UMR 7282

Enzymologie Interfaciale et Physiologie de la Lipolyse

Enzymology at Interfaces and Physiology of Lipolysis

Yarrowia lipolytica LIP2 Lipase, a Potent Drug Candidate for Enzyme Replacement Therapy in Pancreatic Exocrine Insufficiency

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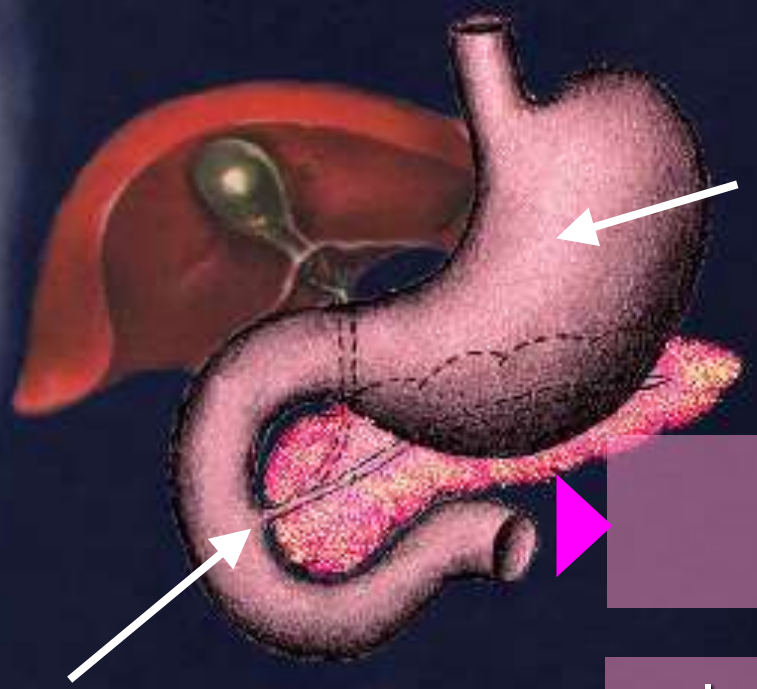
Fat digestion in humans, exocrine pancreatic insufficiency (EPI) and enzyme replacement therapy

Digestion of dietary fat in humans

(≈100 g/day in western countries)



Gastric lipase (HGL)

Pancreatic Lipase (HPL) + colipase

Pancreatic lipase-related protein 2

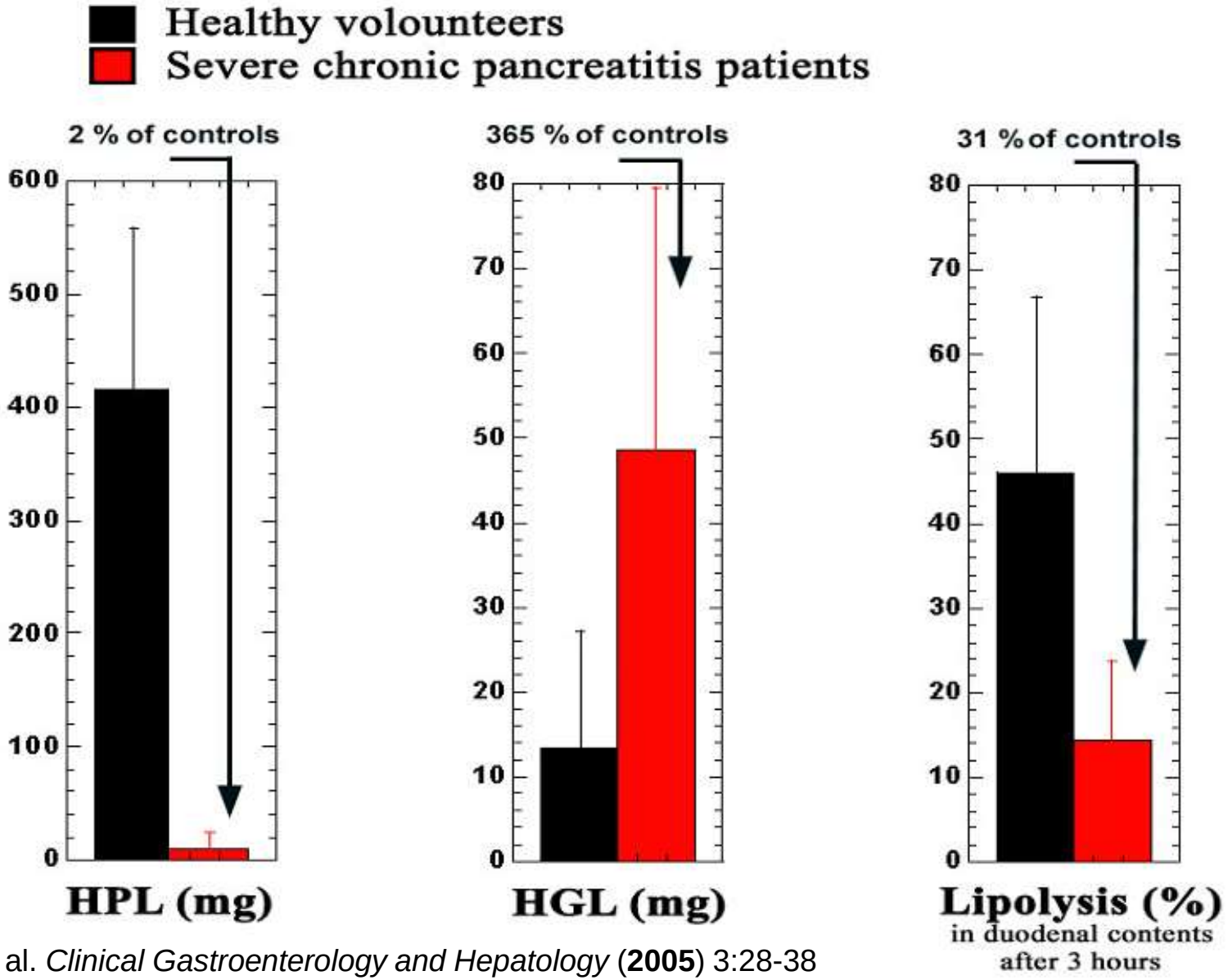
Carboxylester hydrolase

Gastrointestinal lipolysis

Intestinal absorption of lipolysis products

Less then 4% ingested fat is found unabsorbed in healthy subjects

Digestive lipase levels and gastrointestinal lipolysis in EPI



Carrière et al. *Clinical Gastroenterology and Hepatology* (**2005**) 3:28-38

Exocrine pancreatic insufficiency (EPI)

In severe **chronic pancreatitis** or **cystic fibrosis**,
pancreatic enzymes are found at low levels or are missing.

Digestion is not efficient → malabsorption → malnutrition

There is a need for oral exogenous enzymes
for enzyme replacement therapy in EPI

Porcine pancreatic extracts (current treatment)

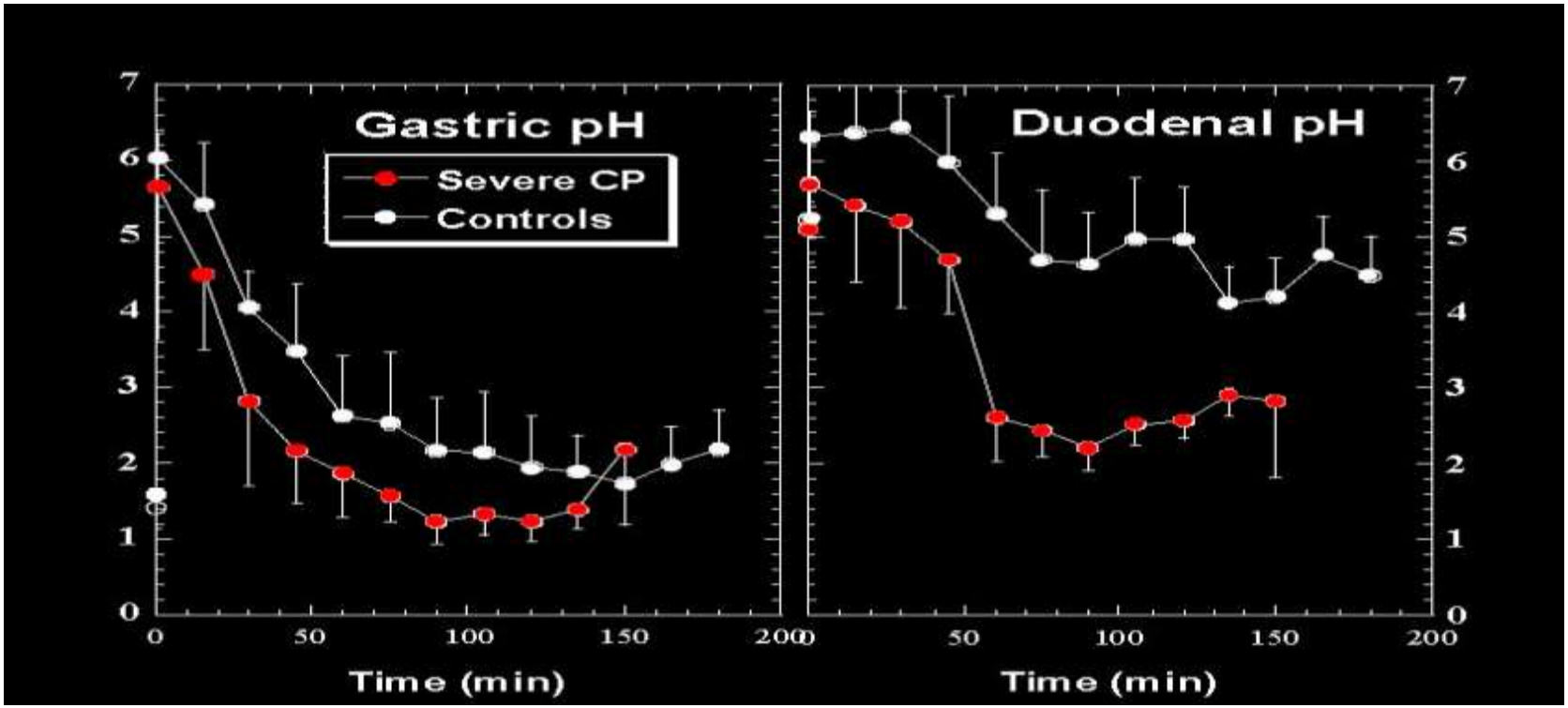
There is a need for novel recombinant enzymes

- to avoid risks of viral transmission from animal products to humans
- to improve enzyme stability and efficacy in the GI tract
- to reduce the daily amounts of drug products

Carrière et al. *Clin. Gastroenterol. Hepatol.* (**2005**) 3:28-38

Parameters to be considered for the development of substitutive enzymes for the treatment of EPI

- Enzymes have to be stable and active in a large pH range, including **acidic pH**



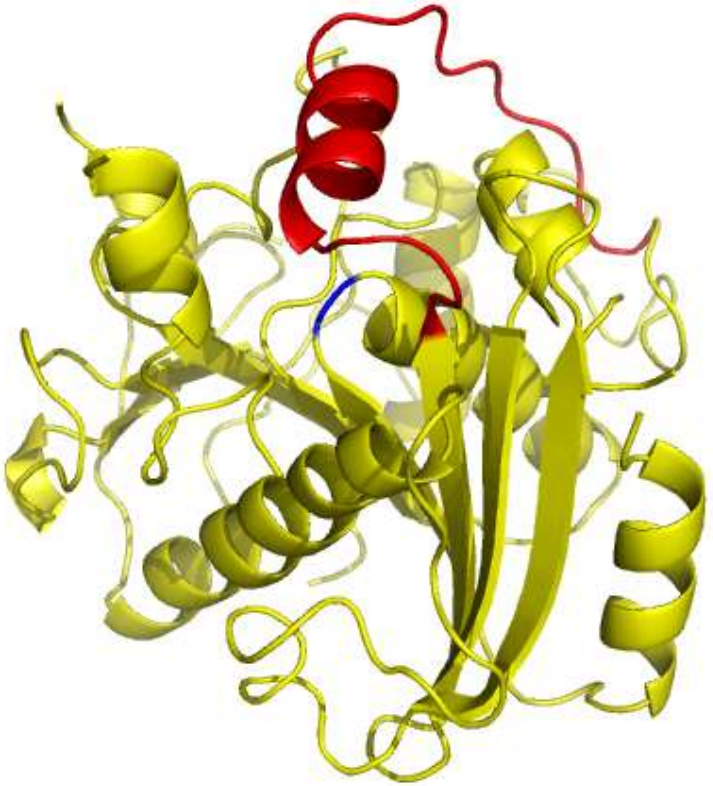
- These enzymes should be rather resistant to proteases, including **pepsin**
- Lipases must remain active in the presence of **bile salts** (intestine)

→ The LIP2 lipase from *Yarrowia lipolytica* was found to be a good candidate

The LIP2 lipase from *Yarrowia lipolytica*

Yarrowia lipolytica is a non-conventional filamentous yeast. This is an oleaginous yeast that can store large amounts of lipids (> 20% of cell mass).
Its genome encodes for many proteins involved in the use of hydrophobic substrates (n-alkanes, triglycerides, fatty acids...).
It is naturally found in various lipid-rich environments (Foods including cheeses, sausages, Waste waters, Soils, Oil fields...)

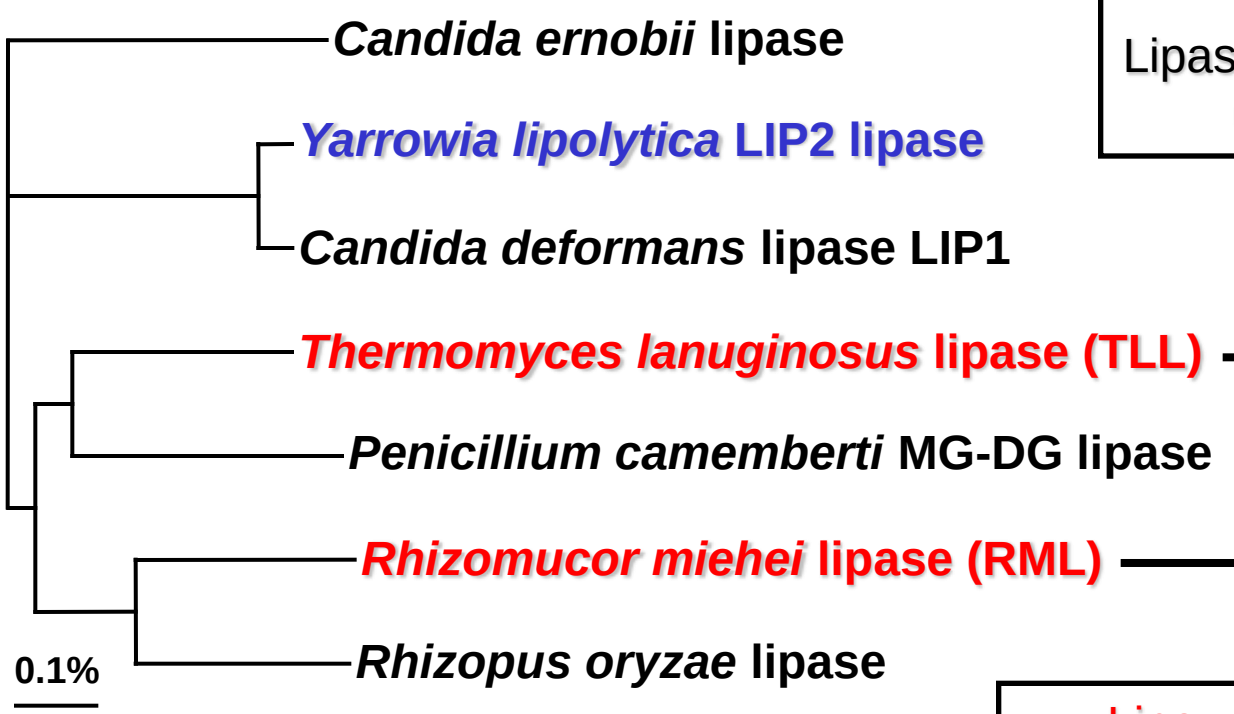
It is a GRAS microorganism (Generally Recognized As Safe)



- a single globular domain
- α/β-hydrolase fold
- the active site is covered by a lid of 22 residues (T88-D109)

Aloulou *et al. EJLST* (**2013**) 115:429–441; Bordes *et al. Biophys. J.* (**2010**) 99:2225–2234

YLLIP2 is related to major industrial lipases from fungal origin



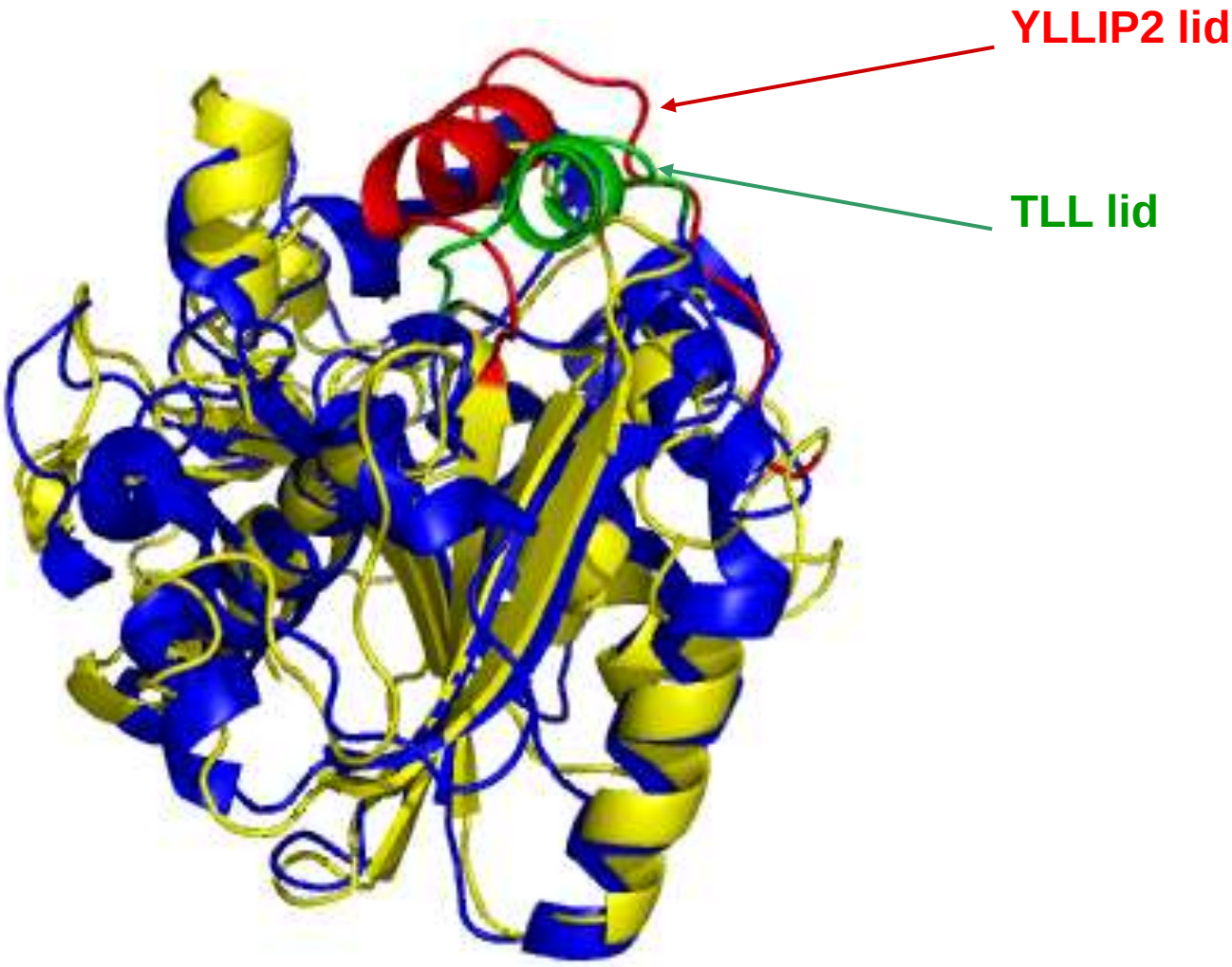
Lipolase™ (Novozymes), first recombinant Lipase on the market (1988), used in detergents

Lipozyme™ (Novozymes), the main lipase used in industrial processes of transesterification

Superimposition of YLLIP2 and TLL 3D structures

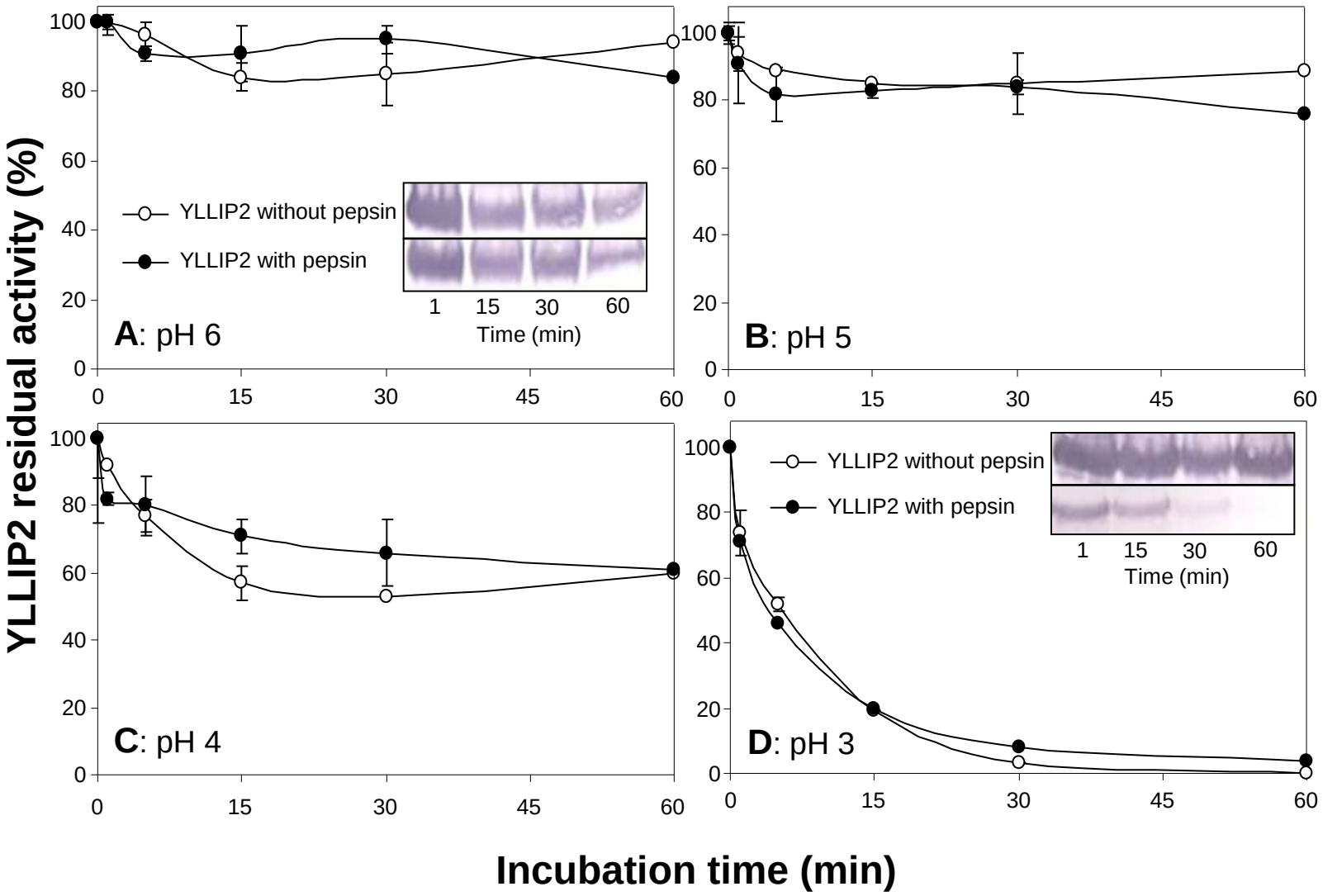
YLLIP2

TLL

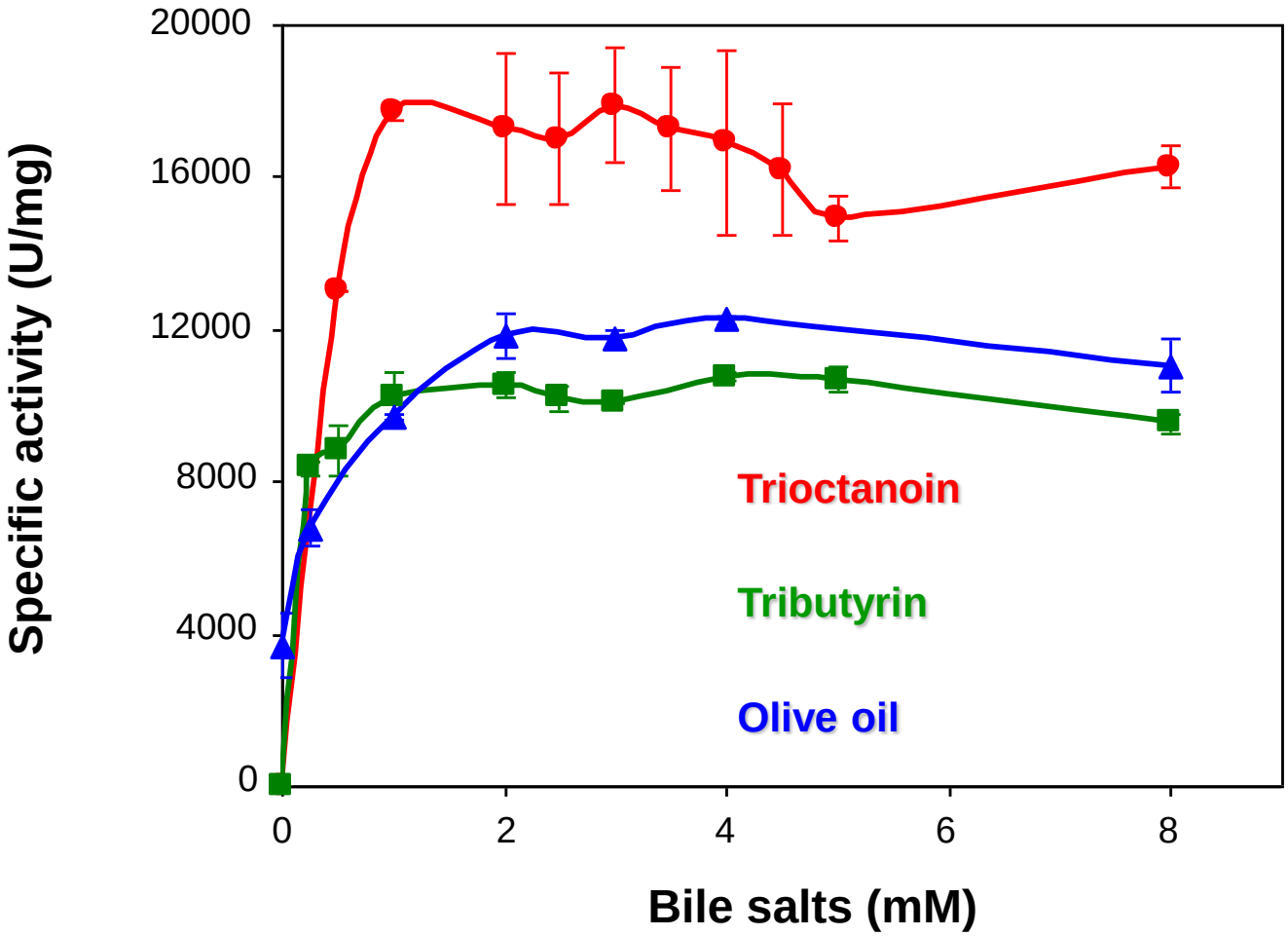


- **Insertion of 8 residues within the lid of YLLIP2**
- **2 disulfide bridges are conserved (C30-C299 et C120-C123)**

Resistance of YLLIP2 to pepsin at various pH (37°C)



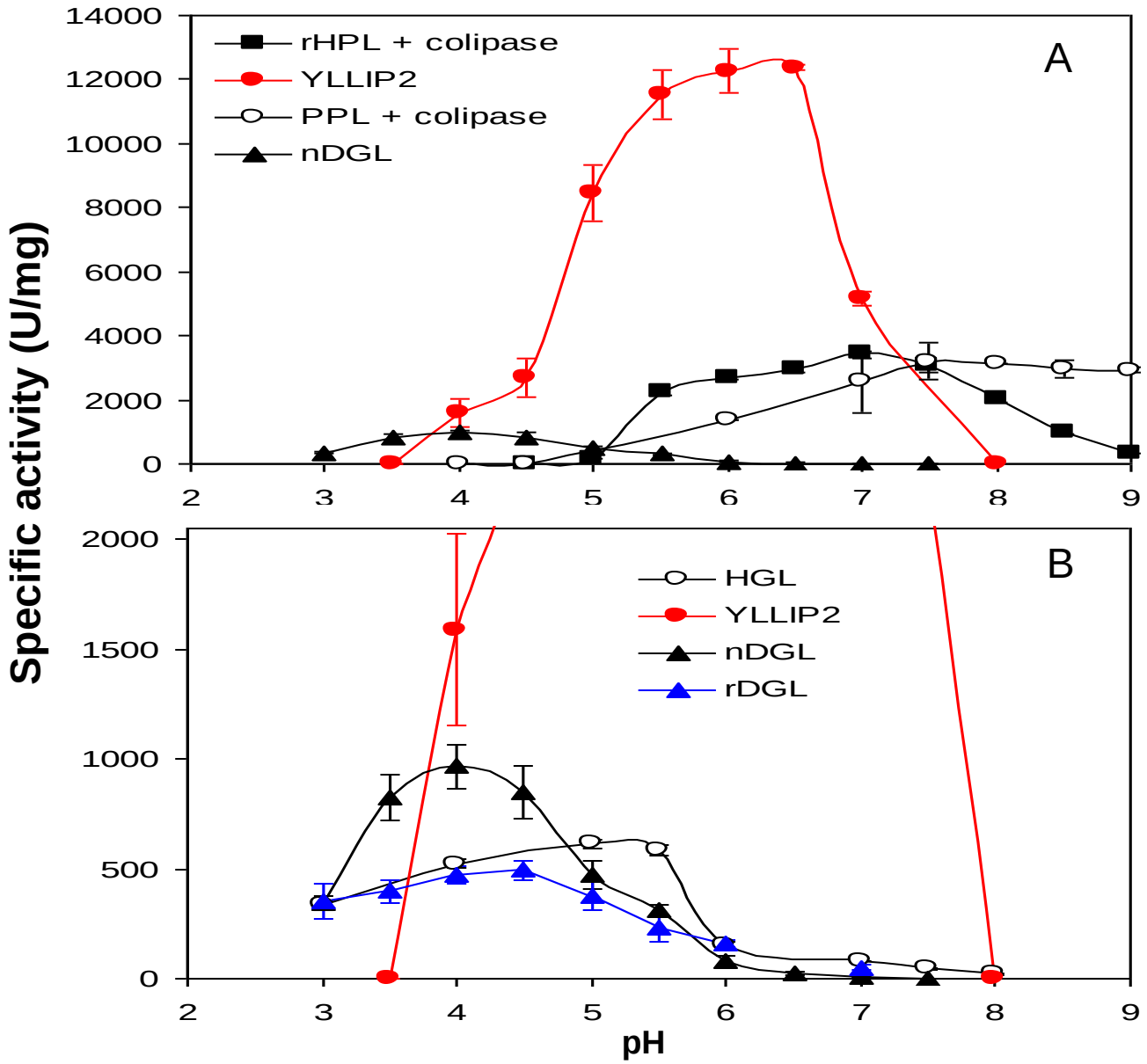
Effects of bile salts on YLLIP2 activity on various TAG (pH 6)



✓ **YLLIP2 is not inhibited by bile salts, on the contrary to most microbial lipases**

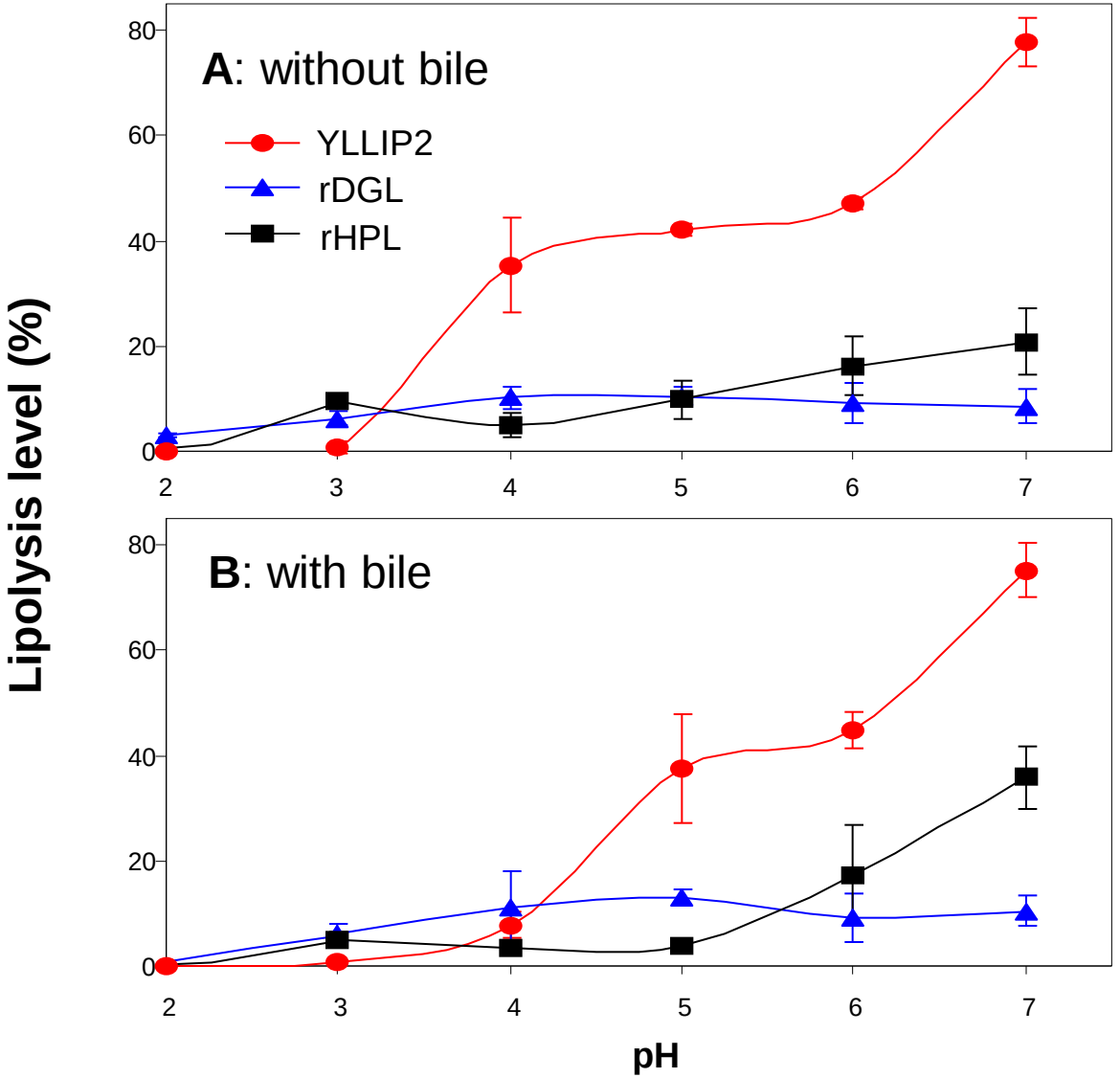
Aloulou *et al. BBA-Mol Cell Biol Lipids* (**2007**) 1771:228–237 and (**2007**) 1771:1446–1456

pH-dependent activity of YLLIP2 and digestive lipases on long chain TAG (optimized assay conditions)



pH-dependent lipolysis of solid-liquid test meal TAG by YLLIP2 and digestive lipases

Lipolyis levels (%FFA versus total fatty acids in TAG) were recorded after 60-min incubations of 1 mg enzyme with 15 mL test meal containing 0.9 g TAG, with and without bile



Comparison of YLLIP2, digestive lipases and pancreatin specific activities (U/mg*) on test meal TAG

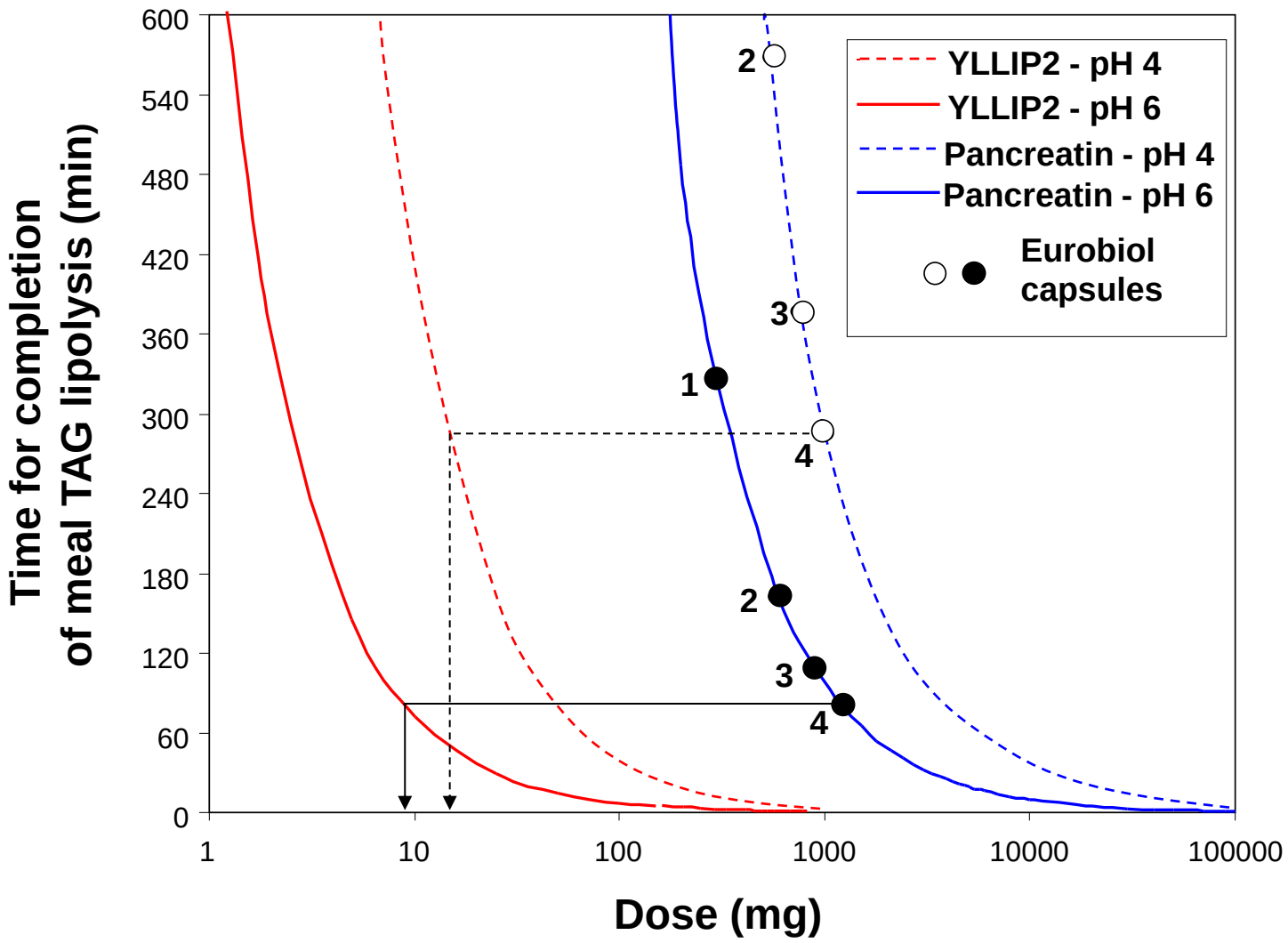
Lipase	*Optimized in vitro pH-stat assays with long-chain TAG*			*Test meal TAG*	
	pH 4	**pH 6**	**pH 9**	**pH 4**	**pH 6**
YLLIP2	**1,588 ± 438**	**12,260 ± 700**	0	**16 ± 2.1**	**94 ± 34**
nDGL	966 ± 99	78 ± 23	0	27	4.8
rHPL	0	2,706 ± 81	351 ± 41	9 ± 0.1	29 ± 1
PPL	0	1,347 ± 10	2,931 ± 96	7.8 ± 0.8	28 ± 1
Eurobiol® 25,000	0	23 ± 0.7	52 ± 3	0.12 ± 0.01	0.42 ± 0.01
Crude pancreatin	0	**48**	**87**	**0.2**	**0.7**

*1 U = 1 µmole FFA released per min

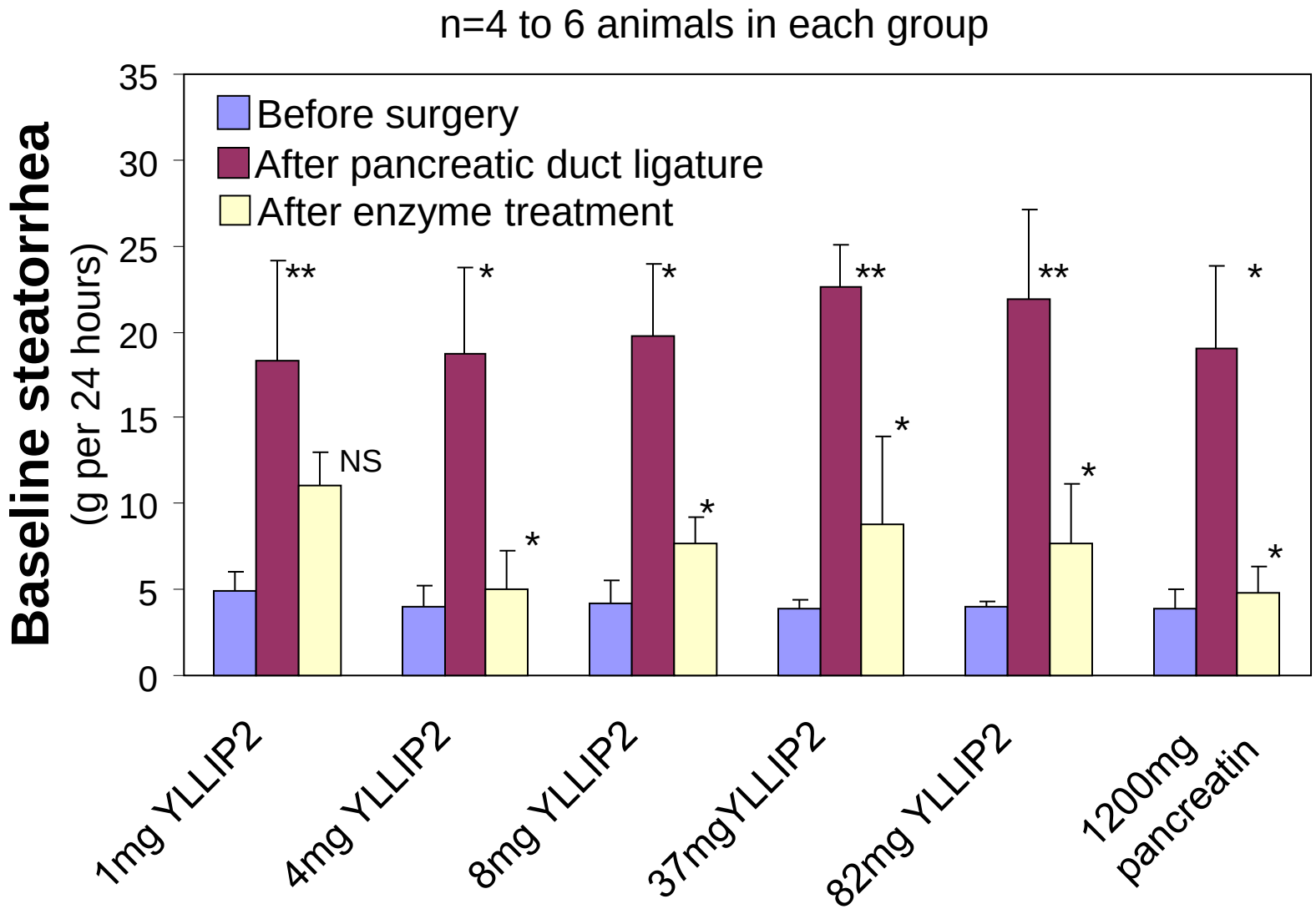
YLLIP2 is **80-fold** to **134-fold** more active than **pancreatin** on a mass basis

Estimation of YLLIP2 and pancreatin doses (mg) required for the completion of test meal lipolysis

based on their respective specific activities on test meal TAG and the amounts of TAG present in a meal (30g)



Dose-dependent effects of YLLIP2 in a minipig model of EPI and comparison with a single dose of pancreatin



Statistics: *P<0.05 and **P<0.01, significant differences vs. the previous period; NS, not significant (P>0.05)

Conclusions

- YLLIP2 is the lipase showing the highest specific activity on test meal TAG in the 4 to 7 pH range, in the presence of bile salts

- YLLIP2 was found to be highly stable and poorly degraded by pepsin in this pH range.

- The doses of YLLIP2 to be administered to PEI patients were deduced from YLLIP2 specific activity on meal TAG and compared to the doses of pancreatin currently given to these patients.

- These doses were then tested in a minipig model of PEI and a dose of 4mg YLLIP2 was found to be equivalent to 1200mg pancreatin in reducing steatorrhea induced after pancreatic duct ligation

- These results strongly support the use of YLLIP2 in enzyme replacement therapy and phase IIa clinical trials in humans are now in progress

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