Breath tests as diagnostic tests in gastroenterology

basics and clinical applications

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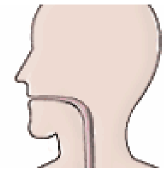
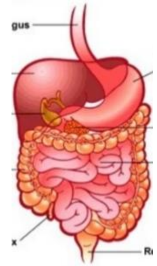
9 juni 2020

Basic principles of breath tests

Basic principle of breath testing



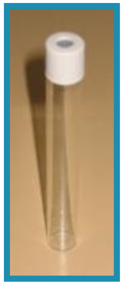
$^{13/14}$C-substrate

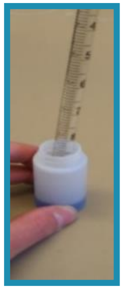


$^{13/14}$C-subgroup

$^{13/14}CO_2$

$^{13/14}CO_2$



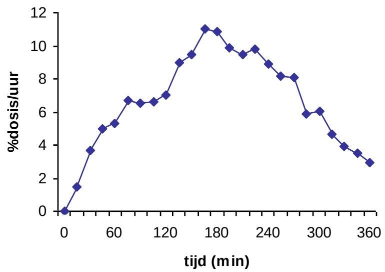


$^{13/14}CO_2$

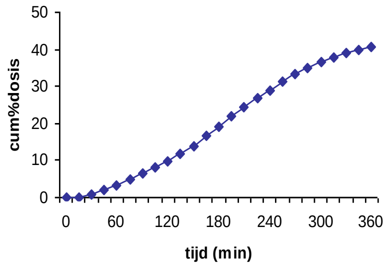
Enzymatic reaction
Digestion/absorption

Rate-limiting step

PDR = percent dose recovery
= %dose/h



cPDR = cumulative percent dose recovery

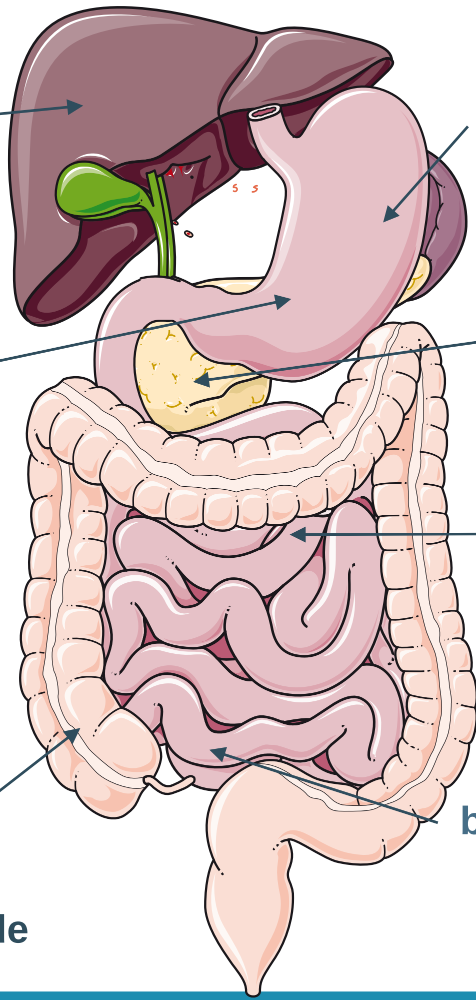


^{13}C or ^{14}C-labelled substrates

- ^{13}C is preferred because of lack of radiation burden
    - Safe in children and pregnant women

- Only ^{14}C tests
    - ^{14}C-glycocholic test
    - Gastric emptying of solid and liquid phase

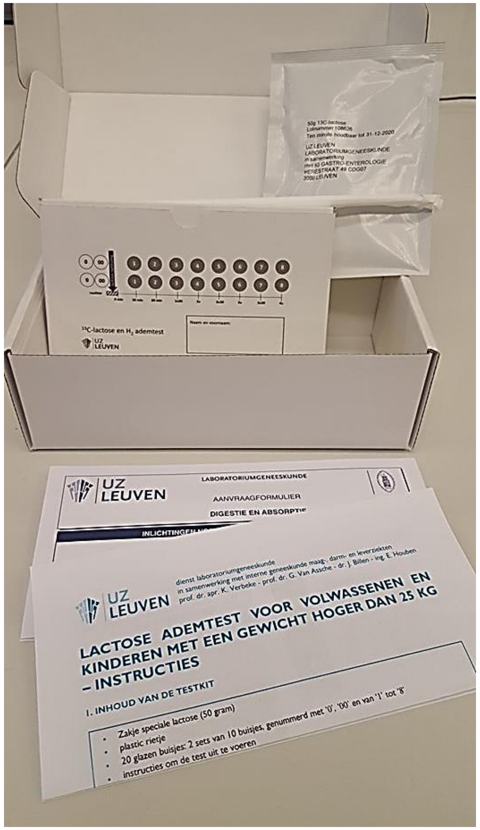
Breath tests in routine clinical diagnose

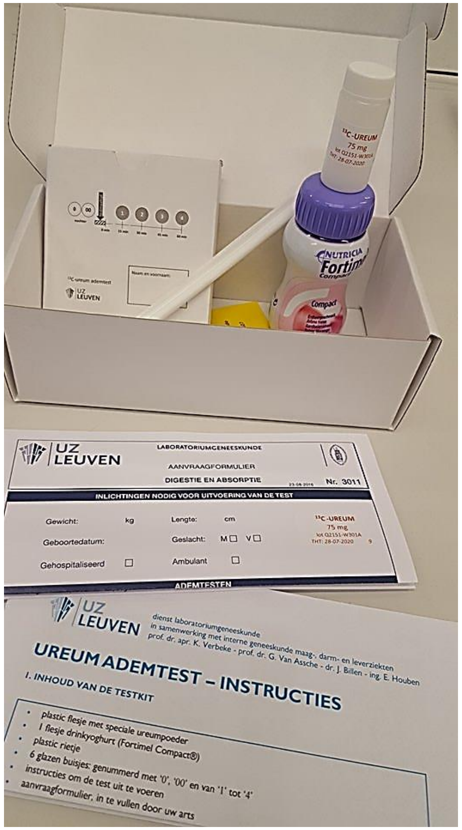


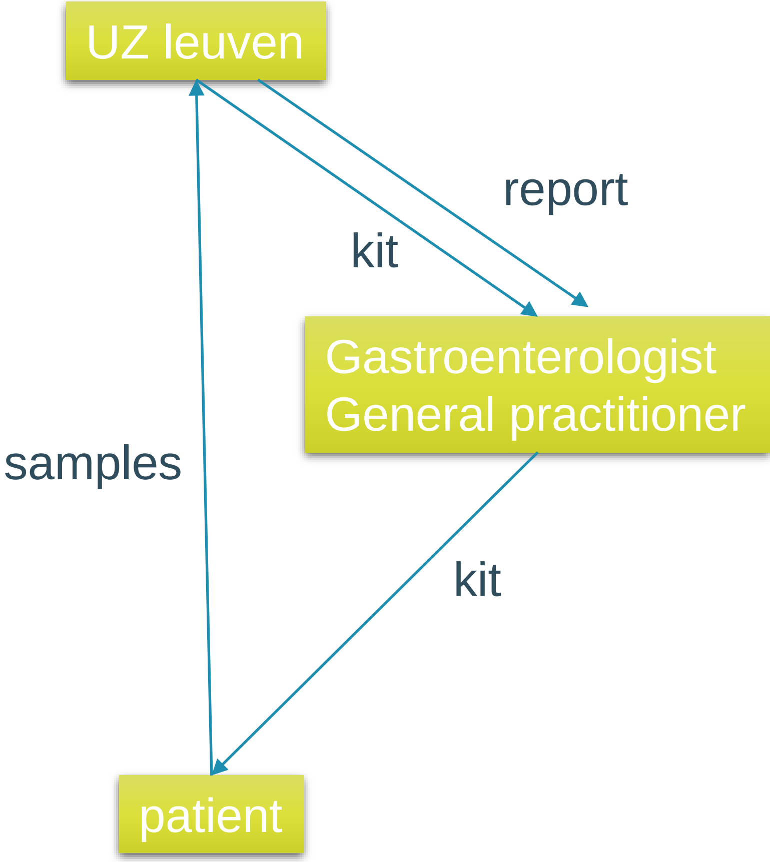
Carbohydrate malbsorption: combined with measurement of H_2 in breath

- Human enzymes do not produce hydrogen
- Hydrogen in breath indicates contact of bacteria with the substrate
- If it happens in the small intestine -> indication of small bowel bacterial overgrowth
- If it happens in the large intestine -> indication of carbohydrate malabsorption

Test kits







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Interpretation: normal values

test	parameter	description
mixed triglycerides	cum%/6h	cumulative percent of administered dose excreted after 6h
lactose	cum%/4h	cumulative percent of administered dose excreted after 4h
	peak excretion	maximal excretion
	H_2 excretion	reflects bacterial metabolism of lactose
glycocholic acid	cum%/6h	cumulative percent of administered dose excreted after 6h
gastric emptying	$t_{1/2}$	half-emptying time (min)

normal values have been established for each of these parameters, using a specific test meal and a specific test duration

Isotope ratio mass spectrometer

- Ratio of $^{13}C/^{12}C$
- always compared to external standard e.g. PDB limestone (Pee Dee Belemnite)
- correction for oxygen isotope effect
- results: expressed in δ^{13} value in per mill (‰)

$$\delta^{13} = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{ref}}{(^{13}C/^{12}C)_{ref}} \times 1000$$

$^{13}C/^{12}C$ in PDB = 0.0112372 ⇨ $\delta^{13} = 0$

$\delta^{13} < 0$: lower enrichment than PDB reference

$\delta^{13} > 0$: higher enrichment than PDB reference

$\delta^{13}{}_{breath}$ at starvation= -28 ⇨ $^{13}C/^{12}C = 0.0108045$

Underlying assumptions

- CO_2-production is known and is stable during the test

excess ^{13}C- atoms exhaled/h

excess ^{13}C-atoms/ mmol CO_2 — mmol CO_2/h

$$\% \text{ dosis/h} = 100 \times \frac{\left(\dfrac{AP_t - AP_{to}}{100}\right) \times 300 \times BSA}{\left(\dfrac{AP_s - AP_{to}}{100}\right) \times \dfrac{\text{amount of substrate (mg)}}{\text{molar mass}} \times \text{number of } ^{13}\text{C positions/molecule}}$$

$BSA = W^{0.5378} \times H^{0.3964} \times 0.024265$

Haycock et al. J Pediatr 1978;93:62

atom percent of substrate (AP_s)

mmol substrate

atom percent excess of substrate

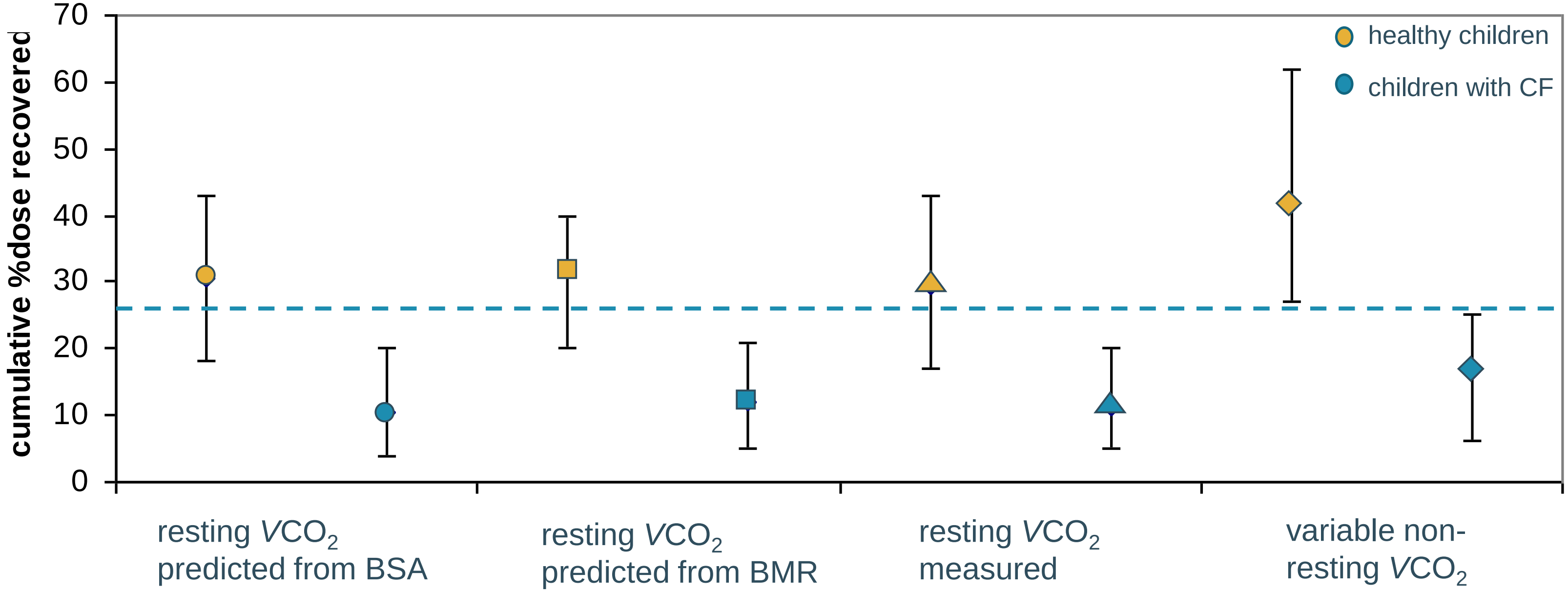
mmol of (potential) ^{13}C atoms administered

effective mmol excess ^{13}C-atoms administered

⇨ Test needs to be done in resting conditions

Influence of CO_2-production

- specificity can be improved using non-resting CO_2-production



Slater C et al. Eur J Clin Nutr 2006; 60:1245-1252

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Underlying assumptions

- CO_2-production is known and is stable during the test
- No contribution to ^{13}C-enrichment from other sources
  - ! Naturally enriched compounds (< C4-plants)
    e.g. glucose < corn starch
    - Dialysis
    - TPN
    - Glucose-infusions
- Test is performed under standardised conditions (test meal, test duration, ...)

Clinical applications of breath tests

Additional information on other breath tests:
www.uzleuven.be/ademtest

^{13}C-MTG

Indication

- Functional assessment of pancreas in case of chronic pancreatic insufficiency
  - grading the stage of the disease
  - evaluate the need for pancreatic enzyme replacement
  - degree of functional impairment may contribute to the decision of surgery or conservative management

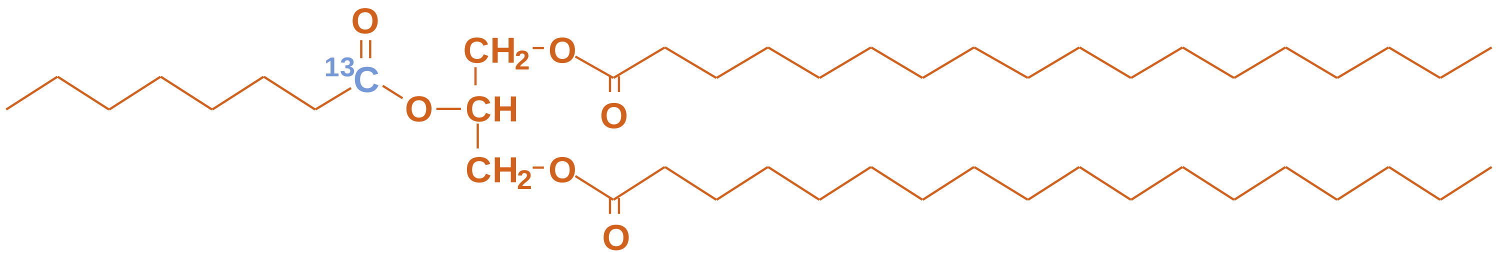
- based on evaluation of fat malabsorption

! Lipid assimilation = lipid digestion + lipid absorption

⇨fat malabsorption occurs when one or both processes fails

^{13}C-mixed triglyceride test

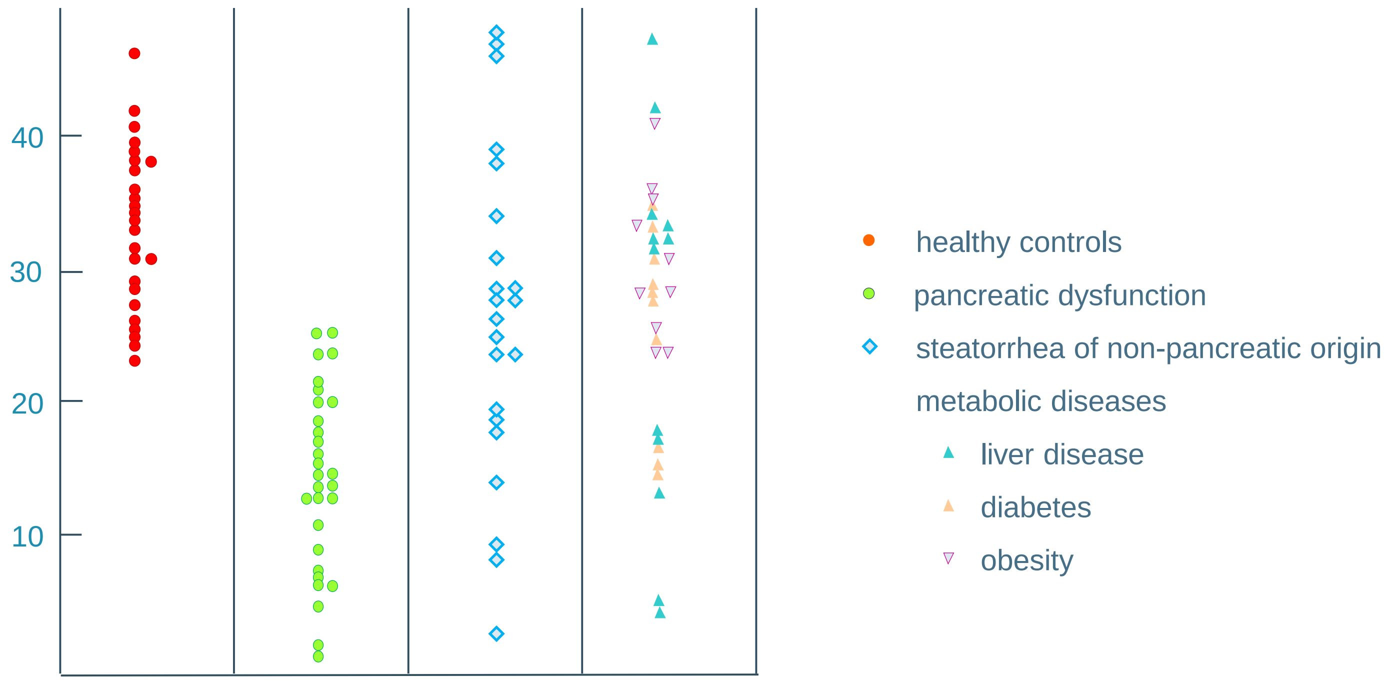
- substrate: 1,3-distearyl,2[^{13}C-carboxyl]octanoyl glycerol



- hydrolysis of long chain fatty acids (position 1 and 3) by pancreatic lipase
- ^{13}C-octanoic acid is hydrolysed non-enzymatically
- medium chain fatty acid: rapidly and completely absorbed (independently of bile acids) and rapidly oxidized in the liver
- little octanoic acid in normal diet: no dilution by unlabelled substrate

Normal versus decreased

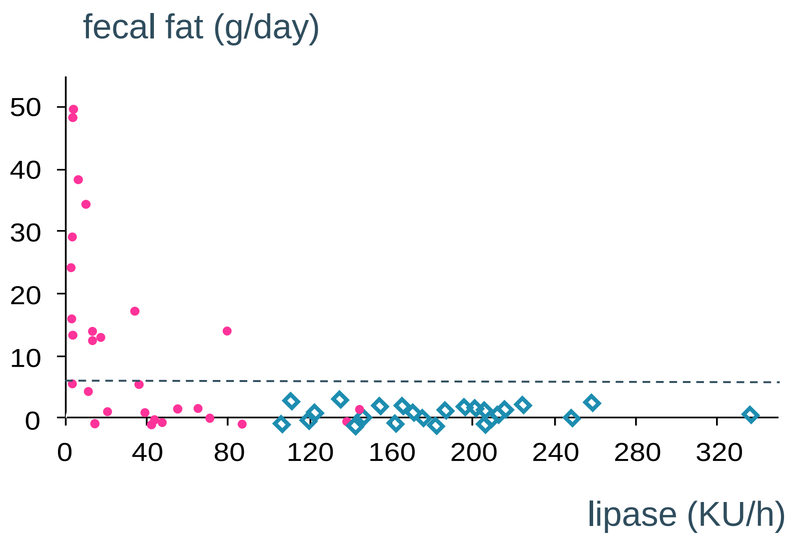
cumulative percent of administered dose excreted after 6h

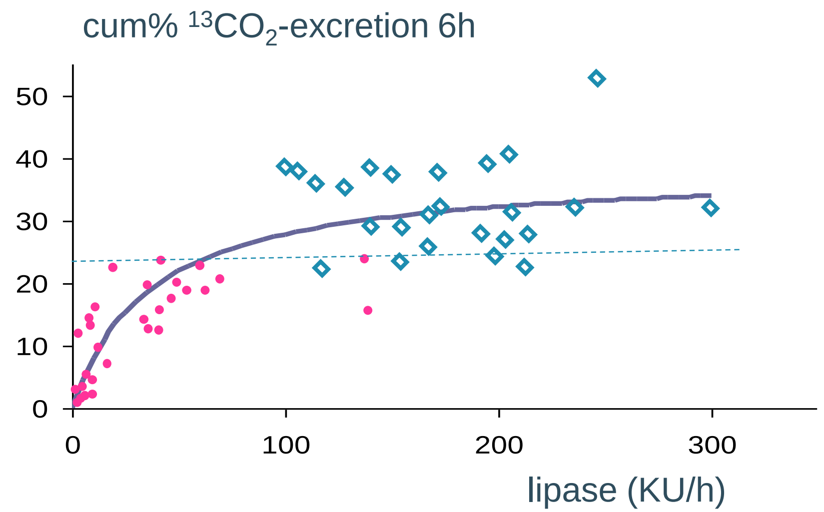


⇨ ^{13}C-MTG-test does not correlate well with fat malabsorption:
⇨ steatorrea can have other causes than pancreatic insufficiency

Vantrappen et al. Gastroenterology 1989; 96:1126-1134

^{13}C-MTG more sensitive than fat malabsorption





steatorrhea when lipase output < 40 KU/h

^{13}C-MTG detects lipase output <90 KU/h (sensitivity 89%)

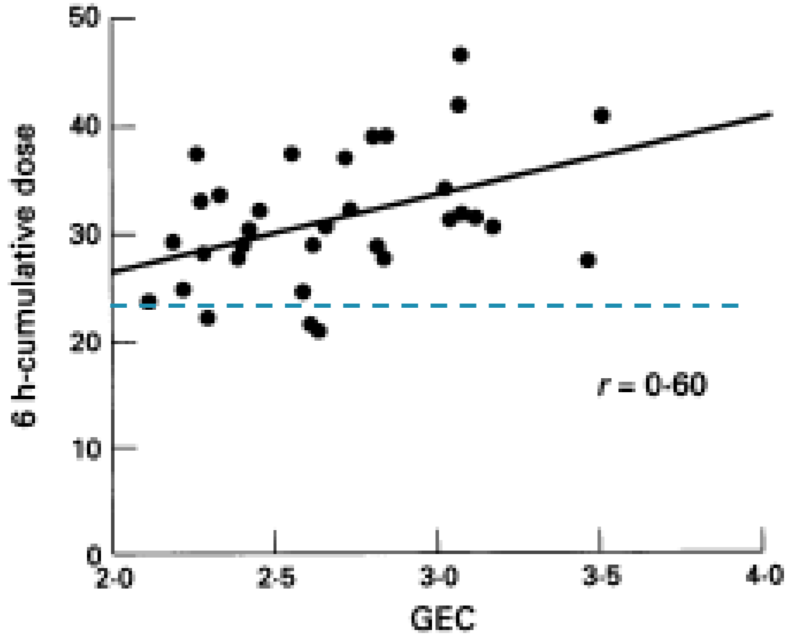
Gastric emptying rate

Relation between gastric emptying rate and rate of intraluminal lipolysis

B D Maes, Y F Ghoos, B J Geypens, M I Hiele, P J Rutgeerts

- subjects without pancreatic insufficiency: clear correlation between gastric emptying rate and rate of intraluminal lipolysis

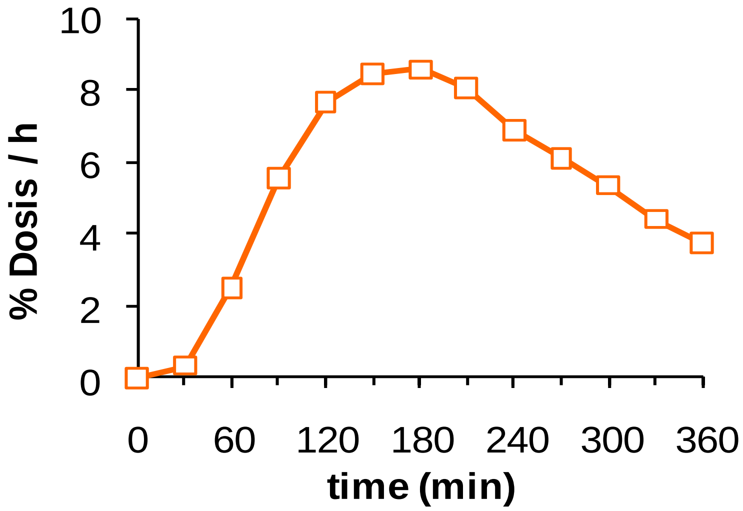
- patients with pancreatic insufficiency: gastric emptying rate had no impact on the rate of intraluminal lipolysis

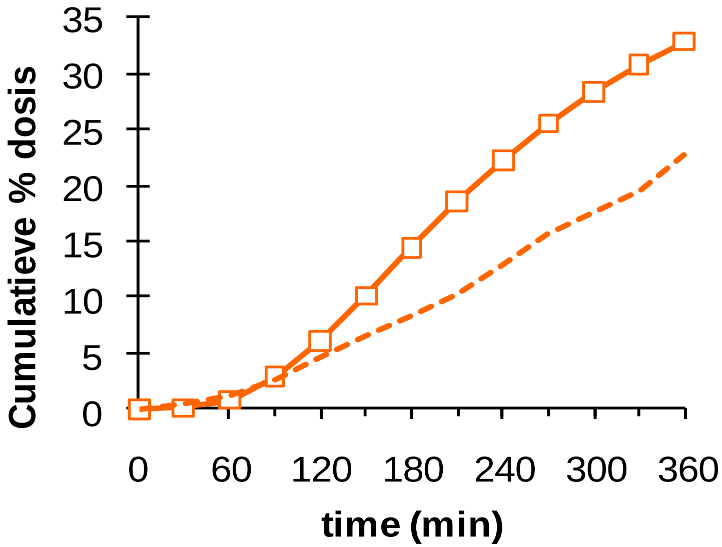


Gut 1996; 38:23-27

Examples

1. Normal lipase activity

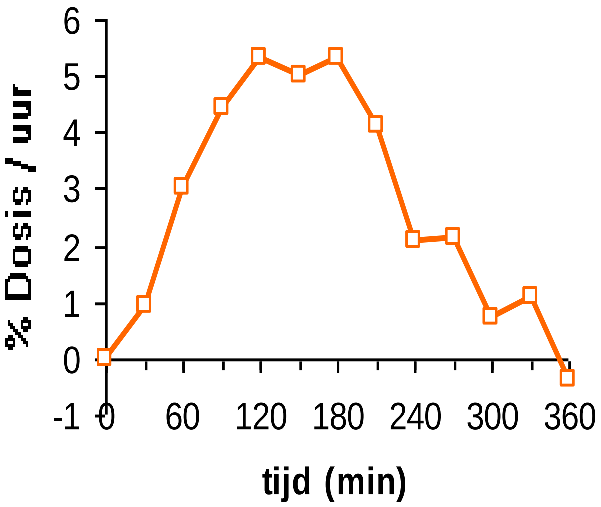


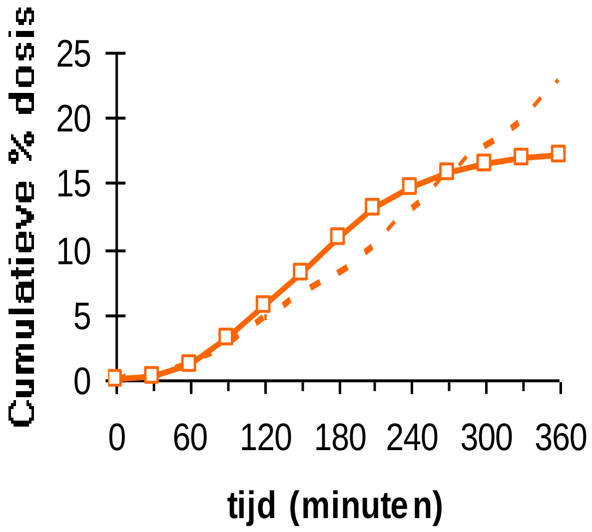


criterium: > 23% of administered dose excreted in breath

Examples

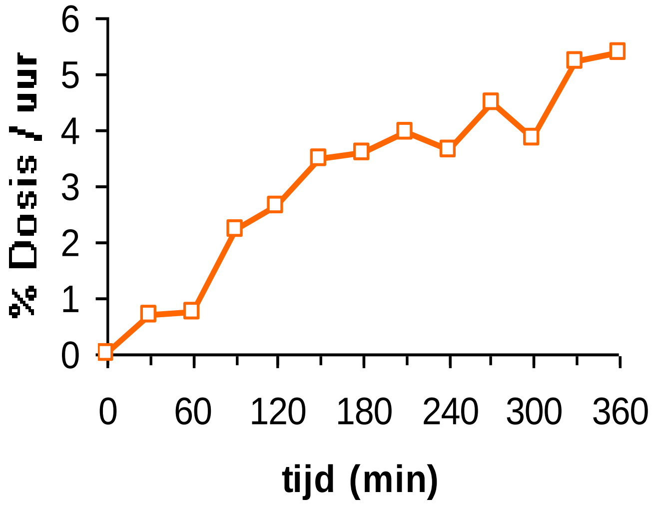
2. low lipase activity

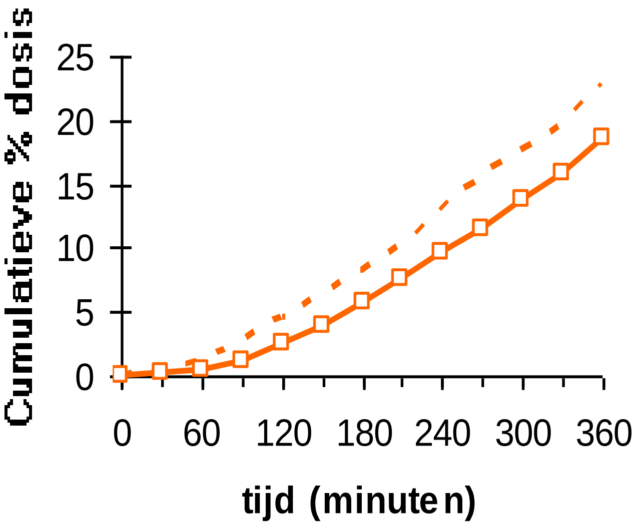




Examples

low lipid digestion, possibly false positive, may be caused by delayed gastric emptying





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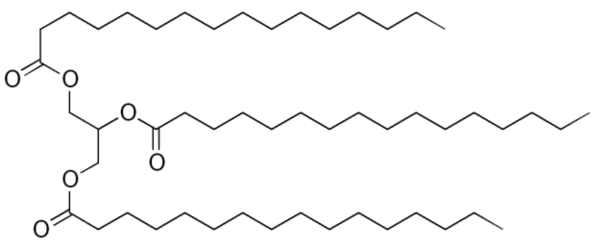
“False” positive tests

- Extensive intestinal mucosal damage (e.g. coeliac or Crohn’s disease)
  - impairment of gut-mediated stimulatory effect of the meal on the pancreas
  - MTG-test normalises if patients are pretreated with CCK-pancreozymin
  - MTG-test normalises in coeliac patients after gluten withdrawal (repair of mucosal damage)
- extremely short contact time
  - e.g. gastrectomy
- patients with diabetes
  - probably due to delayed gastric emptying

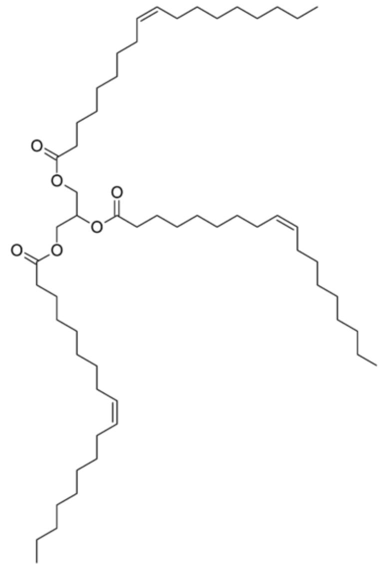
Ghoos Y, et al. Digestion 1981, 22:239-247
Perri F, et al. Gastroenterology 1997, 112:A393

Other breath test substrates

^{13}C-tripalmitin



^{13}C-triolein



^{13}C-hiolein

TABLE I *Fatty acid composition of Hiolein*

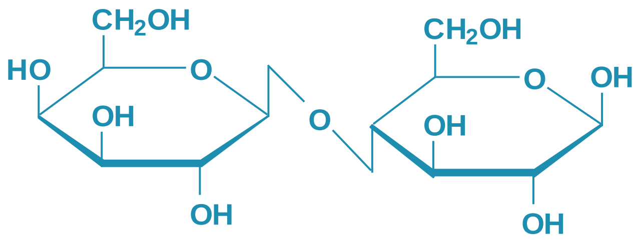
Fatty acid	*Common name*	*Proportion (%)*
18:1 ω-6	Oleic acid	50·6
16:0 ω-6	Palmitic acid	16·9
18:2 ω-6	Linoleic acid	19·6
18:3 ω-3	α-Linolenic acid	3·0
18:0 ω-6	Stearic acid	2·3
16:1 ω-9	Palmitoleic acid	1·8
16:2 ω-6	No common name	1·4
16:3 ω-3	No common name	1·2

- long chain fatty acids that require bile acids for absorption
- not equivalent to ^{13}C-MTG breath test
- practical alternative for measurement of steatorrhea

Lactose: combined $^{13}CO_2/H_2$ breath test

- Indication
  - suspicion of osmotic diarrhoea on the basis of lactose-malabsorption
  - complaints are usually flatus, ructus, postprandial cramps, bloating

- Substrate

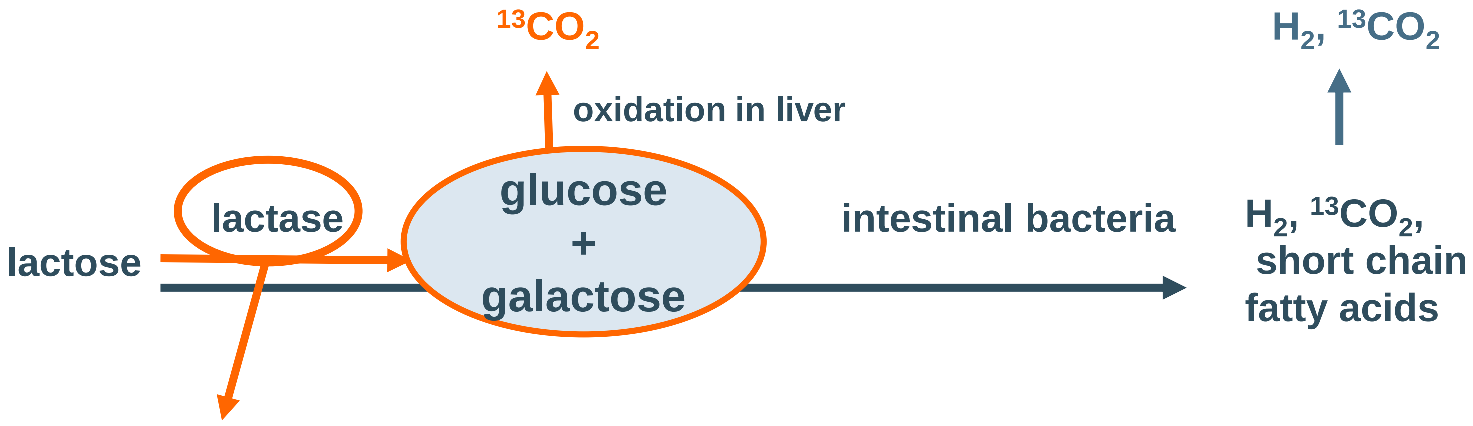


50g ⇨ 25g

- Normal values
  - cum%4h > 14.5%
  - H_2-excretion: (max. value – value t_0) < 20 ppm

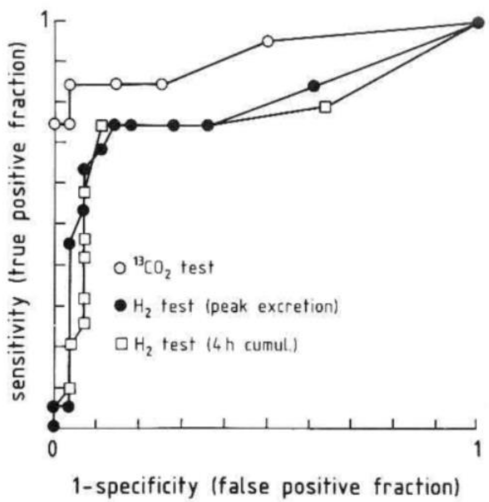
Lactose: combined $^{13}CO_2/H_2$ breath test

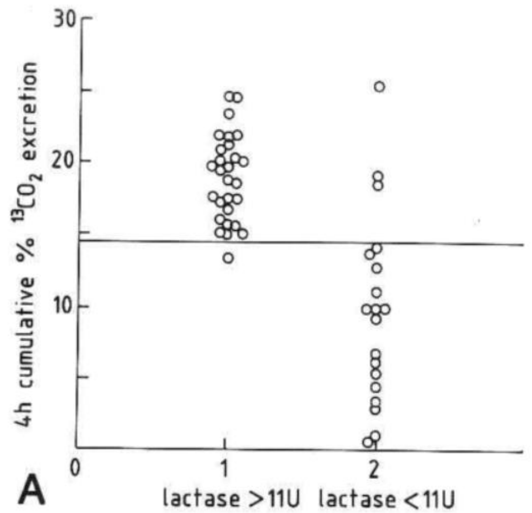
- principle



- located in the brush border membrane
- age dependent
- limited capacity
- varies between various populations
- vulnerable, can be used as indicator of the membrane status

Validation towards jejunal lactase activity

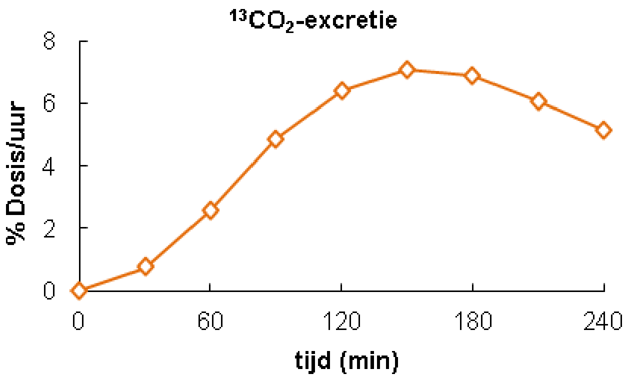


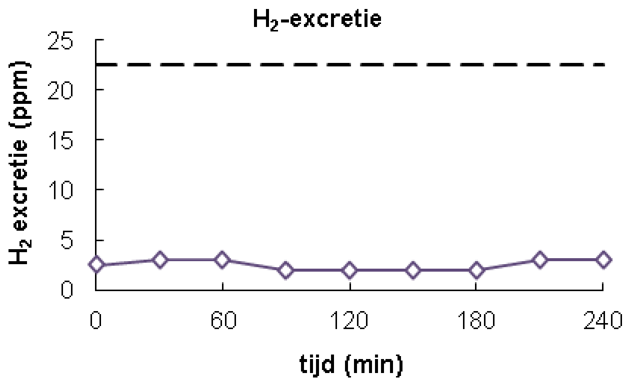


	$^{13}CO_2$-test 4-h cum excr	H_2-test Peak excr	 4-h cum excr
sensitivity	0.84	0,68	0,73
specificity	0,96	0,89	0,89
Pos pred value	0,94	0,81	0,82
Neg pred value	0,90	0,81	0,83
accuracy	0,91	0,81	0,83

Lactose breath test: examples

1. normal lactose test

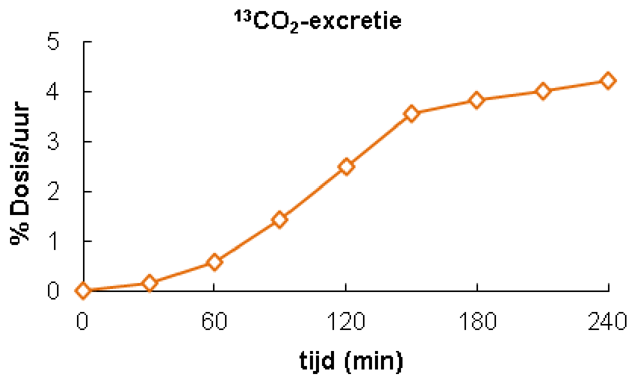


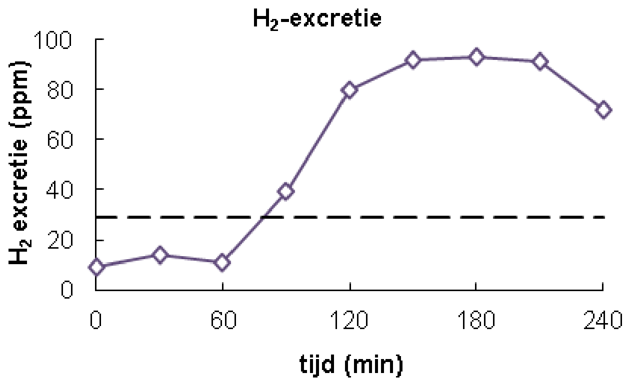


Parameters:		
	normaal	patiënt
piekexcretie	>6.5	7,1
cumulatieve excretie na 4 uur	>14.5	18,62
H_2 excretie (ppm)	<20	1
klachten	geen	?

Lactose breath test: examples

2. lactase deficiency



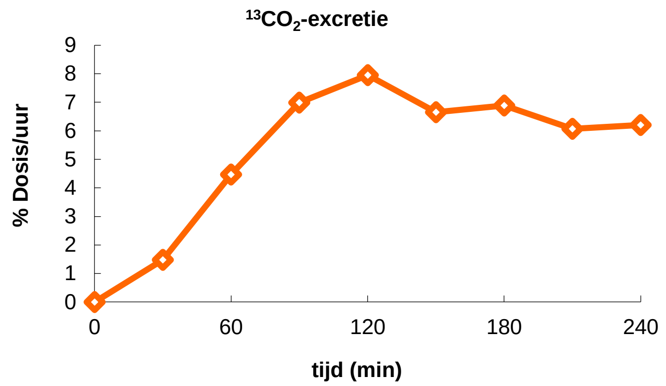


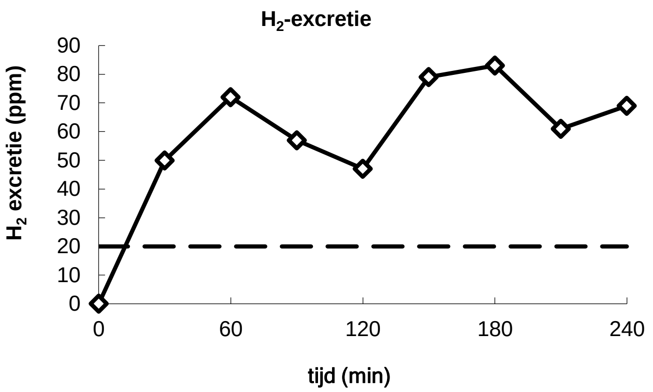
Parameters:

	normaal	patiënt
piekexcretie	>6.5	4,2
cumulatieve excretie na 4 uur	>14.5	9,09
H_2 excretie (ppm)	<20	84
klachten	geen	diarree-buikpijn-opgeblazen gevoel

Lactose breath test: examples

2. Bacterial overgrowth / lactose malabsorption

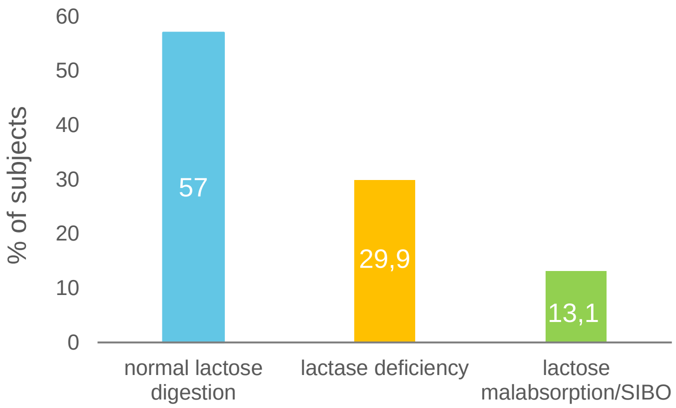


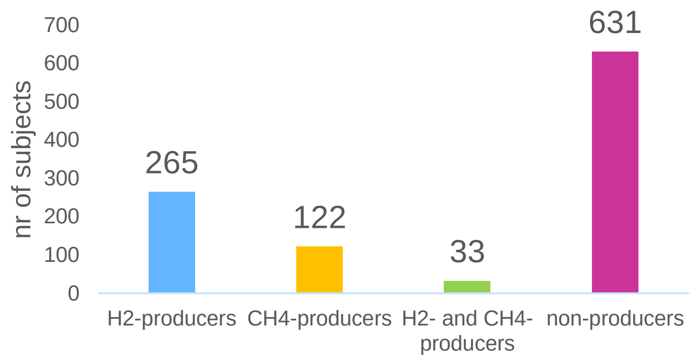


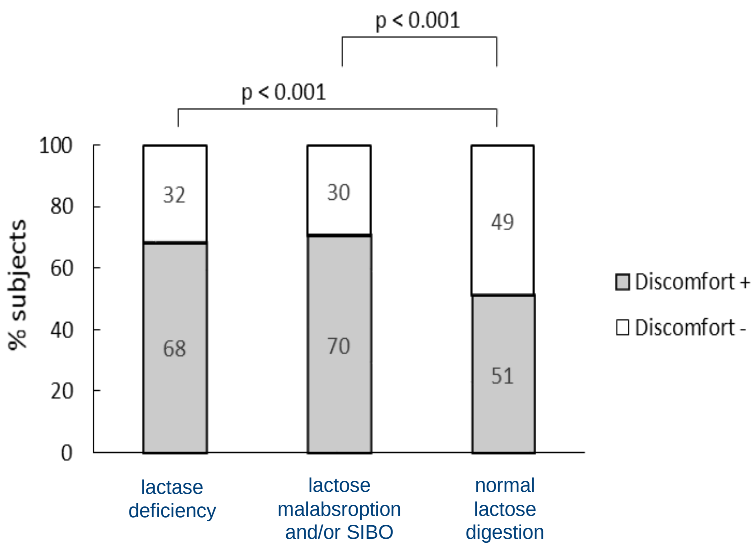
Parameters:

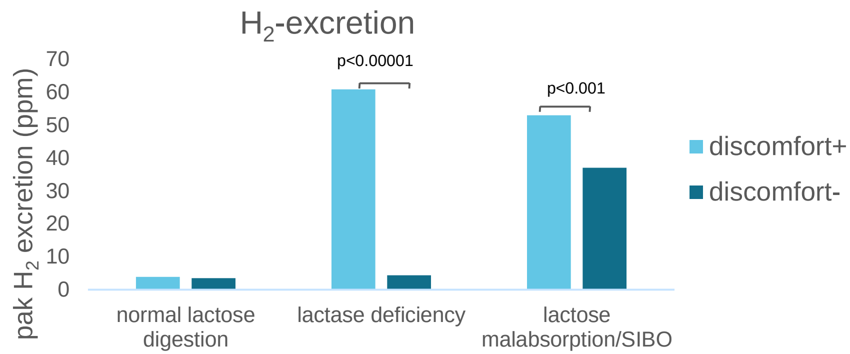
	normaal	patiënt
piekexcretie	>6.5	8,0
cumulatieve excretie na 4 uur	>14.5	21,80
H_2 excretie (ppm)	<20	83
klachten	geen	krampen

Retrospective analysis of 1051 lactose tests









- 93% of complaints were gastrointestinal symptoms (cramps, flatulence, diarrhea, nausea, abdominal pain, bloating)
- Other symptoms: headache and tiredness

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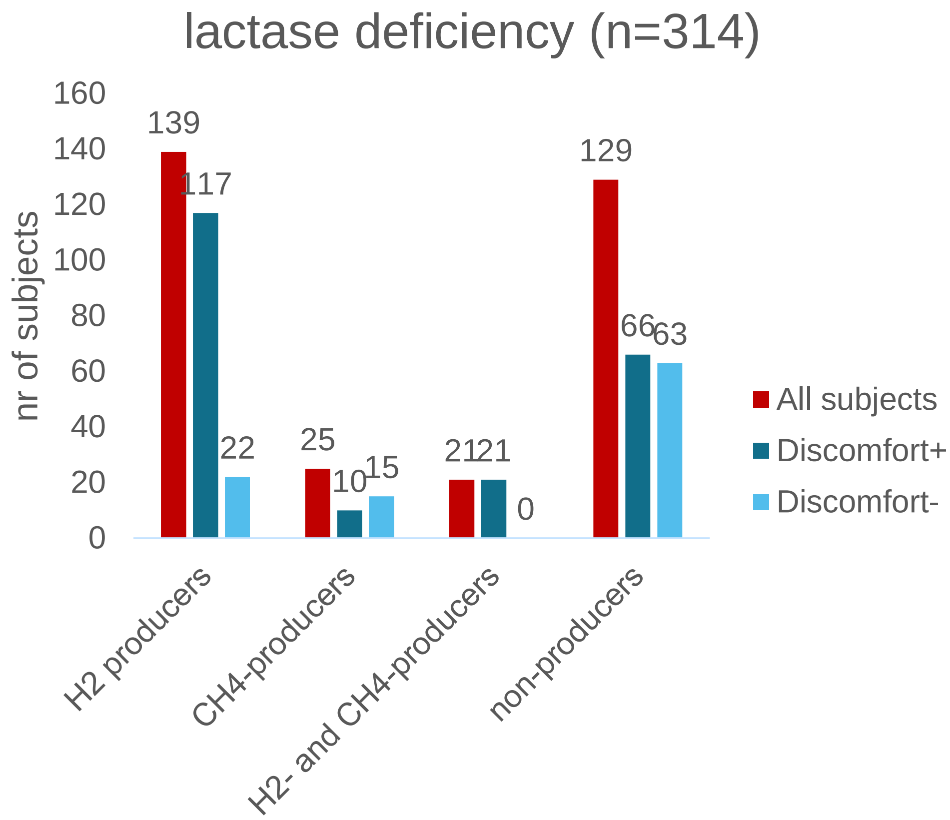
Retrospective analysis of 1051 lactose tests



- Cum. ^{13}C-excretion after 4h <14.5%
- Whether or not increased H_2-excretion >20 ppm

154 subjects would have been diagnosed as normal lactose digesters based on H_2-measurements alone

- proportion of non-H_2 producers after lactulose in most studies <10%
- test too sensitive?
  - reduction of cut-off to 13.5% and 12.5% reduces the non-producers to 37% and 35%
- colon adaptation?
  - breath H_2 excretion decreases in subjects with lactose malabsorption after chronic consumption of lactose

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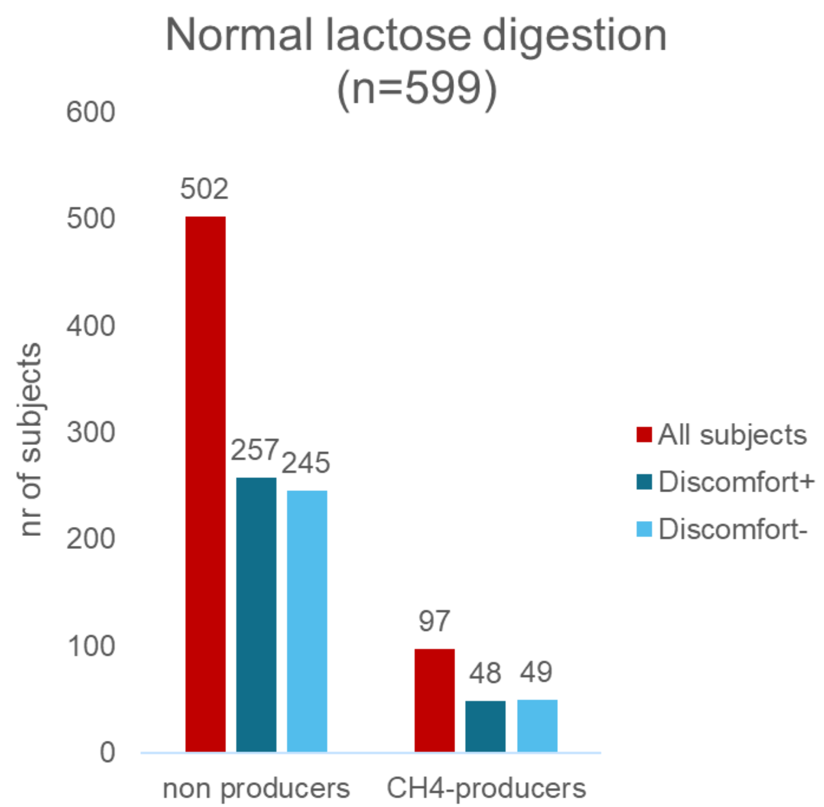
Retrospective analysis of 1051 lactose tests



- Cum. ^{13}C-excretion after 4h >14.5%
- H_2-excretion < 20 ppm

➡ 97 subjects should have been diagnosed as subjects with lactose malabsorption/SIBO (?)

- criticism on CH_4-excretion:

  false negative tests

  - only when the production reaches a threshold, it appears in the breath
  - breath CH_4 excretion is not responsive to changes in the diet

  false positive tests

  - release of CH_4 entrapped in stool due to mixing of the intestinal content (mainly in constipated subjects)

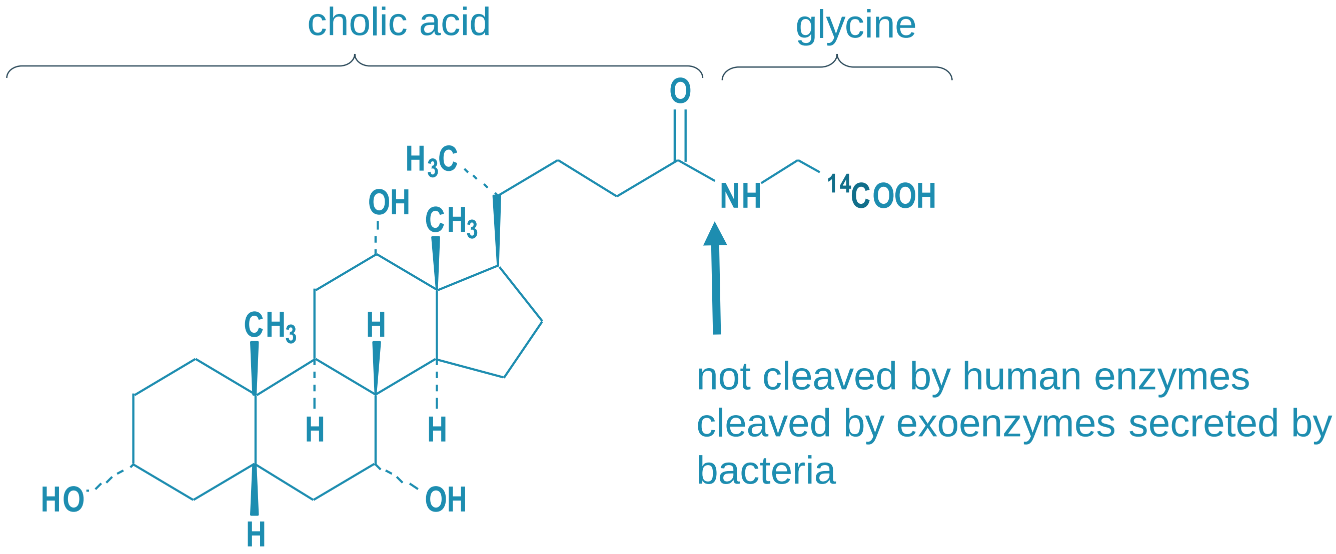
Ongoing study

- New validation study
    - Reduction of dose of lactose to 25g
    - Confirmation of normal values
    - Use of genetic test for lactase deficiency as standard

^{14}C-glycocholic acid

- indication
  - diagnosis of bacterial overgrowth and/or ileal malabsorption

- Principle
  - substrate: ^{14}C-glycocholic acid = conjugated bile acid



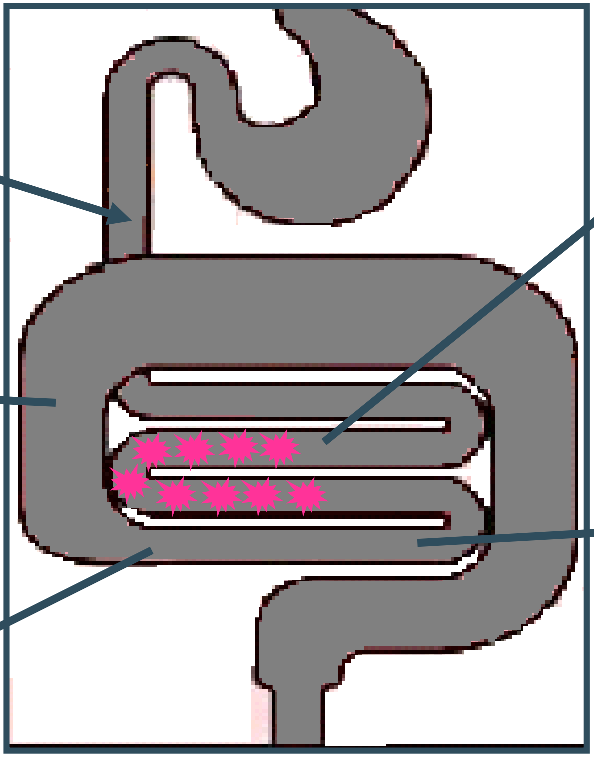
^{14}C-glycocholic acid

bile acids are secreted in conjugated form

ileal malabsorption of bile acids: conjugated bile acids pass into colon
⇨rapidly deconjugated
⇨^{14}C-glycine → $^{14}CO_2$
⇨increased $^{14}CO_2$-excretion

some deconjugation of bile acids

⇨ bile acid is absorbed and reconjugated
⇨ ^{14}C-glycine → $^{14}CO_2$



bacterial overgrowth:
more rapid deconjugation of glycocholic acid
⇨ rapid increase in $^{14}CO_2$-excretion

active absorption of bile acids in conjugated form
⇨bile acid pool

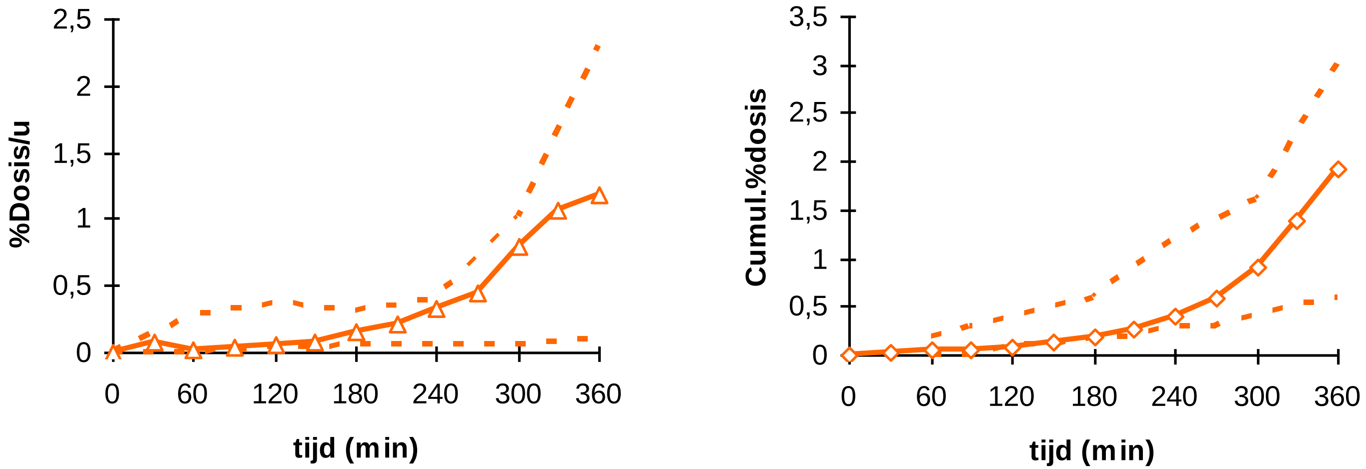
^{14}C-glycocholic acid

- Practical
  - test meal: substrate in gelatin capsule, taken with normal breakfast
  - breath samples: every 30 min
  - total test duration: 6h

- normal value
  - especially the moment at which $^{14}CO_2$-excretion increases is important in the differential diagnosis between bacterial overgrowth and ileal malabsorption

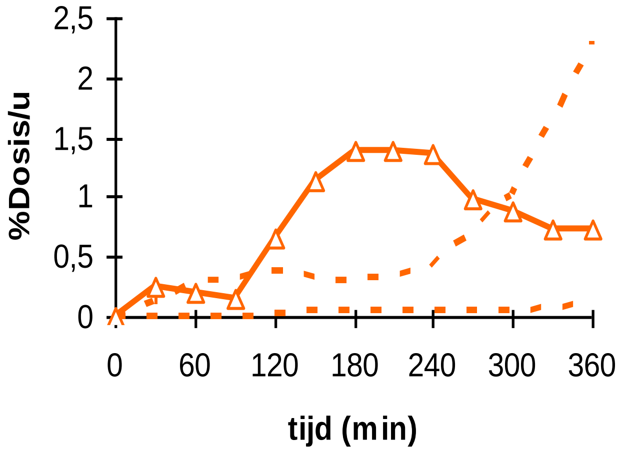
^{14}C-glycocholic acid: examples

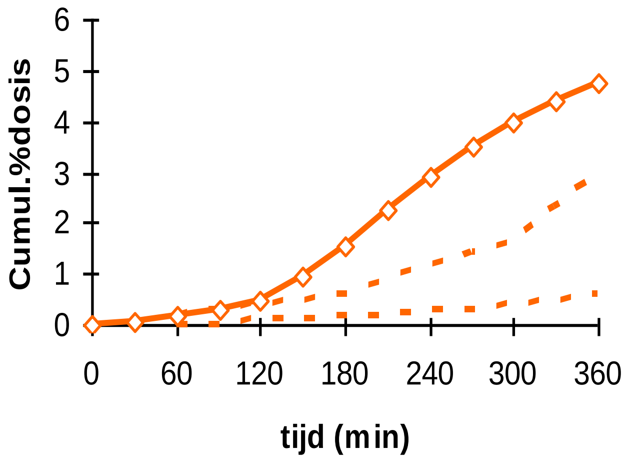
1. normal ^{14}C-glycocholic acid test



^{14}C-glycocholic acid: examples

2. increased metabolism of ^{14}C-glycocholic acid between 1h and 4h

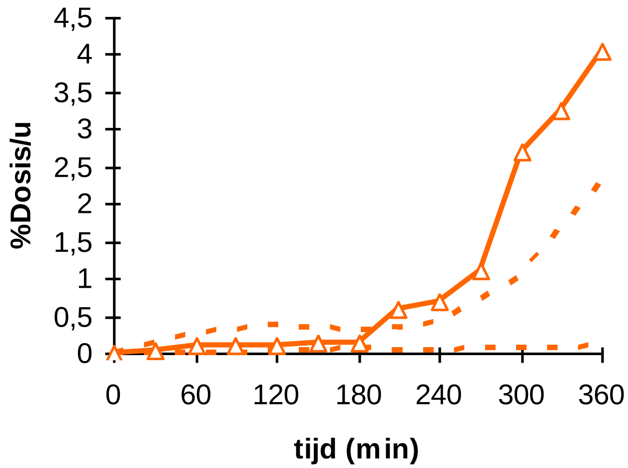


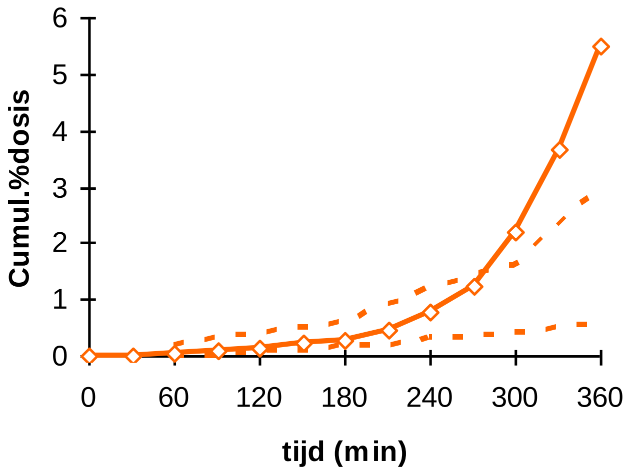


- deconjugation in small intestine because of bacterial overgrowth
- bacterial metabolism in the colon: malabsorption in case of short bowel or fast transit

^{14}C-glycocholic acid: examples

3. increased metabolism of ^{14}C-glycocholic acid after 4h





- colonic metabolism of ^{14}C-glycocholic acid: ileal malabsorption
- metabolism in the small intestine: bacterial overgrowth in case of slow transit
- important distal bacterial overgrowth

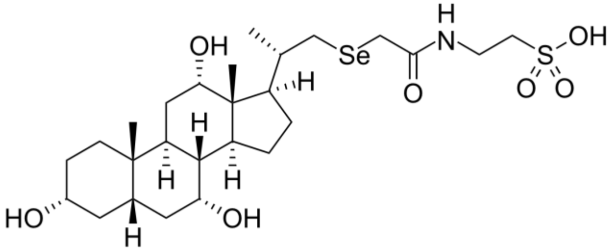
Alternative for bacterial overgrowth

- Glucose-H_2 breath test
  - 75g glucose
  - Rise in H_2 > 20ppm within 90 min suggest SIBO

- Sensitivity is limited (42%)
- Specificity is reasonably good (84%)

Erdogan, Neurogastroenterol Motil. 2015; 27:481-9.

Alternative for ileal malabsorption: ^{75}SeHCat imaging



Principle

- Oral administration of a capsule with 370 kBq (10 µCi); 0.1 mg SeHCAT (^{75}Se-homocholic acid taurine)
- Radiation dose: 0.26 mSv
- Whole body image after 1h and after 7days
- Fraction retained after 7d: >15% of initial activity
- Retention <10% predictive for succesful response to therapy

Conclusions

- Safe
- Non-invasive
- Easy to perform
- Easily available

- underlying assumptions
- Need for standardised conditions

KU LEUVEN

Thank you!