HIGHLIGHT ARTICLE

Does the Pancreas Really Produce Much More Lipase than Required for Fat Digestion?

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Summary

Thirty years ago, it was reported that a linear relationship does not exist between the amounts of human pancreatic lipase secreted in chronic pancreatitis and the degree of steatorrhea, which was considered to appear only after more than 90% of the pancreatic secretory capacity had been lost. From these observations, it was generally thought that the lipolytic potential of the pancreas is much higher than required.

In recent years, however, it has been noted that: 1) the level of inhibition of digestive lipases and gastrointestinal lipolysis by the lipase inhibitor orlistat were almost linearly correlated with the amount of excreted fat; 2) in minipigs with experimentally-induced pancreatic exocrine insufficiency, the amounts of enteric-coated pancreatic extracts needed for restoring fat digestion to normal levels were estimated to be much higher than those usually administered; 3) human pancreatic lipase specific activity on meal triglycerides is 3 orders of magnitude lower than the very high specific activity usually measured under experimental *in vitro* conditions which are far from physiological conditions; 4) in patients with reduced human pancreatic lipase secretion, gastric lipase plays a significant role in fat digestion.

This last observation might explain the absence of a linear relationship between human pancreatic lipase secretion in chronic pancreatitis and steatorrhea. From the low specific activity displayed by human pancreatic lipase on meal triglycerides, one can better understand why more lipase than expected is needed, why fat digestion lasts for more than a few minutes and, finally, why there is not such an excess secretory capacity for lipase as had been previously thought.

Introduction

Since 1973 when the well-known paper by DiMagno *et al*. was published [1], it has generally been recognized by the gastroenterological community at large that the human pancreas produces much greater amounts of enzymes than those required for digestion. These authors studied the relationships between pancreatic enzyme output and malabsorption in patients with severe pancreatic insufficiency, and reported that no steatorrhea was observed until the lipase output was as low as 10% or less of the normal level. Likewise, azotorrhea occurred only when the trypsin output had decreased to 10% of the normal level. It was concluded that the exocrine pancreas has a very large reserve capacity for enzyme secretion and, since then, this finding has been recognized as a fundamental property of the pancreas. The conclusions of DiMagno *et al*. have greatly
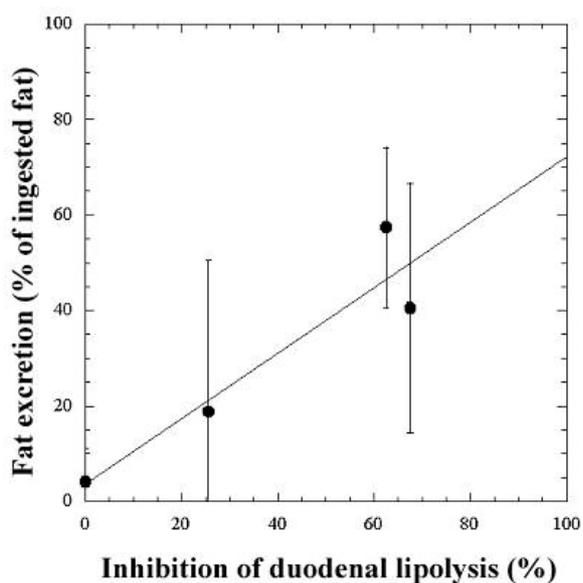
Figure 1. Artificial steathorrea induced by the lipase inhibitor orlistat in humans. Fat excretion (% of ingested fat) plotted as a function of duodenal lipolysis inhibition (%). The stools of volunteers were collected quantitatively for 30 hours prior to and for 100 hours after the test meal. The amount of fat excreted was quantified gravimetrically after stool homogenization and extraction from aliquots with chloroform-methanol [50]. The fat excretion levels recorded after the test meal were corrected in each individual experiment based on the baseline fat excretion determined during the pre-meal stool collection period. The fat excretion levels were expressed as a percentage of the fat ingested with the respective meals. Duodenal lipolysis levels were estimated from cumulated output of lipolysis products at the angle of Treitz during the digestion of a solid-liquid test meal given with and without 120 mg orlistat. The maximum duodenal lipolysis level measured in control subjects without orlistat corresponds to 0% inhibition. These data are from [10].

influenced the development and use of pancreatic extracts (PEs) for the treatment of pancreatic insufficiency; the amounts of active pancreatic lipase present in the current dosages of commercial PEs are much lower than the amounts of pancreatic lipase produced during the digestion of a meal. When PE were not able to adequately treat steatorrhea in chronic pancreatitis (CP) patients, more attention was paid to the destruction of added lipase by gastric acid [2, 3, 4, 5], disturbance in the synchronization of the meal and gastroprotected PE delivery [4, 6, 7], and the delayed release of lipase from enteric-coated tablets [4], rather than to too

low levels of lipase in PEs. DiMagno's hypothesis was supported by a subsequent study in which 10% of the normal lipolytic activity was administered directly into the duodenum and steatorrhea was abolished [8]. However, similar experiments performed by another group did not confirm these results and steatorrhea was not found to be corrected by the duodenal administration of 40% of the normal lipolytic activity [9]. Several later studies have indicated that the large amounts of pancreatic lipase produced by the human pancreas are required for normal fat digestion.

Relationship Between Steatorrhea and the Inhibition of Pancreatic Lipase and Gastrointestinal Lipolysis

The gastrointestinal lipolysis of a mixed solid-liquid test meal was investigated in the presence of the lipase inhibitor orlistat mixed into butter [10]. Total lipase secretory output during the whole meal was measured by the ELISA test and was expressed in mg, whereas the output of active lipases were estimated by activity measurements using tributyrin as a substrate. The level of lipase inhibition was estimated by comparing the output of active lipase with the total output of lipase. It was established that 200-250 mg of human pancreatic lipase (HPL) were produced during a meal (700 mL), these amounts of pancreatic lipase corresponding to 1,600,000-2,000,000 international lipase units (U) measured with tributyrin as a substrate (HPL specific activity equal to 8,000 U/mg of pure enzyme) and 600,000-750,000 Fédération Internationale Pharmaceutique (FIP) units measured with olive oil as a substrate (HPL specific activity equal to 3,000 FIP units per mg of pure enzyme). When half (51.2±34.6%) of the secreted HPL was found to be inactivated by orlistat, duodenal lipolysis of the meal triglycerides was reduced in the same order of magnitude (62.4%) and the level of fat excretion was also found to be 57.4±16.8% of the ingested fat [10]. A good correlation was therefore observed between the levels of pancreatic lipase activity in the duodenal contents and the levels of fat excretion. In
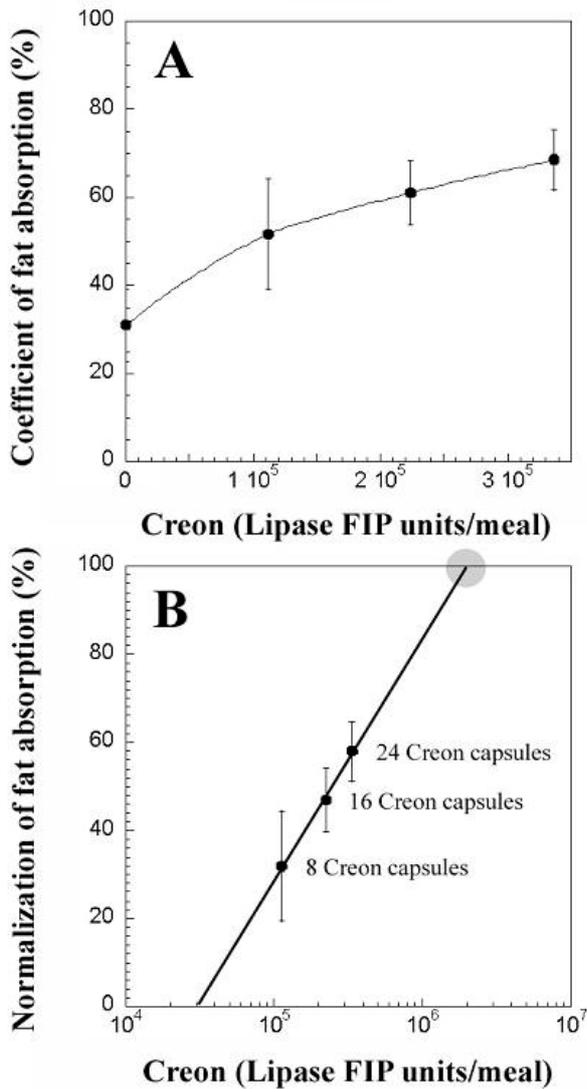
Figure 2. Effects of pancreatin (Creon[®]) on fat absorption in pancreatic exocrine insufficient minipigs. **A.** Coefficient of fat absorption (CFA) as a function of pancreatin dose (lipase FIP units). Values were determined after a 5-day total feces collection from 3 pancreatic exocrine insufficient minipigs (given 0, 8, 16 or 24 capsules of Creon[®] 10,000 minimicrospheres[TM]/meal) and 3 control minipigs, fed twice daily with a 250 g meal for 7-10 days prior to and during feces collection. CFA (%) = 100 x ((g/day in feed – g/day in feces) / g/day in feed). **B.** Log dose-response effects of pancreatic lipase. The doses of pancreatic lipase which would have been necessary for complete normalization of the fat digestion process were estimated by extrapolating to 100% from the log dose-response curve, depending on the fecal CFA obtained. One hundred and 0% effects correspond to the CFA observed in control minipigs (95.5±0.9%) and pancreatic exocrine insufficient minipigs (31.5±8.1%), respectively. These data are from [27].

addition, this study showed the existence of a rather linear and positive correlation between duodenal lipolysis inhibition levels and fat excretion levels (Figure 1).

It is worth noting here that gastric lipase was fully inhibited in these experiments [10] and, therefore, its contribution to lipolysis could not interfere with the relationship established between pancreatic lipase activity, duodenal lipolysis inhibition and fat excretion. The contribution of gastric lipase will be discussed later on however.

Normalization of Fat Absorption by Pancreatic Extracts in a Minipig Model of Pancreatic Exocrine Insufficiency

Studies have been performed on the digestion/absorption of nutrients in pancreatic exocrine insufficient pancreatic duct ligated minipigs fed a high-fat diet (75 g fat per meal), with and without the administration of enteric-coated pancreatin (Creon[®], Solvay Pharmaceuticals GmbH, Hannover, Germany; 10,000 minimicrospheres[TM]). The pigs were also fitted with an ileo-cecal re-entrant fistula in order to determine the prececal digestibility. Complete pancreatic exocrine insufficiency (PEI) decreased the intestinal pH and resulted in severe and roughly equal malabsorption of fat and protein, and a more moderate malabsorption of starch. The coefficient of whole GI tract fat absorption (95.5±0.9% in control animals) was reduced to 31.5±8.1% in PEI minipigs. The administration of Creon[®] at a dose of 8-24 capsules/meal (10,000 units according to the European Pharmacopea (Eur. Pharm.) or FIP units per capsule) resulted in a dose-dependent improvement in fat absorption (Figure 2A). By extrapolating from the log dose-response curve shown in Figure 2B, it was estimated that about 1 million FIP units per meal lipase would have to be administered to normalize fat digestion in the PEI minipig. This figure is only an approximation and the dose of enzymes required to achieve normalization will certainly depend on the

dietary fat load which was very high in the present study (75 g fat/meal; 2 meals/day), but it does indicate that higher doses of lipase than formerly expected may be needed in order to completely normalize the fat absorption levels. No attempts have been made so far to determine the pancreatic secretion rate in the minipig, but a lipase secretion rate of about 650,000 lipase U/12 h has been reported to occur in 17-25 kg pigs [11]. This lipase output corresponds to about 200 mg of pancreatic lipase (see "Specific Activity" in the following section) and it is of the same order of magnitude as the lipase output recorded in humans during test meals [12, 13, 14]. If similar rates of secretion occur in the minipig, this would mean that about 100% of the normal amount of pancreatic lipase secreted would have to be returned to the PEI minipig which was fed a high-fat diet in order to completely normalize the fat digestion/absorption process. Similar results have also been obtained in dogs with induced PEI [15]. This does not match the human data showing that the symptoms of PEI occur only after approximately a 90% loss of pancreatic secretory capacity [1]. One of the factors possibly responsible here is the fast inactivation of the lipase from pancreatin which occurs during small intestinal transit [3]. Another potential explanation based on the delayed release of lipase from enteric-coated microspheres which occurs at low intestinal pH levels, can probably be ruled out in the case of these studies, since the pH was already sufficiently high in the jejunum (1 m from pylorus) to be compatible with rapid enzyme release (a fast release occurs at pH values greater than 5.5). Furthermore, the pancreatin used was administered in enteric-coated minimicrospheres of a size (sieve fraction 0.7-1.6 mm) which seems to be optimum for mixing and emptying with the meal [16], so that inappropriate emptying of the enzymes relative to the meal is also unlikely to have intervened here. Moreover, studies in which uncoated pancreatin was infused directly into the duodenum showed that steatorrhea was far from abolished in CP patients with PEI, even with an infusion rate

of up to 40% of the normal pancreatic lipase secretion rate [9].

In addition, it was noted that complete normalization of protein digestion in PEI minipigs (measured at the ileum) would require the addition of about 40,000 FIP protease units for the digestion of meals containing 40 g protein [17]. This suggests that also the pancreas does not produce a surplus of proteases; i.e. probably the level of exocrine secretion is adequate for normal digestion of dietary proteins.

Specific Activities of Pancreatic Lipase on Meal Triglycerides Versus Optimized Triglyceride Emulsions

The hypothesis that the human pancreas produces a much greater amount of lipase than that required for fat digestion purposes has always been supported by the extremely high specific activity of pancreatic lipase measured *in vitro* using standard triglyceride (TG) emulsions. Pancreatic lipase shows its highest specific activity with tributyrin emulsion (8,000 to 12,500 U/mg of enzyme; 1 U equal to 1 μmole of free fatty acid released per minute). Using the standard assay from the European Pharmacopea (olive oil emulsified with gum arabic), the maximum specific activity towards long chain TG is 3,000 Eur. Pharm. units or FIP units per mg of pure enzyme [18].

If lipase actually acts at such a fast rate during a meal, a simple calculation based on the amounts of the dietary TG ingested and pancreatic lipase output shows that only a few seconds would be required for the lipolysis to be completed [14]. Intubation studies in which samples were taken every 15 minutes during test meals have shown, however, that the process of lipolysis is far from being complete when measured at the angle of Treitz. In addition, *in vitro* and *in vivo* studies of the effect of pancreatic lipase on dietary TG have both shown much lower specific activities than those measured with optimized emulsions [14]. Enzymatic lipolysis is sensitive to the specific surface available at the oil-water interface. Standard assays for measuring lipase activities are usually carried

out with fine emulsions stabilized by various emulsifiers such as gum arabic. Under these conditions, lipolysis occurs at a high rate. Such conditions are not found with a meal in which lipids occur under various forms. In addition, emulsification of fat does not occur immediately in the GI tract. As a result, the specific activity of HPL on the TG of a mixed solid-liquid meal (12-15 U/mg at pH 6.25) was found to be 3 orders of magnitude lower than the maximum specific activity of HPL (12,500 U/mg) recorded with a tributyrin emulsion [19]. With low specific activities of this kind, the pancreatic lipase produced during a meal (200-250 mg/3 h) would theoretically be able to digest all the fat content of a meal in about 20-25 minutes (given a constant pH and a constant specific activity) [14]. *In vivo*, the specific activity cannot be expected to be constant in the GI tract due to pH variations, and pancreatic lipase will probably need a longer time to complete the fat digestion process. It therefore seems likely that the pancreas produces enough lipase to digest all the dietary fat during the digestion period but, contrary to what has been previously suggested, the amounts produced do not seem to be in great excess.

Contribution of Gastric Lipase to Gastrointestinal Lipolysis

When DiMagno *et al.* investigated the relationship between the pancreatic lipase secretion level and steatorrhea, the fact that other lipases might be significantly involved in fat digestion was not taken into account. It has now been established that human gastric lipase (HGL) can release from 10 to 25% of the dietary triglyceride acyl chains in the stomach and continue its action in the small intestine together with pancreatic lipase [18, 20].

In cases of PEI, compensation of HPL insufficiency by HGL was suggested by several studies. During test meals, the lipolytic activity of the gastric contents was sometimes higher in cystic fibrosis (CF) patients than in control subjects [21]. It has

been shown that the secretory output of HGL under pentagastrin stimulation was significantly increased in the late stage of alcoholic CP [22]. We have recently shown that HGL output is clearly higher (3-4 fold vs. controls) in the late stage of CP during test meals, and HGL can achieve a significant lipolysis of the dietary triglycerides (30% of controls) in the complete absence of pancreatic lipase [23]. These data agree with the minipig data in which 30% fat digestibility was observed in the complete absence of pancreatic enzymes [17]. Such a contribution of HGL has therefore to be taken into account when studying steatorrhea levels in PEI. Gastric lipase might be able to compensate for some pancreatic lipase deficiency in the early stages of PEI but not in the late stages.

Discussion

It has been known for a very long time that fibrosis of the pancreas, such as that which develops in the late stages of chronic calcifying pancreatitis is associated with chronic diarrhea and malnutrition. The malnutrition was subsequently explained by PEI and, more specifically, steatorrhea was found in patients presenting a dramatic decrease of lipase secretion. In the 1970s, DiMagno *et al.* [1] established that PEI occurs progressively in alcoholic CP patients and that symptoms of PEI such as steatorrhea and azotorrhea only develop after some 90% of the secretory capacity of the pancreas has been lost (as judged from the response to a maximum stimulus of secretin plus cerulein/CCK, in the absence of a test meal). Decrease in lipase activity is one of the most critical events in the course of CP [24]. Therapy with pancreatin has been found to successfully improve the symptoms of PEI, but complete normalization of the fat digestion process has nevertheless proved to be difficult to achieve in many of these patients [25], whether by administering large doses of enzymes or co-administering acid secretion blockers, which may not only improve fat digestion but can also severely

reduce protein digestibility [26]. These difficulties have led to the quest for other lipases with more suitable stability and activity properties such as new therapeutic alternatives (or as add-ons) to pancreatin. For this purpose, animal models for PEI have been developed to investigate the changes in the digestive process occurring in response to PEI and the problems involved in the corresponding therapeutic strategies.

The pancreatic duct-ligated minipig has often been used as a model for induced PEI. As previously observed in human PEI, it was difficult to normalize the fat absorption process with pancreatin therapy in PEI minipigs (Figure 2). In any event, it now seems obvious that complete normalization of steatorrhea in PEI can require supplementation by far more than 10% and perhaps even more than 100% of the normally secreted enzyme levels [27]. This hypothesis is strongly supported by the results of experiments on the inhibition of gastrointestinal lipolysis [10]. Administration of the lipase inhibitor orlistat (tetrahydrolipstatin; 120 mg *tid*) can lead to about a 40-60% malabsorption of dietary fat by inhibiting postprandial duodenal lipolysis by 60% (Figure 1).

At the same time the effects of pancreatin were reviewed, pancreatic enzyme secretion in the course of a test meal was re-investigated using a more quantitative approach and lipase output was expressed in terms of mg of enzyme [20] instead of lipase units, since these units and the substrate used in the lipase assay often differed from one laboratory to another [28, 29, 30]. Moreover, lipase assays based on enzyme activity were accompanied by independent assays such as the ELISA assay [10, 20]. It was thus possible to estimate both the overall enzyme secretion levels (i.e., the mass of the enzymes secreted) and the stability of the enzymatic activity (in lipase units per mg of enzyme). Since enzyme assays only show the active enzymes, the overall secretion levels can be easily underestimated when the enzyme is unstable and partly inactivated. In the presence of a meal and bile, digestive lipases are highly stable in the duodenal contents and the lipase output deduced from activity measurements has been found to reflect the overall secretion rates quite accurately [10]. In the absence of stabilizing compounds such as meal constituents, enzyme stability is of great importance when it is proposed to estimate lipase output by performing activity measurements. This is particularly true in studies on the secretion of gastric lipase in response to hormonal stimulation. Pentagastrin infusion stimulates both gastric acid and gastric lipase secretion in humans and dogs, but due to the very low pH values, gastric lipase is largely destroyed [31, 32]. Likewise, pancreatic lipase is rapidly degraded by other constituents of pancreatic juice, namely proteases, in the absence of a meal [33] and, furthermore, HPL is highly sensitive to acidic conditions such as would be found in the duodenum of PEI patients [23], and which may be especially severe in the absence of the buffering effects of food. This points to the conclusion that the digestive enzyme output cannot accurately be estimated by applying non-physiological hormonal, neuronal or chemical stimulation in the absence of any protective agents (protease inhibitors, proton pump inhibitor, etc.). The older studies on pancreatic secretion were "capacity" tests using non-physiological or pharmacological stimuli. This should be taken into consideration when comparing the results with those obtained with more physiological test meals. It is very difficult nowadays to check the lipase output obtained in the experiments performed back in the 1970s, but the pancreatic lipase output recorded in response to cerulein/CCK stimulation might have been underestimated. In fact, the investigators were no doubt aware of the poor pancreatic enzyme stability occurring under these conditions, as suggested by their subsequent studies on the preservation of pancreatic enzymes in samples collected from the GI tract [34].

Another hypothesis for explaining the non-linear relationship observed by DiMagno *et al*. between the appearance of steatorrhea and pancreatic lipase secretion might be the fact

that the significant role of another digestive lipase, gastric lipase, was ignored at that time. It has now been shown that gastric lipase secretion is increased in CP [22, 23] and this enzyme can achieve approximately 30% of the meal TG lipolysis observed in healthy volunteers [23]. It is worth noting that observations like those of DiMagno *et al.* are not restricted to adult patients with CP. In CF patients, steatorrhea is also considered to be present when more than 90% of the pancreatic secretory capacity has disappeared [35]. It was also suggested that gastric lipase can partly replace pancreatic lipase in CF patients with PEI, 20 to 80% of the ingested TG remaining absorbed [36, 37, 38, 39]. During a test meal, the lipolytic activity and lipolysis in gastric contents were sometimes found to be higher in CF patients than in control subjects [21, 40]. It therefore seems unlikely from all these observations that a direct relationship can be established between steatorrhea and pancreatic lipase secretion levels. The conclusion is that a common symptom of PEI such as steatorrhea does not only reflect the secretory capacity of the pancreas. Because of the increased contribution to lipolysis of gastric lipase in PEI, one can understand however why high steatorrhea only appears in the late stage of PEI, and why it was considered that 10% residual pancreatic lipase secretion was sufficient to digest dietary fat.

From the various observations reported in this paper, as well as in previous reports [9, 41], it seems that the well-accepted concept according to which the pancreas produces lipase in a much greater amount than that required for complete fat digestion has to be revised. Pharmacological treatments with pancreatic extracts have to be reconsidered in the light of these observations because patients with PEI are probably undertreated. Although modern enteric-coated pancreatin microsphere preparations give better results, fat digestion cannot be completely normalized in most patients with severe PEI (fecal fat excretion greater than 15 g/day). The administration of 25,000 to 40,000 FIP lipase units per meal using pH-sensitive pancreatin microspheres is currently recommended [42]

whereas 600,000-750,000 FIP lipase units are normally secreted during a meal in adults. A lack of dose-response effects is often observed [9, 43], except when higher lipase amounts are used [44]. It is already recognized that the most useful PE are those with high lipase contents [44, 45, 46, 47] and it is recommended to increase the PE dose in case of treatment failure.

High dose lipase therapy was however associated with colonic complications (strictures). From the recent reviews on this topic, it seems that colonic strictures might be related to the late release of pancreatic enzymes in the colon or to the adjuvants used for the "gastroprotection" of pancreatic enzymes. Under normal conditions, large amounts of pancreatic enzymes are delivered to the upper part of the small intestine but these enzymes are also "digested" during their transit through the small intestine and only low amounts of these enzymes reach the colon. In severe pancreatic insufficiency, the pH of the small intestine contents is so low that an important part of the enzymes present in enteric-coated pellets might only be released in the colon. It is probably not the high enzyme amount however which leads to strictures. Not all enzyme products seem to carry the same risk of colonic complications. In the UK, the Creon® brand has about 80% of the market, yet cases of fibrosing colonopathy have occurred with other products, including standard-strength products. It seems possible that one of the excipients may be responsible for the colonic strictures, rather than the enzymes *per se*. There is indeed an animal study in which metacrylic co-polymer (Eudragit®, Röhm Pharma Polymers GmbH, Darmstadt, Germany) was shown to cause a similar pathology to that seen in fibrosing colonopathy [48]. Furthermore, this hypothesis is supported by cases of fibrosing colonopathy in patients who had never received pancreatic enzymes but rather a different metacrylic co-polymer coated drug [49].

In conclusion, the concepts developed in this article suggest that much higher amounts of

lipase should be administered to patients with PEI. It is, however, difficult to administer lipase amounts equivalent to those normally produced during a meal because an enormous number of the capsules currently available will be required (for instance, a Creon® 25,000 capsule only contains 1.5% w/w of active pancreatic lipase). Therefore, research efforts should focus on the development of enriched PE extracts or purified enzymes.

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