dotFIT™ DigestiveEnzymes

Goal

The goal of this product it to improve the digestive process to help mitigate non-clinical digestive issues and enhance nutrient absorption. It is designed to supply a digestive enzyme complex that contains five forms of naturally occurring digestive enzymes, α-amylase, lactase, lipase, cellulase and a neutral protease to support healthy digestion which is often compromised by western lifestyles including diet, stress, chemicals, etc., and aging. Digestive enzymes are necessary for the proper breakdown of carbohydrates, proteins, milk-based products, oils, fats, fibers, and other food components to yield the nutritional constituents that are indispensable in human structural development, function and maintenance throughout life. By consuming these five supplemental enzymes, which are obtained from gastric-resistant microbial sources, the objective is to add to the body's natural production to assist in proper digestion of ingested foods to improve non-clinical digestive irregularities such as bloating, gas, cramps, and constipation while enhancing extraction/absorption of the nutrients (amino acids, vitamins, minerals, glucose, etc.) contained in foods. Additionally, some evidence supports the use of digestive enzyme supplementation (primarily proteases) for attenuating inflammation and enhancing exercise-induced muscle recovery.

Rationale

Digestive enzymes are necessary to breakdown food into the essential nutrition that humans need to develop, grow, reproduce and maintain life.[1] The primary digestive enzymes are produced and secreted by the gastrointestinal system including the pancreas and are capable of digesting lipids, carbohydrates, proteins and nucleic acids. The three main types of digestive enzymes are 1) Proteases to break down protein into small peptides and amino acids. 2) Lipases to break down lipids (fats/oils) into fatty acids and glycerol. 3) Amylases to break down carbohydrates (CHO) into simple sugars.[1,2] While vitamins, minerals and other essential nutrition is liberated from the digestion of foods, digestive enzymes cannot breakdown fiber, and therefore it is not absorbed into the bloodstream.

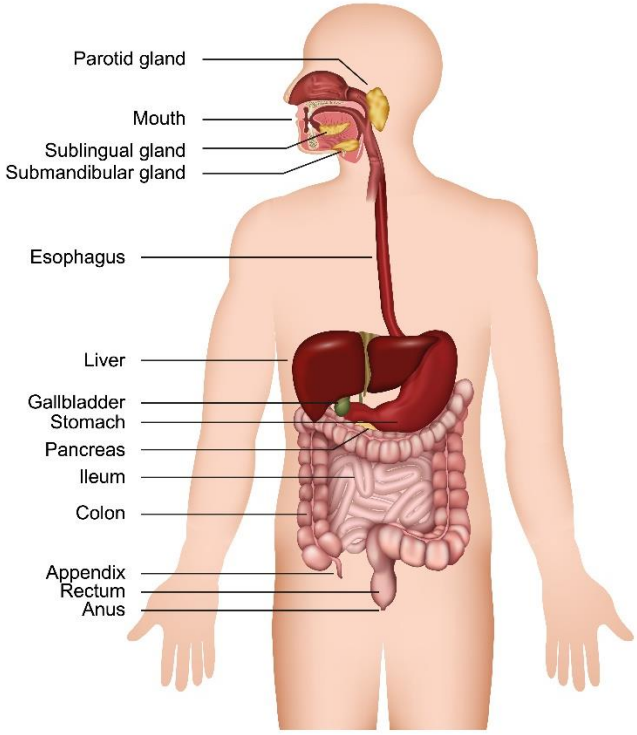
Digestion Overview

After diet selection, everything that happens to human development, maintenance and overall structure starts with digestion of the food stuffs into their essential and usable nutritional components (e.g. amino acids, vitamins, minerals, glucose, etc.).[1,2] The digestion of food takes place in the alimentary canal which encompasses the entire food passage area from mouth thru the anus (Figure 1). - Other than a cephalic response (gastric secretion that occurs before food enters the stomach), digestion begins as food enters the canal. Digestion starts with ingested food being broken down mechanically by chewing and stomach grinding actions leaving smaller particles to be denatured by stomach acids and predigested by α-amylase, lipases, and proteases now present in the stomach and small intestines. The products from this first stage of digestion now enter the small intestines where most of the final breakdown into the individual nutrients and other bio-active components takes place using the digestive enzymes produced by the pancreas and small intestines. When this process works efficiently, the body can properly absorb and utilize the end products for overall metabolism. In essence, the gastrointestinal system (including the alimentary canal), has extracted the essential nutrition from the ingested foods to be converted to usable/absorbable molecules that the body can use to develop and maintain life while discarding what cannot be absorbed and utilized as fecal matter.[1,2]

The better the digestive process, the greater the potential for essential nutrition to perform their respective and synergistic activities to create and maintain the human structure, function and health. Therefore, the rationale for supplementing digestive enzymes is to support the body's natural production if for any reason the production is less than optimal, such as with aging, including aiding in the typical age related decrease in lactase,[3,4] diet changes (introduction of different foods, good or not so good that your gastrointestinal tract [GI] hasn't had consistent/regular exposure to),[4,5] lifestyle alterations or medications.[6,7,8] Additionally, although not part of this paper's proposed application but from a clinical standpoint, the drug treatment of choice for the management of exocrine pancreatic insufficiency (EPI) in chronic pancreatitis, cystic fibrosis (CF) or diabetes is pancreatic enzyme supplementation.[8,9,10,11]

However, for our non-clinical purposes, oral over the counter (OTC) digestive enzyme supplementation, like DigeZyme, may improve overall digestion including important nutrient extraction and subsequent transport to help manage minor common and sporadic digestive problems such as gastrointestinal discomfort, bloating, gas, lactose intolerance, etc. or to support any other "better nutrient uptake related outcome" [12,13,14,15,16,17,18]

Figure 1 – Digestive Organs



Supporting Digestion

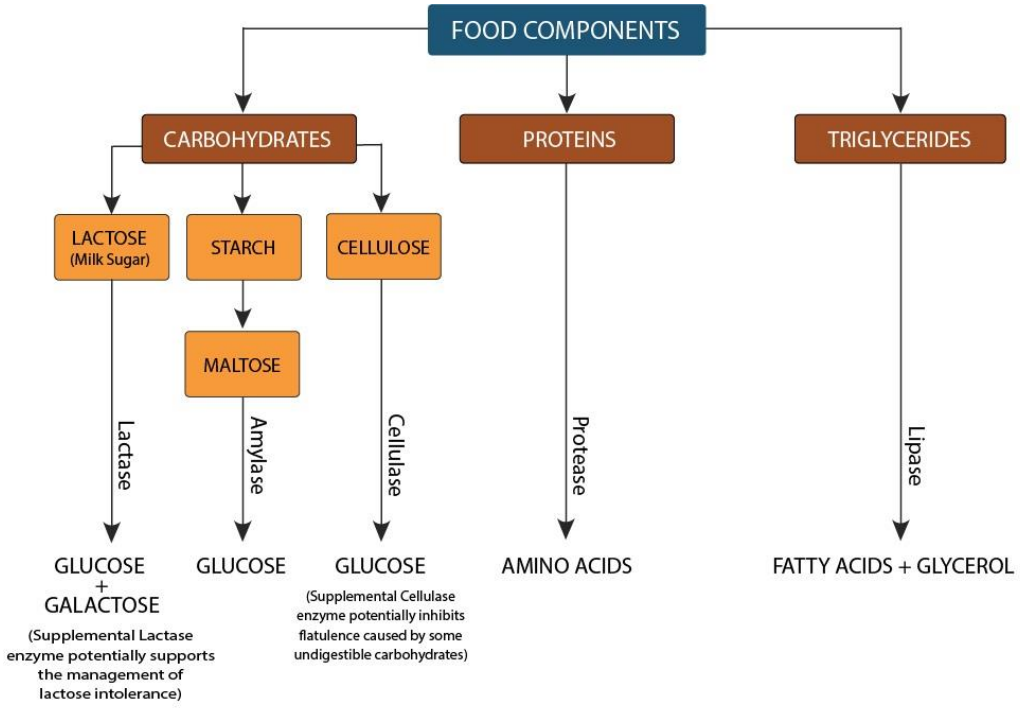
According to the National Institutes of Health (NIH) department of digestive diseases, approximately 60-70 million people are affected by digestive-related health conditions.[19] Correcting minor digestive enzyme insufficiency (or adding to normal production) with supplementation may serve to improve overall digestion including the liberation of essential nutrients and other bio-actives from ingested foods, subsequently decreasing GI discomfort and enhancing nutritional uptake into systemic circulation to potentially improve health including exercise induced inflammation and recovery.[20,21,22] To be sure, digestive enzyme supplementation continues to be used with success in animal nutrition to increase the normal extraction of essential nutrition from ingested feed and enhance the subsequent nutrient/bio-active constituents' uptake into the animal's body to improve growth and health.[23,24,25,26]

Robust scientific evidence is lacking for many human health claims related to the non-clinical use of digestive enzyme supplementation (DES).[27] Therefore, at this time we support the logic in using DES for its ability in certain individuals to improve digestion including extraction of essential nutrition from foods to subsequently enhance nutrient/bio-active uptake into the body to gain related potential benefits such as relief from mild non-clinical digestive disturbances as described above and under certain exercise conditions to enhance recovery. Further, if someone is to use the dotFIT DigestiveEnzymes product solely for GI discomfort, and if after 30-days of proper use there is no change, there may be no reason to continue use for this goal.

Enzymes in Formula

The enzymes, from microbial fermentation (also considered plant derived) contained in this formula are neutral protease, α-amylase, lipase, lactase and cellulase with Figure 2 showing their actions.

Figure 2 – Depiction of Food Components, Their Respective Enzymes and End Products



Neutral Protease

Proteases (also called proteinases or peptidases) are biologically active enzymes found in mammals and involved in all areas of metabolism.[1,2] Protease enzymes participate in many physiological processes, primarily the breakdown of proteins to their constituent peptides and amino acids and activation and deactivation of proteins. They are also involved in managing inflammation and immune functions. [1,2,28] The latter functions give rise to the use of proteases in exercise recovery and osteoarthritis, meaning some of the supplemented enzyme would have to remain whole as it enters the systemic circulation.[29,30]

Basic Mechanism of Action

Proteases initiate protein catabolism through hydrolysis of the peptide bond that connect the amino acids of the protein molecule so that they can be absorbed efficiently by the intestines into the body.[1,2] Buford et al. and Shing et al. have proposed protease exercise recovery mechanisms to be related to their ability to regulate the post-exercise inflammatory response. Buford proposed that proteases reduce the biosynthesis of eicosanoids and decrease edema by enhancing mobilization of inflammatory cells from tissues.[28] Shing suggested the mechanisms to be from reduction of inflammation but also mentions proteases' ability to digest damaged skeletal muscle components,[31] which would speed repair and restore muscle function sooner following exercise. Shing et al. also found protease supplementation during endurance cycling to be associated with maintenance of testosterone concentration and reduction in self-reported fatigue.[20] Studies of protease supplementation compared to placebo have demonstrated success in attenuating strength loss following exercise and decreasing soreness.[20,21,22,28,32] The dosages, which should be measured as "activity units," and protease forms were different in these studies and therefore if someone was to use

protease alone as a supplement for its potential to enhance exercise recovery, it may be confusing to determine the correct amount and not what we're supporting in this paper. Neutral protease is used in this formula because it delivers a broader application in overall utilization.[33] The enzyme activity is 6000 PC/gm (see definition from manufacturer below).

α-Amylase

Amylases are a group of enzymes that breakdown carbohydrates/starches into sugars. Carbohydrates are digested in the mouth**,** stomach and small intestine**.**[1,2] Alpha-amylase, (α-amylase) is a protein enzyme that hydrolyzes alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. α-amylase is the major form of amylase found in humans and other mammals.

Basic Mechanism of Action

Carbohydrates (CHO) from food are polysaccharides that need to be separated into usable sugar units such as glucose. Saliva in the mouth contains amylase, which is why if you chew a piece of bread long enough, the bread polysaccharide is digested to sugar, and yields a sweetness. Amylase is also produced and secreted into the small intestines by the pancreas where most CHO digestion takes place.[1,2] Like most enzymes acting through chemical attraction, amylase adds a water molecule to the bond that connects the glucose units within the polysaccharide. Called hydrolysis, this action breaks the polysaccharide chain into smaller units. If all bonds are broken, the process yields glucose units, the body's preferred energy source.[1,2] Alpha-amylase is used in this formula because it delivers a broader application in overall CHO utilization. Enzyme activity is 24000 DU/gm (see definition from manufacturer below).

Lipase

Lipase is a water-soluble enzyme that catalyzes the breakdown of fats/lipids to fatty acids and glycerol or other alcohols. In mammals, lipases are secreted by digestive organs, liver, and pancreas.[34]

Basic Mechanism of Action

Lipase enzymes are found in the blood, gastric juices, pancreatic secretions, intestinal juices, and adipose tissues. Lipases hydrolyze triglycerides into their component fatty acids and glycerol molecules.[34] After gastric lipase actions, the initial lipase phase happens in in the lumen of the small intestine. Bile salts alter the surface of the fat particles so the lipases can degrade the triglyceride (fats) molecules into the constituent fatty acid and glycerol components that can now be taken up into the epithelial cells in the intestinal wall and resynthesized into triglycerides for transport to muscles and adipose tissue. Lipase is included in this formula to enhance the breakdown of dietary fats as individually necessary.[15,16,17] Enzyme activity is 200 FIP/gm (see definition from manufacturer below).

Lactase

Lactase is an enzyme that catalyzes the hydrolysis and subsequently the breakdown of lactose into glucose and galactose including the sugar (lactose) in milk.

Basic Mechanism of Action

Lactases are secreted in the kidneys, small intestines and liver in most mammals and found primarily at the brush border membrane of the enterocytes along the villi of the small intestines in humans.[35] Lactose is hydrolyzed at the β-glycosidic bond by lactase to form galactose and glucose, which can be absorbed through the intestinal walls and into the bloodstream to be used effectively by the body.[36]

Lactase Insufficiency

Lactose malabsorption or intolerance (LI) are fairly common in individuals with lactase insufficiency or deficiency, and commonly increases with age. In normal conditions, the milk sugar lactose (a disaccharide) is broken down in the small intestine, by the enzyme lactase, to glucose and galactose but as the production of lactase decreases, affected

individuals are not able to cleave this disaccharide and become symptomatic when ingesting lactose (e.g. dairy products).[37] The undigested lactose is fermented by the colonic flora initiating symptoms like diarrhea, bloating, nausea and abdominal pain.[1,2,37] Lactase is included in this formula to supplement declining natural production.[38] Enzyme activity is 4000 ALU/gm (see definition from manufacturer below).

Cellulase

Cellulase is any group of enzymes, mainly produced by bacteria or fungi, that break down cellulose and other related polysaccharides.[39,40] Humans do not produce cellulase, which is why we cannot extract significant energy from fiber.[1,2] Humans can partially break down cellulose through fermentation and we get limited cellulase from the consumption of plants.[41]

Basic Mechanism of Action

Like other enzymes discussed here, cellulase breaks down the bonds between, in this case, the sugars that make up cellulose. Cellulases work synergistically to attack cellulose since it's a difficult task.[41] Cellulase is included in this formula because without enough cellulase, cellulose, from fruits, vegetables, etc., can adversely affect our GI system and manifest as upset stomach, bloating, acid reflux, gas, indigestion, constipation, or diarrhea.[42,43]

Table 1 - Activity Units of Digestive Enzymes (Source - Sabinsa[44])

Enzyme	Activity Units as per FCC	Enzyme Activity (Unit/g)
α-Amylase	DU	24000 U/g (Dextrinizing Unit/g)
Cellulase	CU	1100 CU/g (Cellulase Unit/g)
Lipase	FIP	200 FIP/g (Fédération Internationale Pharmaceutique Unit/g)
Lactase	ALU	4000 ALU/g (Acid Lactase Unit/g)
Neutral Protease	PC	6000 PC/g (Protease Unit on L-tyrosine basis/g)

Unlike other nutritional supplements, the potency or efficacy of an enzyme is not measured by weight or concentration, because the number of milligrams of any enzyme would not determine its true efficacy. Hence, "activity" is the determining factor of potency of an enzyme—in other words, the "effect" it has on proteins, fats, or carbohydrates. The enzyme activity is measured using "activity units" and is generally determined by various assays (test methods) that are performed under specific conditions.[44]

The accepted national standards of the U.S. Food and Drug Administration (testing methodologies) for determining enzyme potency are defined in the Food Chemical Codex (FCC) and published by the National Academy Press, Washington, D.C.[45] This system establishes activity levels and potency for enzymes.[45]

Sabinsa Microbial Fermentations in DigestiveEnzymes (DigeZyme®)

The following microbial enzymes from bacterial and fungal origin are used in DigeZyme®, and are produced by fermentation method.

Table 2 - Sources of Digestive Enzymes

Enzyme	Origin	Source
α-Amylase	Fungal	*Aspergillus oryzae*
Lactase	Fungal	*Aspergillus oryzae*
Lipase	Fungal	*Rhizopus oryzae*
Cellulase	Fungal	*Trichoderma longibrachiatum* (formerly known as *Trichoderma reesei*)
Neutral Protease	Bacterial	*Bacillus subtilis*

Advantages of Microbe Derived Enzyme Preparations:

The advantages of microbe-derived enzymes are the requirement of a lower dosage to be effective and a broader pH range of activity than animal-based counterparts (example: 4800 lipase units from microbe derived lipase was found to be equal to 60,000 units of conventional animal derived lipase[46]).[5,8,33,47] The multi enzyme complex (DigeZyme®) consists of broad acting enzymes obtained from the fermentation process with Aspergillusoryzae. This enzyme group is resistant to the action of gastric juices, while retaining their digestive activity.

Other advantages compared to animal sourced:

- Microbe-derived enzymes offer effective digestive support[48] and can work synergistically with the animal derived supplement, or as a vegan-friendly alternative[5,8]
- Microbe-derived enzymes provide a broad-spectrum of digestive enzymes, such as protease, lipase, amylase, lactase, cellulase, etc.[5,8,49]
- Microbe-derived enzymes may be used at a lower dosage and possess a broader pH range (i.e. 2–11) of activity compared to animal-based enzymes, which function in a limited alkaline pH range[5,8,33,47,50]
- Stable throughout passage: unusually high stability and activity throughout a wide range of pH conditions enables microbe-based enzymes to function more consistently for a longer distance through the digestive tract[5,8,33,46,47,48,50]
- Microbe-based counterparts are highly active at higher temperatures[5,8]
- Micro-organisms as a source of enzymes for nutritional supplements are more cost effective with a sustainable supply[50,51]
- Enzyme mixtures have a wider range of therapeutic advantage than individual enzymes, according to Dr. Peter Streichhan, a world-renowned enzyme researcher[52]

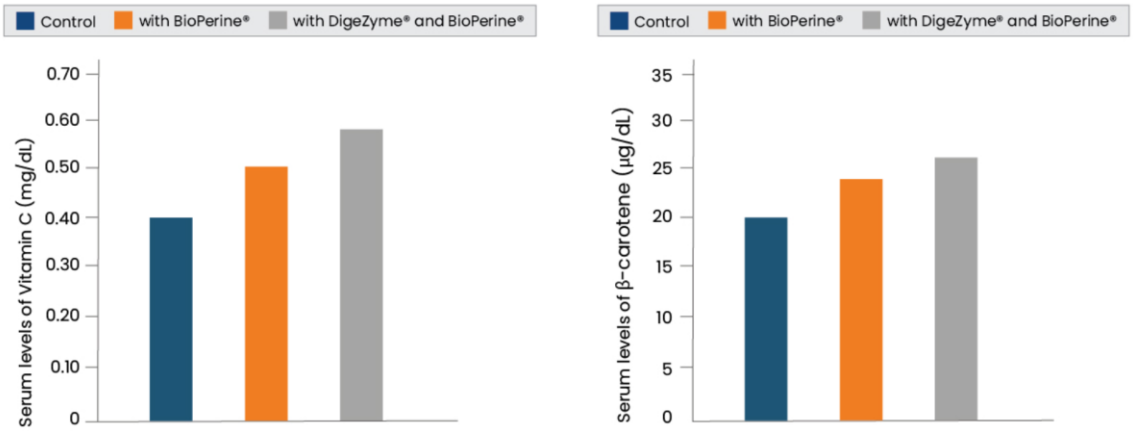
Clinical Data on DigeZyme®

Company Pilot Study on Nutrient Absorption Enhancement Potential

Using healthy volunteers in a five-day pilot study by the DigeZyme manufacturer, Sabinsa Corporation, the authors determined that the enzyme product enhanced the absorption of vitamins and minerals compared to control subjects and subjects using the nutrient enhancer BioPerine.* Figure 3 depicts the results.

**BioPerine is a Sabinsa product also used to enhance absorption[53] and as Figure 3 shows, DigeZyme added to BioPerine further improved the nutrient absorption*

Figure 3 – Results from Sabinsa Pilot Study



Relative bioavailability of micronutrients vitamin C and β-carotene in the presence of BioPerine® plus DigeZyme® or BioPerine® alone. Subjects were provided multivitamin/mineral capsule with or without BioPerine® alone or BioPerine plus DigeZyme and matched to controls. Conclusion: pilot study provided sufficient data to establish that DigeZyme® enhanced absorption of minerals and vitamins even in the presence BioPerine®.

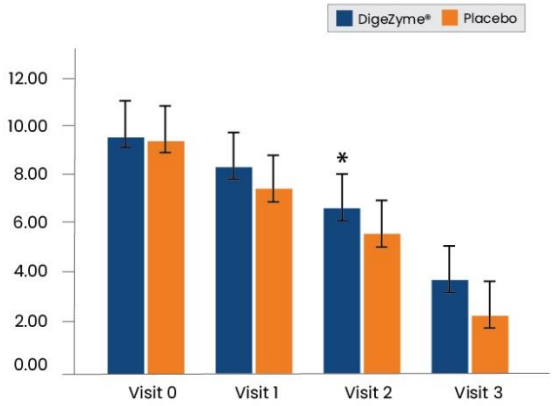
Published Clinical Trial on DigeZyme (DZ) in Management of Delayed Onset Muscle Soreness (DOMS)

Majeed et al. compared the treatment of DigeZyme (multi enzyme complex described here) with a matching placebo in reducing pain associated with DOMS induced by eccentric exercise in 20 male subjects taking 50 mg of DZ or placebo three times daily for three days.[22] DOMS symptoms were analyzed throughout the study period as the primary efficacy measure. Table 3 captures the statistically significant change in symptoms in the primary efficacy parameters from baseline to final visits between the placebo and DZ groups. Figures 4 and 5 graph the soreness and pain ratings. Further, there was a reducing trend in biomarkers of muscle damage (creatine kinase and lactate dehydrogenase). Compared to placebo, DZ subjects showed significant improvement in subjective pain and tenderness along with a trend in displaying less muscle damage leading the authors to conclude the following: "The multi-enzyme complex (DZ) contains enzymes that are indicated for relieving the symptoms of DOMS. The findings of this study suggest that multi enzyme complex can have several potential clinical applications. Protease supplementation with exercise can result in a more rapid recovery of damage caused by exercise-induced DOMS."[22] The study was only 3-days with positive trends in some of the non-significant results in certain endpoints such as muscle damage markers and strength, which suggests that over a normal lengthy training period, there would be an eventual recovery benefit, thus higher performance potential compared to a non-supplemented state. These results are in line with previous similar trials.[20,21,28,32]

Table 3 – Effects of DigeZyme vs. Placebo on Measures of Delayed Onset Muscle Soreness

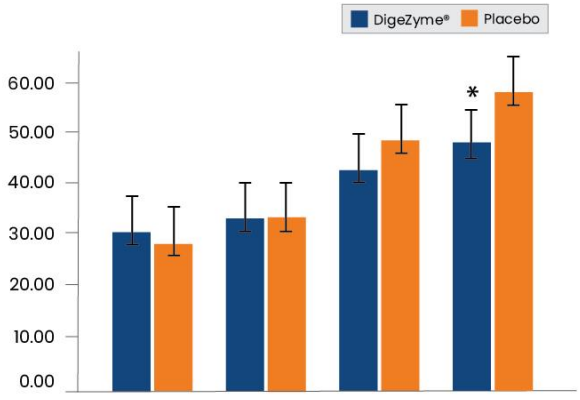
Subjective Parameters					
Measure	**Investigational Products**				**Significance**
Hand held Dynamometer readings result [grip strength] kg	**DigeZyme®**		**Placebo**		0.4956
	Baseline	**72 hours Post Exercise**	**Baseline**	**72 hours Post Exercise**	
	36.2 (7.86)	37.6 (8.32)	35.2 (6.84)	36.2 (5.26)	
Thigh muscle (point of pain) Algometer Reading's kg/cm²	9.5 (0.58)	5.3 (1.18)	9.3 (1.03)	3.9 (1.45)	0.0436*
Calf muscle (point of pain) Algometer Reading's kg/cm²	9.1 (1.09)	3.9 (1.20)	9.4 (1.11)	3.3 (1.03)	0.1397
Total Time Taken Illinois Agility Run test (Seconds)	25.6 (3.43)	24.6 (4.11)	25.2 (1.69)	23.8 (2.91)	0.7246
The McGill pain questionnaire (total pain score)	29.5 (2.55)	48.7 (8.86)	28.1 (1.45)	61.3 (7.07)	0.0061*
Objective Parameters (Serum markers (U/L)					
Serum Creatine Kinase	42.4 (11.69)	45.8 (10.49)	36.7 (4.30)	40.5 (5.42)	0.5735
Serum Lactate De-hydrogenase	161.1 (35.56)	164.2 (31.17)	162.6 (28.40)	161.4 (25.44)	0.2556

Figure 4



Aglometer readings to quantify pressure pain threshold (ppt) of thigh muscle. *p<0.05 between the treatment groups

Figure 5



The muscle soreness questionanaires (MSQ). *p<0.05 between the treatment groups

Results by Majeed et al. suggested that DigeZyme® was able to decrease the associated pain and tenderness induced by exercise. Decrements were also observed in McGill Pain Questionnaire showing high significance in the active arm (Figures 4 and 5).[22]

Summary

The body's natural digestive enzymes are responsible for the breakdown of foods into the molecules that can be absorbed into the body in order to create, develop and maintain life. The primary enzymes included in the DigestiveEnzymes complex are proteases (protein digestion), amylases (CHO digestion) and lipases (fats/lipid digestion) with sub-categories such as lactase (lactose digestion) and although not produced by the body, cellulase (cellulose digestion). Natural enzyme production is often compromised by genetics, age and lifestyle including diet and environmental insults. Supplementing digestive enzymes may add to normal production or help correct non-clinical enzyme insufficiencies to improve digestion and subsequent nutrient (e.g. amino acids, vitamins, minerals, essential fats, glucose, etc.) extraction and absorption. The result of better digestion may be relief from minor digestive

disorders such as bloating, gas, cramps, etc., and/or improved health or daily recovery outcomes from enhanced essential nutritional uptake.
The advantages of microbe-derived enzymes over animal-based counterparts are the requirement of a lower dosage to be effective, and a broader pH range of activity making it remarkably operative throughout the GI tract. Additionally, there is evidence that supplementing digestive enzymes may decrease inflammation, improve exercise-induced soreness and concomitant recovery.

Typical Use

- For individuals attempting to improve overall digestion including important nutritional extraction from ingested foods and transport to help manage minor common and sporadic digestive problems such as gastrointestinal discomfort, bloating, gas, lactose maldigestion, etc. and/or offer any other "better nutrient/bio-active uptake related outcome."
    - Not recommended if contraindicated by an existing health condition or discouraged through qualified medical advice.
    - If using solely for GI discomfort, and if after 30-days of proper use there is no change, there may be no reason to continue use for this goal.
- For exercisers to potentially reduce exercise-induced DOMS and improve nutrient uptake to enhance recovery
- Take three (3) capsules total daily. Take one (1) capsule with each of three main meals or as directed by a health professional

Precautions

Proper use of the dotFIT DigestiveEnzymes complex is considered safe for the general population.[22,54]

Contraindications

Digestive enzyme supplementation should be avoided during pregnancy or lactation because of lack of data or should only be used as recommended by the attending physician. According to the Natural Medicine Data Base, proteases may be contraindicated when taking anticoagulant/antiplatelet drugs and Amoxicillin (Amoxil, Trimox).[55]

Adverse Reactions

However unlikely, a user may experience abdominal cramps or diarrhea from related change in digestion management.[55]

Upper Limit/Toxicity

None established or known when taken as directed

Summary

Purpose

- Deliver an enzyme complex that can add to the body's natural production of the primary enzymes assisting in proper digestion of ingested foods to improve non-clinical digestive irregularities (if necessary) such as bloating, gas, cramps and constipation including from lactose maldigestion
- Enhance extraction/absorption of the nutrients/bio-actives (amino acids, vitamins, minerals, glucose, flavones, etc.) contained in foods to glean related benefits of improved nutrient utilization throughout the body
- Some evidence supports the use of digestive enzyme supplementation (primarily proteases) for decreasing inflammation and exercise-induced DOMS and improving muscle recovery

Unique Features

DigestiveEnzymes 5-enzyme complex (DigeZyme) containing neutral protease, α-amylase, lipase, lactase and cellulase is from Sabinsa Corporation, a manufacturer and supplier of herbal extracts, cosmeceuticals, minerals and specialty fine chemicals. Sabina has more than 120 scientists working full time conducting ongoing research both in India and the United States. Sabinsa continues to develop and patent beneficial nutrients for the world market.

- Contains microbe-derived enzymes, which have distinct advantages over animal-based enzymes
  - Survives the different pH levels in the GI tract for more activity throughout passage
  - Lower dosages with more potency
  - Heat stable - can operate in high temperatures
  - Economical and sustainable source
  - Vegan friendly and gluten free
- Multi-enzyme mixture has greater therapeutic value than individual enzymes
- Specifications adhere to the most stringent international standards and regulatory norms – Food Chemicals Codex, an acceptable standard for the US FDA
- Beyond the typical three common enzymes (protease, α-amylase, lipase), DigestiveEnzymes contains lactase to help breakdown lactose from dairy and other foods and cellulase to specifically assist in helping manage the digestion of fibers
- This formula considers use of other dotFIT products to help the user maintain a safe and optimal range of total nutrient intake
- Manufactured in a regularly inspected NSF certified facility, in compliance with Good Manufacturing Practices (GMPs) exclusively for dotFIT, LLC

Supplement Facts Panel

Supplement Facts

Serving Size: 1 Capsule

	Amount Per Serving	% DV
Digestive Enzyme Complex (Digezyme®)	50 mg	*
Alpha Amylase (1200 DU), Protease (300 PC), Cellulase (55 CU), Lactase (200 ALU), Lipase (10 FIP)		

* Daily Value not established.

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