KU LEUVEN

Breath tests for the assessment of gastrointestinal function

TARGID
Translational Research Center for Gastrointestinal Disorders

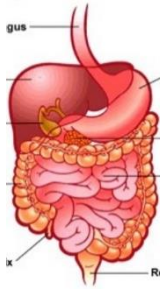
Kristin Verbeke

Laboratory Medicine, Digestion and Absorption

UZ Leuven

Basic principle of breath testing

$^{13/14}$C-substrate



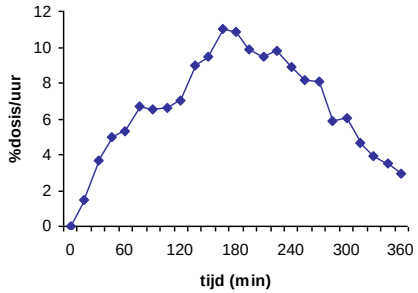
$^{13/14}$C-subgroup

$^{13/14}CO_2$

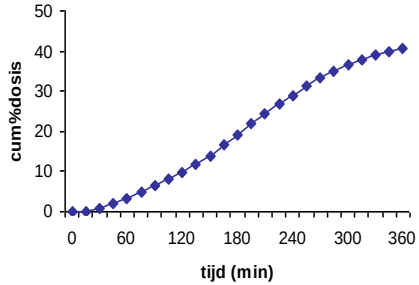
$^{13/14}CO_2$

$^{13/14}CO_2$

PDR = percent dose recovery = %dose/h



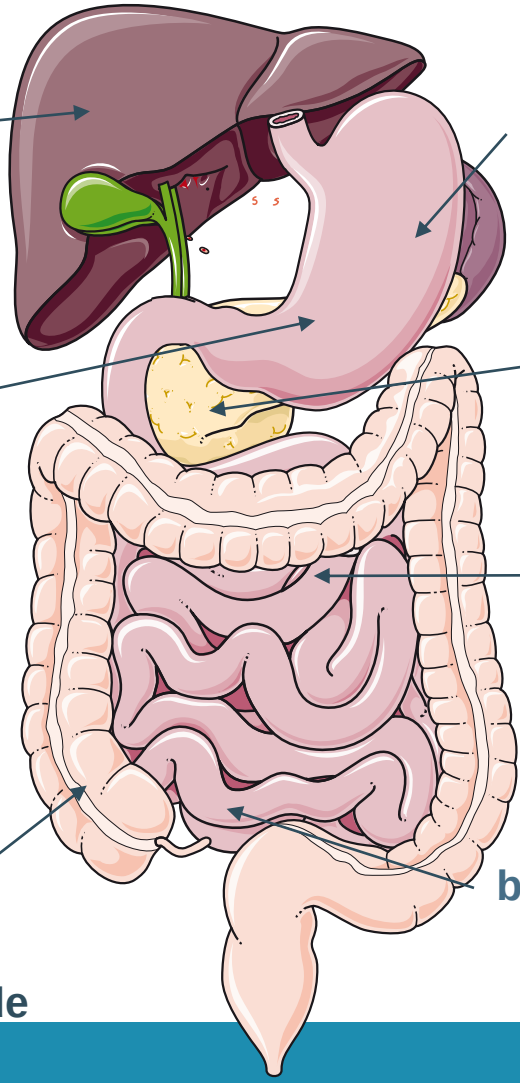
cPDR = cumulative percent dose recovery



^{13}C or ^{14}C?

^{14}C	^{13}C
radiation dose: e_{50} = 0,11 mSv/185 kBq (ICRP-68, 1994, f = 1, $t_{1/2}$ biol = 28d) not in children (-18y) nor in pregnant women	no radiation dose safe in children and pregnant women can be repeated several times
$t_{1/2}$ ^{14}C: 5730 y ⇨ radiation burden for the environment	
	possible interference from naturally enriched compounds e.g. glucose (TPN and glucose infusions)
β-scintillation	isotope ratio mass spectrometry
simultaneous measurement of gastric emptying with solid and fluid test meal glycocholic acid breath test	all other breath tests

Breath tests in routine clinical diagnose



liver function

^{13}C-aminopyrine

helicobacter pylori ^{13}C-urea

digestion and absorption

transit

fat

gastric emptying

^{13}C-mixed triglyceride

^{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

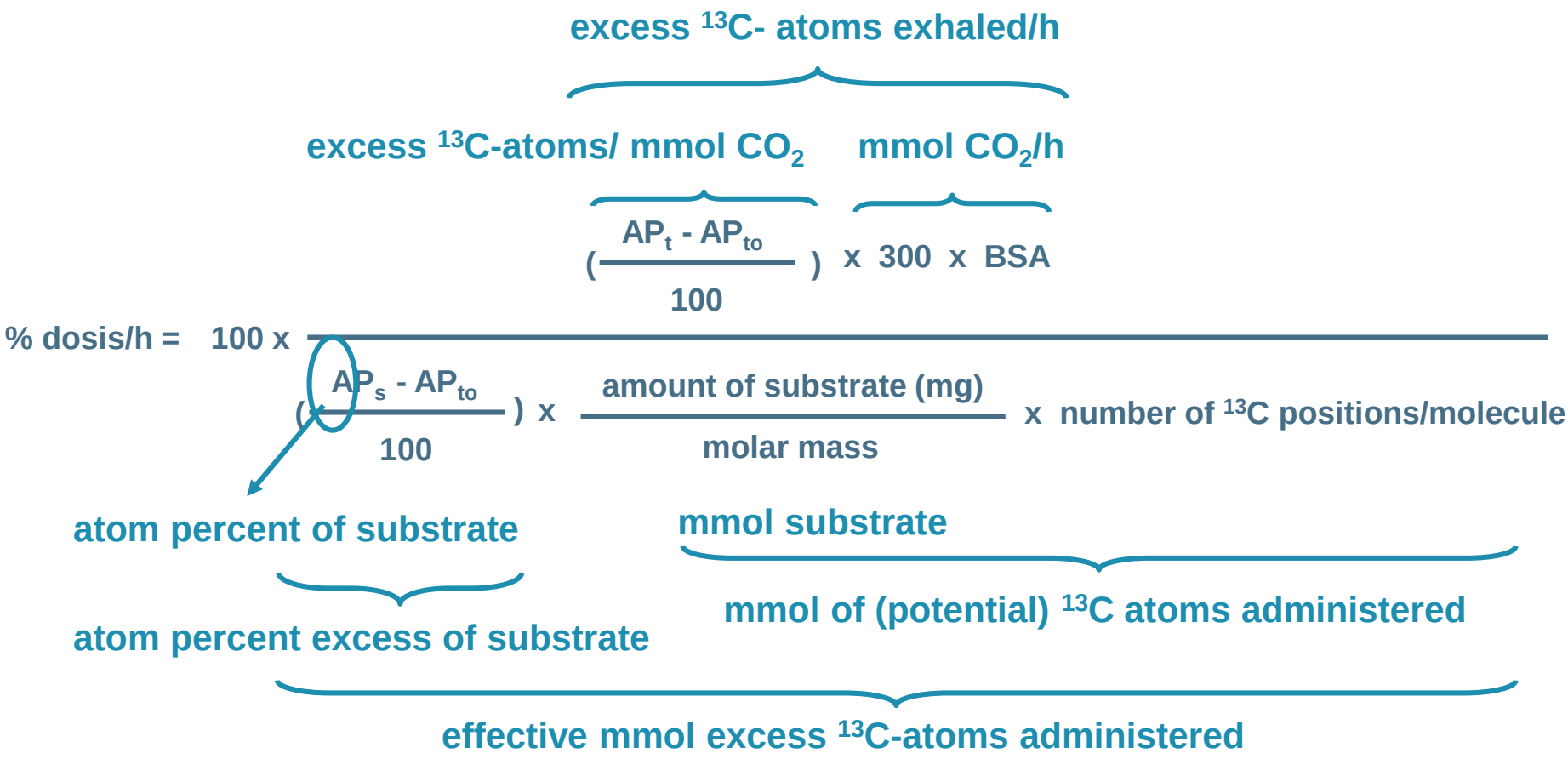
Interpretation of the results: normal values

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

results of a breath test have to be compared to the normal values

Calculation of results: ^{13}C-substrates



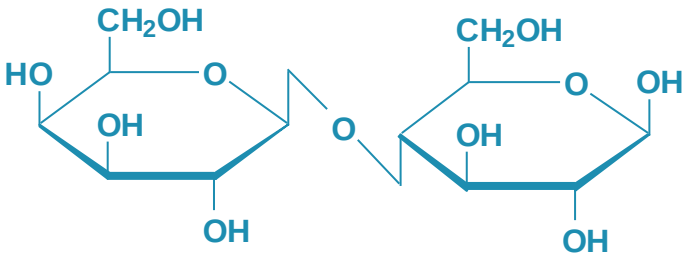
Additional information

Ademtest Lactose	
Uitvoerfrequentie	elke werkdag, tijdens de diensturen
Antwoordtijd	<1w
Aanvraagnummer (formuliernummer)	11000 (3011)
Acceptabele stalen - Recipiënt	Labco Exetainer
Verantwoordelijke	Kristin Verbeke
Uitvoerend lab	Laboratoria UZ Leuven
Procedure	**1. Bijzonderheden** Info algemeen: https://w1.uzleuven.be//labo/LAG/LAG_Procedureboek/LAG/LBG_ademE.pdf Info kinderen algemeen: http://w1.uzleuven.be/labo/LAG/LAG_Procedureboek/LAG/LBG_adem_kindE.pdf Instructiefilmpje: https://www.uzleuven.be/ademtest-lactose-fructose-sucrose Interpretatie ademtest: https://w1.uzleuven.be/labo/LAG/LAG_Procedureboek/LAG/LBG_11000E.pdf

Lactose breath test

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

- Substrate

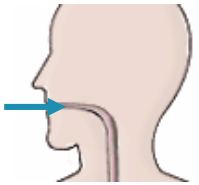


naturally enriched lactose
AP = 1.01099

Lactose hydrogen breath test

False-negative tests in case of hydrogen non-producers

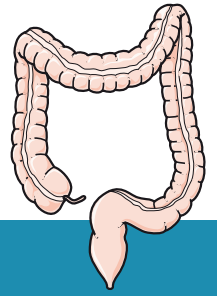
lactose



Hydrolysis to glucose and galactose by intestinal lactase

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

In case of incomplete digestion



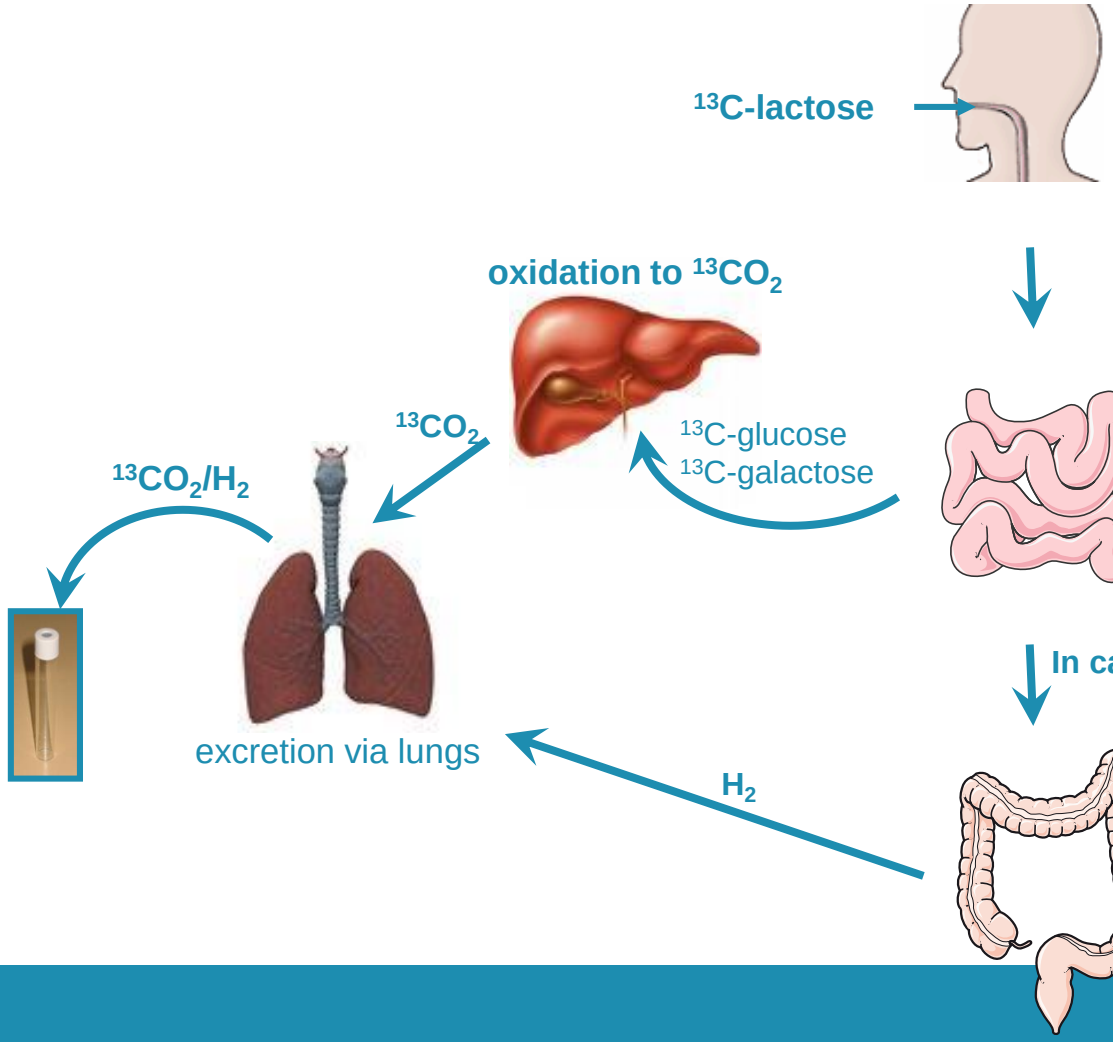
Microbial fermentation with production of H_2

H_2

excretion via lungs

H_2

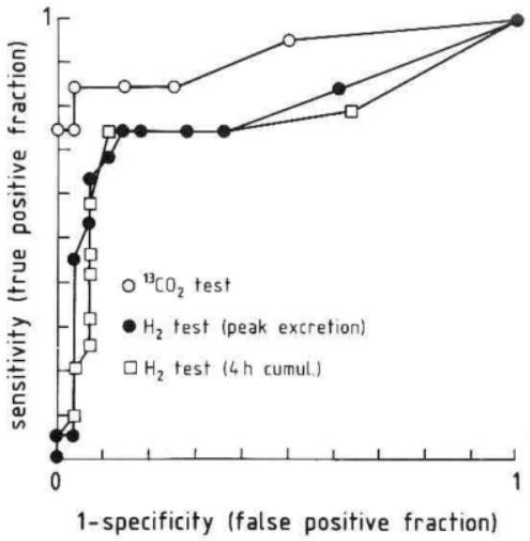
Combined $^{13}CO_2/H_2$ lactose breath test

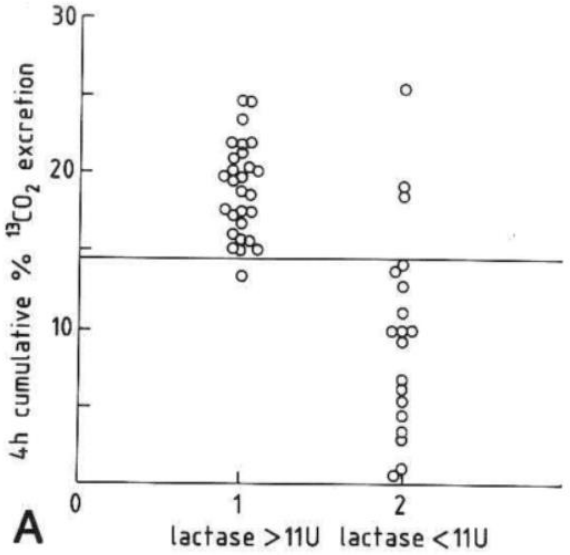


- located in the brush border membrane
- age dependent
- limited capacity
- varies between various populations
- vulnerable, can be used as indicator of the membrane status

KU LEUVEN

Validation towards jejunal lactase activity





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

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

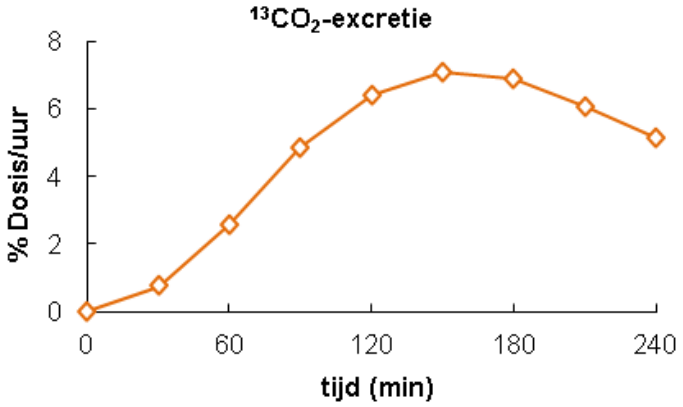
Lactose: combined $^{13}CO_2/H_2$ breath test

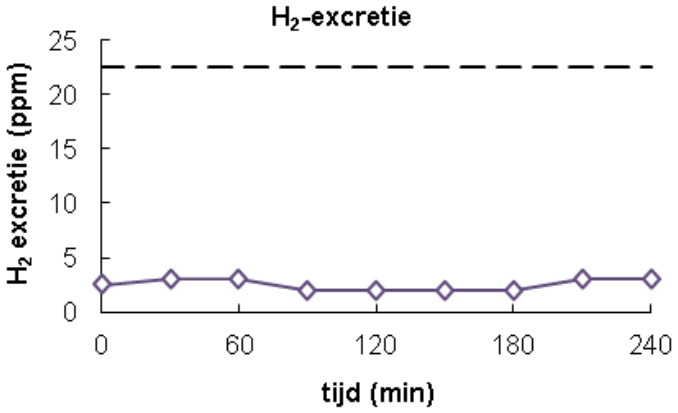
- Practical
  - testdosis: 50 g ^{13}C-lactose: corresponds to the amount of lactose in 1 L milk
  - breath samples: every 30 min
  - total test duration: 4h
  - avoid a fibre rich meal on the evening before the test

- Normal value
  - peak excretion > 6.5%
  - cum%4h > 14.5%
  - H_2-excretion: (max. value – value t_0) < 20 ppm

Lactose breath test: examples

1. normal lactose test

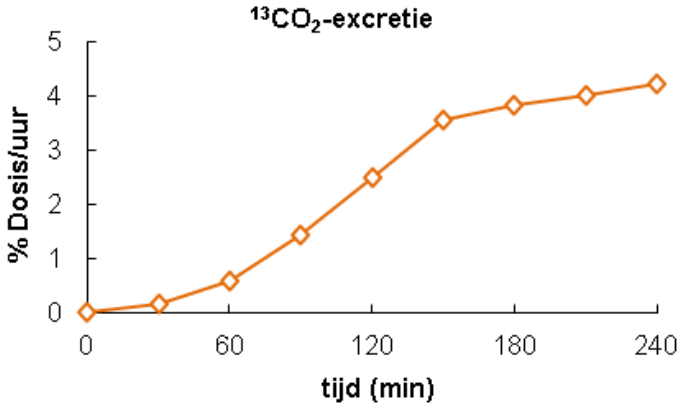


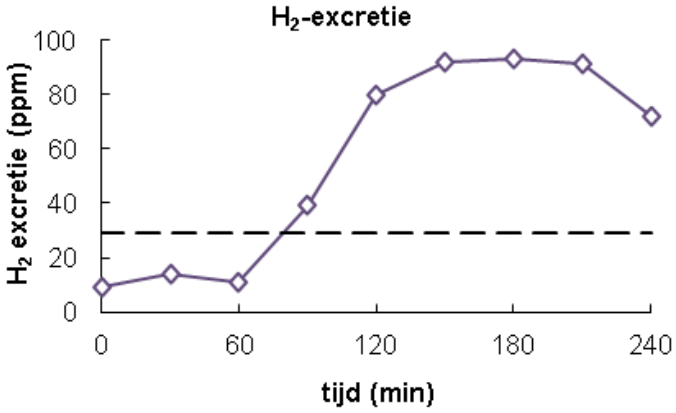


Parameters:		
	normal	patiënt
piekexcretion	>6.5	7,1
cumulative excretion after 4 h	>14.5	18,62
H_2 excretion (ppm)	<20	1
complaints	none	?

Lactose breath test: examples

2. lactase deficiency

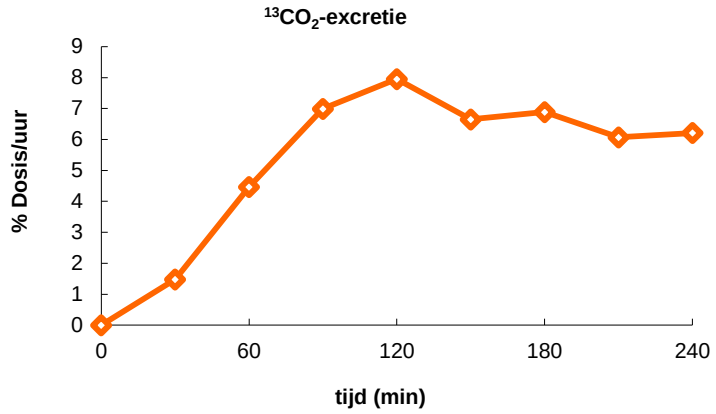


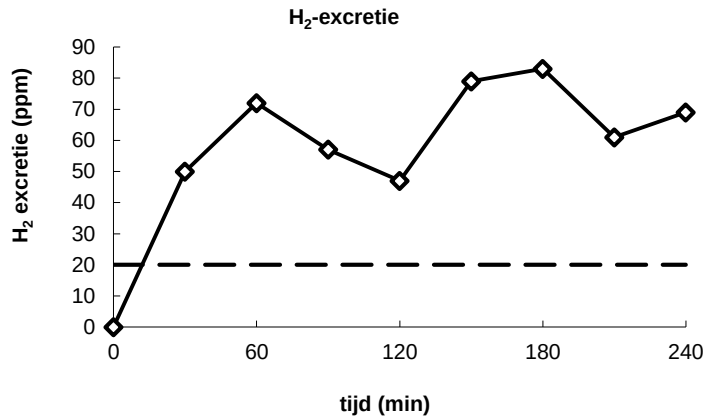


Parameters:		
	normal	patiënt
piekexcretion	>6.5	4,2
cumulative excretion after 4 h	>14.5	9,09
H_2 excretion (ppm)	<20	84
complaints	none	Diarrhea, bloating, abdominal pain

Lactose breath test: examples

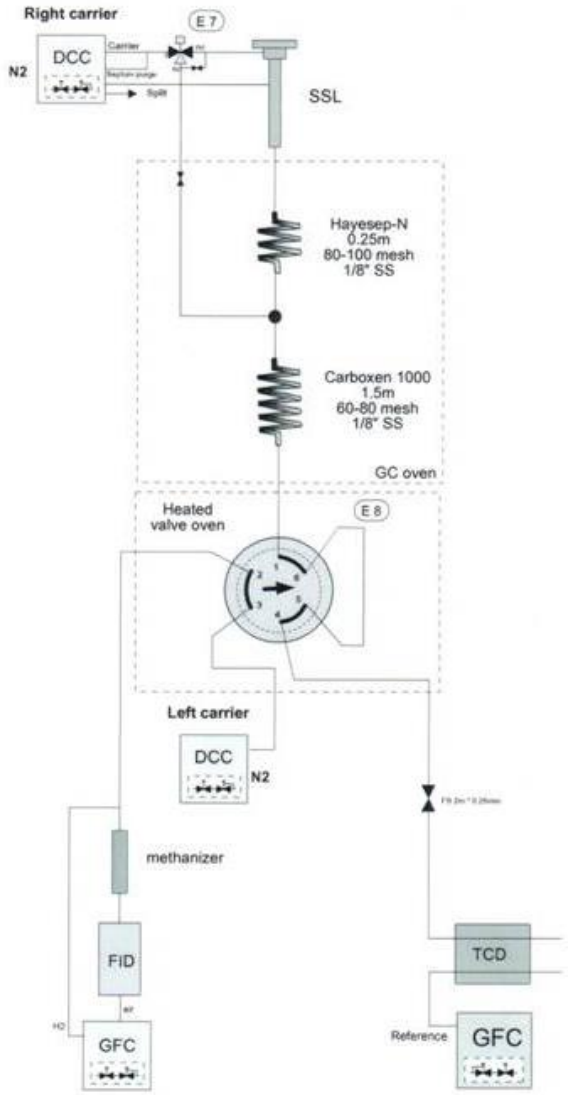
2. Bacterial overgrowth / lactose malabsorption



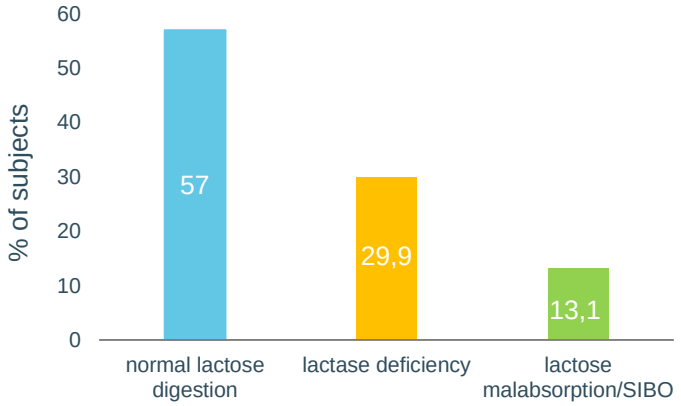


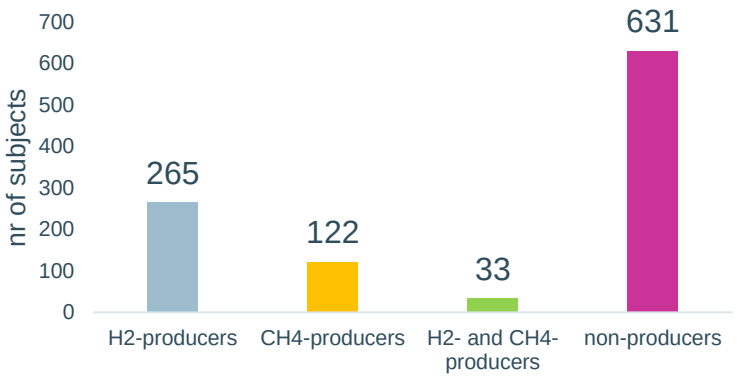
Parameters:	normal	patiënt
piekexcretion	>6.5	8,0
cumulative excretion after 4 h	>14.5	21,80
H_2 excretion (ppm)	<20	83
complaints	none	cramps

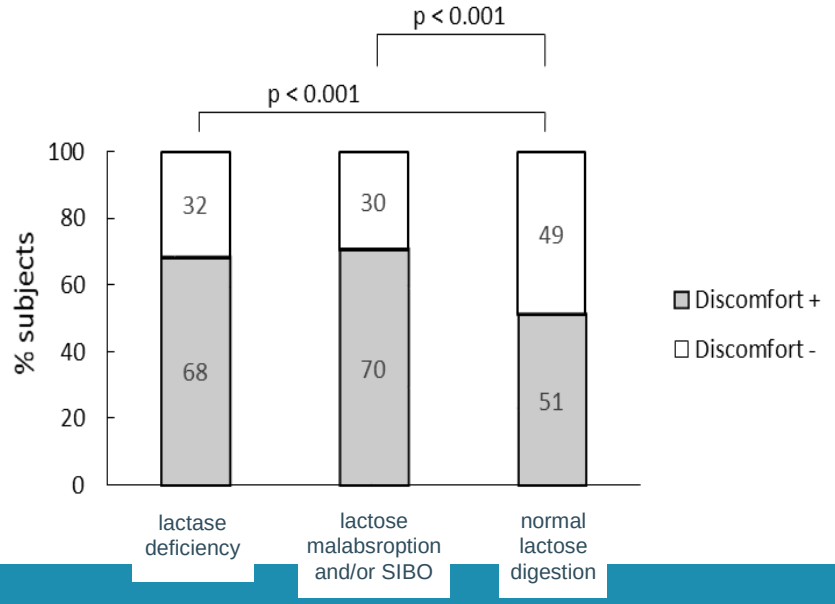
Measurement of H_2, CH_4 and CO_2 in 1 run

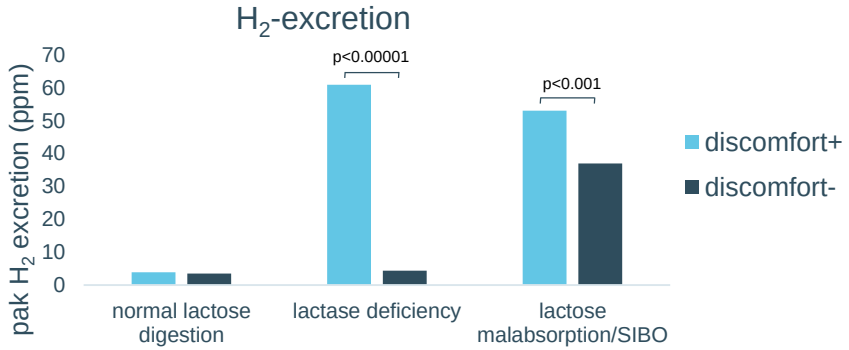


Retrospective analysis of 1051 lactose tests





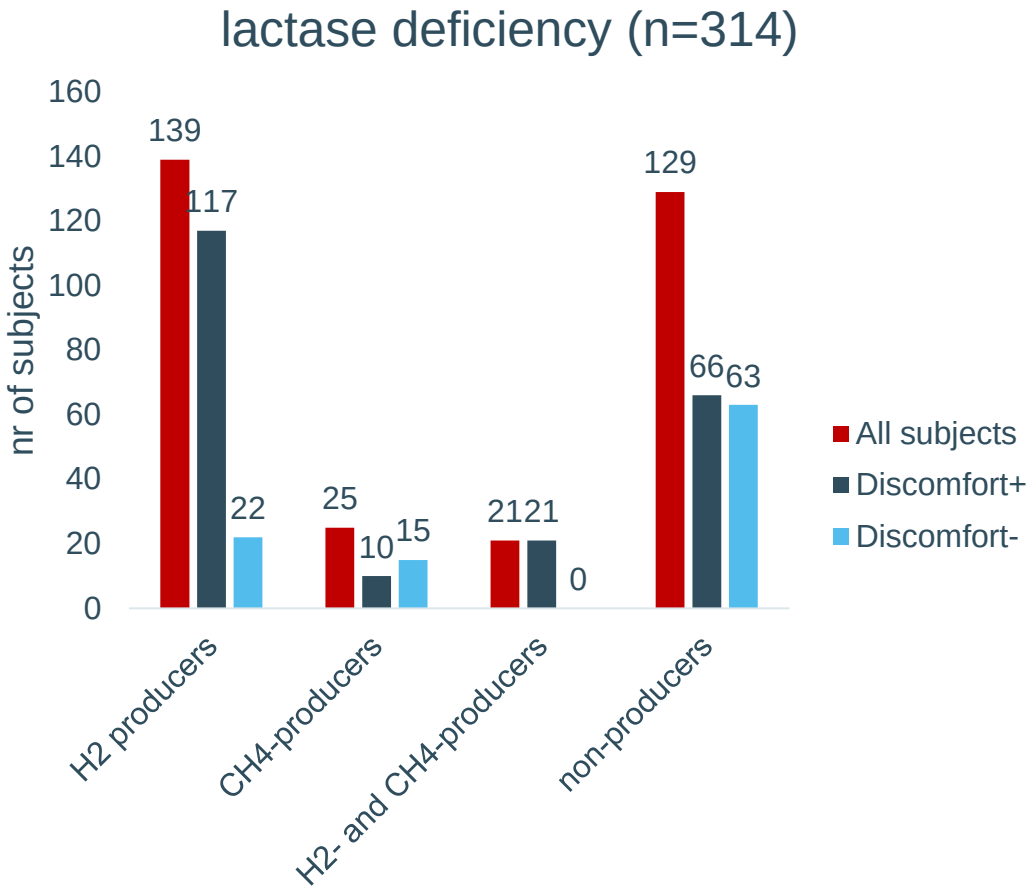




- 93% of complaints were gastrointestinal symptoms (cramps, flatulence, diarrhea, nausea, abdominal pain, bloating)
- Other symptoms: headache and tiredness

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

Retrospective analysis of 1051 lactose tests

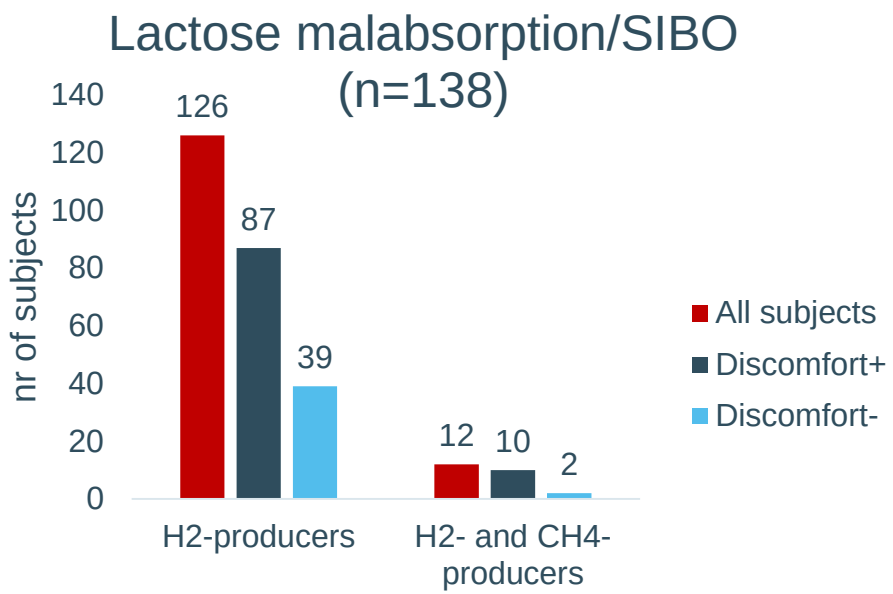


- Cum. ^{13}C-excretion after 4h <14.5%
- Whether or not increased H_2-excretion >20 ppm

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 (cut-off of 14.5% too high)?
  - 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

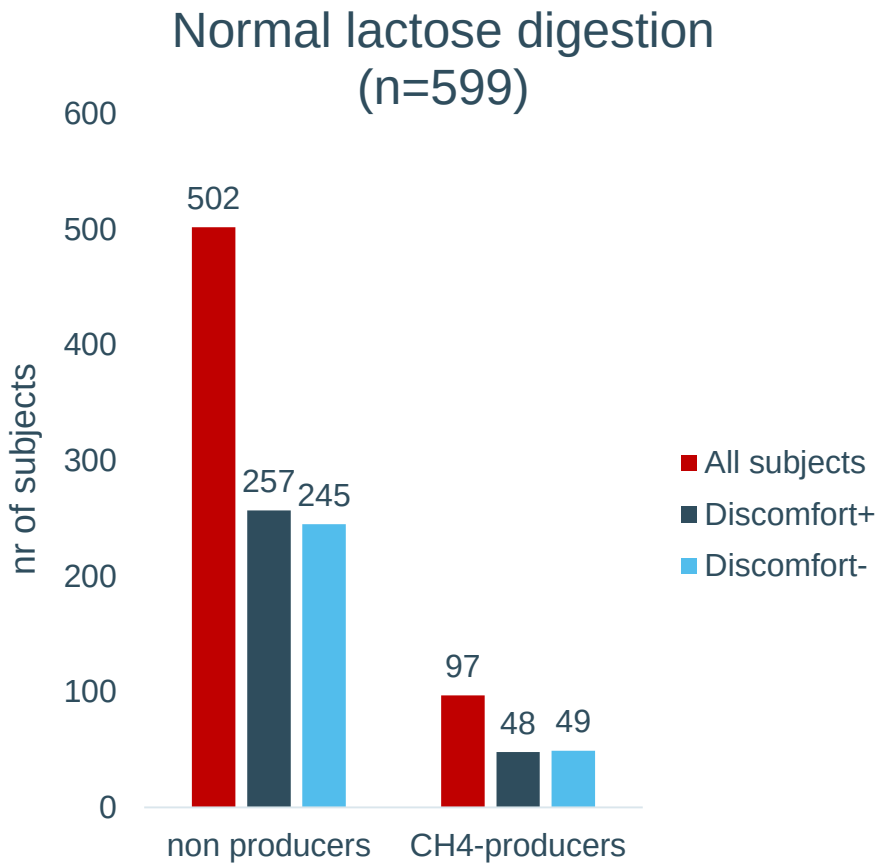
Retrospective analysis of 1051 lactose tests



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

- Malabsorption induced by high dose of lactose?
- Cut-off of 14,5% is too low?
  - Subjects are lactase deficient?

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)

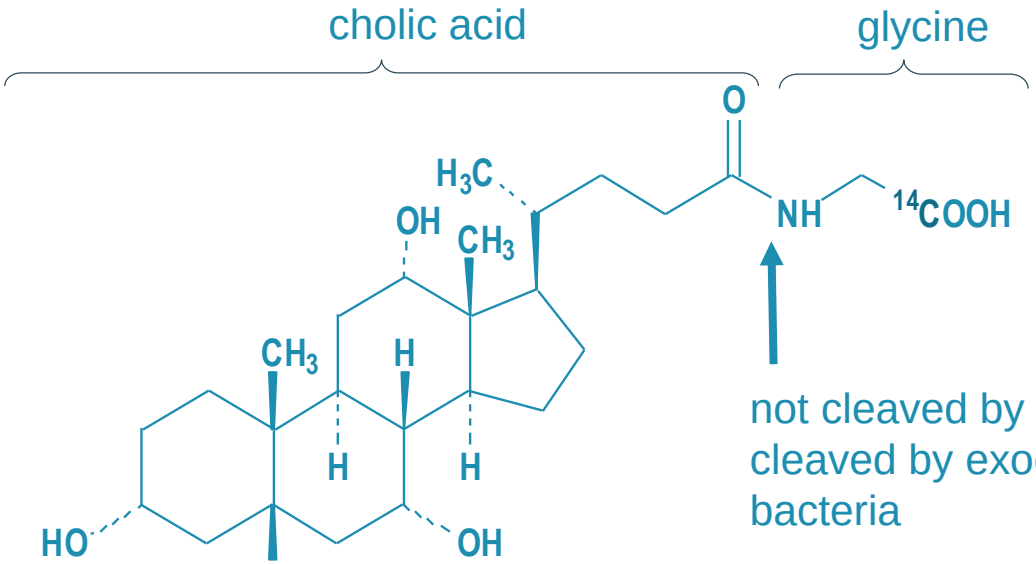
Future plans

- New validation study
  - Reduction of dose of lactose to 25g
  - Establishment of new normal values
  - Use of genetic test for lactase deficiency as standard
  - Analysis of the microbiota composition/activity

^{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

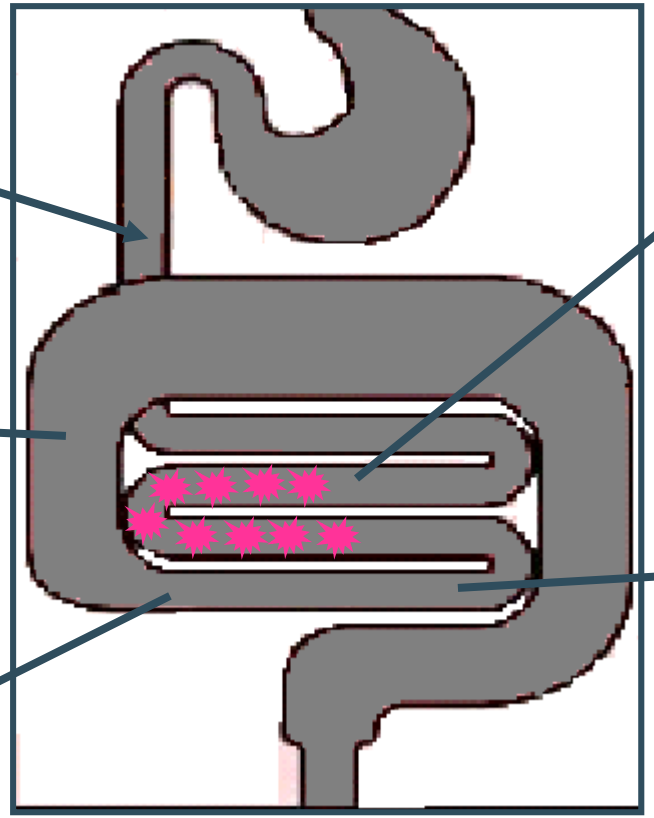
^{14}C-glycocholic acid

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

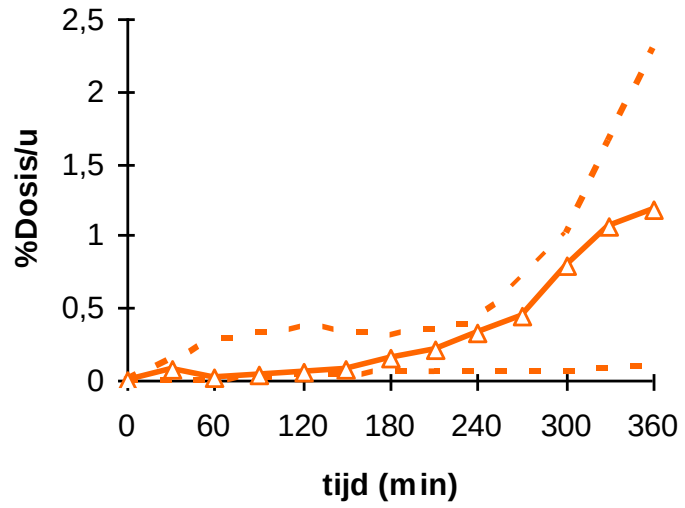
^{14}C-glycocholic acid

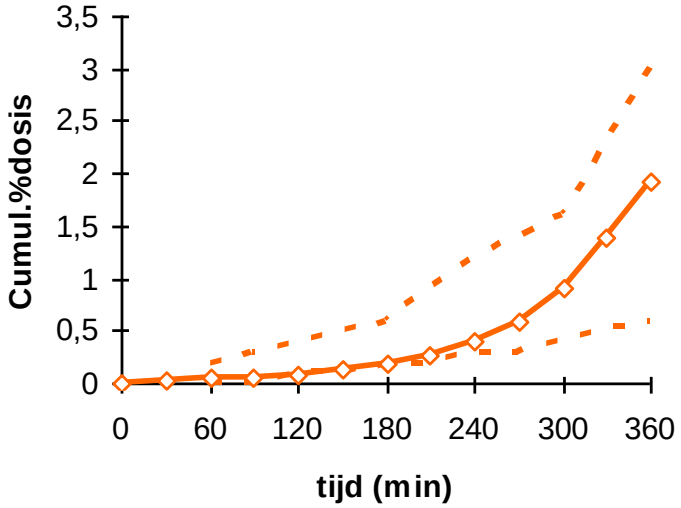
- Practical
  - test meal: substrate in gelatin capsule, taken with normal breakfast
  - breath samples: every 30 min
  - total test duration: 6h

- 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

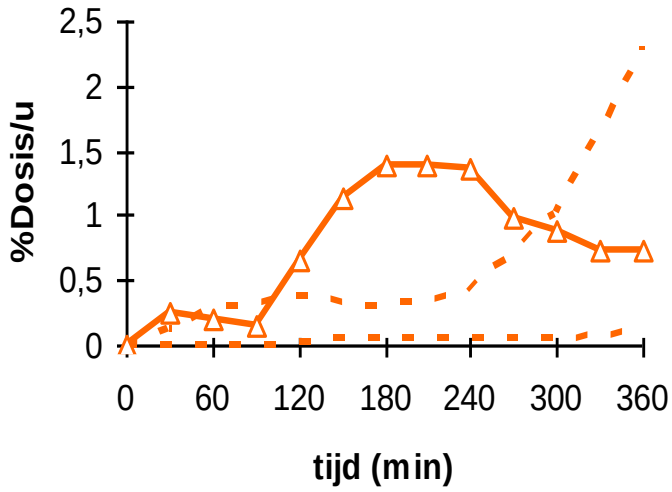
1. normal ^{14}C-glycocholic acid test

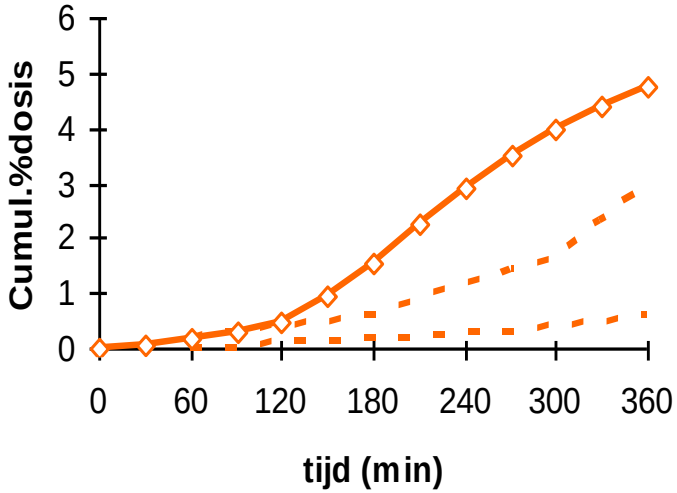




^{14}C-glycocholic acid: examples

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

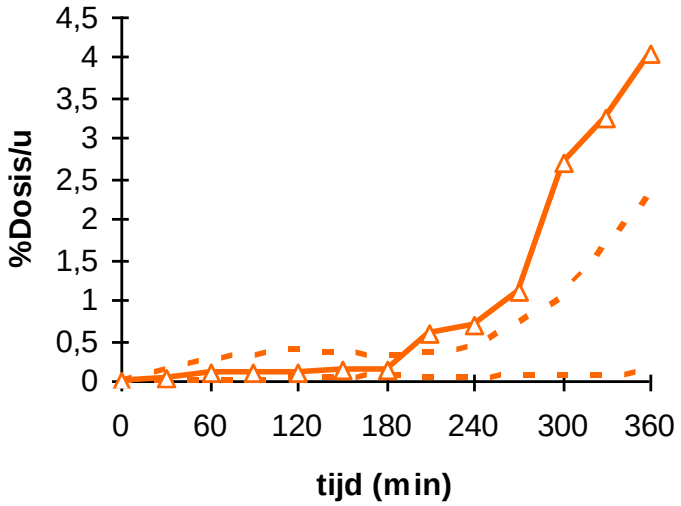


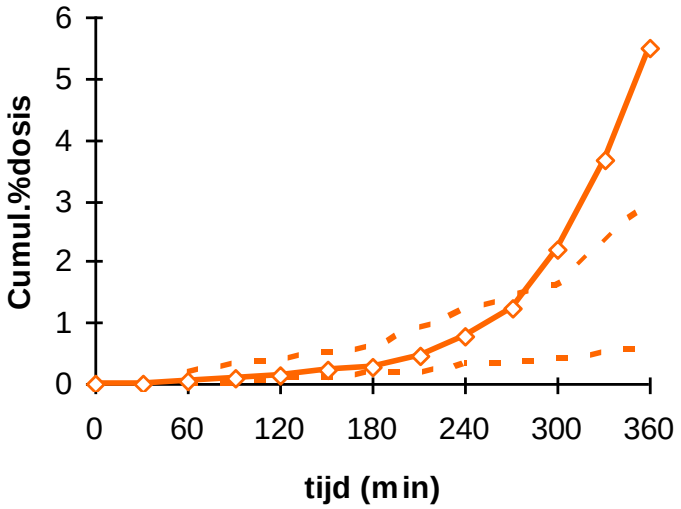


- deconjugation in small intestine because of bacterial overgrowth
- bacterial metabolism in the colon: malabsorption in case of short bowel or fast transit

^{14}C-glycocholic acid: examples

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

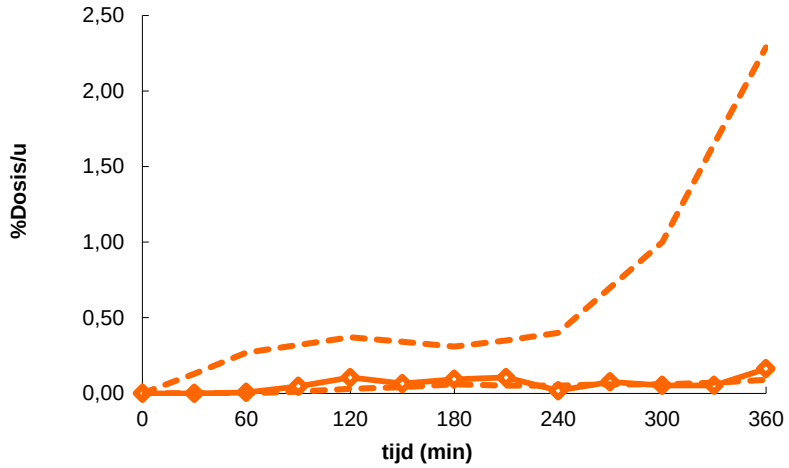


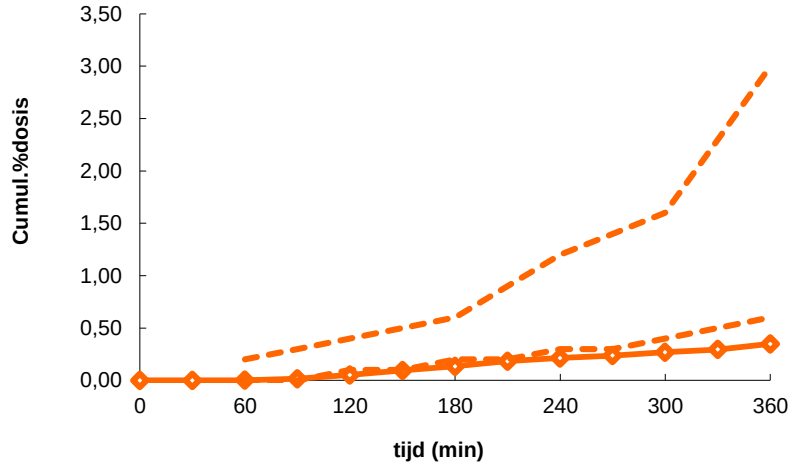


- colonic metabolism of ^{14}C-glycocholic acid: ileal malabsorption
- metabolism in the small intestine: bacterial overgrowth in case of slow transit
- important distal bacterial overgrowth

^{14}C-glycocholic acid: examples

3. Low metabolism during the 6h of the test





- Normal test
- Unreliable due to antibiotics or colon preparation
- False negative due to massive fecal bile loss or too short contact time with bacteria

Combination with fecal analysis

- 72-h collection of all feces starting on the moment of glycocholic breath test
  - Fecal output (g/day)
  - % of dry weight (%)
- Recovery of exogenous ^{14}C (^{14}C-glycoch
  - Liquid scintillation after oxidation to ^{1}
- Transit: recovery of orally administered ^{3} dose)
  - Liquid scintillation after oxidation to ^{3}
- Fecal fat excretion (g/day)
  - Continuous extraction using Soxhlet
  - Gravimetric quantification of extracte
- (endogenous) bile acids (mmol/day)
  - Enzymatic reaction - spectrofotomet

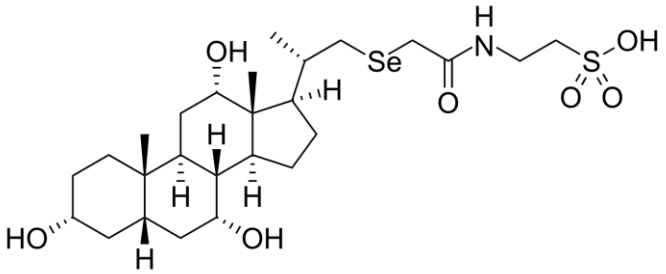
Combination with feces collection

	patient	normal value
g feces per day	1233	<200g
Percent dry weight	8.17	<15.0%
^{14}C-excretion day 1	60.2	<5.0%
^{14}C-excretion day 1+2	70.4	
^{14}C-excretion day 1+2+3	71.8	
^{3}H-excretion day 1	62.6	0.0-25.0%
^{3}H-excretion day 1+2	81.4	25.0-65.0%
^{3}H-excretion day 1+2+3	82.4	65.0-90.0%
g fat per day	29.6	<7g
mmol bile acids per day	4.15	<1.11 mmol

Limitations of the ^{14}C-glycocholic acid breath test

- No normal values
- A low ^{14}C-excretion does not exclude (massive) fecal bile acid loss
- ^{14}C-substrate not available in many countries ⇨ low number of (recent) studies, test considered as obsolete in many reviews
- Analysis of fecal ^{14}C-excretion is considered cumbersome
- ^{14}C-substrate is no longer reimbursed in Belgium (sept 2015)
  ⇨move to ^{13}C-glycocholic acid?

Alternatives: ^{75}SeHCat



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

Advantages

- Easier (?)

Limitations

- Expensive (not reimbursed in Belgium)

Walters, Therap Adv Gastroenterol 2010; 3:349-357

Alternatives: quantification of C4

- 7α-OH-4-cholesten-3-one (C4): bile acid precursor
- Hepatic bile acid synthesis is increased in case of fecal bile loss
- Fasting plasma C4 levels are increased
- Analysis is time consuming and requires HPLC-analysis ⇨ not widely applicable