Breath tests as diagnostic tests in gastroenterology

basics and clinical applications

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Basic principles of breath tests
Basic principle of breath testing

13/14C-substrate → 13/14C-subgroup → 13/14CO₂ → 13/14CO₂ → 13/14CO₂

Enzymatic reaction
Digestion/absorption

Rate-limiting step

PDR = percent dose recovery
= %dose/h

cPDR = cumulative percent dose recovery

Translational Research in Gastrointestinal Disorders
$^{13}\text{C}$ or $^{14}\text{C}$-labelled substrates

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

- Only $^{14}\text{C}$ tests
  - $^{14}\text{C}$-glycocholic test
  - Gastric emptying of solid and liquid phase
Breath tests in routine clinical diagnosis

**Digestion and absorption**
- $^{13}$C-lactose
- $^{13}$C-fructose
- $^{13}$C-sucrose
- $^{13}$C-mixed triglyceride
- $^{13}$C-urea
- Liver function: $^{13}$C-aminopyrine

**Transit**
- Orocaecal transit: $^{13}$C-lactose ureide
- Gastric emptying: $^{13}$C-octanoic acid, $^{13}$C-glycine
- Helicobacter pylori: $^{13}$C-urea
- Bacterial overgrowth/ ileal malabsorption: $^{14}$C-glycocholic acid

**Fat**
- Carbohydrates: $^{13}$C-lactose, $^{13}$C-fructose, $^{13}$C-sucrose
Carbohydrate malabsorption: 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 $\rightarrow$ indication of small bowel bacterial overgrowth
- If it happens in the large intestine $\rightarrow$ indication of carbohydrate malabsorption
Test kits

UZ leuven

report

kit

samples

Gastroenterologist

General practitioner

kit

patient
## Interpretation: normal values

<table>
<thead>
<tr>
<th>test</th>
<th>parameter</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mixed triglycerides</td>
<td>cum%/6h</td>
<td>cumulative percent of administered dose excreted after 6h</td>
</tr>
<tr>
<td>lactose</td>
<td>cum%/4h</td>
<td>cumulative percent of administered dose excreted after 4h</td>
</tr>
<tr>
<td></td>
<td>peak excretion</td>
<td>maximal excretion</td>
</tr>
<tr>
<td></td>
<td>H₂ excretion</td>
<td>reflects bacterial metabolism of lactose</td>
</tr>
<tr>
<td>glycocholic acid</td>
<td>cum%/6h</td>
<td>cumulative percent of administered dose excreted after 6h</td>
</tr>
<tr>
<td>gastric emptying</td>
<td>t₁/₂</td>
<td>half-emptying time (min)</td>
</tr>
</tbody>
</table>

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

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

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

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

$\delta^{13}_{\text{breath at starvation}} = -28 \implies ^{13}\text{C}/^{12}\text{C} = 0.0108045$
Underlying assumptions

- CO₂-production is known and is stable during the test

\[ \text{BSA} = W^{0.5378} \times H^{0.3964} \times 0.024265 \]


\[ \% \text{dosis/h} = 100 \times \frac{(\text{AP}_t - \text{AP}_o)}{100} \times \frac{\text{amount of substrate (mg)}}{\text{molar mass}} \times \frac{\text{number of } ^{13}\text{C positions/molecule}}{\text{mmol substrate}} \]

\[ \text{effective mmol excess } ^{13}\text{C-atoms administered} \]

⇒ Test needs to be done in resting conditions
Influence of CO$_2$-production

- specificity can be improved using non-resting CO$_2$-production

Underlying assumptions

- \( \text{CO}_2 \)-production is known and is stable during the test
- No contribution to \(^{13}\text{C}\)-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
**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

\[
\begin{align*}
\text{substrate: } & 1,3\text{-distearyl,2}\left[^{13}\text{C-carboxyl}\right]\text{octanoyl glycerol} \\
\end{align*}
\]

- 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:
⇒ steatorrhea 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
Examples

1. Normal lipase activity

criterium: > 23% of administered dose excreted in breath
Examples

2. low lipase activity
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

Other breath test substrates

- 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**

- **Normal values**
  - cum%4h > 14.5%
  - H$_2$-excretion: (max. value – value $t_0$) < 20 ppm

50g $\Rightarrow$ 25g
Lactose: combined $^{13}$CO$_2$/H$_2$ breath test

- **principle**

![Diagram showing lactose metabolism.]

- 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

<table>
<thead>
<tr>
<th></th>
<th>$^{13}$CO$_2$-test</th>
<th>H$_2$-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-h cum excr</td>
<td>Peak excr</td>
</tr>
<tr>
<td>sensitivity</td>
<td>0.84</td>
<td>0.68</td>
</tr>
<tr>
<td>specificity</td>
<td>0.96</td>
<td>0.89</td>
</tr>
<tr>
<td>Pos pred value</td>
<td>0.94</td>
<td>0.81</td>
</tr>
<tr>
<td>Neg pred value</td>
<td>0.90</td>
<td>0.81</td>
</tr>
<tr>
<td>accuracy</td>
<td>0.91</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Hiele M, J Lab Clin Med 1988; 112:193-200
Lactose breath test: examples

1. normal lactose test

### Parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>peak excretion (ppm)</td>
<td>&gt;6.5</td>
<td>7.1</td>
</tr>
<tr>
<td>cumulative excretion 4 hr</td>
<td>&gt;14.5</td>
<td>18.62</td>
</tr>
<tr>
<td>H₂ excretion (ppm)</td>
<td>&lt;20</td>
<td>1</td>
</tr>
<tr>
<td>symptoms</td>
<td>none</td>
<td>?</td>
</tr>
</tbody>
</table>
Lactose breath test: examples

2. lactase deficiency

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normaal</th>
<th>Patiënt</th>
</tr>
</thead>
<tbody>
<tr>
<td>piekexcretie</td>
<td>&gt;6.5</td>
<td>4.2</td>
</tr>
<tr>
<td>cumulatieve excretie na 4 uur</td>
<td>&gt;14.5</td>
<td>9.09</td>
</tr>
<tr>
<td>H₂ excretie (ppm)</td>
<td>&lt;20</td>
<td>84</td>
</tr>
<tr>
<td>klachten</td>
<td>geen</td>
<td>diarree-buikpijn-opgeblazen gevoel</td>
</tr>
</tbody>
</table>
Lactose breath test: examples

2. Bacterial overgrowth / lactose malabsorption

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak excretion</td>
<td>&gt;6.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Cumulative excretion after 4 hrs</td>
<td>&gt;14.5</td>
<td>21.80</td>
</tr>
<tr>
<td>H₂ excretion (ppm)</td>
<td>&lt;20</td>
<td>83</td>
</tr>
<tr>
<td>Symptoms</td>
<td>No</td>
<td>Abdominal pain</td>
</tr>
</tbody>
</table>
Retrospective analysis of 1051 lactose tests

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

Retrospective analysis of 1051 lactose tests

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

- Cum. $^{13}$C-excretion after 4h <14.5%
- Whether or not increased H$_2$-excretion >20 ppm
Retrospective analysis of 1051 lactose tests

- Criticism on CH$_4$-excretion:
  - False negative tests:
    - Only when the production reaches a threshold, it appears in the breath
  - False positive tests:
    - Release of CH$_4$ entrapped in stool due to mixing of the intestinal content (mainly in constipated subjects)

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

- Cum. $^{13}$C-excretion after 4h >14.5%
- H$_2$-excretion < 20 ppm
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

[Diagram showing the structure of $^{14}$C-glycocholic acid, with notes that it is not cleaved by human enzymes but is cleaved by exoenzymes secreted by bacteria.]
\( ^{14}\text{C-glycocholic acid} \)

Bile acids are secreted in conjugated form.

Ileal malabsorption of bile acids: conjugated bile acids pass into colon
- Rapidly deconjugated
- \( ^{14}\text{C-glycine} \rightarrow ^{14}\text{CO}_2 \)
- Increased \( ^{14}\text{CO}_2 \)-excretion

Some deconjugation of bile acids:
- Bile acid is absorbed and reconjugated
- \( ^{14}\text{C-glycine} \rightarrow ^{14}\text{CO}_2 \)

Bacterial overgrowth: more rapid deconjugation of glycocholic acid
- Rapid increase in \( ^{14}\text{CO}_2 \)-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%)

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 7 days
- Fraction retained after 7d: >15% of initial activity
- Retention <10% predictive for successful response to therapy

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

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

- underlying assumptions
- Need for standardised conditions
Thank you!