Digestive Enzyme Supplementation in Gastrointestinal Diseases

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Abstract: *Background:* Digestive enzymes are able to break down proteins and carbohydrates and lipids, and their supplementation may play a role in the management of digestive disorders, from lactose intolerance to cystic fibrosis. To date, several formulations of digestive enzymes are available on the market, being different each other in terms of enzyme type, source and origin, and dosage.

Methods: This review, performed through a non-systematic search of the available literature, will provide an overview of the current knowledge of digestive enzyme supplementation in gastrointestinal disorders, discussion of the use of pancreatic enzymes, lactase (β-galactosidase) and conjugated bile acids, and also exploring the future perspective of digestive enzyme supplementation.

Results: Currently, the animal-derived enzymes represent an established standard of care, however the growing study of plant-based and microbe-derived enzymes offers great promise in the advancement of digestive enzyme therapy.

Conclusion: New frontiers of enzyme replacement are being evaluated also in the treatment of diseases not specifically related to enzyme deficiency, whereas the combination of different enzymes might constitute an intriguing therapeutic option in the future.

Keywords: Bile acids, celiac disease, enzyme supplementation, gastrointestinal disease, lactose intolerance, pancreatic insufficiency.

INTRODUCTION

Digestive enzymes are produced and secreted by the gastrointestinal system to degrade fats, proteins, and carbohydrates, to accomplish the digestion and, afterwards, the absorption of nutrients. Their supplementation, when indicated, may provide a reliable help as an adjuvant treatment of several disorders characterized by an impairment of digestive functions. To date, various formulations of enzyme supplementation are available on the market, and they are currently used in clinical practice for the management of several digestive diseases, especially those involving organs designated to the production of digestive enzymes, including the exocrine pancreas (which produces pancreatic enzymes) and the small intestinal brush border (which produces lactase).

Pancreatic enzyme supplementation is the therapy of choice for the management of exocrine pancreatic insufficiency (EPI) in chronic pancreatitis, pancreatic cancer, cystic fibrosis (CF) or diabetes [1-6].

Another relevant application of enzyme supplementation in the clinical practice is the management of lactose intolerance. It is estimated that 75 percent of individuals worldwide experience hypolactasia, or some decrease of lactase activity, especially during adulthood [7].

Recent evidence suggests that digestive enzymes may be useful also in celiac disease, but they are far from being used in the routine management of the disease. In celiac disease a lifelong gluten-free diet may bring about difficulties as avoiding gluten completely is problematic owing to the contamination with gluten of presumably gluten free foods [8]. New therapeutic approaches include enzyme supplementation, correction of the intestinal barrier defect against gluten entry, blocking of gliadin presentation by human leukocyte antigen blockers and tissue transglutaminase inhibitors [9].

Finally, conjugated bile acids, even if are not classifiable as enzymes, are able to promote absorption of dietary lipids by emulsifying them in micelles, so we included them in this review.

This paper will provide an overview of the current knowledge of digestive enzyme supplementation in gastrointestinal diseases and include also publications with animals and *in vitro* studies. We did a non-systematic but thorough review of the available literature. Respectively, indications, biochemical features and dosages of pancreatic enzymes, lactase (β-galactosidase), conjugated bile acids and endopeptidases will be reviewed. Finally, our hypothesis for a possible scenario of digestive enzyme supplementation in the next future will be presented.

PANCREATIC ENZYME SUPPLEMENTATION

Indications

EPI is a life-threatening condition associated to several pancreatic and extra-pancreatic diseases (chronic pancreatitis, acute pancreatitis, cystic fibrosis, pancreatic cancer, Schwachman syndrome and as a consequence of gastrointestinal and pancreatic surgery). Patients with EPI who lose weight, those with daily fecal fat excretion higher than 15 g under a diet including 100 g fat per day, and those with relevant steatorrhea-related symptoms are classically considered as requiring enzyme substitution therapy [5].

Furthermore, pancreatic enzyme supplementation could be used to relief abdominal pain in chronic pancreatitis, since the introduction of exogenous enzymes is supposed to play a negative feedback regulation on endogenous enzyme secretion, with consequent reduction of pancreatic duct pressure. Notwithstanding, their use in clinical practice remains controversial [1] and different studies are looking for criteria predicting a clinical response in this subset of patients [2].

Enzyme Features

Pancreatic enzymes can be divided into three groups, according to their respective function: proteolytic enzymes (mainly trypsinogen and chymotripsinogen and their active forms trypsin and chymotripsin), amylolitic enzymes (pancreatic amylase), and lipolitic enzymes (principally lipase) [10].

Exogenous pancreatic enzymes are primarily extracted from porcine or bovine sources. Lipase may also be synthesized from microbial sources, such as Aspergillus oryzae and Rhizopus arrhizus [11].

As described in animal studies, 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 [12];

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however, porcine pancreatin, which contains trypsin, amylase and lipase, is actually the only pancreatic enzyme replacement therapy (PERT) available in the UK [13].

Commercially available formulations are both non-enteric-coated and enteric-coated: this latter preparation has been developed to facilitate the passage of ingested enzymes through the hostile acid milieu of the stomach and duodenum, because the efficacy of exogenous enzyme supplementation is decreased by low pH; lipase is indeed irreversibly denatured when exposed to pH ≤4 13, 14].

Until April 2010, pancreatic replacement therapy did not require safety and efficacy data to be submitted to FDA. Since April 2010, FDA required clinical trials and Investigational New Drug Application submission for the approval of pancreatic enzymes preparations in the United States, thus leading to the removal of previously available products from the market [3]. Six products have obtained FDA approval in US: Creon and Zenpep (2009) Pancreaze (2010), Ultresa, Viokace and Pertzye (2002) [15].

Liprotamase is a novel biotechnology-derived, non-porcine enzyme replacement therapy containing three purified and stable enzymes: cross-linked crystalline lipase, crystalline protease and amorphous amylase. Since the stability (resistance against proteolysis and stability at acid pH) is an intrinsic characteristic of the individual enzyme, coating is not required. In a phase III trial, a dose of one capsule per meal (5 capsules per day) was well tolerated, increased fat and protein absorption and significantly decreased stool weight in patients with cystic fibrosis [4].

Recommended Dosages and Daily Posology for the Formulation

The required daily dose of pancreatin is variable, being related to the etiology and severity of pancreatic insufficiency and clinical features of the patient, such as age and body weight, and, for cystic fibrosis, also genotype and intestinal factors affecting absorption. Preparations of pancreatic enzyme are dosed by lipase content. However, many evidences suggest that a minimal dose of 25 000–50 000 U of lipase per meal is generally required to reduce steatorrhoea to <15 g fat per day in adults [16-18]. When dealing with cystic fibrosis, 500–3000 U lipase / kg per meal are recommended, and <6000 or 10 000 U lipase / kg / day in children. Children aged >4 years tend to eat less fat per kilogram than at ages <4 years requiring fewer enzyme dosage (500 vs. 1000 U lipase / kg / meal respectively) [19].

Enzymatic Activity and Relevance of Enzymes Contained in the Formulation

The activity and concentration of these enzymes are determined by multiple factors, including animal's species, age and sex, as well as husbandry practices. Pancreatic physiology of hogs is more similar to humans than any other animal species. Enzymatic activity levels from pork sources are approximately 30- to 50-percent higher than beef sources [5].

However, commercially available formulations differ from each other in terms of enzyme (lipase, amylase, protease) content. In Table **1**, a non-comprehensive list of exogenous pancreatic enzyme formulations available to date in Europe is shown (Table **1**, adapted from MIMS [6]).

Table **2** compares enzymatic activity of pancreatic and fungal-based enzymes [5].

LACTASE (β-GALACTOSIDASE) SUPPLEMENTATION
Indications

Lactase deficiency represents the main cause of lactose malabsorption. Lactase is an enzyme produced by intestinal villi, which is able to hydrolyze lactose into galactose and glucose. High lactase concentrations are normally present in neonates, but, after weaning,

its activity decrease in most people in a genetically-based fashion, driving to the so-called primary lactose malabsorption. Secondary hypolactasia, instead, can result from any damage of the small intestinal mucosal brush border or increase of the gastrointestinal transit time. Lactose intolerance is defined when lactose malabsorption causes gastrointestinal symptoms [20].

Even if, strong evidences suggest usefulness of lactase supplementation in lactose intolerance, also in infants, this issue is not covered by available guidelines.

Enzyme Features

Replacement of native lactase through the use of exogenous enzymes, derived from yeast or fungi, with microbial exogenous lactase (obtained from yeasts or fungi) may be considered a reliable therapeutic option. Exogenous lactase can be administered with milk, or as capsules/tablets before eating dairy products. The latter formulations are widely available on the market, and several studies have investigated and confirmed their efficacy [21-24].

Enzymatic Activity and Recommended Dosages

At the same dose, enzymes obtained from different microorganisms display different efficacy in hydrolyzing lactose. Comparative studies showed that lactase derived from K. lactis displays higher efficacy than lactase from A. niger [25, 26]. Enzymatic activity depends on features of commercial formulations. Table **3** shows some common lactase brands, widely used in US and Europe, with each own enzymatic activity.

Moreover, in a study from Lin *et al*, three different lactase formulations (Lactogest -soft gel capsule, Lactaid –caplet-, and DairyEase -chewable tablet-), compared with placebo, were fed to lactose intolerants with either 20 g or 50 g of lactose; the trial was performed with 6000 IU (respectively four capsules of Lactogest - two caplets of Lactaid or two tablets of DairyEase) and 3000 IU (two capsules of Lactogest) of lactase. All enzyme preparations were able to decrease the peak as well as total breath H2, when a 20g-dosage of lactose was administered. 6000 IU of lactase treatment reduced total hydrogen production significantly (P < 0.05) below that observed with 3000 IU dosage. Symptoms improved significantly (P < 0.05) with all the products. When a dosage of 50 g of lactose was administered, neither 3000 nor 6000 IU of beta-gal were able to improve the digestion and absorption of lactose. Results from these studies demonstrate the relative equivalency of chewable, caplet, and soft-gel beta-gal products, based on IUs of enzyme fed [27].

CONJUGATED BILE ACIDS
Indications and Features

Conjugated bile acids are amphipathic molecules that emulsify the lipolysis product of dietary triglycerides and fat-soluble vitamins. In particular, ursodeoxycholic acid (UDCA) is a tertiary bile acid widely used in the treatment of different cholestatic diseases [28]. Several Cochrane reviews evaluated beneficial and harmful effects of UDCA in patients with non-alcoholic fatty liver disease/steatohepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, after liver transplant [29-32]. Nevertheless, none of them lead out a significant evidence to support or refuse the use of bile acids in such diseases, often because of the small sample size of the studies reviewed, except in the case of primary biliary cirrhosis where a benefit on survival was excluded [30]. Furthermore, a meta-analysis by Manley and colleagues showed that UDCA can prevent gallstone formation in patient undergone to bariatric surgery [33]. Bile acids have been evidenced to be useful also in progressive familial intrahepatic cholestatis (PFIC), with a decrease of cholestasis and hepatocytonecrosis markers, and an improvement of hepatic functional test, with a dose of 20-30 mg/kg/day, over a period ranging from 2 to 4 years [34].

Table 1.   List of exogenous pancreatic enzyme formulations available in Europe.

Trade Name	Lipase (U)	Amylase (U)	Protease (U)
Non-enteric coated			
Pancrex V powder (100/250/300g)	25000	30000	1400
Pancrex granules	5000	4000	300
Pancrex V capsules	8000	9000	430
Pancrex V tablets	1900	1700	110
Enteric coated			
Creon 10	10 000	8000	600
Creon 25	25 000	18 000	1000
Creon Micro	5000	3600	200
Nutrizym 10	10 000	9000	500
Nutrizym 22	22000	19800	1100
Pancrease HL	25000	22500	1200
Pancrex V Forte tablets	56000	5000	330
Enteric coated [porcine derived]:			
Pertzye	8,000 USP units	30,250 USP units	28,750 USP units
Pertzye	16,000 USP units	60,500 USP units	57,500 USP units
Enteric coated [porcine derived]:			
Pancrelipase (Lip-Prot-Amyl)	5000 USP units	27,000 USP units	17,000 USP units
Zenpep	3000 USP units	16,000 USP units	10,000 USP units
Zenpep	5000 USP units	27,000 USP units	17,000 USP units
Zenpep	10,000 USP units	55,000 USP units	34,000 USP units
Zenpep	15,000 USP units	82,000 USP units	51,000 USP units
Zenpep	20,000 USP units	109,000 USP units	68,000 USP units
Zenpep	25,000 USP units	136,000 USP units	85,000 USP units
Cotazym	10,000 USP units	40,000 USP units	35,000 USP units
Cotazym	10,800 USP units	42,000 USP units	45,000 USP units
Cotazym	25,000 USP units	100,000 USP units	100,000 USP units
Enteric coated [porcine derived]:			
Creon	3000 USP units	15,000 USP units	9500 USP units
Creon	6000 USP units	30,000 USP units	19,000 USP units
Creon	12,000 USP units	60,000 USP units	38,000 USP units
Creon	24,000 USP units	120,000 USP units	76,000 USP units
Creon	36,000 USP units	180,000 USP units	114,000 USP units
Lipram	10,000 USP units	30,000 USP units	30,000 USP units
Lipram	16,000 USP units	48,000 USP units	48,000 USP units

Table (1) continued

Trade Name	Lipase (U)	Amylase (U)	Protease (U)
Lipram	18,000 USP units	58,500 USP units	58,500 USP units
Lipram	16,000 USP units	48,000 USP units	48,000 USP units
Lipram	20,000 USP units	65,000 USP units	65,000 USP units
coated [porcine derived]:			
Pancreaze	4200 USP units	17,500 USP units	10,000 USP units
Pancreaze	10,500 USP units	43,750 USP units	25,000 USP units
Pancreaze	16,800 USP units	70,000 USP units	40,000 USP units
Pancreaze	21,000 USP units	61,000 USP units	37,000 USP units
Pangrol	10,000 Ph.Eur.U	9,000 Ph.Eur.U	500 Ph.Eur.U
Pangrol	20,000 Ph.Eur.U	12,000 Ph.Eur.U	900 Ph.Eur.U
Pangrol	25,000 Ph.Eur.U	22,500 Ph.Eur.U	1,250 Ph.Eur.U
Panzytrat	25,000 Ph.Eur.U	22,000 Ph.Eur.U	1,250 Ph.Eur.U
Ozym	40,000 Ph.Eur.U	25,000 Ph.Eur.U	1,500 Ph.Eur.U
Enteric coated [porcine derived]:			
Ultresa	13,800 USP units	27,600 USP units	27,600 USP units
Ultresa	20,700 USP units	41,400 USP units	41,400 USP units
Ultresa	23,000 USP units	46,000 USP units	46,000 USP units
Non-enteric coated [porcine derived]:			
Viokace	10,440 USP units	39,150 USP units	46,000 USP units
Viokace	20,880 USP units	78,300 USP units	78,300 USP units

Table 2.   Comparison between pancreatic and fungal-based enzymatic activity. SKB: Sandstedt, Keen and Blish, Cereal Chemistry 12, 172, 1939, based on the digestion of starch over time

Enzyme	Pancreatin	Microbe-derived
Amylase units	≈89 USP	100 SKB (4800 USP)*
Protease units	≈197 USP	500 HUT (3250 USP)**
Lipase units	≈80 USP	100 LU***

HUT: Hemoglobin Units; based on enzymatic hydrolysis of denatured hemoglobin. LU: Lipase Units; based on lipolytic activity utilizing olive oil. USP: U.S. Pharmacopoeia units. *1 SKB=48 USP;**1 HUT = approximately 6.5 USP; *** No conversion available to USP

Table 3.   Common lactase brands, widely used in US and Europe, with each own enzymatic activity.

Trade Name	Lactase (U)
Silact	>30.000
Lacdigest	2250
Lactaid	9000
Digerlat	100000
Dairy-Ease	3000

Recommended Dosages and Daily Posology for the Formulation

In prolonged use, the mean daily posology is about 5-10 mg/Kg, or rather 300-600 mg/die in the majority of cases, in the treatment of biliary lytiasis. To treat dyspepsia, 300 mg/die, divided in 2-3 administrations, are considered an effective dosage.

In the retard formulation, daily posology is 450 mg/die, but in obese patients, or in presence of important risk factors for lithiasis, it is beneficial to raise dose to 675 mg/die. In dyspepsia, a smaller dose (225 mg/die) is recommended [34].

ENZYME SUPPLEMENTATION IN CELIAC DISEASE

Celiac disease (CD) is a multifactorial disease featured by an inflammatory response to ingested gluten in the small intestine; gluten peptides rich in proline and glutamine (from wheat, barley, rye), elicit an immune reaction in genetically predisposed subjects. Actually, gluten-free diet is the only accepted treatment for celiac disease [35].

Prolyl endopeptidases (PEPs) are a group of serine proteases that break down proline remnants in peptides [36, 37]. Recently PEPs have been evaluated as a possible therapy for celiac disease, because of their capacity for enhance the degradation of gluten peptides in the gut, as shown through both *in vitro* and *in vivo* studies, emphasizing also the hypothesis of a combination enzyme therapy (endopeptidase plus another protease) [8, 36-38].

Even if these reports are promising, actually there is not yet a role for PEPs for the treatment of CD, neither commercial preparations are available. Further and larger studies are needed to confirm these interesting results.

RATIONAL DESIGN OF AN ENZYME COMBINATION THERAPY

As seen in this review, each exogenous enzyme plays a relevant role in the treatment of digestive disorders. Such evidence theoretically suggest that a "super-enzyme", containing digestive enzymes (except those still being tested and not available for clinical practice, such as prolyl endopeptidase), may be of interest in a selected number of conditions, such as severe pancreatic insufficiency other causes of severe malabsorption syndrome, conditions of severe malnutrition, "fragile" patients, such as the great elderly or infants. This hypothetic formulation should contain, for each enzyme, at least its lower dosage when used alone. Other interesting associations come out from several evidences of pathophysiology of digestive enzymes: in patients with pancreatic insufficiency the bicarbonate secretion, necessary for neutralizing the duodenal acid chyme, could be severely impaired, forbidding the correct working of exogenous pancreatic enzymes, so that addition of PPI is actually recommended in refractory steatorrhea. Following this evidence, a formulation including a PPI in association with pancreatin may be useful in some cases of severe pancreatic failure.

Moreover, according to Gass *et al*, conjugated bile acids, not only promote lipid absorbtion, but could also accelerate the hydrolysis of dietary proteins by pancreatic proteases, so that a possible association should be useful in pancreatic disorders, especially in biliary etiology [39, 40]. In addition, UDCA plays a role in liver disease of cystic fibrosis, improving biochemical markers of cholestasis, nutritional/general status and histologic pattern: a unique preparation including UDCA plus pancreatin may be of interest in cystic fibrosis with liver involvement.

Finally, the impairment of gut microbiota can worsen or cause alterations of digestive functions, so the restoration of the microbial homeostasis represents a reliable therapeutic option for the management of several digestive disorders [41].

The presence of bacterial overgrowth in human EPI has been studied using non-invasive breath tests or by duodenal juice sampling and culture [42-47]. These studies have shown that small intestinal bacterial overgrowth can complicate from a quarter to a half of cases of EPI, suggesting that it might contribute to development or persistence of diarrhea in patients with EPI and adequate pancreatic enzyme supplementation [44, 47-49].

Moreover, bacterial overgrowth is often observed, in experimental models of EPI, but also in dogs with naturally occurring pancreatic failure [50, 51].

In addition, in a pig model of pancreatic insufficiency – with a previous clonation of the Staphylococcus hyicus lip gene in Lactococcus lactis - the coefficient of fat absorption was higher after consumption of lipase-expressing L. lactis than that after consumption of the inactive control strain [52].

Furthermore, fermented milk derivatives are able to increase the absorption of lactose, and to reduce the symptoms of intolerance in patients with lactose malabsorption, since yogurt microbes display an intrinsic lactase activity. Yogurt derives from the incubation of milk together with two lactic acid bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophiles,* which play an active role in the hydrolysis of lactose during the fermentation (which reduces the content of dietary lactose by 25-50%) as well as after the consumption of lactose [53-57].

So, according to these data, a probiotic addition, respectively to lactase in the treatment of lactose intolerance, and to pancreatic enzymes in the treatment of pancreatic insufficiency, may constitute a new, intriguing, convenient formulation in the therapy of these diseases, but always providing a tailored choose of the probiotic strain to be used [58].

The combination of digestive enzymes and probiotics may be an interesting option also in the field of CD: *Aspergillus niger*, which produces one of the endopeptidases studied for this aim (AN-PEP), has been studied also for the use of another product called aspergillopepsin, which is not specific for gluten epitopes. This protein, in conjunction with dipeptidyl-peptidase IV, can have a role in degrading larger proteins into smaller fragments, exposing the residues to more specific endopeptidases or exopeptidases [59, 60].

Nevertheless, further studies are needed to support this hypothesis.

CONCLUSION

As reviewed in this paper, enzyme supplementation therapy may play an important role in several digestive and malabsorption disorders, such as EPI and lactose intolerance. Currently, the animal-derived enzymes represent an established standard of care, however the growing study of plant-based and microbe-derived enzymes offers great promise in the advancement of digestive enzyme therapy.

New frontiers of enzyme replacement are being evaluated also in the treatment of diseases not specifically related to enzyme deficiency, such as CD, whereas the combination of different enzymes might constitute an intriguing therapeutic option in the future. Furthermore, a tailored probiotic addition to the enzyme supplement, for example to lactase in the treatment of lactose intolerance, and to pancreatic enzymes in the treatment of pancreatic insufficiency, seems to offer an advantage to the therapeutic management of such disorders.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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