ was 50μg/ml. When enzyme concentration was <1000 μg/mL, ERCC1™ proceeded at a rate that was dependent of reactant concentration (initial rate of reaction). Optimal concentrations of ERCC1™ were selected between 100-1000μg/mL for further experiments. When AstraGin® was added to ERCC1™ in bovine hemoglobin hydrolysis, AstraGin®'s involvement is independent of ERCC1™ activity. The enzyme activity between ERCC1™ and ERCC1™ +AstraGin® had no statistical differences. However, AstraGin® is safe to be included in ERCC1™. The pH study indicated ERCC1™ worked well in a wide range of pH and most high enzymatic activity appeared in acidic environment. This characteristic allows ERCC1™ to work well in human digestive system.

By gel electrphoresis, we observed that hemoglobin bands almost completely disappeared on the electrophoresis gel when ERCC1™ was involved in hemoglobin digestion. The smaller smeared bands were mainly produced forms of ERCC1™'s digestion. ERCC1™ adapted well to a wide range of pH, and ERCC1™ had high digestive capacity to digest >90% of the hemoglobin in 60 minutes over wide range of pH. In another time-course digestion test, we also observed that ERCC1™ digested greater than 95% of the hemoglobin within 60 minutes, and percent of the undigested hemoglobin continually decreased as time pass.

Bovine hemoglobin was not absorbed in Caco-2 cells due to its large size. ERCC1™ hydrolyzed bovine hemoglobin to peptides and amino acids by cleaving the peptide bonds. After hydrolysis by ERCC1™, AstraGin® was shown to increase the total amount of absorption (AUC) of bovine hemoglobin-derived peptides by 41% and 83% in 45 minutes with 1X and 10X

strength and the rate of absorption by 30% and 66% in 45 minutes at 1x and 10x strength. AstraGin® was also shown to increase the total amount of absorption (AUC) of bovine hemoglobin-derived amino acids by 70% and 125% in 15 minutes with 1X and 10X strength and the rate of absorption by 61% and 110% in 15 minutes at 1x and 10x strength.

In conclusion, this study demonstrated that AstraGin® significantly increased the amount and rate of transport of amino acids and peptides across the Caco-2 cell monolayer. In our previous studies, we demonstrated that AstraGin® conferred significant and parallel alterations in mRNA and carrier transport protein levels to increase various nutrients absorption in Caco-2 cells. This study demonstrated that adding AstraZyme® which is a combination of ERCC1™ and AstraGin® can not only effectively breakdown proteins but also increase the bioavailability and absorption of peptides and amino acids in human intestinal cells.

7. References

1. Picariello G, Iacomino G, Mamone G, Ferranti P, Fierro O, Gianfrani C, Di Luccia A, Addeo F. (2013) Transport across Caco-2 monolayers of peptides arising from in vitro digestion of bovine milk proteins. *Food Chem.*, 139(1-4):203-12.

2. Takeda J, Park HY, Kunitake Y, Yoshiura K, Matsui T. (2013) Theaflavins, dimeric catechins, inhibit peptide transport across Caco-2 cell monolayers via down-regulation of AMP-activated protein kinase-mediated peptide transporter PEPT1. *Food Chem.,* 138(4):2140-5.

3. Su RX, Qi W, He ZM. (2007) Identification and release kinetics of peptides from the process of peptic hydrolysis of bovine hemoglobin by LC-ESI-MS/MS. *Prep Biochem Biotechnol.* , 37(2):123-38.

4. Kaur L, Rutherfurd SM, Moughan PJ, Drummond L, Boland MJ. (2010) Actinidin enhances protein digestion in the small intestine as assessed using an in vitro digestion model. *J Agric Food Chem.,* 58(8):5074-80.

AstraGin® is a registered trademark of NuLiv Science USA, Inc.

AstraZyme® is a registered trademark of Enzymology Research Center Catalysts, Inc.

ERCC1™ is a trademark of Enzymology Research Center Catalysts , Inc.

Enzymology Research Center Catalysts, Inc.
215 Main Street, Miltona, MN 56354 U.S.A.
Phone: 1.218.943.7904

BENEFITS OF DIVINE DIGESTION

Taking the information presented so far, here is how these products could potentially fit into your daily life.

Picture yourself at a beautiful social gathering taking in the scene, mingling with other guests when suddenly you are snapped back into the reality of … digestion. Your thoughts plummet to the familiar worries of what to eat or more importantly, what not to eat. Then your lovely time digresses to thinking about bodily functions!

Instead, what if you had the tool(s) to allow you to experience this social setting and any other with confidence, the confidence that comes with knowing that your digestive system is well cared for and that your supplements are performing at maximum efficiency, freeing your concentration for more significant endeavors?

In order to maintain optimal health, millions of transactions must take place at a cellular level. We cannot consciously make these happen, but we can ensure that our body is performing these actions as effectively and efficiently as possible. Clearly, we need to make quality food and supplement choices. Just like a rocket, your body, requires specialized fuel to successfully accomplish its mission. AstraZyme® creates this fuel by maximizing the digestion and absorption of proteins, so they can be used to rebuild and refuel your body.

Taking AstraZyme® daily along with your healthy lifestyle delivers the following benefits:

1. Provides more energy
2. Decreases food consumption because you are getting more out of the foods you eat
3. Relieves occasional gas, bloating and gastrointestinal discomfort
4. Enhances vitamin and mineral absorption
5. Promotes initial weight loss of 5-10 pounds
6. Slows the aging process

ASTRALOG™

We would like to acknowledge all of the ground-breaking research that has been made available since the discovery of enzymes. The following researchers were utilized as sources in the writing of this publication:

John Beard, D.Sc.
Nicholas J. Gonzalez, M.D.
Edward Howell, M.D.
William Donald Kelley, D.D.S., M.S.
Rev. Viktoras Kulvinskas, M.S.
D.A. Lopez, M.D.
M. Miehlke, M.D.
Hans A. Nieper, M.D.
Hiromi Shinya, M.D.
R.M. Williams, M.D., Ph.D
Max Wolf, M.D.
Harun Yahya

Final Note:

Supplementing each meal with AstraZyme® will transform normal food into super fuel thus keeping your magnificent rocket machine operating through time and space with AstraNomical levels of energy!

To the Stars,

Viktor, Troy and Lee