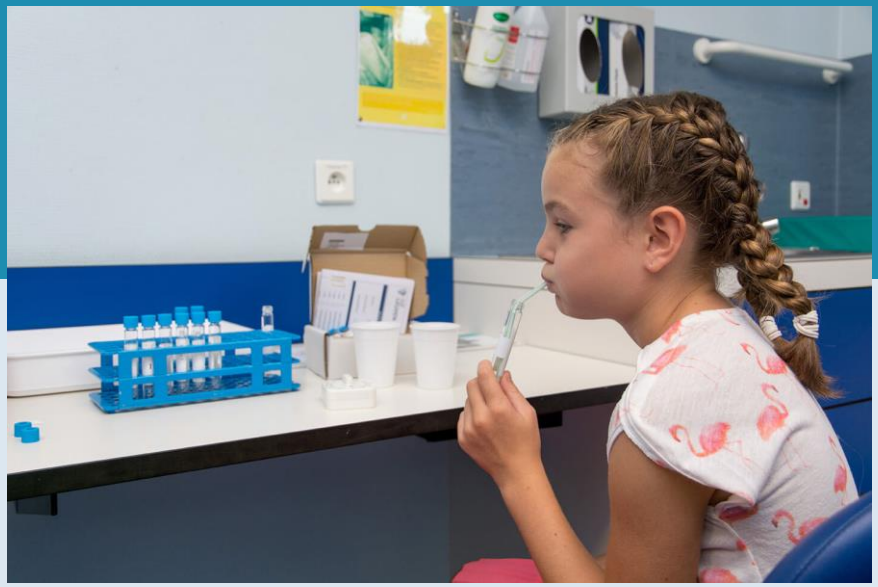
Breath tests as diagnostic tests in gastroenterology

basics and clinical applications



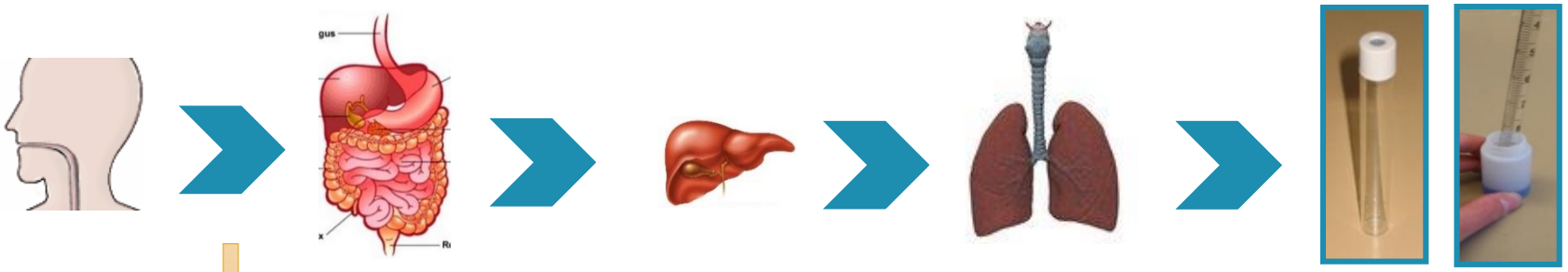
Kristin Verbeke

KU Leuven, Belgium

9 juni 2020

Basic principles of breath tests

Basic principle of breath testing

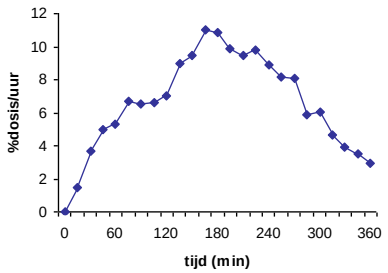


$^{13/14}$C-substrate → $^{13/14}$C-subgroup → $^{13/14}CO_2$ → $^{13/14}CO_2$ → $^{13/14}CO_2$

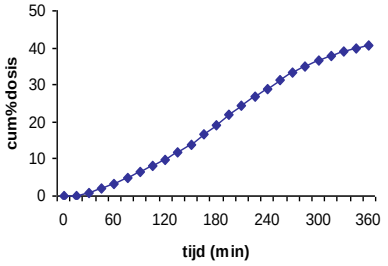
Enzymatic reaction
Digestion/absorption

Rate-limiting step

PDR = percent dose recovery = %dose/h



cPDR = cumulative percent dose recovery

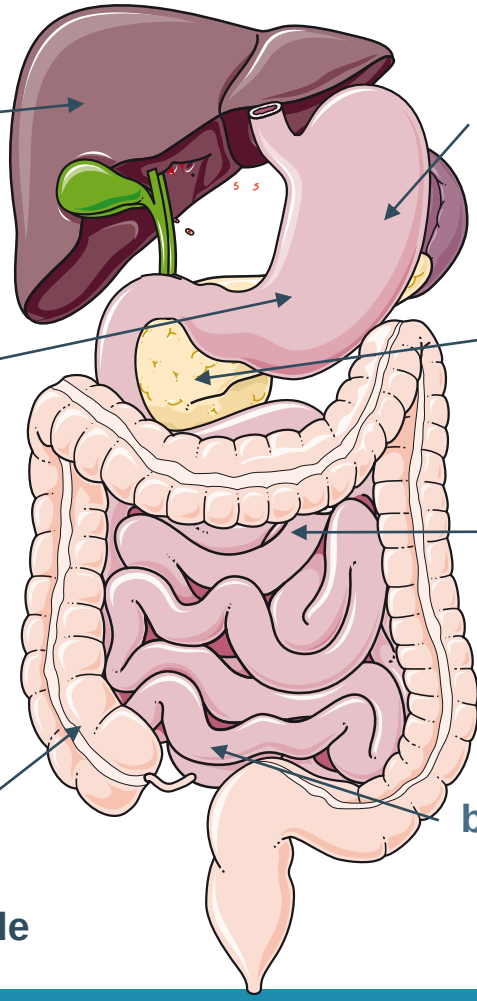


^{13}C or ^{14}C-labelled substrates

- ^{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



liver function

^{13}C-aminopyrine

helicobacter pylori ^{13}C-urea

digestion and absorption

transit

fat

^{13}C-mixed triglyceride

gastric emptying

^{13}C-octanoic acid

^{13}C-glycine

carbohydrates

^{13}C-lactose

^{13}C-fructose

^{13}C-sucrose

bacterial overgrowth/ ileal malabsorption

^{14}C-glycocholic acid

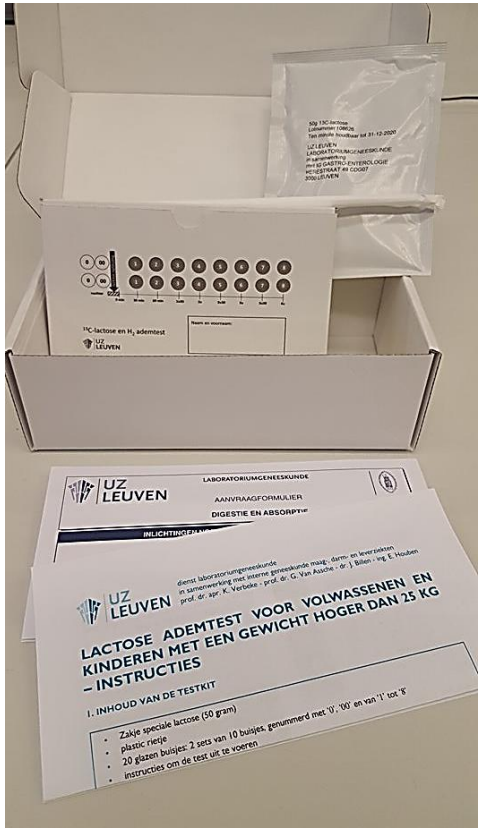
orocaecal transit

^{13}C-lactose ureide

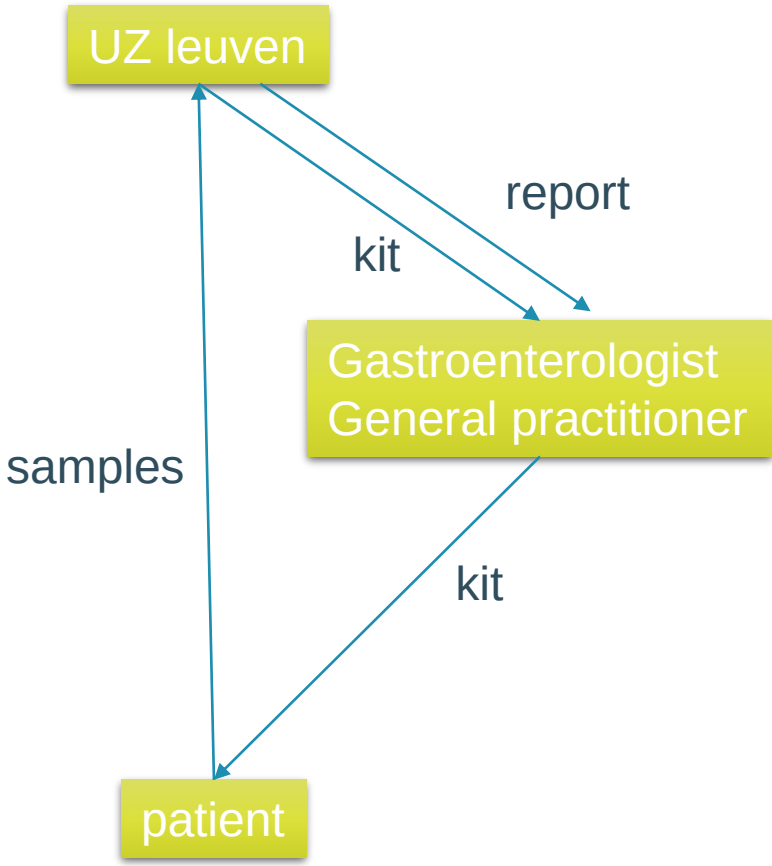
Carbohydrate malbsorption: combined with measurement of H_2 in breath

- 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


UZ LEUVEN
LABORATORIUMGENEESKUNDE
AANVRAAGFORMULIER
DIGESTIE EN ABSORPTIE
LACTOSE ADEMTEST VOOR VOLWASSENEN EN KINDEREN MET EEN GEWICHT HOGER DAN 25 KG – INSTRUCTIES
I. INHOUD VAN DE TESTKIT
Zakje speciale lactose (50 gram)
plastic rietje
20 glazen buisjes: 2 sets van 10 buisjes, genummerd met '0', '00' en van '1' tot '8'
instructies om de test uit te voeren

UZ LEUVEN
LABORATORIUMGENEESKUNDE
AANVRAAGFORMULIER
DIGESTIE EN ABSORPTIE
Nr. 3011
INLICHTINGEN NODIG VOOR UITVOERING VAN DE TEST
Gewicht:
Lengte:
Geboortedatum:
Geslacht:
Gehospitaliseerd
Ambulant
ADEMTESTEN
UREUM ADEMTEST – INSTRUCTIES
I. INHOUD VAN DE TESTKIT
plastic flesje met speciale ureumpoeder
1 flesje drinkyoghurt (Fortimel Compact®)
plastic rietje
6 glazen buisjes: genummerd met '0', '00' en van '1' tot '4'
instructies om de test uit te voeren
aanvraagformulier, in te vullen door uw arts


UZ leuven
report
kit
Gastroenterologist
General practitioner
samples
kit
patient

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 ⇨ $\delta^{13} = 0$

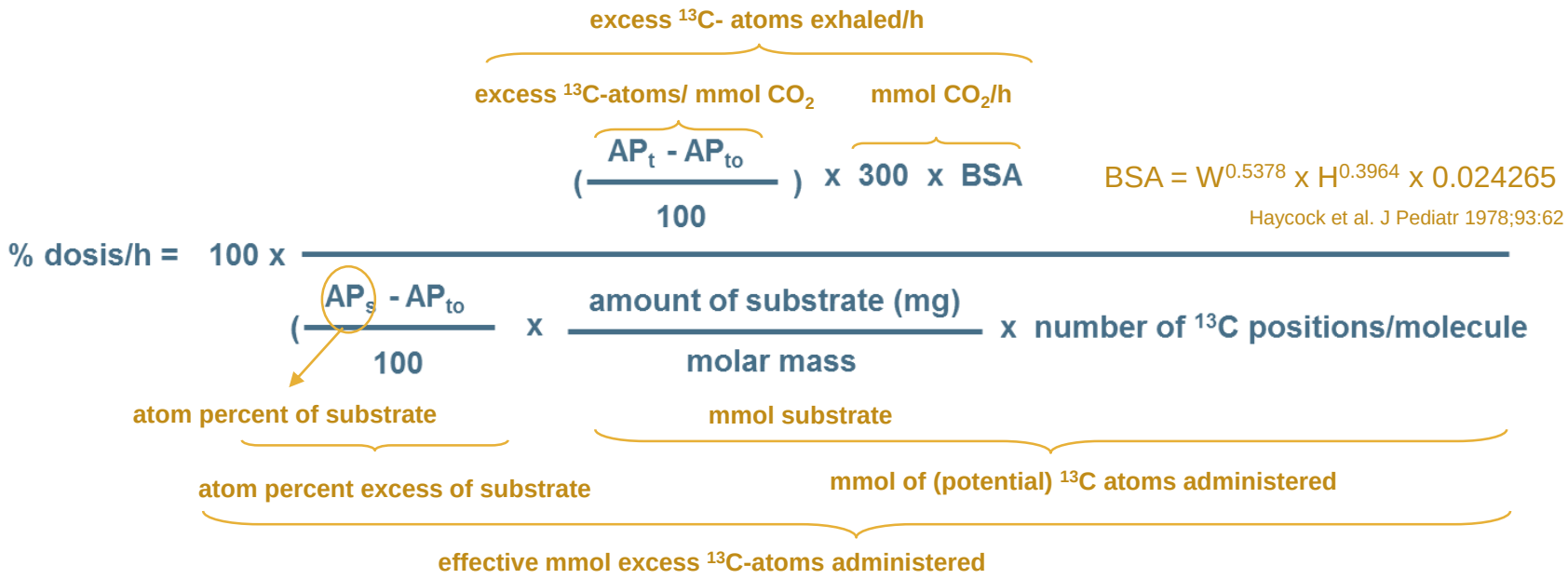
$\delta^{13} < 0$: lower enrichment than PDB reference

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

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

Underlying assumptions

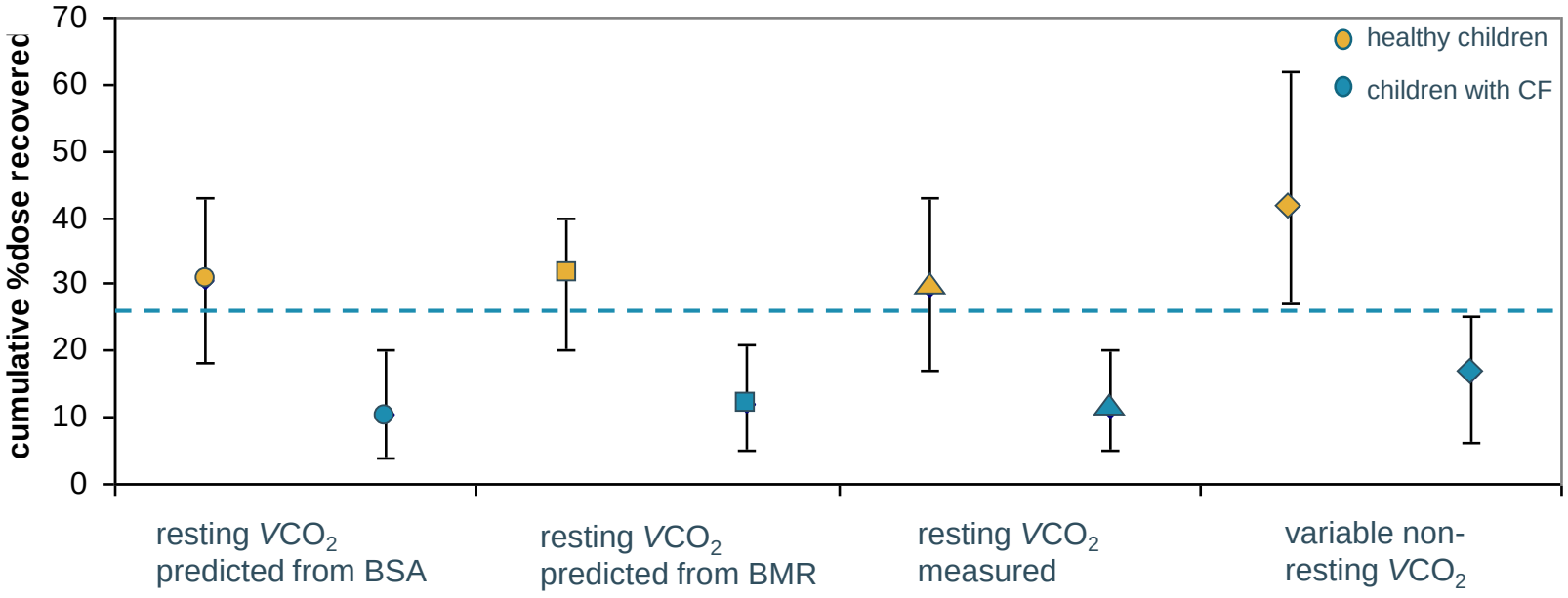
- CO_2-production is known and is stable during the test



⇨ 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

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

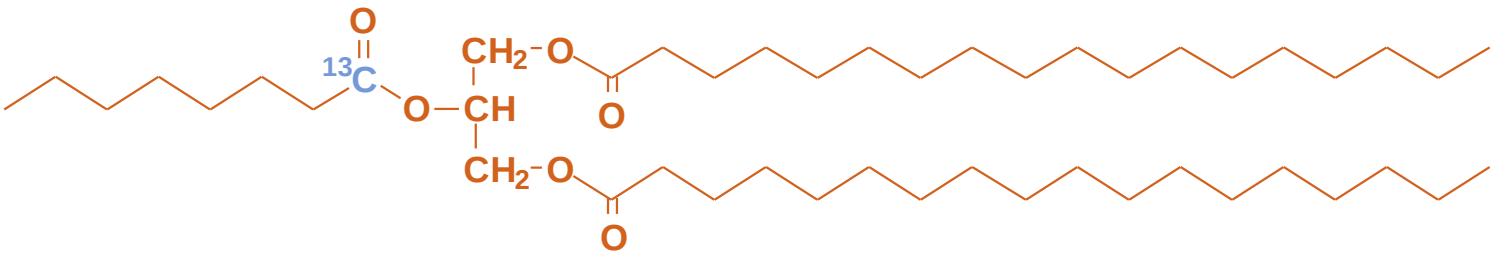
- based on evaluation of fat malabsorption

! Lipid assimilation = lipid digestion + lipid absorption

⇨fat malabsorption occurs when one or both processes fails

^{13}C-mixed triglyceride test

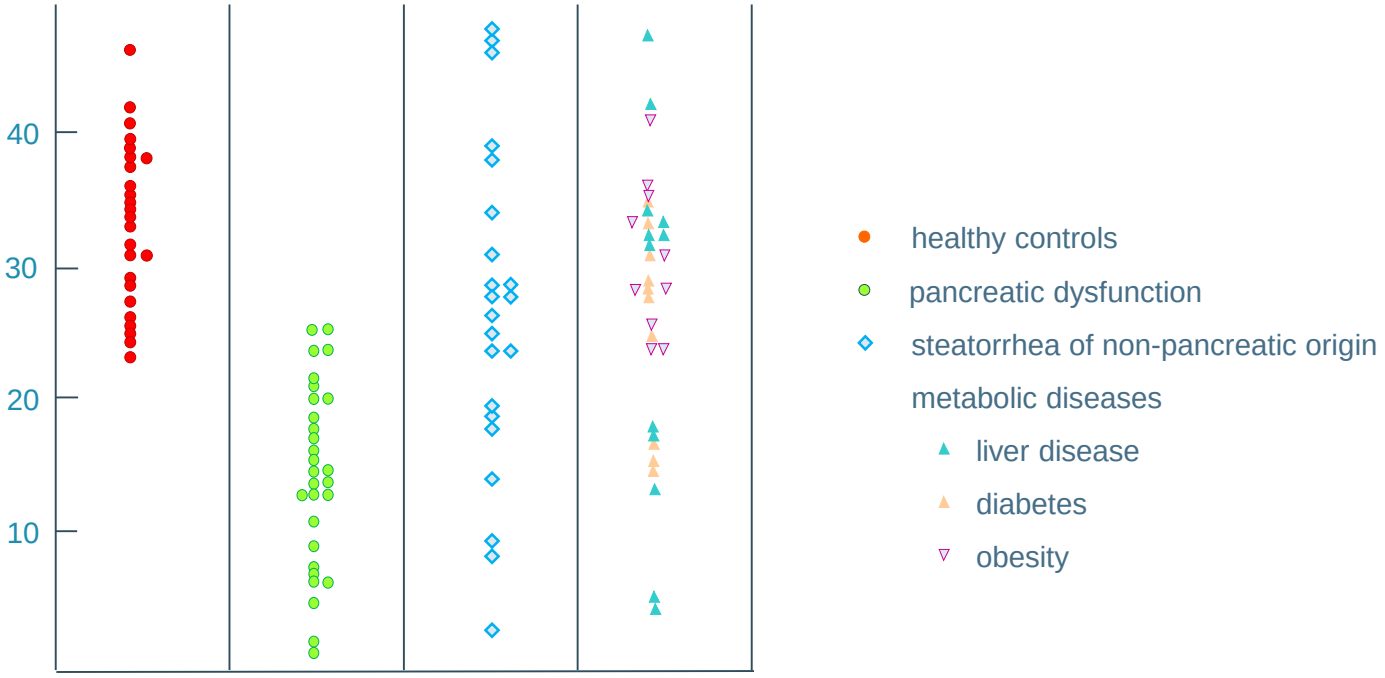
- substrate: 1,3-distearyl,2[^{13}C-carboxyl]octanoyl glycerol



- 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

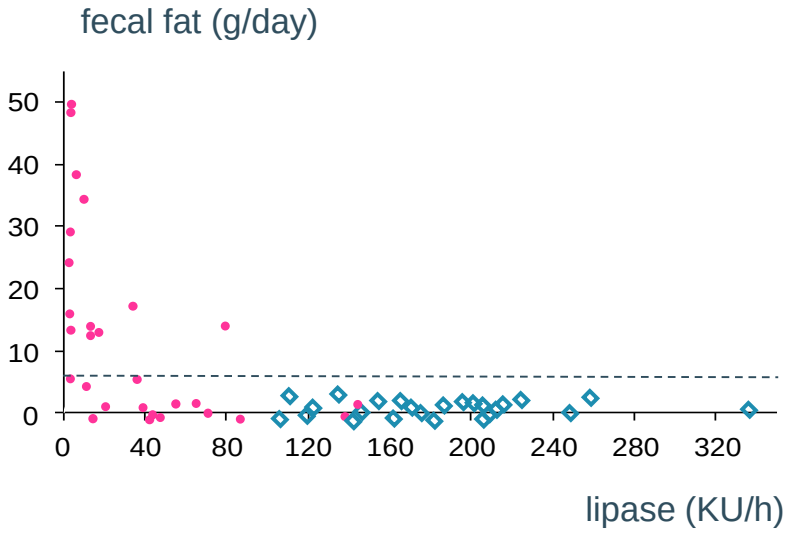
cumulative percent of administered dose excreted after 6h



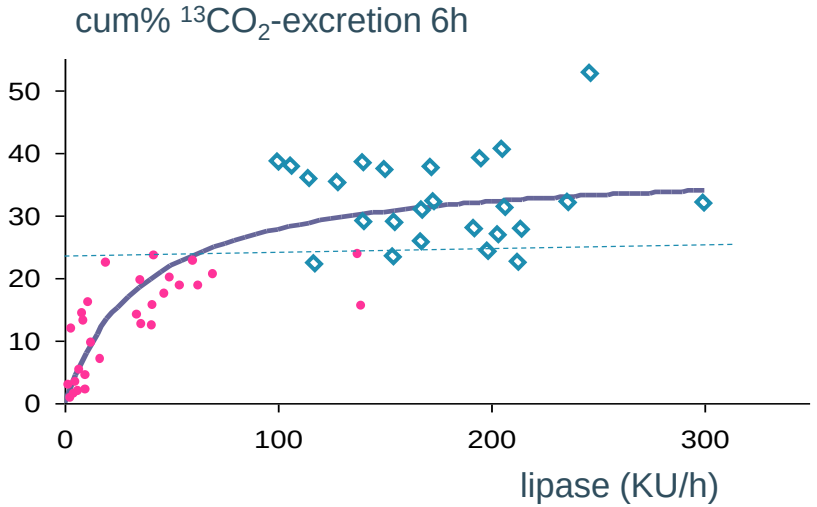
⇨ ^{13}C-MTG-test does not correlate well with fat malabsorption:
⇨ steatorrea can have other causes than pancreatic insufficiency

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

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



steatorrhea when lipase output < 40 KU/h



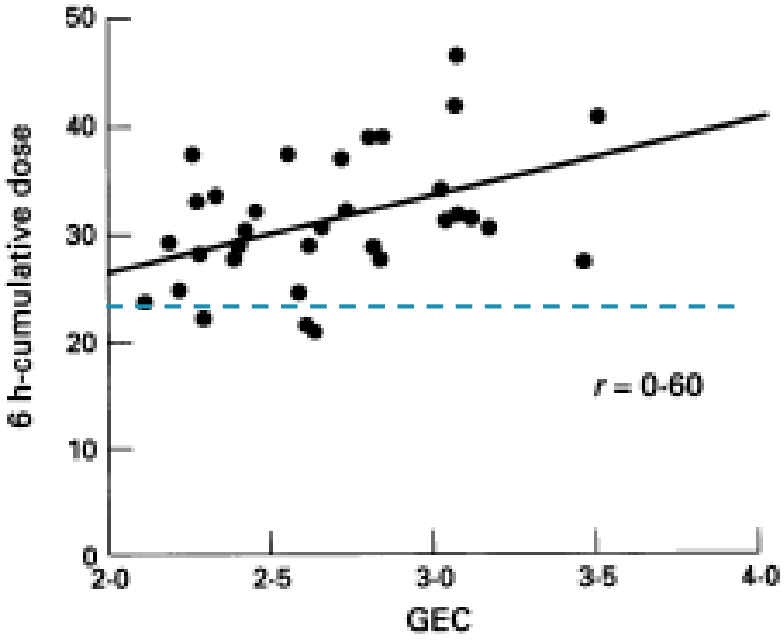
^{13}C-MTG detects lipase output <90 KU/h (sensitivity 89%)

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

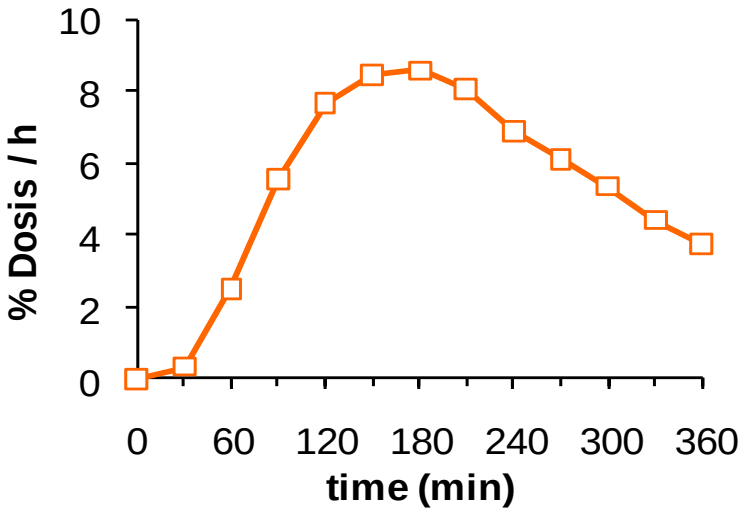
- subjects without pancreatic insufficiency: clear correlation between gastric emptying rate and rate of intraluminal lipolysis
- patients with pancreatic insufficiency: gastric emptying rate had no impact on the rate of intraluminal lipolysis

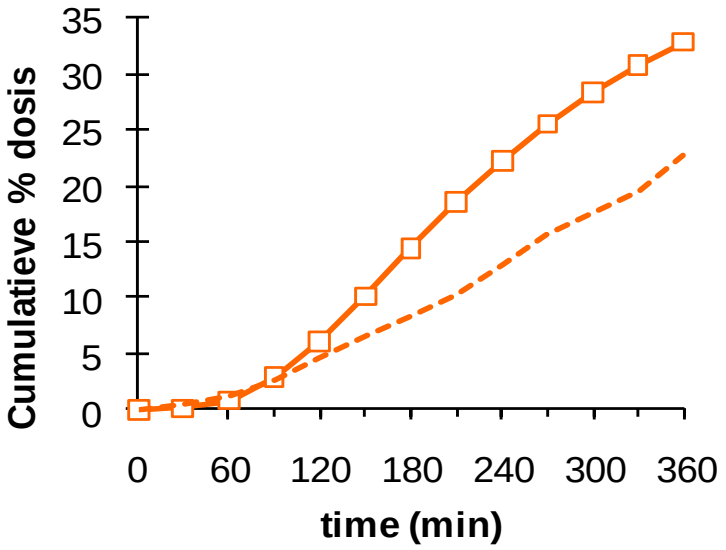


Gut 1996; 38:23-27

Examples

1. Normal lipase activity

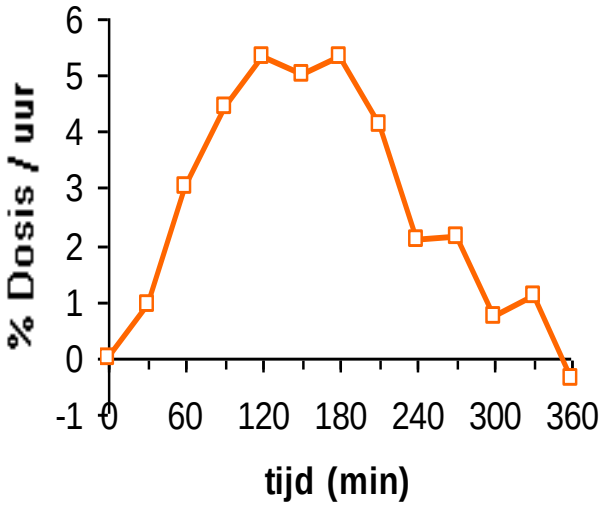


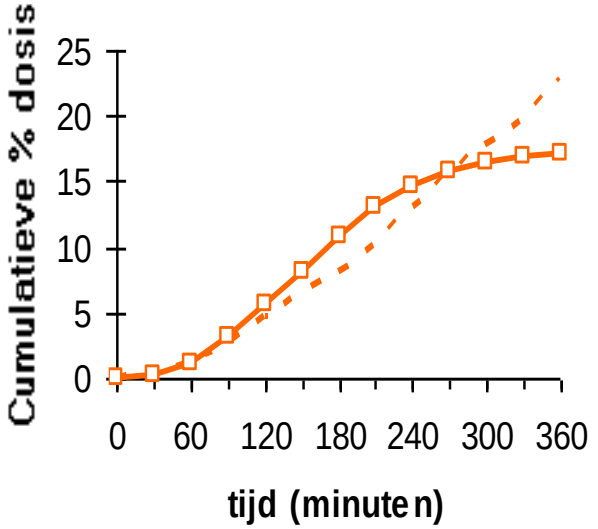


criterium: > 23% of administered dose excreted in breath

Examples

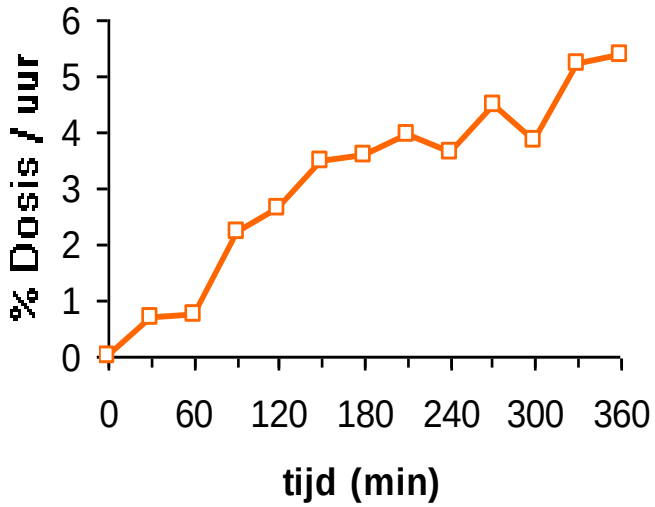
2. low lipase activity

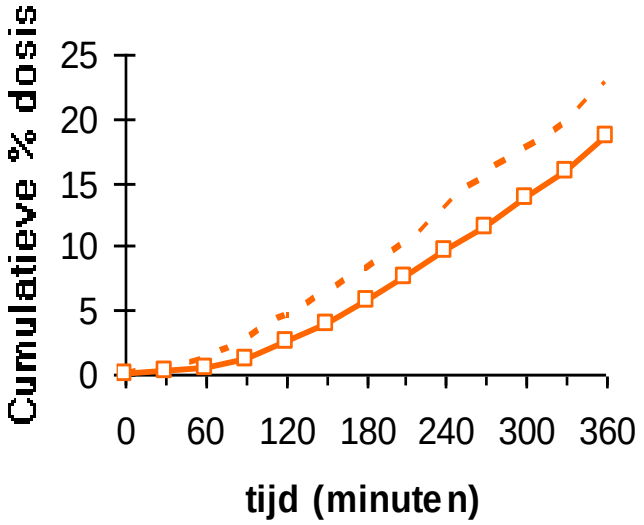




Examples

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





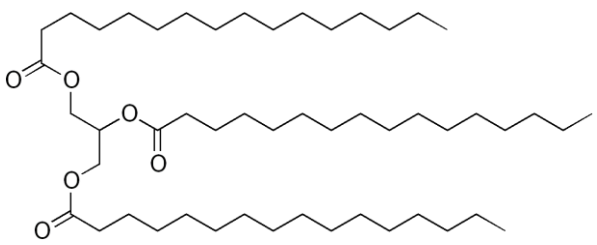
“False” positive tests

- 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

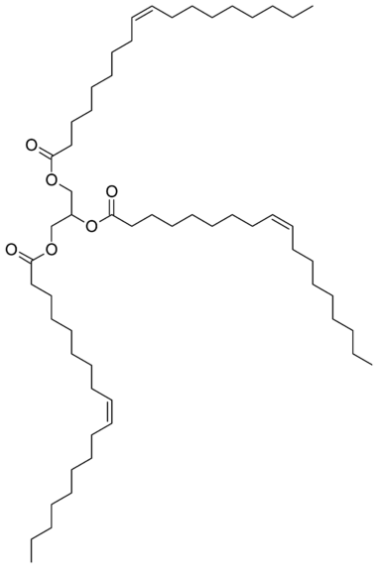
Ghoos Y, et al. Digestion 1981, 22:239-247
Perri F, et al. Gastroenterology 1997, 112:A393

Other breath test substrates

^{13}C-tripalmitin



^{13}C-triolein



^{13}C-hiolein

TABLE I *Fatty acid composition of Hiolein*

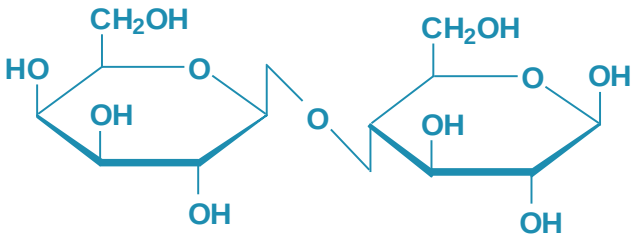
Fatty acid	*Common name*	*Proportion (%)*
18:1 ω-6	Oleic acid	50·6
16:0 ω-6	Palmitic acid	16·9
18:2 ω-6	Linoleic acid	19·6
18:3 ω-3	α-Linolenic acid	3·0
18:0 ω-6	Stearic acid	2·3
16:1 ω-9	Palmitoleic acid	1·8
16:2 ω-6	No common name	1·4
16:3 ω-3	No common name	1·2

- 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

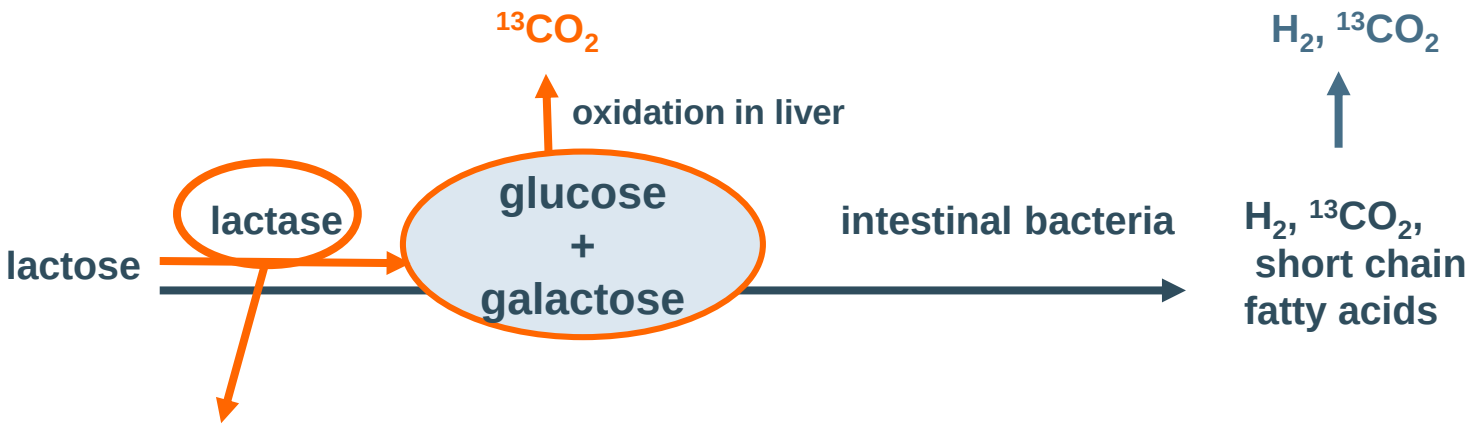


50g ⇨ 25g

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

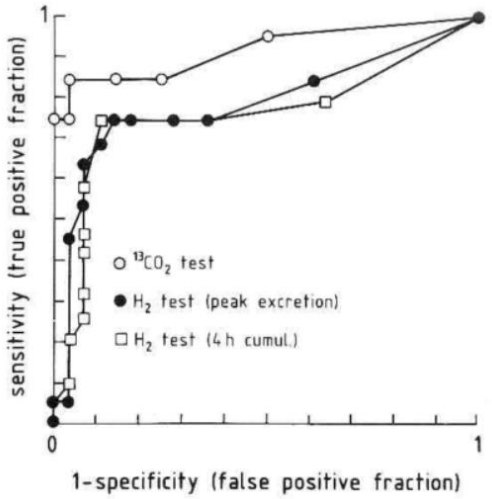
Lactose: combined $^{13}CO_2/H_2$ breath test

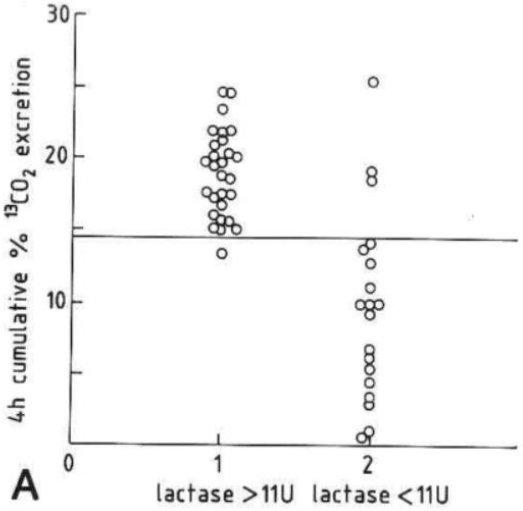
- principle



- 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	H_2-test 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

Hiele M, J Lab Clin Med 1988; 112:193-200

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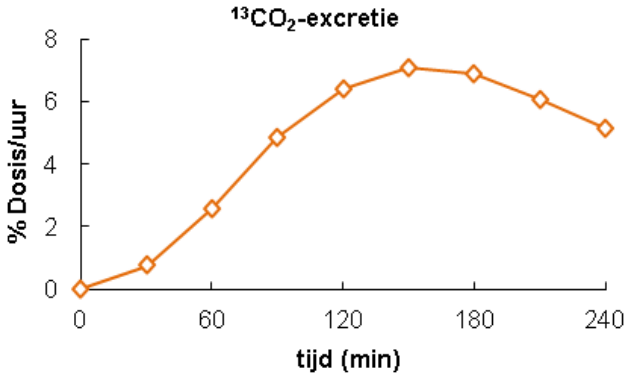
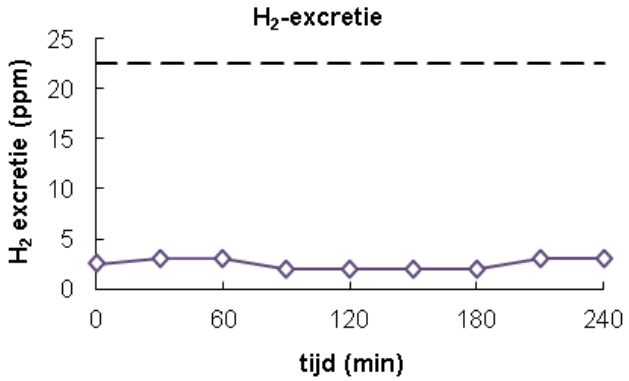
Lactose breath test: examples

1. normal lactose test

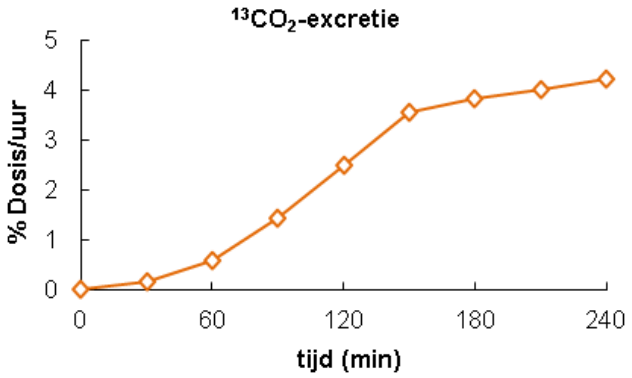


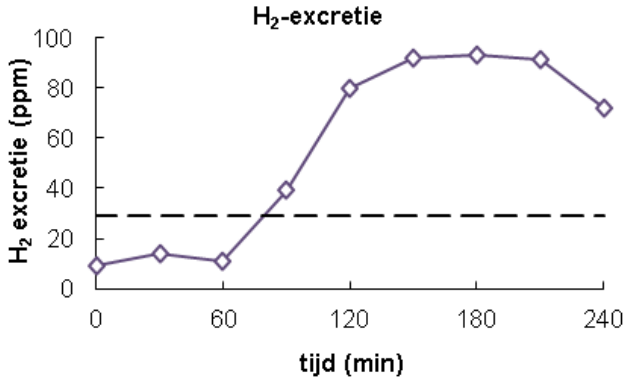


Parameters:		
	normaal	patiënt
piekexcretie	>6.5	7,1
cumulatieve excretie na 4 uur	>14.5	18,62
H_2 excretie (ppm)	<20	1
klachten	geen	?

Lactose breath test: examples

2. lactase deficiency

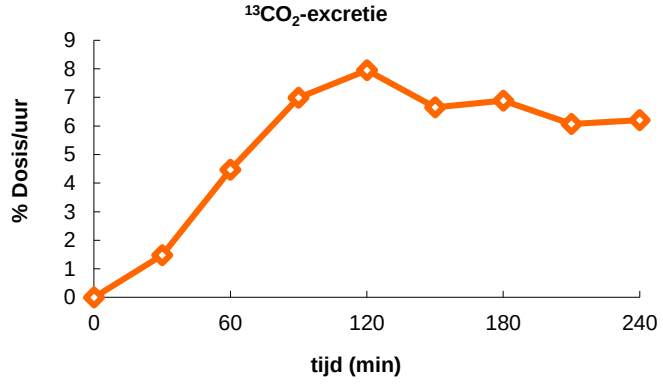


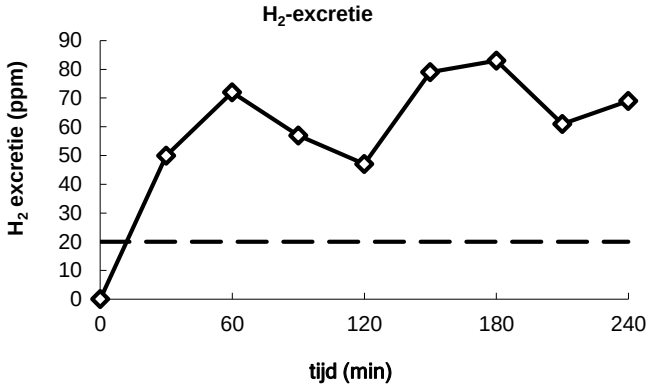


Parameters:		
	normaal	patiënt
piekexcretie	>6.5	4,2
cumulatieve excretie na 4 uur	>14.5	9,09
H_2 excretie (ppm)	<20	84
klachten	geen	diarree-buikpijn-opgeblazen gevoel

Lactose breath test: examples

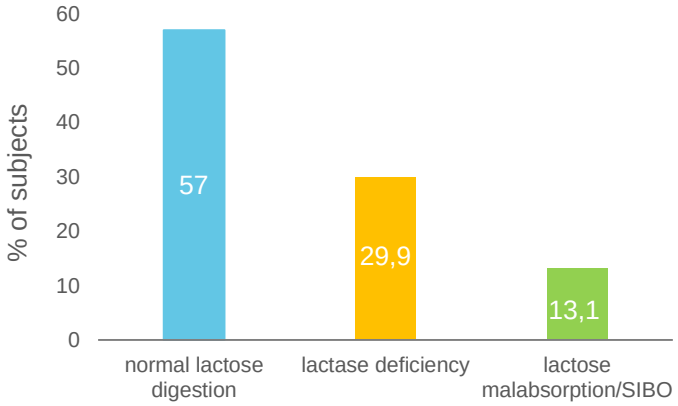
2. Bacterial overgrowth / lactose malabsorption

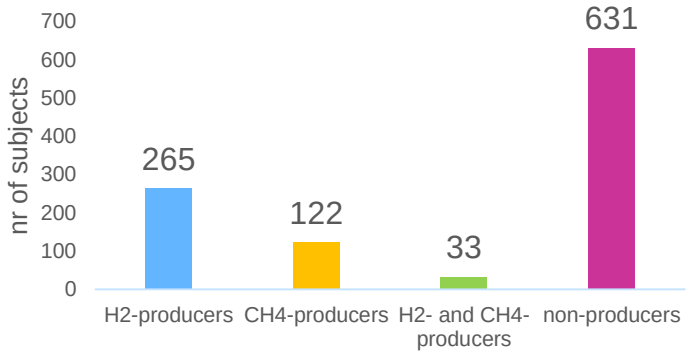


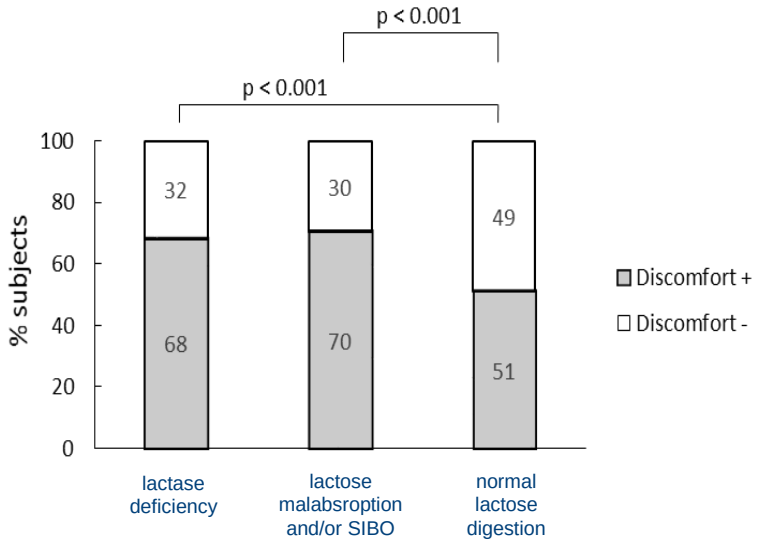


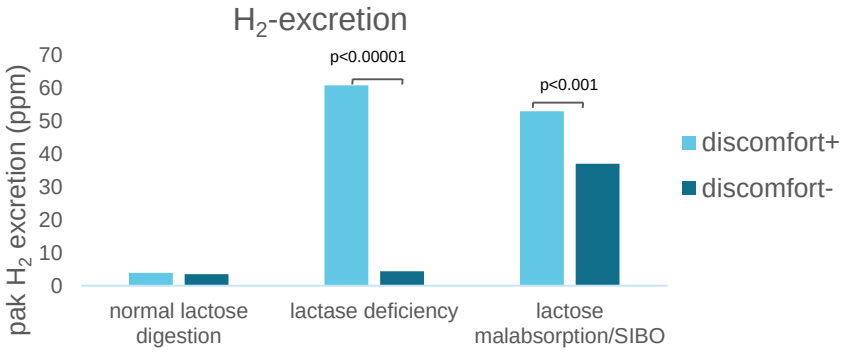
Parameters:		
	normaal	patiënt
piekexcretie	>6.5	8,0
cumulatieve excretie na 4 uur	>14.5	21,80
H_2 excretie (ppm)	<20	83
klachten	geen	krampen

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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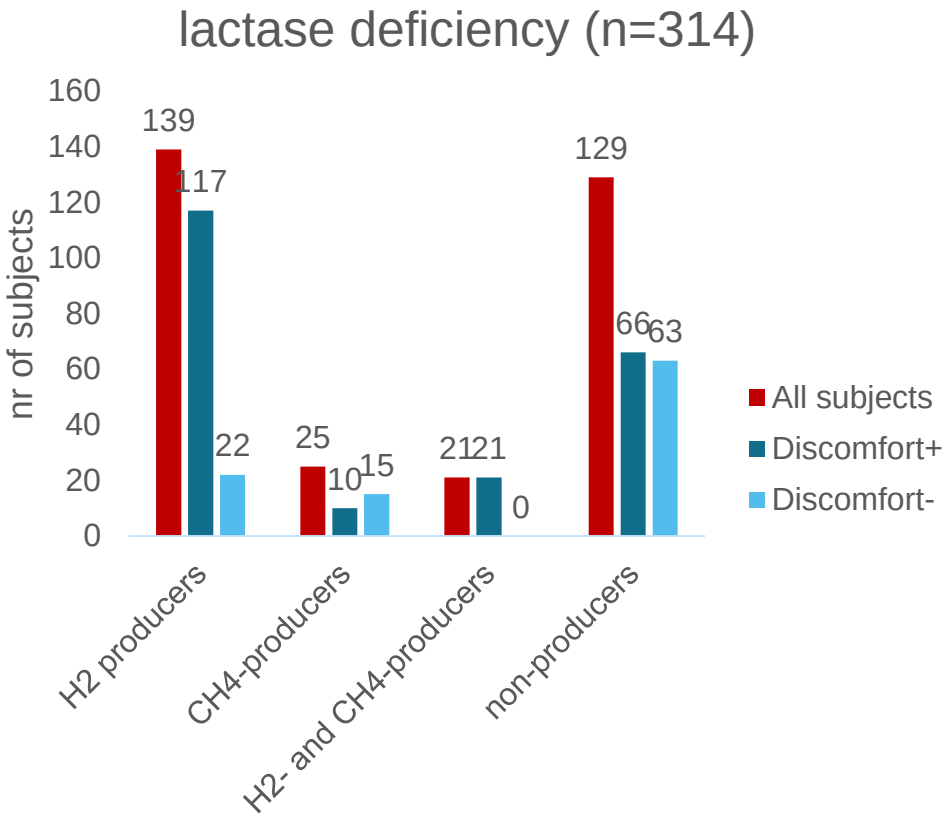
Houben et al. *Nutrients 2015; 7:7469-7485.*

Retrospective analysis of 1051 lactose tests



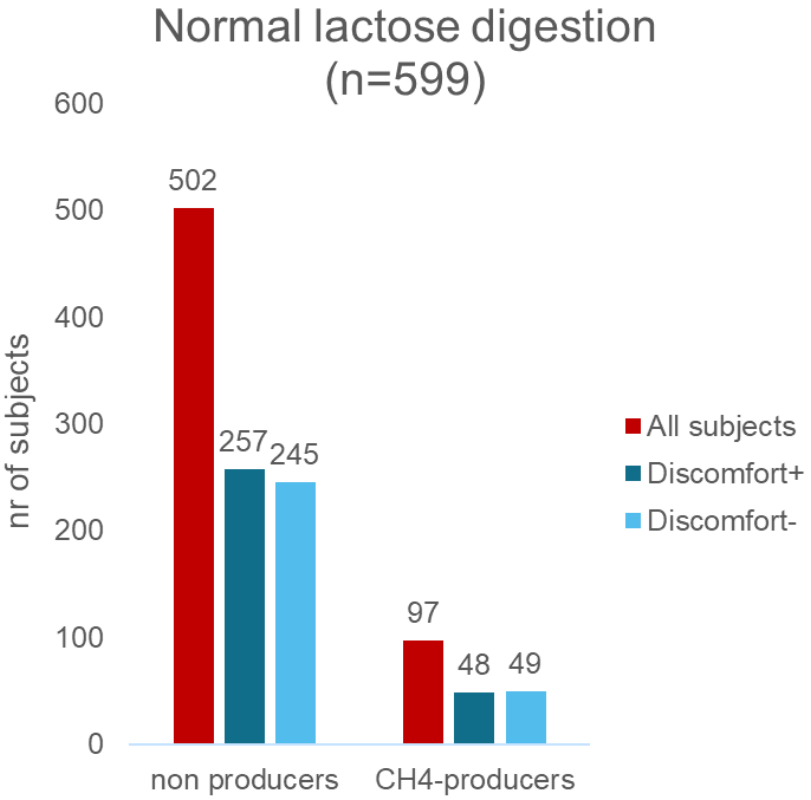
- Cum. ^{13}C-excretion after 4h <14.5%
- Whether or not increased H_2-excretion >20 ppm

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

KU LEUVEN

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)

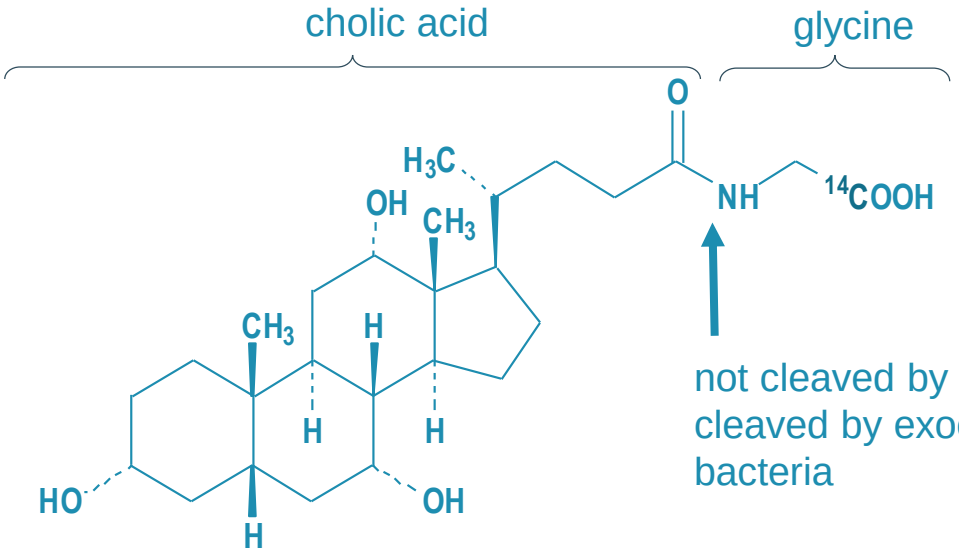
Ongoing study

- 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



not cleaved by human enzymes
cleaved by exoenzymes secreted by bacteria

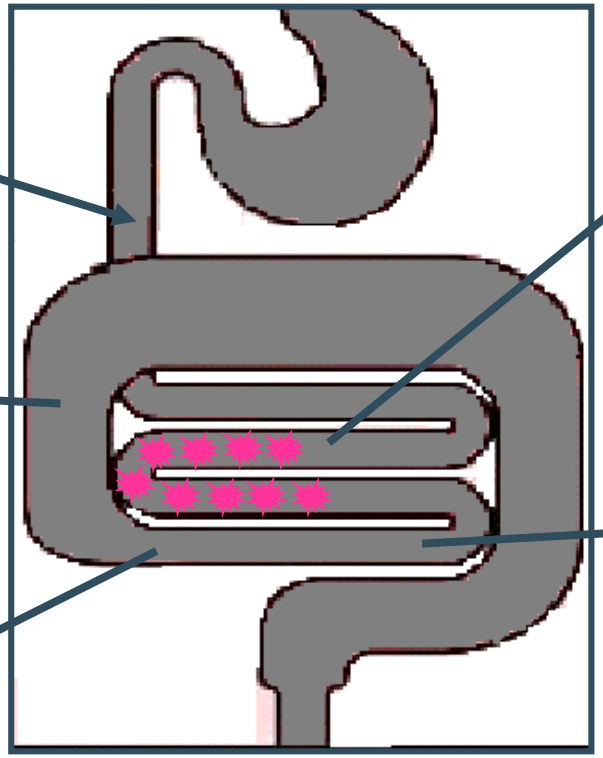
^{14}C-glycocholic acid

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

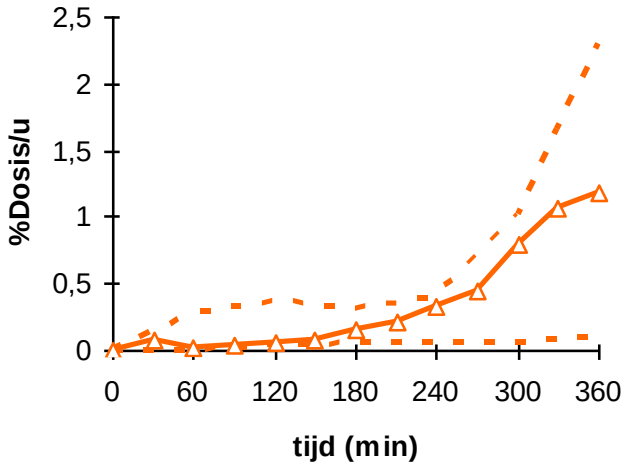
^{14}C-glycocholic acid

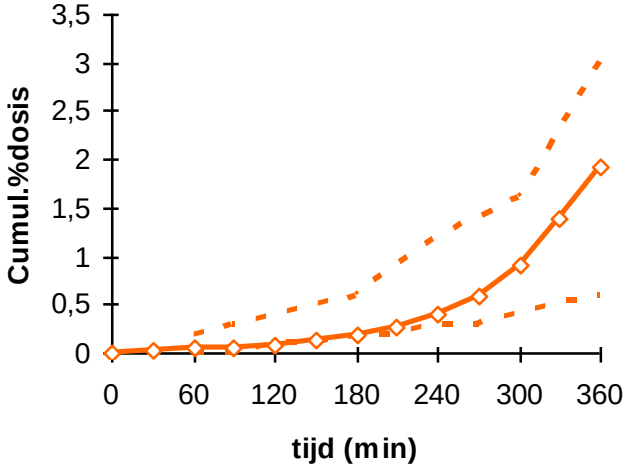
- Practical
  - test meal: substrate in gelatin capsule, taken with normal breakfast
  - breath samples: every 30 min
  - total test duration: 6h

- 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

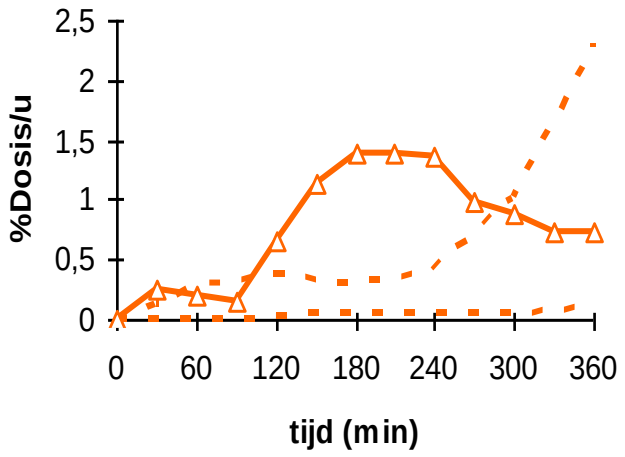
1. normal ^{14}C-glycocholic acid test

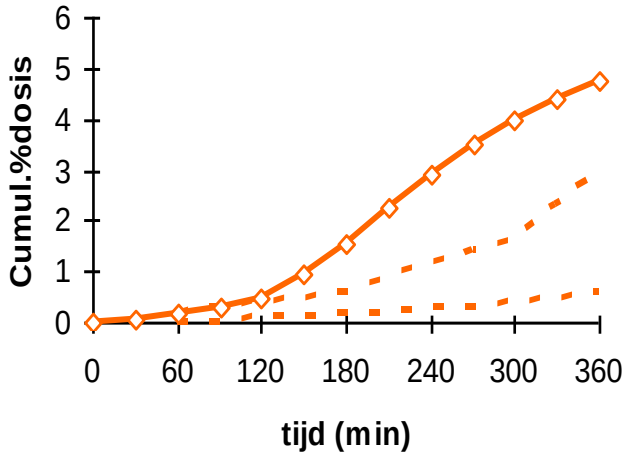




^{14}C-glycocholic acid: examples

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

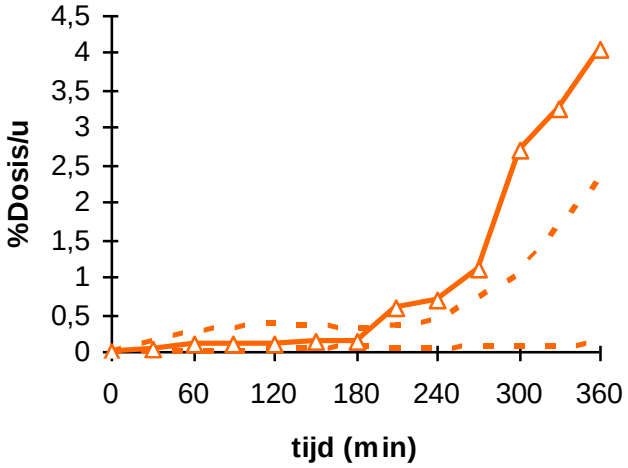


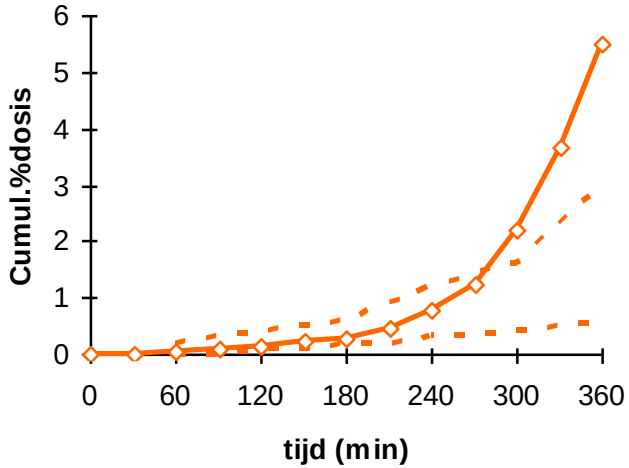


- deconjugation in small intestine because of bacterial overgrowth
- bacterial metabolism in the colon: malabsorption in case of short bowel or fast transit

^{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

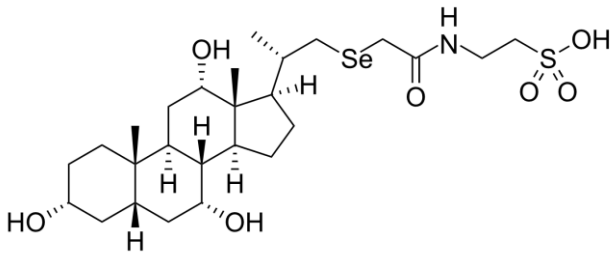
Alternative for bacterial overgrowth

- 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

Thank you!