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SYSTEMIC PROTEOLYTIC ENZYMES: A REVIEW OF MECHANISMS OF ACTION AND THE RESULTING HEALTH BENEFITS

Transformation Enzyme Corporation (TEC) is a nutritional supplement company specializing in enzyme therapy since 1991. Our goal is to educate health care professionals and the public on the fundamental health benefits of enzyme and probiotic supplementation. We believe a healthy diet and lifestyle, along with optimal digestion and a strong immune system, is the foundation of wellness and therefore enzyme therapy is the Genesis of Good Health™.

Enzymes Defined

Enzymes are protein molecules that catalyze chemical reactions. In the human body, digestive enzymes catalyze or facilitate the breakdown of food molecules within the gastrointestinal tract. Metabolic enzymes are those found in the cells and tissues that are responsible for all chemical reactions in the body. Supplemental proteolytic enzymes or "proteases" are digestive enzymes that specifically digest proteins into small peptides or amino acids.

As digestive aids, when taken with meals they facilitate complete digestion of animal and plant proteins. When taken between meals, proteolytic enzymes are absorbed into the blood stream. In this sense, they are known as "systemic enzymes" as they impact the entire body in a positive way. This literature review will look at the key characteristics and research available on the proteolytic enzymes used by TEC and discuss the mechanisms of action and systemic health benefits they have on the immune system, circulatory system, and ultimately the entire body.

Key Characteristics of Supplemental Proteases

There are several sources for supplemental enzymes available on the market today. They are animal (pancreatic, trypsin, chymotrypsin), plant (bromelain, papain), and microbial (fungal and bacterial). TEC uses microbial and plant enzymes for their long history of safety, quality, purity and efficacy.

Stability

Gastric stability is very important when selecting supplemental enzymes. As protein molecules, it is logical to think they would be denatured in the harsh environment of the stomach. For example, endogenous pancreatic enzymes are secreted into the small intestines, bypassing the stomach all together. So when pancreatic enzymes are taken orally as supplements they must have an enteric coating in order to survive the acid in the stomach. Microbial and plant enzymes on the other hand are not as susceptible to the acid (Mamadou 2005).

Table 1 shows the various proteases TEC uses in its formulations currently, their sourcing and active pH range. TEC uses only enzymes that have proven their ability to withstand the varying pH levels, bile salts and endogenous enzymes that occur during digestion (Borowitz 2012; Ehren 2009; Fieker 2011; Griffin 1989; Layer and Keller 2003; Mitea 2008; Pierzynowski 2012; Schneider 1985; Stepniak 2006; Zentler-Munro 1992).

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>SOURCE (controlled fermentation)</th>
<th>Effective pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromelain</td>
<td>Ananas comosus 6</td>
<td>4.0 - 9.0</td>
</tr>
<tr>
<td>Papain</td>
<td>Carica papaya (tropical plant)</td>
<td>3.0 - 10.5</td>
</tr>
<tr>
<td>Peptidase</td>
<td>Aspergillus oryzae</td>
<td>3.25 - 7.5</td>
</tr>
<tr>
<td>Protease 3.0</td>
<td>Aspergillus niger</td>
<td>2.75 - 4.7</td>
</tr>
<tr>
<td>Protease 4.5</td>
<td>Aspergillus oryzae var</td>
<td>2.75-6.25</td>
</tr>
<tr>
<td>Protease 6.0</td>
<td>Aspergillus oryzae</td>
<td>2.75 - 7.0</td>
</tr>
<tr>
<td>DPP-IV (Dipeptidyl Peptidase)</td>
<td>select Aspergillus species</td>
<td>2.0 - 8.0</td>
</tr>
</tbody>
</table>
Graph 1 illustrates the enzyme activity of select proteases measured at varying stages of simulated digestion. The bromelain maintains 47%, while the microbial proteases maintain activity at 89% and 125%. While TEC incorporates bromelain into some of its protease blends, the vast majority of enzymes used are microbial. This is beneficial because it provides a product with broader functionality, greater enzyme concentration, and requires no enteric coating meaning a much cleaner product free from “other ingredients.”

Activity
The units of activity indicate the potency of the enzyme and its effectiveness to breakdown its target molecules. The system for determining enzyme activity used by the American food industry is derived from the Food Chemical Codex (FCC) and is accepted as the standard by the FDA. The Food Chemical Codex is published by the National Academy Press and is an FDA funded project through the Food and Nutrition Board of the National Research Council.

The FCC establishes specifications for foods and food substances used in food processing. It is the only national standard for the evaluation of microbial enzymes. FCC units establish activity levels and procedures for determining enzyme potency. Weight measurements are not appropriate since there is no direct relationship between weight and units of activity. Not all manufacturers use FCC units and are not required to do so by law. The activity of some of the protease enzymes currently found in dietary products are often reported in FCC units as follows:

- HUT (Hemoglobin Unit Tyrosine base)
- PU (Papain Unit)
- SAPU (Stable Acid Protease Unit)
- MCU (Milk Clotting Unit)
- FU (Fibrinolytic Unit)
- GDU (Gelatin Digesting Unit)

Safety
We are often asked about the safety of enzymes derived from microbial sources. There is research starting as early as 1970’s along with observational data for the past 100 years showing no occurrence of toxicity or infection from oral supplemental intake of microbial enzymes (Barbesgaard 1992; Baur 1994; Dixon and Walsh 1992; Dvorackova 1990; Godfrey and West 1992; Guyton 1991; Linz and Pestka 1992; Losada 1992; Pier and Richard 1992; Quirce 1992; Reed 1975; Richard and Cole 1989; Schechtman 1992; Schwimmer 1981; Wei and Johg 1986).

Even with this proof, TEC and its manufacturers take this question seriously. The fermentation and extraction of microbial enzymes is a very controlled and precise process that uses only non-toxigenic strains of microorganisms that are continually screened for possible mutations every 2nd generation. Additionally, the extraction of the enzyme is limited to only a “pure-isolated” protein molecule, therefore microorganisms and toxins are not extracted and do not become a part of the final enzyme product. Even still, the enzymes are quarantined and tested to further ensure their safety and purity. Our manufacturers are GMP certified which means they meet or exceed the standards for good manufacturing procedures.

Another common concern is “will the supplemental enzymes cause an inhibition of endogenous digestive enzymes, creating dependence with long term use?” The answer is no, they do not cause inhibition of endogenous enzyme secretion. The secretion of pancreatic juices and enzymes is regulated by the hormones secretin and cholecystokinin, not the presence or concentration of enzymes. In fact, supplemental enzymes work synergistically with your endogenous enzymes, taking the load off the pancreas while supporting optimal digestion. The next section will discuss the absorption and circulation of systemic proteases explaining how they are utilized by the immune system as needed, recycled back to the liver for digestive support or degraded to amino acids. So again, there is no negative inhibition of supplementing with proteases for systemic benefits.

Absorption
Many practitioners recognize that proteolytic enzymes have sustained systemic benefits in animals and humans beyond the gastrointestinal tract that imply absorption, but a comprehensive model detailing how they are absorbed and delivered to the multiple sites of action without damaging healthy tissue has not been clearly illustrated until recent years. Now we have a comprehensive range of digestive and immunology research from many diverse countries. By assembling this research, a more complete picture emerges.

The first step in the absorption process is getting the proteases (exogenous or supplemental) through the intestinal...
mucous and across the intestinal epithelium. Mucous penetration for many proteases is relatively simple because many of them thin mucous by digesting the proteins in it (Chang 2012; Braga 1993, 1990; Majima 1986). Once through the mucous, there are at least four ways for proteases to get across the epithelium (Lorkowski 2012).

First, smaller proteases can be transported by the same carrier-mediated active transport that transports shorter peptides and amino acids. Similarly, the second mechanism involves endocytosis by columnar epithelial cells. Endocytosis is signaled by receptors, and the endocyte is absorbed and transported across the cell, but this mechanism allows for much larger molecules to be transported. The third mechanism involves simply leaking between the endothelial cells through gaps. A final mechanism involves active transport via vesicles across specialized endothelial cells called M-cells. Once transported across the vesicles and into circulation, they immediately complex with the alpha-2 macroglobulin and are actively absorbed by waiting leukocytes. (Lorkowski 2013; Isenman 1995).

Alpha-macroglobulin reacts with nearly all endoproteases, neutralizing them by encasing them in a protein structure that allows only the smallest peptides to reach the active protease site (Lorkowski 2012; Barth 1992; Barrett 1973). When the complex forms, the alpha-macroglobulin undergoes a conformational change that allows it to bind cytokines and interact with certain cellular surface receptors, especially the low density lipoprotein receptor-related protein 1 (LRP-1). LRP-1 regulates the transport of macromolecules into cells by endocytosis. By triggering this receptor, the protease activated alpha-macroglobulin complex is able to enter cells. These receptors are abundant on leukocytes and macrophages. Once they enter the cell, the complex is sorted. If cytokines are bound to them, those are detected by the cellular mechanisms. Otherwise, if not bound to a cytokine, the complex is either degraded and recycled, or stored for later use as a defensive protein (Etique 2013) (see Figures 1,2,3).

This last use is the one that makes protease supplementation useful. Leukocytes are transported throughout the body in search of oxidative stress by both the lymphatic and cardiovascular circulatory systems with guidance from signaling cytokines and circulating active immune complexes. Animal research suggests that when they encounter a peroxide gradient, they sense it through the dual oxidase receptor and actively follow the peroxide gradient towards higher levels of peroxide (Henry 2013). Depending on the type of leukocyte, the dual oxidase receptor may also be using the redox reaction to build up a supply of oxidative compounds such as chloramines (Stief 2000) in preparation for an eventual oxidative burst. When the concentration of...
peroxide reaches a sufficient level, it triggers the anticipated oxidative burst, releasing both the oxidative compounds and the stored defensive proteases complexed with alpha macroglobulin (Henry 2012; Niethammer, 2009; Stief 2000). The oxidative compounds attack whatever molecules are contributing to the inflammation and also neutralize the alpha macroglobulin complexes, releasing and activating the stored proteases. These then act on whatever proteins are present. By oxidizing and digesting the molecules present, the immune response neutralizes most sources of inflammation and any immune complexes and cytokines in the local area. When the oxidative potential subsides, fresh alpha macroglobulin arrives and re-encases the protease if it is still active, and the cycle starts over.

To summarize thus far, we have addressed the most common questions regarding the use of supplemental microbial enzymes. The facts are:

1. Microbial enzymes are GI stable and are not destroyed in the harsh environment of the stomach.
2. They are safe and pose no toxic or infection threat, and do not cause inhibition of endogenous enzymes.
3. They are absorbed intact as functional systemic enzymes into circulation, escorted by alpha 2 macroglobulins and utilized by the leukocytes.

As a defensive protein, protease is directed to the site of inflammation by the immune system, where the immune system then uses it as a tool to protect cells, signal other cells, or to degrade unwanted or invasive proteins. When used in this manner they have antioxidant, immune-stimulatory, immunosuppressive, and anti-inflammatory effects depending on how your immune system is directing them. Basically, they are a tool the immune system uses to interact with cells and proteins to signal or degrade them. Now, let’s explore more closely the functions and mechanisms of action that gives both endogenous and supplemental proteases their systemic benefit.

**Systemic Benefits of Proteases**

It is important to note that most of what we know about proteases’ interaction with the immune system has been learned from the study of endogenous protease re-absorption and systemic use. In an ideal world and healthy body, supply meets demand and all is well. So why does the body need supplemental proteases? The body is a very complex organism that also strives to be very efficient. It is known that many compounds that our body is able to synthesize are absorbed intact from the digestive system to save energy. Consequently, the body has a limited capacity to synthesize proteases quickly. Plants, animals and microbial organisms contain a lot of proteases that are very similar to human proteases. When the body has a need, and recognizes these proteases, it will absorb them from the digestive tract in the same manner as it reabsorbs human trypsin, and uses them for the same purposes. In our fast paced, high stress, processed food society, the need for supplemental proteases to assist the body in maintaining health has never been greater.

The important thing to remember when considering supplemental proteases is they are more like supplemental vitamins for your immune system. They are not like drugs which interfere with some aspect of either your body’s function or of a pathogen’s function. Supplemental proteases save the body the energy of synthesizing a tool it already makes allowing that energy to be used elsewhere. They also provide an additional supply when the body cannot keep up with the demand. And lastly, because they are so similar to naturally occurring molecules there are no negative side effects as commonly seen with medications.

There are numerous ways in which proteases can impart systemic benefits. Some assign the systemic effects completely to the nutritional benefits of more rapid and complete digestion of proteins, making more amino acids available not only for building but also for signaling. Another benefit related to digestion is proteases ability to clear the blood stream of short chain peptides. Others make the point that proteases can eliminate innate immune system triggering proteins before they reach the intestinal lining. Still others suggest that the proteases interact directly with the extensive portion of the immune system that extends along the gastrointestinal tract, suppressing the immune system rapidly via a variety of mechanisms at the surface of the cells. Finally, there are the proponents of action via intestinal absorption and delivery discussed in the previous section. The benefits of protease supplementation are diverse, and it follows that the mechanisms of action are likely to be diverse and multifaceted as well. Recently, scientific research has become available to construct scientific models that coherently explain how proteases might provide systemic benefits via each of these mechanisms.

**Systemic Benefits of Complete Protein Digestion**

It is well established that proteases digest proteins, delivering amino acids to the body to build and repair cells. The faster and more complete this nutritional process occurs, the greater the overall benefit to the body. The fact that amino acids are a necessary component for virtually every cell in our body (enzymes, hormones, neurotransmitters, immune cells, blood cells, muscles, bones, etc.) is enough argument alone to support complete protein digestion with supplemental proteases. Moreover, there are some proteins
more resistant to digestion than others. When these proteins are not completely digested the result is not only a lack of amino acids for building, but worse, an increased potential for “trigger proteins” that initiate oxidative stress, inflammation, and over whelm the immune system.

A key factor in this resistance can be the number and distribution of proline amino acids. Proline bonds create a tight bend in an amino acid chain, causing a protein to fold. This creates the opportunity for cross-linkages to form and numerous folds with cross-linkages make it difficult for endogenous proteases such as pepsin, trypsin and chymotrypsin to digest proteins or portions of proteins. A good example of this is the protein gluten which resists digestion because it is rich in proline bonds. In addition, the most resistant degradation peptides are rich in cross-linking amino acids such as glutamine. When gluten and related peptides reach the intestinal epithelium, the innate immune system recognizes them as threats through the toll-like receptor systems, triggering immune responses (Palová-Jelinková 2013; Junker 2012; Volta 2012; Sapone 2011). In sensitive individuals, repeated exposures to undigested proteins can cause local inflammation in the gastrointestinal tract as well as systemic effects throughout the body (Volta 2012).

The good news is, there are certain microbial proteases, also known as “endo-peptidases,” that are quite effective at digesting proline bonds, working with the body to digest these proteins and eliminate immune triggering proteins. Since there are many other resistant proteins in food capable of triggering similar responses, more complete protein digestion is capable of improving systemic health in sensitive individuals.

Systemic Benefits of Releasing Signaling Amino Acids

Another way proteases may provide systemic benefit via complete protein digestion is by releasing signaling amino acids. By making specific signaling amino acids readily available you are supporting the health of many tissues of the body. Repeated stimulation of the immune system by undigested proteins and other components of the typical western diet may result in loosening of the tight junctions between the cells lining the intestines, allowing some bacterial toxins to “leak” past the epithelial barrier and trigger more intense immune responses (Muniandy 2009). The amino acids glutamine and arginine are known to stimulate the tightening of these junctions and the amino acid leucine stimulates the replication of the intestinal lining cells (Beutheu 2013; Rapin 2010). Properly formulated proteases consumed with protein may support more rapid and complete release of these amino acids, indirectly and rapidly supporting intestinal integrity through better nutrition (Rhoads 2009). Beyond the intestinal epithelium, glutamine is the most important fuel for intestinal immune cells, and is an important factor in T-cell differentiation (Ruth 2013; Waickman 2012).

Furthermore, research has shown that amino acids from protein support muscle development and retention best when the amino acids are released more rapidly from protein. The branched chain amino acid leucine is especially important as increased concentrations of free leucine in mammalian muscle cells signal to the cell that adequate amino acid supplies are available. This initiates increased growth and cell division via stimulation of the mammalian target of rapamycin (mTOR) enzyme. It also inhibits apoptosis (cell death) by inhibiting the 5’ adenosine monophosphate-activated protein kinase (AMPK) enzyme (Li 2011; Kim2009; Kimura 2003). Other amino acids such as isoleucine, valine and glutamine have an additional upstream effect on mTOR and muscle growth, but their roles are not yet as clearly defined by research (You 2012; Nicklin 2009; Wu 2009; Yao 2008). mTOR is also present in many other cell types, regulating many systems. An example is the cells of the hypothalamus, where stimulation of mTOR and suppression of AMPK is being investigated as playing a central role in suppressing hunger (Guzmán-Quevedo 2013; Journel 2012; Cota 2006). Glutamine and tryptophan have been identified as signaling molecules in the neurological system, and the amino acids arginine and glutamine have been identified as having cardiovascular benefits (Albrecht 2010; Dong, 2011; Wu 2009; Stone 2007; Kakoki 2006). Many other signaling amino acid benefits have been recently identified, clarifying the nutritional aspect of proteases as releasing not just the building blocks of the body, but the fuel for the building and the signals that regulate the building (Sousa 2012; Ropelle 2008).

Systemic Benefits of Fibrinolytic Activity

An area in which supplemental proteases have far reaching systemic benefit is their support of healthy blood flow. We have discussed the importance of optimal protein digestion so now let’s review the importance of optimal circulation and the role proteases can play. Red blood cells are responsible for carrying nutrients and oxygen to the cells and carrying waste from the cells. Better blood flow will naturally enhance this process and support cellular nutrition, function, and detoxification.

Earlier in this paper we discussed the fact that endogenous proteases bound to alpha 2 macroglobulin have the ability to break down short chain peptides circulating in the blood. Relative to cardiovascular health, proteases also have the ability to break down excess fibrin and thrombocytes in blood. Blood clots are formed by thrombocytes (small fragmented “platelet” cells) and fibrin (a fibrous protein mesh) that holds the thrombocytes together. Fibrin is activated and controlled by the presence of fibrinogen. During times of
injury and acute inflammation, increased fibrin is necessary to prevent excess bleeding. However, when acute trauma is absent and chronic inflammation is still present, unnecessary fibrin remains and can contribute to thrombocyte aggregation, increased blood viscosity, and ultimately poor blood flow (see Figures 2 and 3). Supplemental proteases, both microbial and plant, have been studied for many years and now are well accepted for their ability to break down fibrin and reduce thrombocyte aggregation (Choi 2001; Kiessling 1970; Roschlau 1968). Flynn et al demonstrated the effects of proteases from A. Oryzae by injecting them into clotting blood observing almost “complete lysis of the clot.”

Dr. DicQie Fuller-Looney, founder of Transformation Enzyme Corp., taught and illustrated the benefits of enzyme therapy with Dark Field Microscopy. In her clinic she showed patients how their diet and lifestyle impacted their blood. Specifically, poor protein digestion or excess fibrin in the blood can be seen as erythrocyte aggregation, rouleau or spicules. The blood samples after implementation of dietary modification and enzyme therapy were markedly improved, appearing similar to Figure 4.

Furthermore, it is hypothesized that fibrinolytic activity of the proteases can also break down the same protein that allows tumors to form. According to M. Mamadou, Ph.D., in a literature review titled “The Role of Proteolytic Enzymes in Tumor Growth Control,” many studies are noted as demonstrating the anti-tumor effects of proteolytic enzymes. Since the 1960s, it has been postulated and later demonstrated that cancer cells are sticky and that the stickiness is a result of excess fibrin covering the cells. This increased presence of fibrin may be due to poor plasma fibrinolytic and thrombolytic activity. In fact, many studies have correlated the metastatic ability of cancer cells to fibrinolysis and thrombolysis. With less thrombolytic activity, there is more prevalence of cancer cells that invade and metastasize within the body (O’Meara 1958; Kojima and Sakai 1964; Clifton and Agostino 1964). The fact that oral proteases enhance thrombolytic and fibrinolytic activities may help attenuate and/or prevent further tumor cell growth (Kokron 1976; Thornes 1968, 1975; Steckerl 1961).

Additionally, the capacity of tumor cells to attach to the endothelium lining of the blood vessels is a necessary step in tumor cell invasion and dissemination to other tissues. The enhanced blood rheology resulting from proteolytic enzymes will inhibit this attachment. The lysis of fibrin covering the tumor cells will also help expose the cancer cells and promote their removal by the immune system and apoptosis. For the complete paper on tumor growth control, contact TEC.

In review, the function of proteases in the digestion of proteins can have far reaching health benefits. The delivery of bioavailable amino acids and peptides supplies all the cells of the body not only with the building blocks but also the signaling molecules that “tell” the cells to build. Secondly, with complete digestion of proteins the opportunity for erroneous peptides to “trigger” an immune response is minimized. And third, the hydrolysis of proteins in circulation improves blood flow and therefore can have a positive impact on cardiovascular health, control of tumor growth and many other areas of health.
Direct Interaction with Gastrointestinal Immune Cells

While proteases have important indirect effects on the immune system by neutralizing immune triggers and releasing amino acids, they may also interact directly with the immune cells on the surface of the gastrointestinal tract. Starting with the dendritic cells under the tongue, the gastrointestinal system is lined with immune cells. The work of Zimmer and others demonstrates that proteases may interact with dendritic immune cells (Mascarell 2011; Zimmer 2011). They discovered that interactions with proteases blocked immune stimulating receptors, digested surface proteins associated with stimulation of the immune system, suppressed expression of inflammatory genes in the cells, and even interfered with the activation of T-cells, suppressing systemic adaptive immune system responses. Suppression of T-cells in the body is not generally a healthy effect, but in the gastrointestinal tract it is important to prevent the development of food intolerances.

Discussion

Studies measuring blood plasma and serum levels of supplemental proteases show only very modest concentrations of proteases, and these are complexed with alpha 2-macroglobulin. This could lead one to conclude that the availability of the proteases is insufficient to support any significant systemic benefits. However, these concentrations are in line with many endogenous proteases in normal circulation. This conclusion misses the point. Proteases are tools of the immune system, whether they are endogenous or exogenous. They do not exert an effect based on mean serum or plasma concentrations. Their effect is more targeted and elegant. They are stored by the immune system in leukocytes, and delivered safely to where they are needed by a very complex immune system that responds to cytokines, antigens, and concentrations of oxidative compounds. When the delivery is complete, it releases the exogenous proteases along with oxidative compounds and a wide variety of other proteases, to quickly neutralize whatever is creating the oxidative signal.

Supplementing with proteases for systemic support of the immune system addresses a natural nutritional deficiency brought about by cooking and processing foods, restoring the natural supply of exogenous enzymes. Like many other forms of nutrition, it supports all systems of our body, allowing them to function normally, in spite of the nutritional deficits of the modern food supply.

Research and Clinical Observations: Anti-inflammatory Benefits of Proteases

The Baylor Study

In 2009, Baylor University conducted a double-blind, placebo-controlled study to assess the effects of Transformation’s proprietary protease blend on acute inflammation (Buford et al). In this study the participants were subjected to a strenuous exercise bout to induce acute inflammation. The laboratory findings for the protease group showed lower levels of pro-inflammatory cytokines (TNFα, IL1β, IL6, and IL12), lower levels of COX2 activity and improved muscle strength when compared to the same values in the placebo group.

Background

Biochemically, physiologically, or clinically, inflammation can be defined as the biological reparation response following any disruption of the cell’s structural or functional integrity by injurious agent(s) (internal or external) to the body. Based on this definition, injury brought about by sports or athletic activities will trigger a biological reparation response. Any therapeutic agent or dietary supplement that could enhance this biological reparation response would not only be helpful in athletic events but also in healthcare, as inflammation remains a common denominator to any health challenge.

Objectives

The study was designed to determine the effects of TEC’s proteolytic enzyme blend on muscle function and inflammation following strenuous exercise.

Methods

The study was conducted in a randomized, double-blinded, placebo-controlled manner. The selection of 29 physically active healthy males with an approximate average age of 22 was by consent and upon approval of the study guidelines by the University of Baylor Institutional Review Board for Human Subjects. Following the baseline testing (T1), the participants were divided into two groups: one group of 14 received 3 capsules of TEC’s protease blend (equivalent to 1,065,051 HUT + 1,800,000 PU) 3 times per day, whereas the other group of 15 received equal dosing of a placebo made up of cellulose. The participants took their respective supplements for 21 days. On day 21 the participants were subjected to another series of tests (T2), followed by an intense physical exercise regimen. Immediately following exercise, the tests were performed again (Post) as well as at 3, 24, and 48 hours. The participants continued taking the supplement until all testing had been completed.
Results
Tumor necrosis factor alpha (TNFα) is a cytokine that plays an important role in the inflammatory response, stimulating the secretion of IL1 and IL6. The secretion of TNFα needs to occur in any type of inflammatory or disease process, but its persistent secretion and long term effects on the body are less desirable. For health and recovery, a controlled synthesis and secretion of TNFα is optimal.

Interleukin 6 (IL6) is a pro-inflammatory cytokine promoting fever and high levels of acute phase liver proteins.

Interleukin 1 beta (IL1β) in high concentrations within the blood circulation induces fever, triggers the inflammatory response of the liver including a blood pro-coagulation effect, and promotes catabolism.

Cyclooygenase 2 (COX2) is an enzyme that is involved in pain and inflammation. The greater its activity, the greater the pain and inflammation, and a decrease in COX2 activity results in less pain. Several pharmaceutical drugs are used to help control its activity; however those drugs are not without serious side effects.
Force production in quadracep flexion and extension was measured to assess the effect of the protease blend on muscle strength and performance.

**Conclusion**
Protease supplementation seems to attenuate muscle strength losses after eccentric exercise by regulating leukocyte activity and inflammation.

**Discussion**
It is a known fact that inflammation is a common denominator in any pathological condition and occurs as long as the body is functioning. The causative agents may be varied, but the body’s basic response is the same. While the Baylor study used strenuous exercise to induce inflammation, the data points are characteristic of any condition that causes cellular injury, such as trauma, infection, stress, environmental toxins, or tumor growth, for example. Additionally, a controlled secretion of TNFα helps pro-inflammatory cytokines IL1 and IL6 respond appropriately, helping to modulate acute inflammation and control chronic inflammation. Modulated inflammation also reduces COX2 levels, which can result in less pain. This combination of decreased inflammation and lessened pain has the potential to enhance muscle performance.

**TEC Clinical Trial: The Effect of Protease on Inflammation**

In May of 2012, Dr. Milton Bastidas began a 12-week clinical trial at the TEC clinic in Houston, TX. The goal was to assess the benefits of TEC’s proprietary protease blend in individuals with varying levels of “silent” inflammation. The inflammatory markers C-reactive protein (CRP), Homocysteine, Fibrinogen, and erythrocyte sedimentation rate (ESR) were chosen as a representative group of blood markers for assessing changes in the level of inflammation in the participants. The optimal ranges for each marker are based on what is seen in healthy individuals rather than pathological ranges seen in disease states.

**Methods**
The participants were recruited through the local Chamber of Commerce and by word of mouth. Qualified participants were male or female between the ages of 30 and 65 with no known health concerns. Those on statins, NSAIDS, or chemotherapy were not accepted for this trial. A total of 22 participants began the trial with 18 completing the trial.

Baseline evaluations (week 1) performed in-house included metabolic and body type questionnaires, body composition analysis (RJL), lipid profile, and dark field microscopy. The in-house parameters were monitored for educational and control purposes. Blood was also drawn through Quest Diagnostics® labs and analyzed for CRP, Homocysteine, Fibrinogen, and ESR. All tests were performed at weeks 1, 6, and 12.

The participants were divided into 4 groups and given the following protocols. They were instructed not to change their diet or exercise patterns during the 12 week trial.

- **Group 1 (low)** = 355,017 HUT / 600,000 PU
  2 x day between meals
- **Group 2 (medium)** = 710,034 HUT / 1,200,000 PU
  3 x day between meals
- **Group 3 (high)** = 1,065,051 HUT / 1,800,000 PU
  4 x day between meals
- **Group 4 (placebo)** = 1 capsule cellulose
  2 x day between meals

**Results**
CRP is an acute phase reactant which increases sharply in response to inflammation. It binds to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system. It is synthesized by the liver in response to cytokines such as IL1β, IL6, IL12, and TNFα released by macrophages and fat cells. Healthy CRP levels are less than 0.55 mg/L. Group 3 demonstrated a significant decrease in CRP.
Fibrinogen is an acute phase reactant which increases sharply in states of inflammation and the presence of IL6. It is a key factor in blood viscosity and promotes the formation of blood clots inside coronary arteries. The optimal fibrinogen range is 200-300 mg/dL. Group 3 demonstrated the greatest improvement.

ESR shows the rate of settlement of whole blood against gravity in a one hour period. It is a rough measure of abnormal concentration of acute phase proteins. This marker is a non-specific indicator of tissue damage and inflammation.

Of the 4 markers of inflammation observed, homocysteine is a non protein amino acid. It is part of a biochemical loop shared with methionine, S-Adenosyl methionine, and S-Adenosyl Hcy with the help of cofactors such as B12, betaine, folate, and B6. When there is a problem with methylation, homocysteine is not recycled into methionine, causing an accumulation which then sets the stage for inflammation as it reduces nitric oxide production, oxidizes LDL, and increases blood viscosity by increasing fibrinogen activity. As all these effects of increased homocysteine persist, it sets the stage for atherosclerotic plaque and the persistent inflammatory immune response which is characteristic of cardiovascular disease. Homocysteine values should be less than 7.2 umol/L. We did not see a correlation between protease supplementation and homocysteine levels.
DNA as efficiently as it once did. If such irreparable damage slows down, and your body is not able to repair damaged muscle mass usually decreases, metabolism function poorly or even die if this occurs, which initiates inflammation and promotes premature aging.

As you get older, several physical changes occur that affect your health. Muscle mass usually decreases, metabolism slows down, and your body is not able to repair damaged DNA as efficiently as it once did. If such irreparable damage occurs, one of three things usually happens. First, the cell might die on its own or the "T" cells of the immune system do their job and eliminate the damaged cell. Secondly, the cell might replicate without restriction and transform into either a benign or a malignant tumor. Third, and very often, the cell will simply not be able to perform the functions for which it was designed. All these factors act as pre-cursors to aging.

So what happens when your cells are not functioning properly? One possibility is that the DNA of the cell is unable to produce the necessary enzymes for the body, or perhaps that the enzymes that it does produce are unusable. Modifying our lifestyle in an effort to reduce the stress on our body and cells is essential. As the cells in our body become less productive, does it not also make sense to supplement our diets with tools that can help it? When the cells in our body are unable to work the way they need to, it is actually possible to supplement our body with digestive enzymes, vitamins, minerals, and antioxidants to support the body in fighting oxidative stress. The digestive system is a vast network of organs and glands that begins at the mouth and ends at the anus. It is one of the first systems in the body to become compromised due to stress. When imbalanced, it often begins a cascade of chronic health challenges in other systems. Repairing, rebuilding, or simply supporting a healthy digestive system is essential to improving the absorption of nutrients, enhancing immune response, and reducing oxidative stress. Thus, healthy digestion equals healthy aging and increased quality of life.

Redox - The Measurable Factor of Aging

Your biological clock is always ticking, but did you know that your chronological age is not necessarily a reflection of your body’s biological age? Instead, there is a measurable biochemical process called "oxidative-reduction." Redox (rH2) is a measurement of oxidation-reduction potential (free radicals) under a specific pH measurement. This calculable factor mirrors your body’s actual aging process.

Oxidation-reduction reactions create high cellular energy in the form of ATP and also oxidize ("burn up") invading pollutants, xenobiotics (foreign materials that get into the body), and microorganisms that lead to degenerative diseases and contribute to premature aging. Measuring the rH2 of body fluids yields information about the body’s ability to produce energy (mitochondrial function), the degree to which excess free radicals are being produced, and ultimately cellular integrity and function.

According to Dr. Robert Greenberg, an rH2 value below 28 is considered “reduced” and has more electron donors than acceptors. A reduced solution is desirable, as increased electron donors in the cell and mitochondria means the TCA cycle is producing electron-rich intermediates that the
mitochondria can readily convert into energy. An rH2 value greater than 28 is considered “oxidized” and has a greater number of electron acceptors which produce unstable molecules (free radicals) in excess.

This is why doctors emphasize the benefits of eating foods rich in antioxidants. The principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, vitamin C, selenium, and zinc. Other antioxidants that are rapidly gaining notoriety are lutein and zeaxanthin. The body cannot manufacture these micronutrients, so they must be supplied through the diet via proper digestion and absorption.

An oxidized solution also indicates a decreased ability to produce high energy cellular fuel. Ultimately, as your rH2 decreases, so does your body’s “potential” biological age with respect to oxidation reduction reactions. Oxidation is not always bad. In fact, it is essential for life. The key is to find that healthy balance where the oxidation meets the body’s needs but is not in excess.

Clinical Findings
Transformation Enzyme Therapy Clinic utilized a Qualitative Fluid Analyzer (QFA) to monitor the biological terrain of our clients. The body fluids used are blood, saliva, and urine. The parameters we analyze are pH, redox, and resistivity. While all the parameters of biological terrain analysis are equally important, the focus of these clinical observations is to look at oxidation reduction (rH2) results.

A total of 106 patient files containing at least two QFA tests were reviewed for comparison of rH2 readings. All clients had been given dietary recommendations, an enzyme protocol, and lifestyle suggestions as per the individual biochemical needs. When we speak of “showing improvement,” it means the redox value moved closer to or into the ideal range for that body fluid. The ideal values are as follows:

- Blood 21.5 - 23.5
- Saliva 21.5 - 23.5
- Urine 22.5 - 24.5

**Results**

- Urine rH2 showed the greatest improvement, with 54.7% of the records reflecting improved values. This indicates greater utilization of electrons for energy production.
- In 51.8% of the records, Saliva rH2 showed improvement. This represents better digestion, absorption, metabolism, and elimination of waste.
- In 46.2% of the records, Blood rH2 showed improvement. This means the blood has a greater number of electrons and is primed with potential energy. The blood is able to create a chemical reaction whether to destroy a pollutant or to provide more energy.
- Another point of interest is the correlation between improved saliva pH and improved saliva rH2. We saw this positive correlation in 62% of our records. Each time, it indicated both better digestion and lowered oxidative stress.

For the complete report please contact TEC and request our Clinical Observation – Nutrition and Aging.

**Implications for Health: The Ripple Effect**

The digestive system is where the body receives the vast majority of nutrients (or non-nutrients in many cases). When nutrition and digestion are good, the body receives nutrients and benefits (see Figure 5). However, if diet and lifestyle choices are poor, the opportunity for toxicity increases.

The circulatory system is the “transportation highway” of the body. The better the blood flow, the better the delivery of nutrients to the cells and removal of waste from the cells. If this system is congested or the red blood cells are aggregated, then the delivery and removal processes are compromised. This can contribute to increased toxicity and will have a negative impact on health overtime.

A toxin can be created internally or received externally and can be anything that interferes with the healthy workings of your cells. In a healthy scenario, the circulatory system is doing its job to keep up with and eliminate toxins in a timely manner. But if our food choices, digestive ability or circulatory system is congested, the level of toxicity rises.
If poor blood flow and increased toxicity persists over time, cellular damage can occur. The toxicity leads to inflammation or oxidative stress and the lack of nutrients impedes timely repair. This is why holistic and integrative practitioners focus on nutrition and chronic inflammation as the root of degenerative diseases.

When toxicity and cellular damage persist at a greater rate than does nutrition and elimination, then the result is what we see as a disease in your patient.

If you believe fundamentally that “nutrition is the foundation of wellness” then you agree proteases play a key role in the nutrition process. Whether it is your own endogenous proteases or with the help of supplemental proteases, healthy blood flow supports delivery of the nutrients and removal of waste for optimal cellular function and integrity. It is this basic concept that drives the clinical application of supplemental proteases in every practice regardless of modality or focus.

Listed below are common areas TEC’s clinicians have successfully used TEC’s proteases in their protocols (see Figure 6).

**Bottom Line: Nutrients IN – Waste OUT**

How does all the above translate into better health for your patients? It is no secret chronic degenerative “lifestyle” diseases continue to rise and the use of prescription medications is following suit. As integrative, holistic healthcare practitioners you are acutely aware that traditional medicine is not working and you know the health of the gut and managing inflammation is the foundation of wellness.

We know 2 major health benefits of proteolytic enzymes are their role in reducing inflammation/oxidative stress and their digestive and fibrinolytic properties helping to improve circulation. Keep in mind neither inflammation nor fibrin are...
bad. It is obvious both the clotting of blood and inflammation are necessary for life. It is when the response is chronic or excessive that we have a problem.

The Ripple Effect of poor choices can easily be reversed by making healthy choices and implementing enzyme therapy. Through better digestion and a healthy gut, nutrients are delivered to the cells rather than toxins. Through improved circulation, toxins are removed in a timely manner and inflammation is reduced. The end result of nutrients in and waste out is health and balance to the entire body (see Figure 5).

While our first priority is to promote a healthy diet and lifestyle, we are all exposed to injurious agents on a regular basis. So our second priority is to support the body in a healthy response that allows it to return to balance. This extensive review of literature highlights the mechanisms of action, showing how the proteases work and how they can be used to complement all modalities and treatment protocols.

**Frequently Asked Questions**

**When is the best time to take a protease supplement?**

For maximum systemic benefit, it is best to take proteases between meals as this allows for faster absorption into circulation. However, if this is not realistic for your patient, then it is ok to take proteases with meals knowing that some of the protease enzymes may be used to digest food proteins. Also, it is better to take small doses several times throughout the day rather than one or two large doses in a day. Common dosing times are first thing in the morning, mid-morning, mid-afternoon, and bedtime.

**Can protease enzymes be taken with NSAIDS and/or prescription medications?**

Digestive enzymes function by breaking down specific chemical bonds in foods. In most cases digestive enzymes can therefore safely be taken with medications. However, it is of course always recommended to let your health care provider know what you are taking.

**Can protease enzymes be taken with prescription blood thinners?**

One area of caution is with prescription blood thinning agents. These types of prescription drugs interfere with the natural blood clotting mechanisms, while proteases break down fibrin allowing for better blood flow. They can be taken in conjunction, but it is recommended to dose them about four hours apart and monitor lab work closely. We recommend notifying and working with the doctor prescribing the medications.

**Can you take protease enzymes when taking prescription “protease inhibitors”?**

More often than not, the term “protease” that describes proteolytic enzymes is used in very general terms. There are many metabolic proteases in our body, each with many different functions. The medications that are designed as protease inhibitors are targeting a very specific viral protease. The supplemental digestive proteases are very different and will not interfere with the medication. In fact, oral supplemental digestive enzymes can be very supportive to those patients with auto-immune disorders.

**Suggested Reading**

*Science Brief – Improved Digestion Supplementing with Oral Digestive Enzymes* for more info on TPP Digest™

*Science Brief – Improved Gluten Digestion with Innovative Enzyme Solutions* for more info on TPP Carbo-G™

*Science Brief – Probiotics: Their Benefit on Human Health* for more info on TPP Probiotic™

**References**

**ENZYME SAFETY AND STABILITY**


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ENZYME STABILITY


ABSORPTION

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ANTI-INFLAMMATORY EFFECTS

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Systemic Proteolytic Enzymes


BLOOD FLOW


TUMOR GROWTH CONTROL


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