Successful treatment of maldigestion due to Pancreatic Exocrine Insufficiency: diagnosis, clinical picture and the challenges of measurements involved

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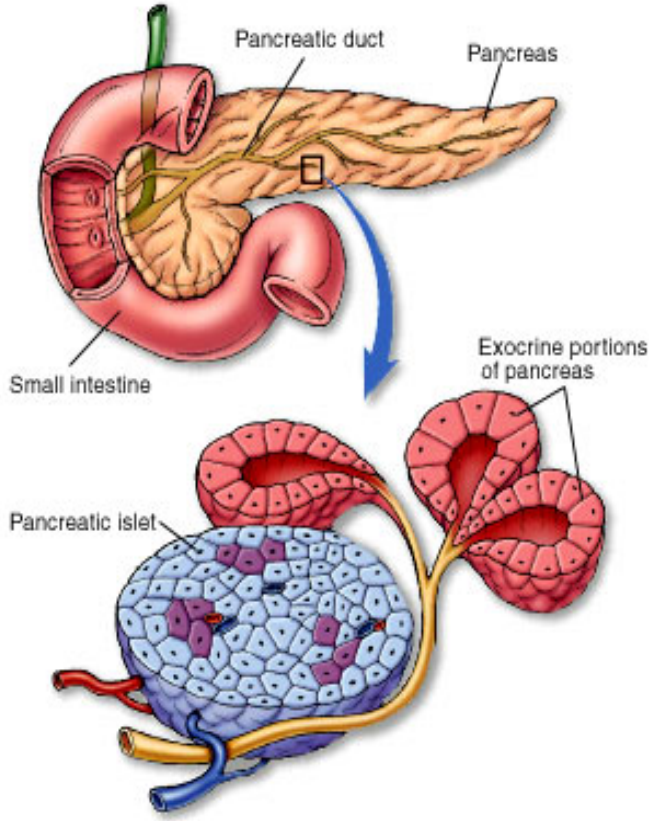
Abbott Established Pharmaceuticals

Pancreas: Physiology

- Compound gland with distinct exocrine and endocrine functions[1]
- Exocrine pancreas secretes enzymes that digest carbohydrates, proteins and fats, and bicarbonates for neutralisation of acidic chyme from the stomach[2]
- Exocrine pancreas: 80% to 85% of the pancreas; rest of the gland constitutes the endocrine portion[1]
- Basic cellular unit of the endocrine pancreas: Islet of Langerhans[1]
- Functional unit of the exocrine pancreas: Acinus[2]



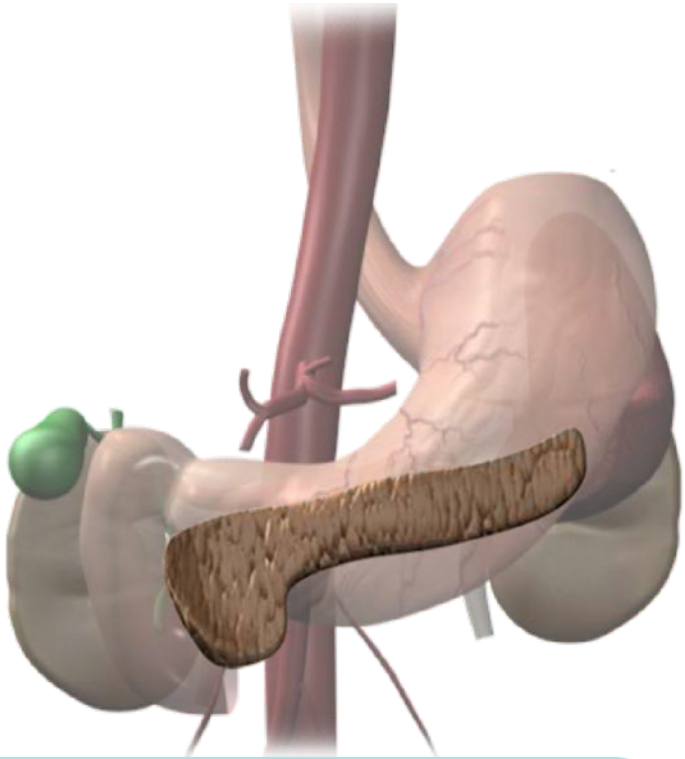
1. Hruban RH et al. The Pancreas. *Robbins and Cotran Pathological basis of disease*.7th ed. Elsevier; 2005.
2. Guyton AC, et al. *Textbook of Medical Physiology.* 11th ed. Elsevier; 2006.

Normal Lipase Secretion From Pancreas

How much enzymes does a normal pancreas secrete?

- Normal pancreatic enzyme secretion (lipase, amylase and proteases) varies according to meal volume

- To avoid fat malabsorption, lipase secretion of greater than 5-10% of normal is needed[1,2]

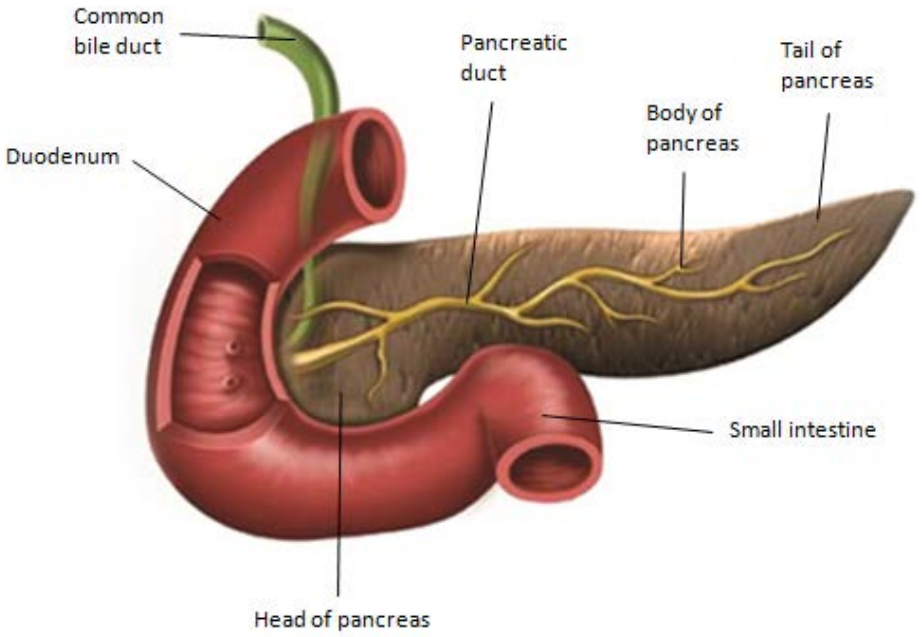


Intraluminal pancreatic activity must be <10% before malabsorption occurs[2]

1. Keller J, Layer P. *Gut.* 2005;54(Suppl VI):vi1-vi28.
2. Layer P et al. *Gastroenterology.* 1986;91(1):41-48.

Pancreatic Exocrine Insufficiency

- Definition: Condition in which quantity of enzymes secreted in response to a meal are insufficient for maintaining normal digestion[1]
- Main reasons for inadequate availability of pancreatic enzymes[1,2]
  - Reduced production and secretion
  - Inadequate stimulation
  - Acid-mediated inactivation
  - Obstruction of the pancreatic duct
- Main clinical consequence of PEI is fat maldigestion and malabsorption resulting in steatorrhoea[1]



1. Australasian treatment guidelines for the management of pancreatic exocrine insufficiency. 2010:1-89.
2. Dominguez-Munoz JE. *Gastroenterol Hepatol.* 2011;7(6):401-403.

PEI, pancreatic exocrine insufficiency.

Pancreatic Exocrine Insufficiency: Causes

- Chronic pancreatitis[2,3]
- **Cystic fibrosis[1,2,3]**
- Pancreatic cancer[2,3]
- Pancreatectomy[2,3]
- Gastrectomy[2,3]
- Pancreaticoduodenectomy[2,3]

1. Sikkens ECM et al. *Best Pract Res Clin Gastroenterol*. 2010:24;337-347.
2. Dominguez-Munoz JE. *Curr Gastroenterol Rep.* 2007;9(2):116-122.
3. Dominguez-Munoz JE. *Gastroenterol Hepatol.* 2011;7(6):401-403.

Clinical Features

Clinically evident PEI with steatorrhoea is noted only when 90% of pancreatic function is lost[1]

- Main clinical consequence of PEI: Fat maldigestion and malabsorption resulting in steatorrhoea[2]
  - Steatorrhoea is characterised by foul smelling, frothy and buoyant stools due to high fat content
  - Other symptoms: Abdominal pain, flatulence and weight loss in adults or lack of weight gain in children
- Advanced maldigestion leads to deficiencies of fat-soluble vitamins (vitamins A, D, E and K), magnesium, calcium, zinc and folic acid[1]
- Body weight loss, failure to thrive in pediatric patients

1. Sikkens ECM et al. *Best Pract Res Clin Gastroenterol*. 2010:24;337-347.
2. Australasian treatment guidelines for the management of pancreatic exocrine insufficiency. 2010:1-89.

Diagnosis of PEI (Faecal Elastase Test)

- Faecal elastase tests are becoming more prevalent in clinical practice[1]
- Cannot be used to measure PEI therapy
- Requires a single stool sample[2]
- Measures the elastase enzyme via ELISA in the stool[1,2]
- Specificity: Approximately 93%
  - Compromised in patients with small bowel disease and type 1 diabetes
  - Risk of false positive result in diarrhoea and other intestinal disorders[2]

>200 µg/g stool: normal value[2]

<200 µg/g stool: mild PEI[1]

<100 µg/g stool: severe PEI[1]

1, Australasian treatment guidelines for the management of pancreatic exocrine insufficiency. 2010:1-89.
2. Sikkens ECM et al. *Best Pract Res Clin Gastroenterol*. 2010:(24);337-347.

Diagnosis of PEI and Therapy

- Diagnosis of PEI is based on intestinal lipase activity
- Calculation of fat excretion based on 72-h faecal fat quantification[1]
- Steatorrhoea is present if the ingested fat excreted is
  - >7% in patients over 6 months of age or
  - >15% in patients under 6 months of age.[2]
- Odious nature of this test makes it very unpopular with both patients and laboratory technicians[1,2]
- Fat intake impacts stool fat excretion
- Coefficient of fat absorption is to be determined

'Gold standard' method for the diagnosis of fat maldigestion[1]

1. Dominguez-Munoz JE et al. *J Gastroenterol Hepatol* 2011;26(2):12-16.
2. Australasian treatment guidelines for the management of pancreatic exocrine insufficiency. 2010:1-89

Coefficient of Fat Absorption

Fat intake (g/day) – fat excretion (g/day) / fat intake (g/day) x 100

A high fat diet (optimally 2g/kg/day or 60 g/m^2/day or 100 g/day planned by a dietician and intake verified

Use of stool dye marker (e.g. FD& C Blue Dye No 2) given at the beginning and at the end of the 72 h controlled diet. The first dyed stool will be discarded. The stool collection should continue including the first blue marked stool after dye intake (72 hours plus, depending on GI transit).

Konstan et al. Clinical Investigation, 3(8), 831-741, 2013.

Coefficient of Nitrogen Absorption

Same collection methodology as for the CFA

Analytics for nitrogen (e.g. combustion) for the calculation of protein excretion

Caveat: bacterial nitrogen and endogenous proteins are included

Konstan et al. Clinical Investigation, 3(8), 831-741, 2013.

Coefficient of Carbohydrate Absorption

Carbohydrate maldigestion not quantifiable in stool due to hindgut metabolism of starch.

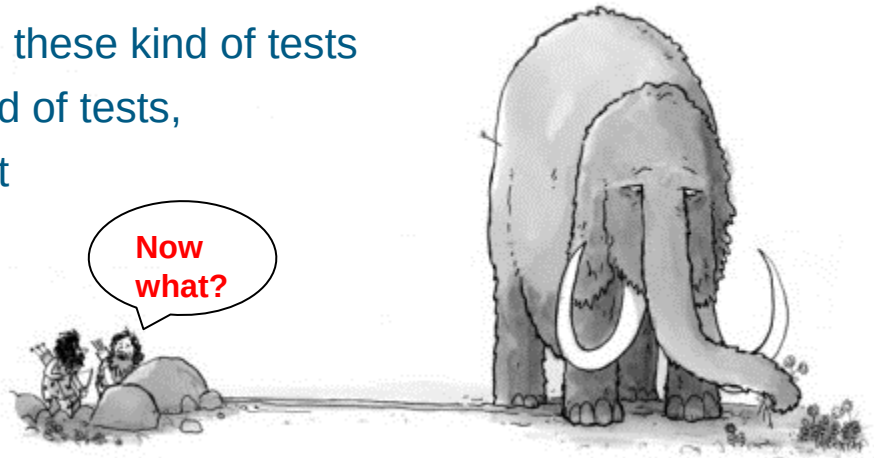
Konstan et al. Clinical Investigation, 3(8), 831-741, 2013.

(BIO)ANALYTICS

Challenges

- There are no (good) standards
- There are no (good) blanks

- Huge amounts of unpleasant inhomogeneous sample material
- (Very) laborious and sometimes a somewhat risky sample workup
- Analytical techniques are often not part of todays skillset of the biomedical analist
- Clinical (central) laboratories are not set up to run these kind of tests
- Bioanalytical laboratories are not set up to run these kind of tests
- Some specialized hospitals may run these kind of tests,
  but they are generally not GxP/BMV compliant



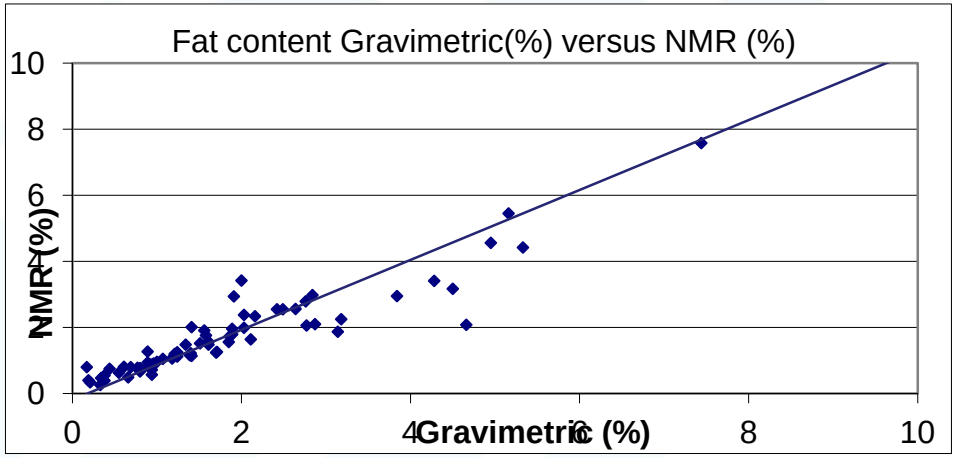
CFA: van de Kamer & Jeejeebhoy "gold standard methods"

van de Kamer[1] (Total Fat)	van de Kamer[1] (Fatty acids & Fat)		Jeejeebhoy[2] (Total Fat)
↓	Homogenize feces		↓
Saponification with KOH in EtOH ↓ Acidify with HCl ↓ Extract with P.E. (+ NaCl & amylalcohol) ↓	Boil with 0.1N HCl ↓ Extract with P.E. (+ NaCl & amylalcohol) ↓ Evaporate to dryness ↓ ↓		Acidify with HCl ↓ Mix/Extract with hexane-EtOH-Et_2O ↓ Centrifuge ↓ Collect upper layer ↓ Repeat with infranatant 2x & Collect upper layers ↓
Titrate with KOH	Titrate with KOH in i-BuOH	Boil with KOH in i-BuOH ↓ Titrate excess KOH with HCl	Evaporate to dryness ↓ Weigh

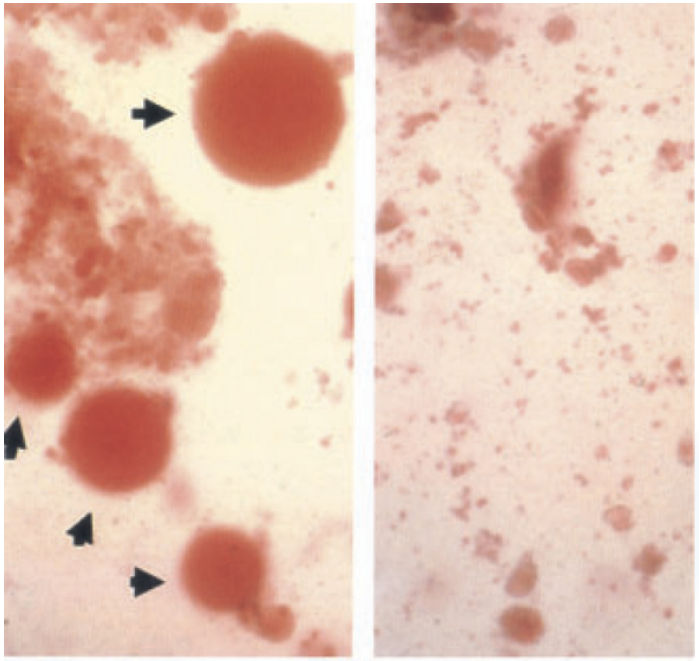
1. JH van de Kamer, H ten Bokkel Huinink, HA Weyers. *J. Biol. Chem.* 177: 347-355 (1949)
2. KN Jeejeebhoy, S Ahmad, G Kozak Clin. Biochem. 3: 157-163 (1970)

CFA: ^{1}H & ^{13}C NMR, NIR & FT-IR, ^{13}C breath test "alternative methods"

NMR (^{1}H & ^{13}C)	Infrared (NIR & FT-IR)	^{13}C breath test
Homogenize feces ↓	↓	Dose with ^{13}C-glyceride together with standardized meal
Spread on teflon sample pad ↓ Dry (microwave at 110 ^{0}C) ↓ NMR	Record NIR or FT-IR spectrum ↓ Calculate fat content using PLS multicomponent analysis and a van de Kamer learning set	↓ Collect exhaled air in exeteiner tube ↓ Measure $^{13}CO_2/^{12}CO_2$ ratio with MS



Stool fat: semi quantitative / screening methods



Positive Negative

Sudan red coloring	Acid steatocrit
Small sample	Homogenize feces
↓ Add glacial AcOH & Sudan Red in EtOH ↓ Spread on microscopic slide and heat twice to boil ↓ Measure red fatty droplets	↓ Mix with $HCLO_4$ ↓ Centrifuge ↓ Measure size of fatty layer

CNA: Dumas (or elemental) or (micro) Kjeldahl

Kjeldahl	Dumas
↓ Homogenize feces ↓	
↓ Destruct sample in K_2SO_4, HgO & H_2SO_4 ↓ Add NaOH & K_2S ↓ Distill of ammonia and trap in boric acid ↓ Titrate	↓ Place sample in combustion analyzer & press start

Methods correlate very well and can be considered equivalent

Table 1. Percent Nitrogen (Wet Basis) by Nitrogen Analyzer and Kjeldahl Methods

	Nitrogen analyzer		*Kjeldahl*		*Difference between methods*
Specimen No.	*Duplicates*	*Average*	*Duplicates*	*Average*	*(% N)*
1	1.76	1.81	1.75	1.75	+0.06
	1.86		1.74		
2	1.20	1.21	1.22	1.21	0.00
	1.22		1.20		
3	1.72	1.76	1.63	1.63	+0.13
	1.80		1.62		
4	1.64	1.69	1.68	1.68	+0.01
	1.73		1.67		
5	2.00	2.03	1.96	1.94	+0.09
	2.06		1.92		
6	0.66	0.64	0.60	0.59	+0.05
	0.61		0.57		
7	1.24	1.23	1.22	1.22	+0.01
	1.22		1.22		
8	0.7(	0.78	0.70	0.72	+0.06
	0.79		0.73		
9	0.53	0.55	0.53	0 54	+0.01
	0.57		0.54		
10	1.33	1.35	1.27	1.26	+0.01
	1.36		1.25		

CFA & CNA: (Bio)analytics overview

Method	Pro	Con	
van de Kamer	Gold standard	Requires 72h stool sample	Very laborious & unpopular
Jeejeebhoy (graphimetric)	Correlates well with vdK		Quite laborious & unpopular
^{1}H (& ^{13}C) NMR	Less laborious. Fast & correlates well with vdK		NMR not common in BA or central labs
NIR & FT-IR	Less laborious. Fast & correlates 'OK' with vdK		High variance
Acid steatocrit & coloring	Easy and fast		Only semiquantitative
^{13}C breath test	Easy and fast. Promising	Not well established yet	
Dumas & Kjeldahl	Well established	Requires 72h stool sample. Bacterial nitrogen & proteins are included	

There is room for improvement

CLINICAL STUDIES WITH PERT IN CYSTIC FIBROSIS

Guidelines for Management of Infants with CF by Cystic Fibrosis Foundation

- 60% of infants with CF will have PEI at birth
- Approximately 90% of infants have PEI at 1 year of age
- CFF recommendations:
  - Pancreatic functional status measured by faecal elastase test or CFA
  - Pancreatic enzyme replacement therapy (PERT) initiated in patients confirmed with PEI in the presence or absence of symptoms

Borowitz D. *J Pediatr* 2009.

CF Infants <24 Months

Objective	To evaluate the efficacy and safety of PERT (Creon® Micro) in infants younger than 24 months with CF
Study Design	Multicenter, open-label, baseline-controlled, single-arm study in 12 CF patients with PEI
End Point	Primary end point was mean change from baseline in the CFA after 2 weeks of treatment, based on 72-hour fat balance assessments

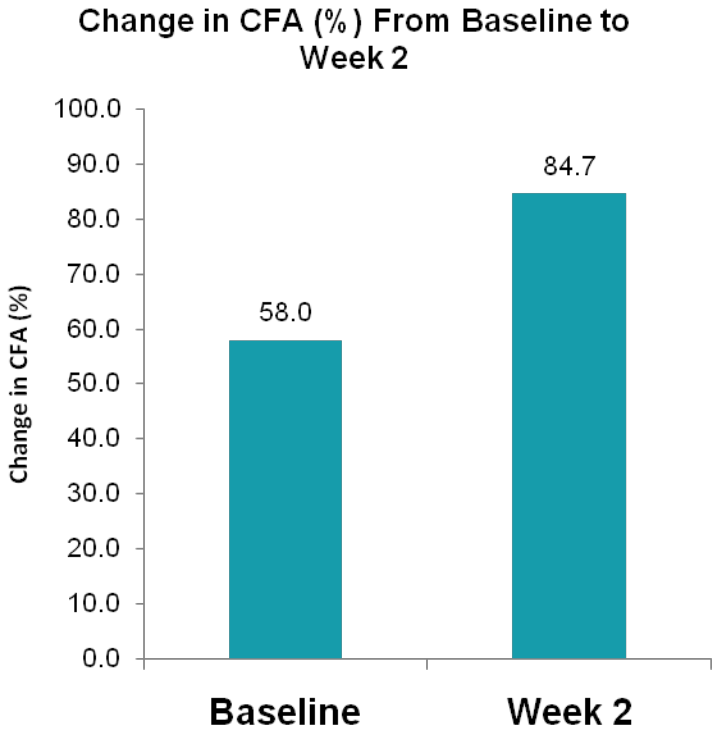
Colombo C, et al. *Pancreas.* 2009;38(6):693-699.

CF, cystic fibrosis.

CF Infants <24 Months

Results

- The CFA significantly increased from a baseline mean of 58.0% to a mean of 84.7% after 2 weeks of treatment
- Subject acceptance of therapy was good in the majority of patients
- Patient weight and height increased over 8 weeks of treatment
- No serious adverse event was reported



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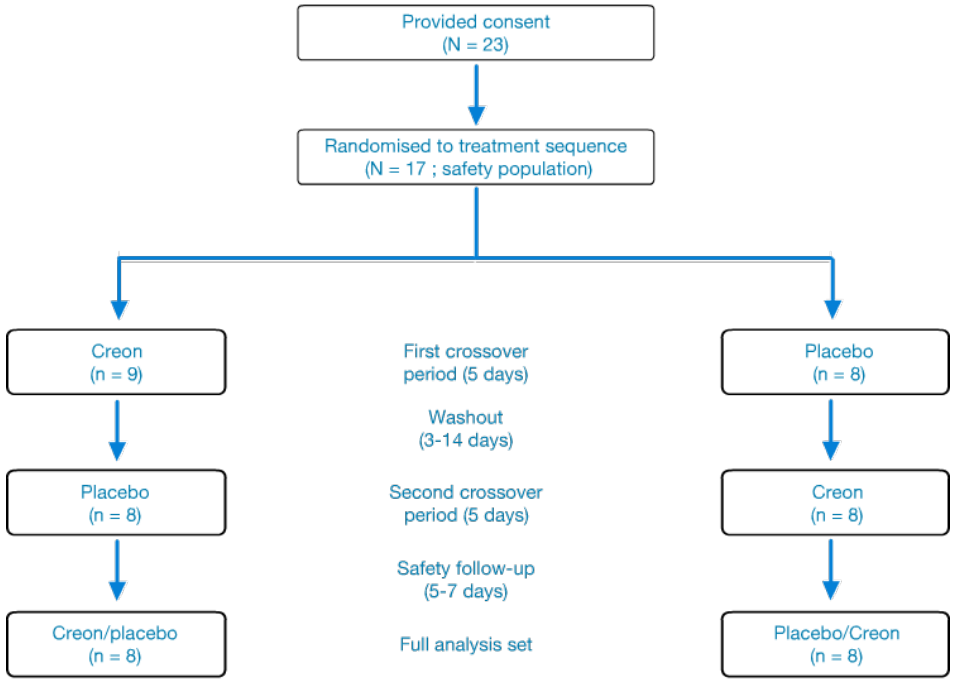
Colombo C, et al. *Pancreas.* 2009;38(6):693-699.

CF, cystic fibrosis.

CF Children Aged 7 to 11 Years

Objective	To study the efficacy and tolerability of PERT (Creon®) compared with the placebo in children aged 7 to 11 years with PEI due to CF
Study Design	Multicenter, randomised, double-blind, placebo-controlled, 2-period crossover, superiority study
End point	Primary outcome measure was CFA. The secondary outcome measures were CNA and clinical symptoms.

Study Design



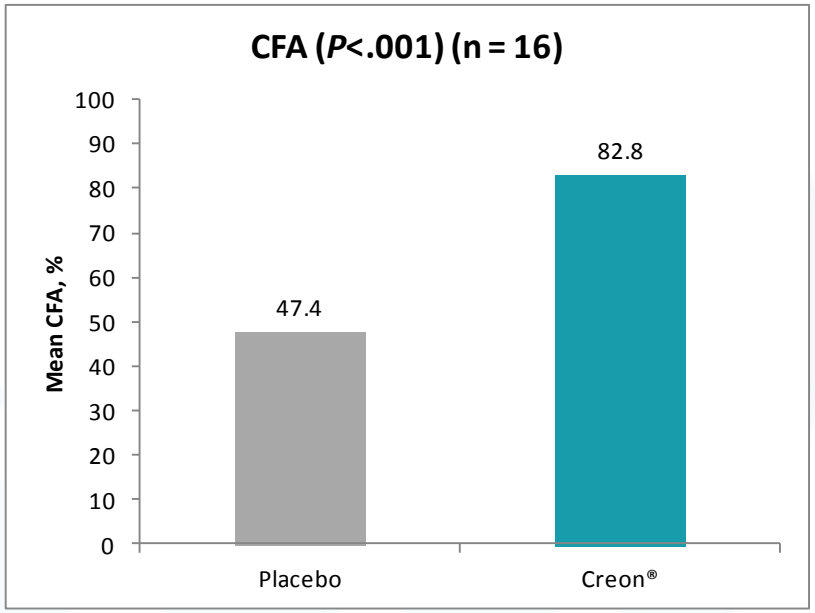
*One patient in the pancrelipase/placebo sequence withdrew consent on day 2 of the first treatment period and was not included in the efficacy analysis.

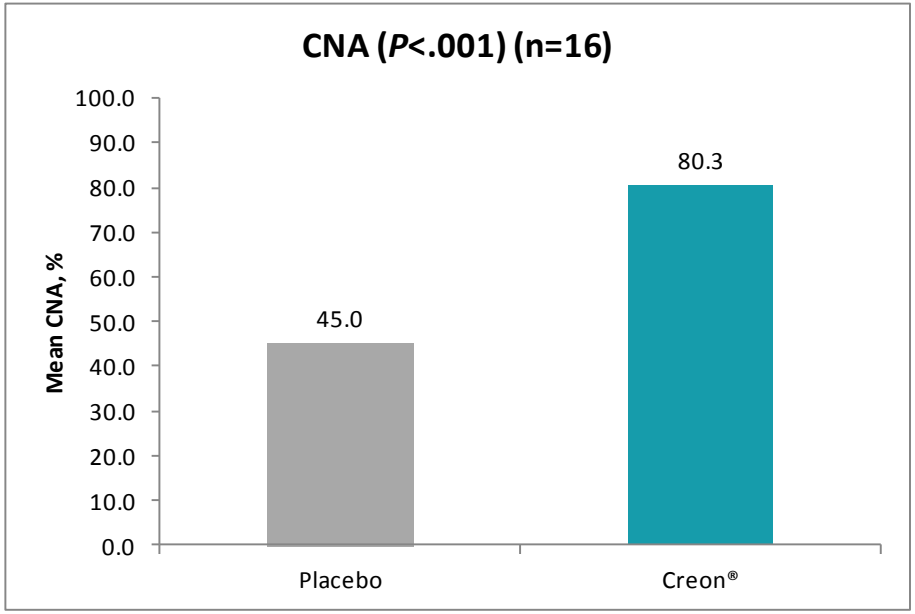
Graff GR, et al. *Clin Ther.* 2010;32(1):89-103.

CF, cystic fibrosis; CFA, coefficient of fat absorption; CNA, coefficient of nitrogen absorption; PEI, pancreatic exocrine insufficiency.

CF Children Aged 7 to 11 Years

Creon® significantly improved both CFA and CNA





Graff GR, et al. *Clin Ther.* 2010;32(1):89-103.

CF, cystic fibrosis; CFA, coefficient of fat absorption; CNA, coefficient of nitrogen absorption.

Summary and Conclusion

PEI causes maldigestion of fat, protein and carbohydrates consequently leading to malabsorption and malnutrition.

The efficacy of pancreatic enzyme supplementation can be shown by the coefficient of fat absorption (CFA).

The CFA determination is cumbersome as it requires 4 day controlled dietary record and 3 day stool collection for the determination of fat intake and fat excretion. Gold standard for quantification of fat maldigestion.

Alternative tests for quantification of maldigestion (not only fat) in PEI are warranted, i.e. for pediatric patients.

THANK YOU