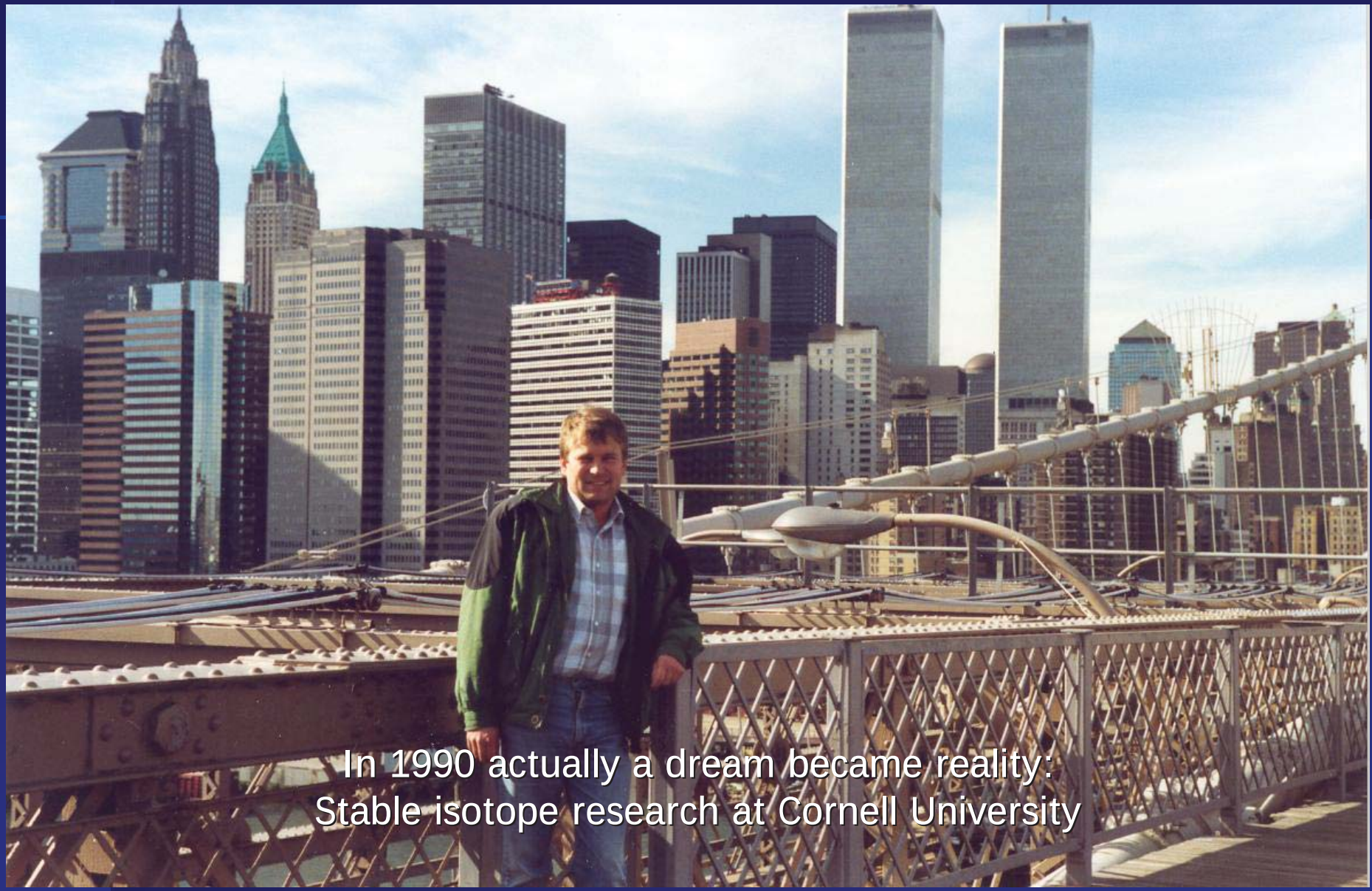
Stable Isotopes in Gastroenterology and Nutrition

Klaus D. Wutzke, Ph.D., D.Sc.

Children‘s Hospital of the University of Rostock
Research Laboratory
www.kinderklinik-rostock.de ‘Forschung‘


In 1990 actually a dream became reality:
Stable isotope research at Cornell University

Headlines

I would like to entertain you with the following items, namely:

- Stable isotopes
- Measuring methods
- $^{13}CO_2$-Breath tests
- ^{13}C-Urea breath test for *Helicobacter pylori* diagnosis
- ^{13}C-Methacetin and $^{13}C_2$-aminopyrine for liver function tests
- Lactose-[^{13}C]ureide breath test for orocoecal transit time measurements
- Impact of L-carnitine on fat oxidation
- Utilisation of doubly labelled *Lactobacillus johnsonii* yoghurt.

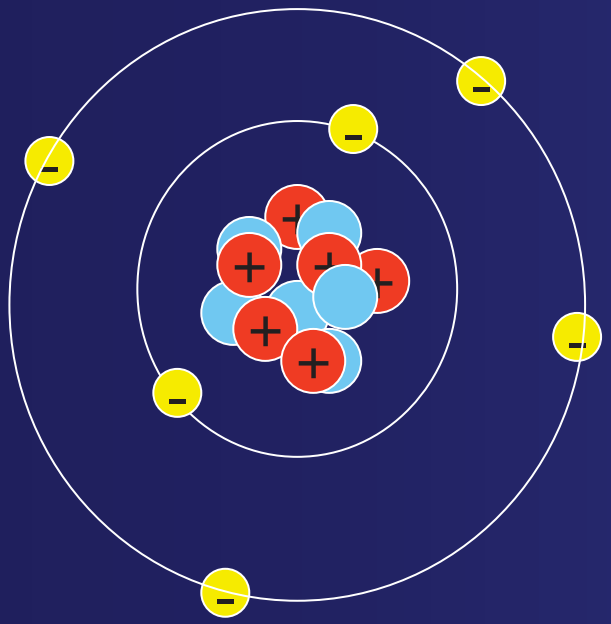
Stable isotopes

- They are rare.
- „Isos topos“ (Greek) „on the same place“ at the periodic system of the elements.
- Features, peculiarities:
- Non radioactive.
- Equal number of protons and electrons
  - identical chemical properties.
- Different number of neutrons
  - different physical properties (mass).

Stable carbon isotope

^{12}C (98,9%)

6 6 6



neutrons

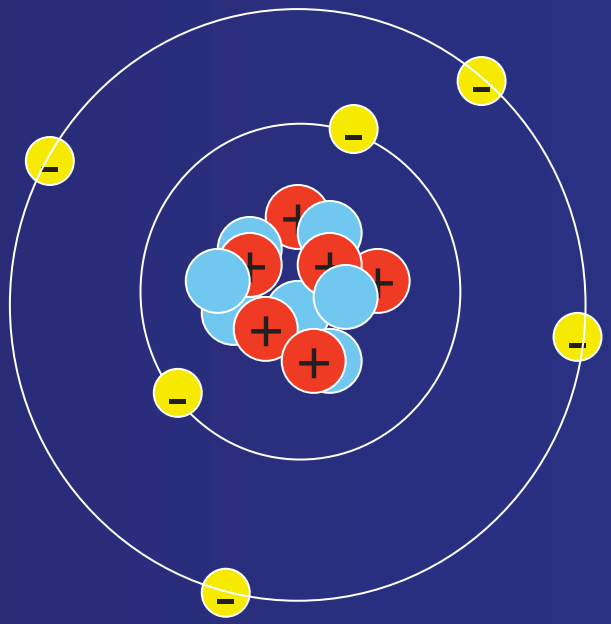
protons

electrons

resemble

^{13}C (1,1%)

7 6 6



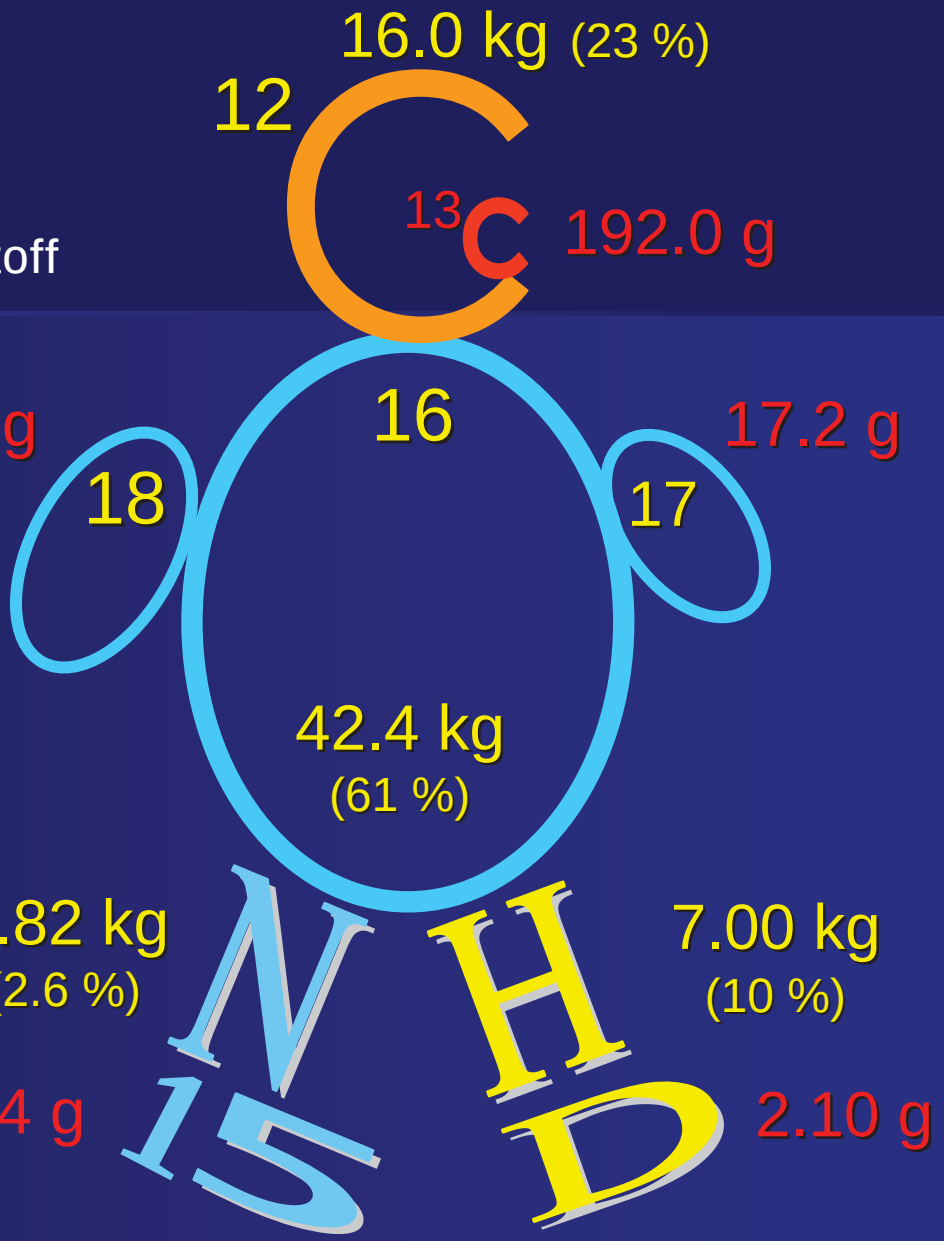
Isotope (Greek ισοσ: same; τοποσ: place)

12				24,305 σ 0,063			~ 95 ms β+ βp 4,16; 3,95	122,5 ms β+ βp 1,94; 1,77...	3,86 s β+ 3,2... γ 583; 74...	11,3 s β+ 3,1... γ 440...
11				Na 22,98977 σ 0,530			Na 19 p	Na 20 446 ms β+ 11,2... βα 2,15; 4,44... γ 1634...	Na 21 22,48 s β+ 2,5... γ 351...	Na 22 2,602 a β+ 0,5; 1,8 γ 1275 σ 29000
10				Ne 20,179 σ 0,038	Ne 16 p	Ne 17 109 ms β+ 8,0; 13,5... βp 4,59; 3,77; 5,12...	Ne 18 1,67 s β+ 3,4... γ 1042...	Ne 19 17,22 s β+ 2,2... γ (1357...)	Ne 20 90,51 σ 0,037	Ne 21 0.27 σ 0,692
9				F 18,998403 σ 0,0095	F 15 p	F 16 p	F 17 64,8 s β+ 1,7 no γ	F 18 109,7 m β+ 0,6 no γ	F 19 100 σ 0,0095	F 20 11,0 s β− 5,4... γ 1634...
8	O 15,9994 σ 0,000270		O 12 p	O 13 8,9 ms β+ 16,7... βp 1,44; 6,44; 0,93...	O 14 70,59 s β+ 1,8; 4,1... γ 2313...	O 15 2,03 m β+ 1,7 no γ	O 16 99,762 σ 0,000178	O 17 0,038 $\sigma_{n,\alpha}$ 0,235	O 18 0,200 σ 0,00016	O 19 27,1 s β− 3,3; 4,7... γ 197; 1357;
7	N 14,0067 σ_{abs} 1,85			N 12 11,0 ms β+ 16,4... γ 4439... βα 0,2...	N 13 9,96 m β+ 1,2 no γ	N 14 99,63 σ 0,075 $\sigma_{n,p}$ 1,81	N 15 0,37 σ 0,000024	N 16 5,3 µs 7,13 s β− 4,3; 10,4... γ 6129; 7115... βα 1,3...	N 17 4,17 s β− 3,2; 8,7... βn 1,17; 0,38... γ 871; 2184	N 18 0,63 s β− 9,4 γ 1982; 165 817; 2467
C 12,011 σ_{abs} 0,0034	C 8 2p	C 9 126,5 ms β+ 3,5... βp 8,24; 10,92...	C 10 19,3 s β+ 1,9... γ 718; 1022	C 11 20,38 m β+ 1,0 no γ	C 12 98,90 σ 0,0034	C 13 1,10 σ 0,0009	C 14 5730 a β− 0,2 no γ	C 15 2,45 s β− 4,5; 9,8... γ 5298...	C 16 0,747 s β− βn 0,79; 1,72	C 17
B 10,81 σ_{abs} 759	B 7 p	B 8 770 ms β+ 14,1... β2α ~ 1,6; 8,3	B 9 p	B 10 20,0 σ 0,5 $\sigma_{n,\alpha}$ 3837	B 11 80,0 σ 0,0055	B 12 20,20 ms β− 13,4... γ 4439... βα 0,2...	B 13 17,33 ms β− 13,4... γ 3684 βn 3,6; 2,4...	B 14 16,1 ms β− 14,0... γ 6090; 6730	B 15	
	Be 6	Be 7 53,29 d	Be 8	Be 9 100	Be 10 $1,6 \cdot 10^6$ a	Be 11 13,8 s	Be 12 24,4 ms		Be 14	

10 7 2002

Isotope-man/woman

deliberately German suffix: ... stoff



A 70 kg-man contains

314 g

heavy **stable** isotopes.

Stakeholders:

^{2}H, ^{13}C, ^{15}N, ^{17}O, ^{18}O

modified from Wada

Basic requirements of the tracer technique

- The application of a stable isotope labelled tracer substance necessarily give rise to an increase of the isotopic enrichment of the organism.
- The metabolic fate of the tracer can be followed by measuring its enrichment.
- It is possible to draw conclusions for the respective unlabelled substance (biologically indistinguishable from the tracee).

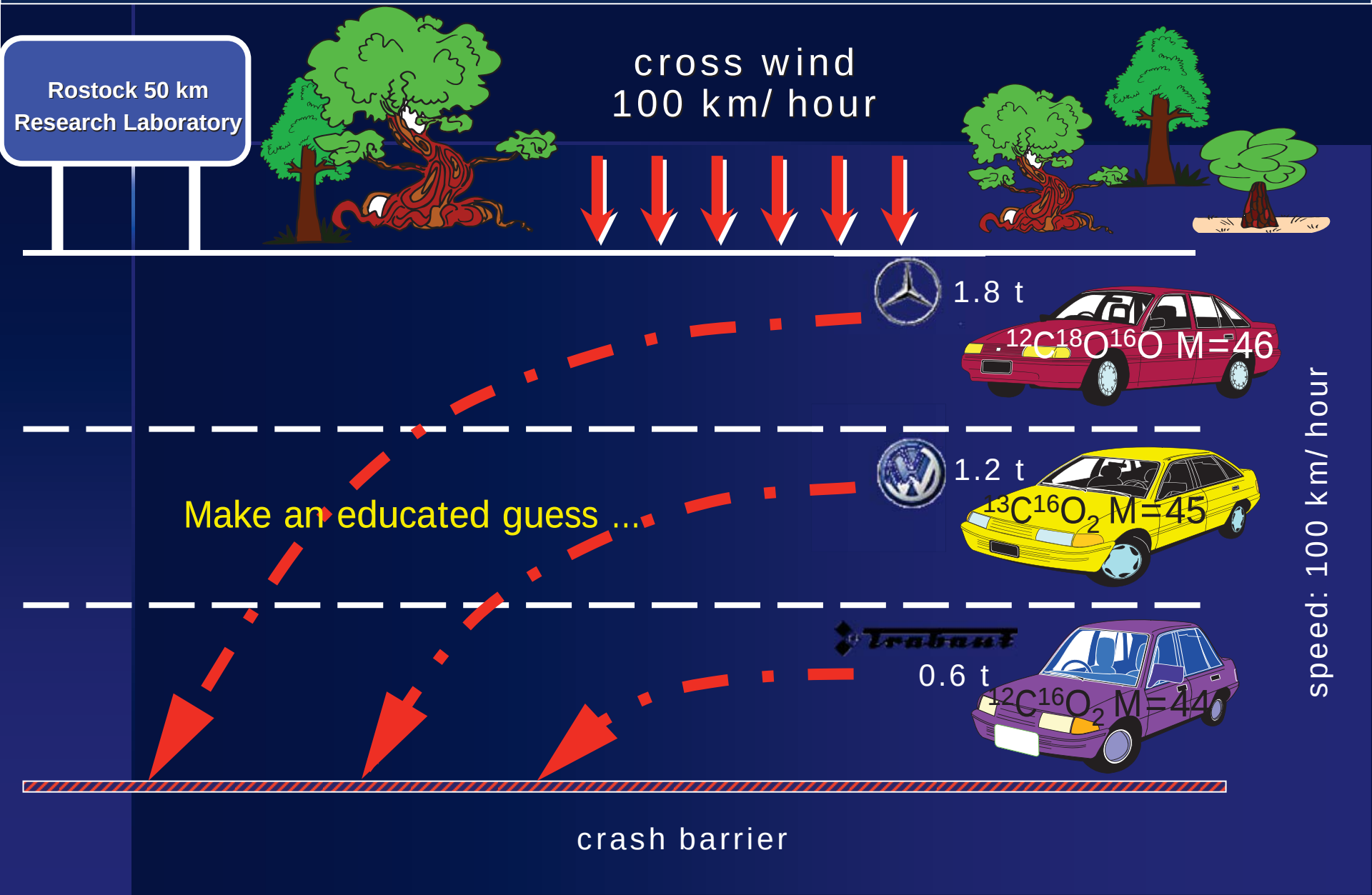
Measuring methods

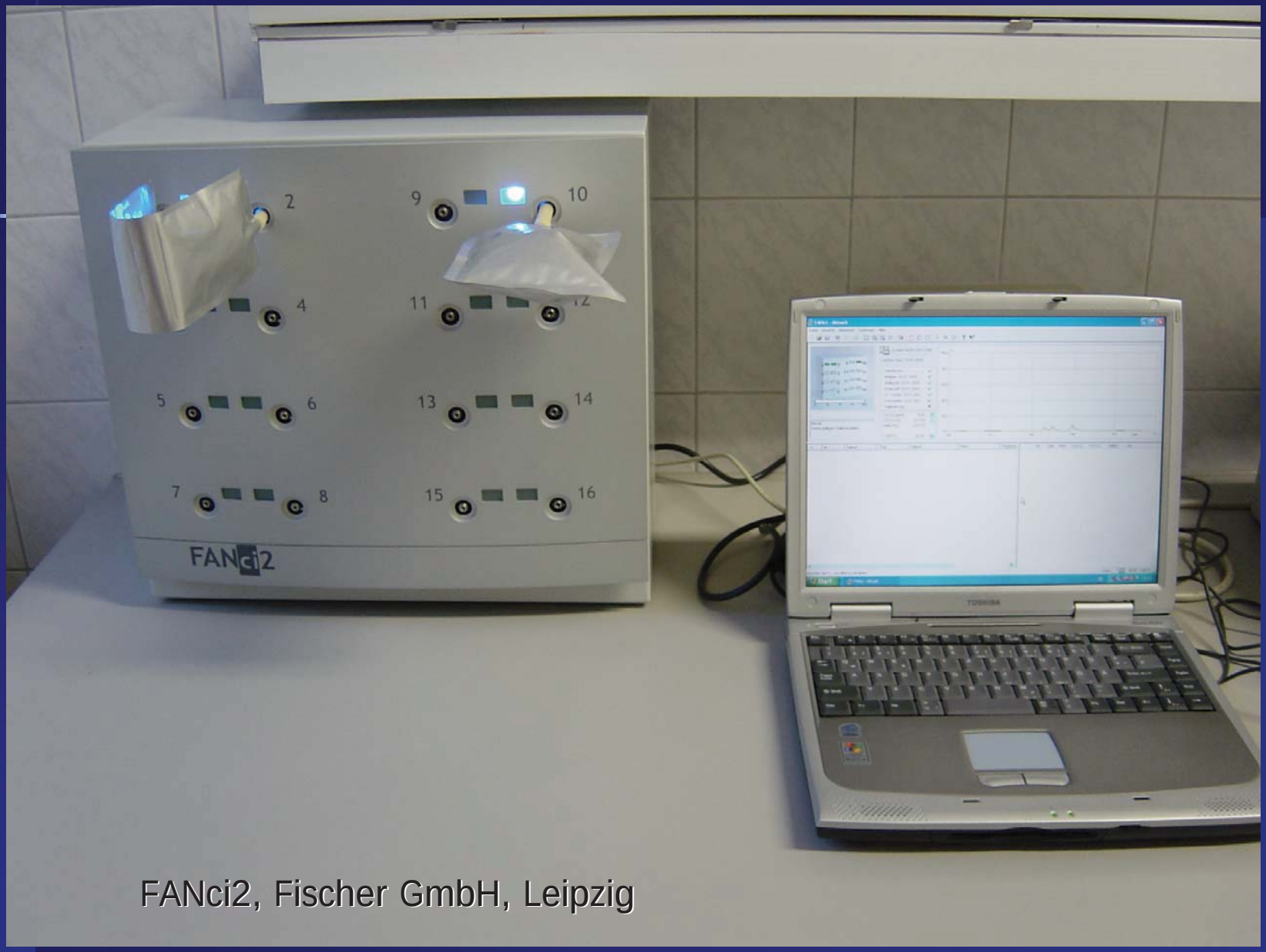
- Analytical method
- mass spectrometry (^{2}H, ^{13}C, ^{15}N, ^{18}O)
- CF-IRMS
- CF-C-IRMS
- nondispersive
- infrared spectroscopy
- nuclear magnetic resonance spectroscopy (NMR)

- Accuracy [atom-%]
- 0.01
- 0.0001
- 0.001

Equipment: Tracer Mass 20-20, SerCon, Crewe, UK

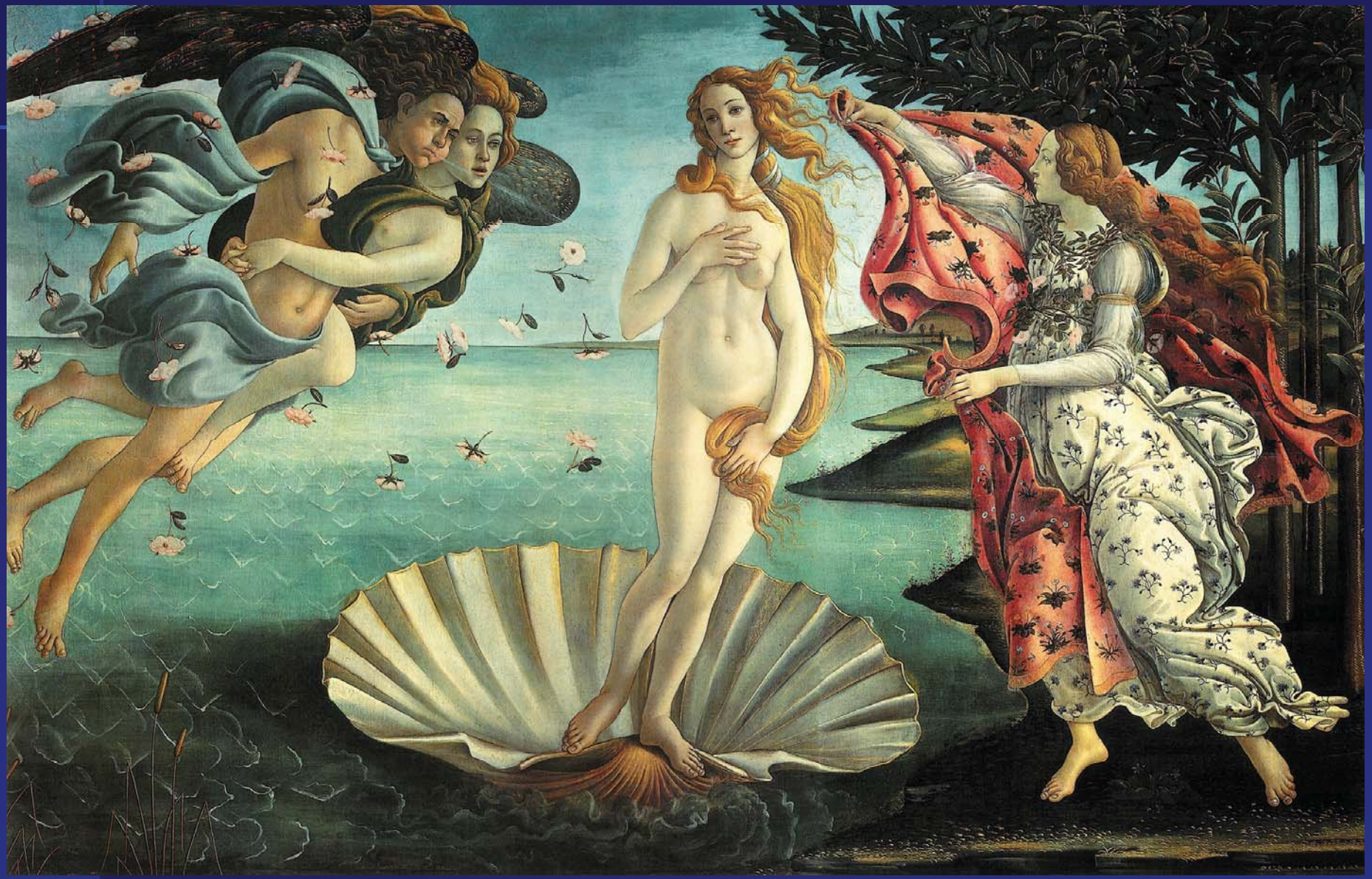
Mass spectrometry on the interstate



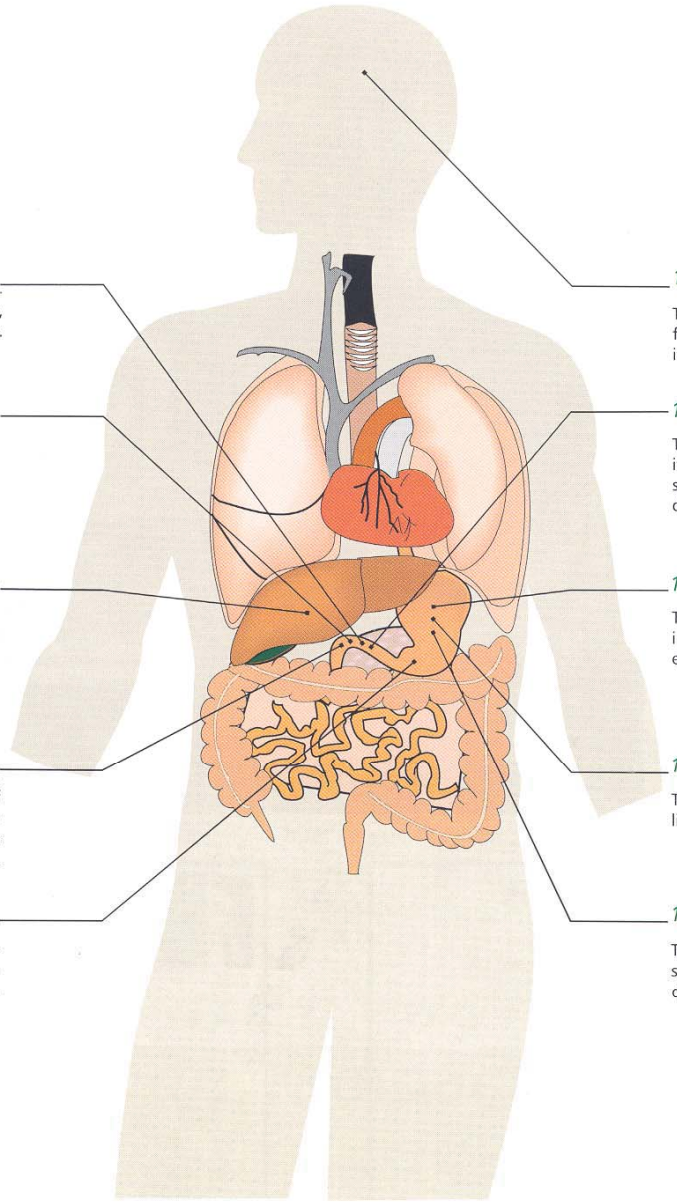


FANci2, Fischer GmbH, Leipzig

$^{13}CO_2$-Breath tests



^{13}C Breath Tests in Action



^{13}C Mixed Triglycerides B.T.
To detect exocrine pancreatic insufficiency, to monitor enzyme replacement therapy.

^{13}C Starch B.T.
To test for chronic pancreatitis. The assimilation of starch significantly is disturbed with exocrine pancreatic insufficiency.

^{13}C Methacetine/ Aminopyrine B.T.
To monitor demethylating and oxidative activities of hepatocytes. To follow up liver transplants and liver diseases.

^{13}C Glycocholic Acid B.T.
To study enterohepatic circulation of bile acids. Indicates bacterial overgrowth in the jejunum and/or bile acid loss by impaired ileal function.

^{13}C Triolein/Hiolein B.T.
To read pancreas' lypolytic activity, and to detect fat malabsorption. May replace stool analysis.

^{13}C Leucine B.T.
To investigate aminoacid metabolism function, kinetics, and incorporation into proteins.

^{13}C Lactose (Sucrose) B.T.
To secure lactase or sucrase deficiencies, if H_2 Test concordant. Adverse H_2 Tests suggest bacterial overgrowth, celiac disease, or gastrectomy.

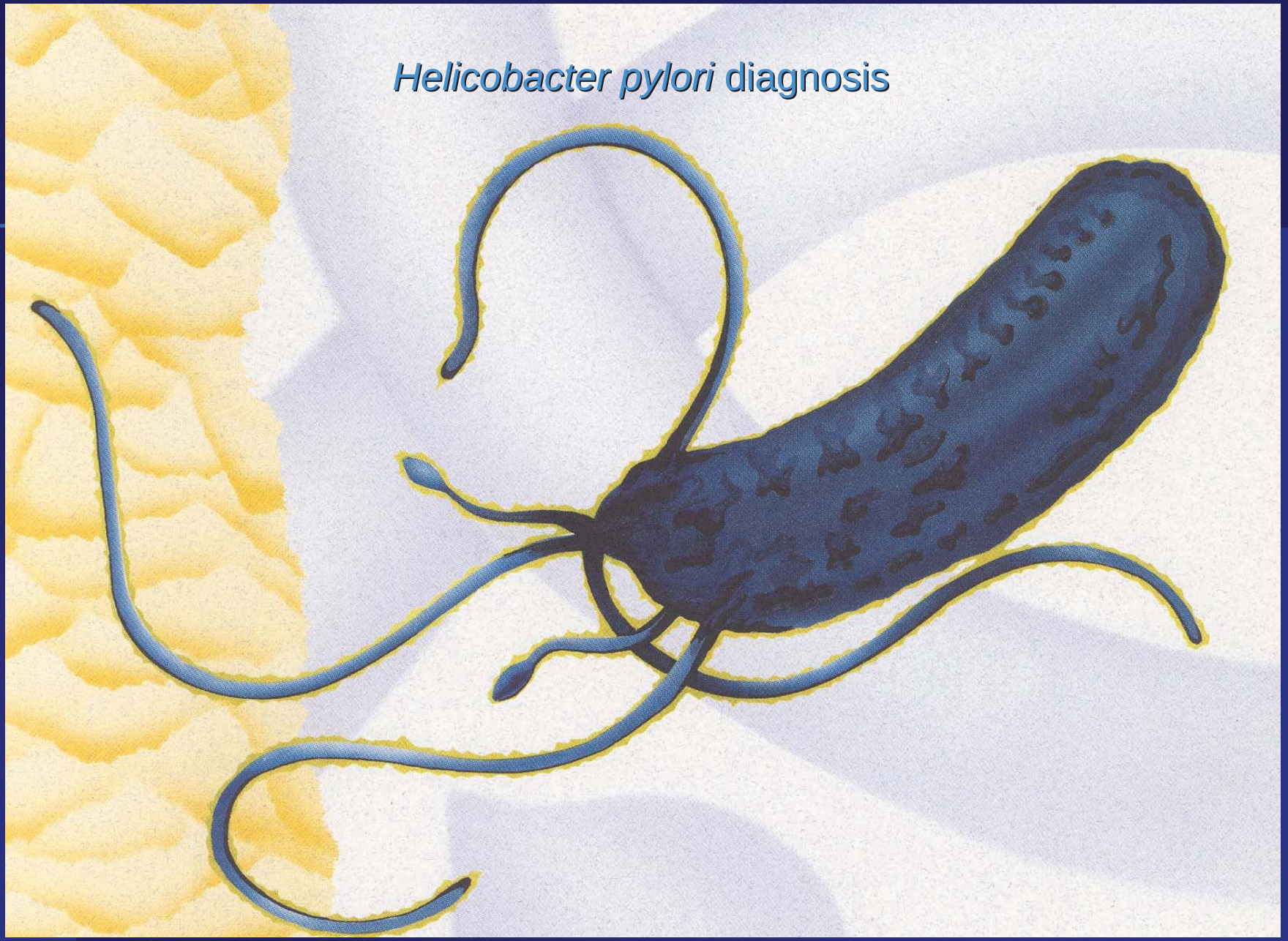
^{13}C Urea B.T.
To determine Helicobacter pylori infective status, and to control eradication therapy success.

^{13}C Acidic Acid B.T.
To determine gastric motility for liquid or half-liquid materials.

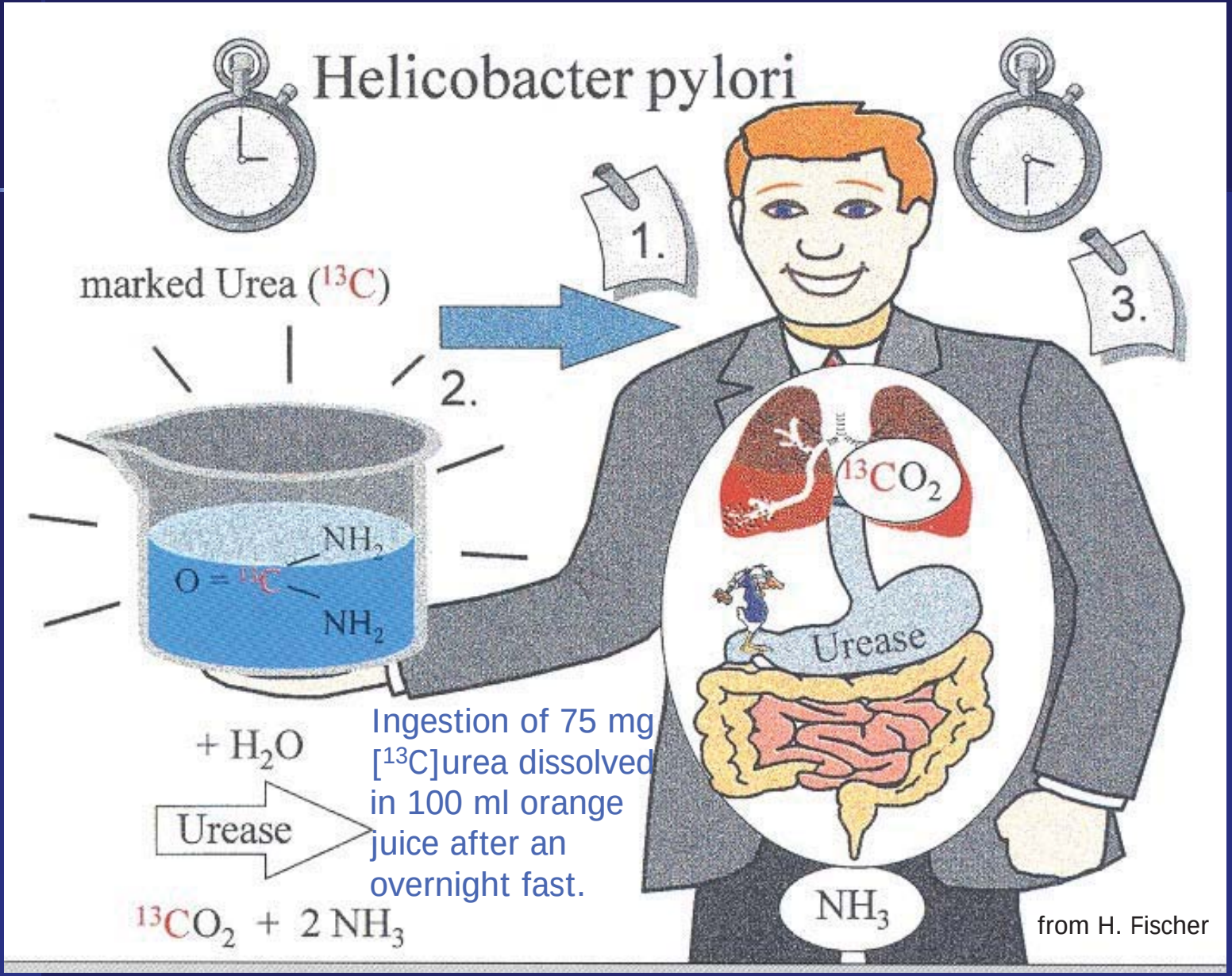
^{13}C Octanoic Acid B.T.
To measure gastric motility for solids, e.g. to calibrate therapy for diabetes mellitus.

from G. Wagner

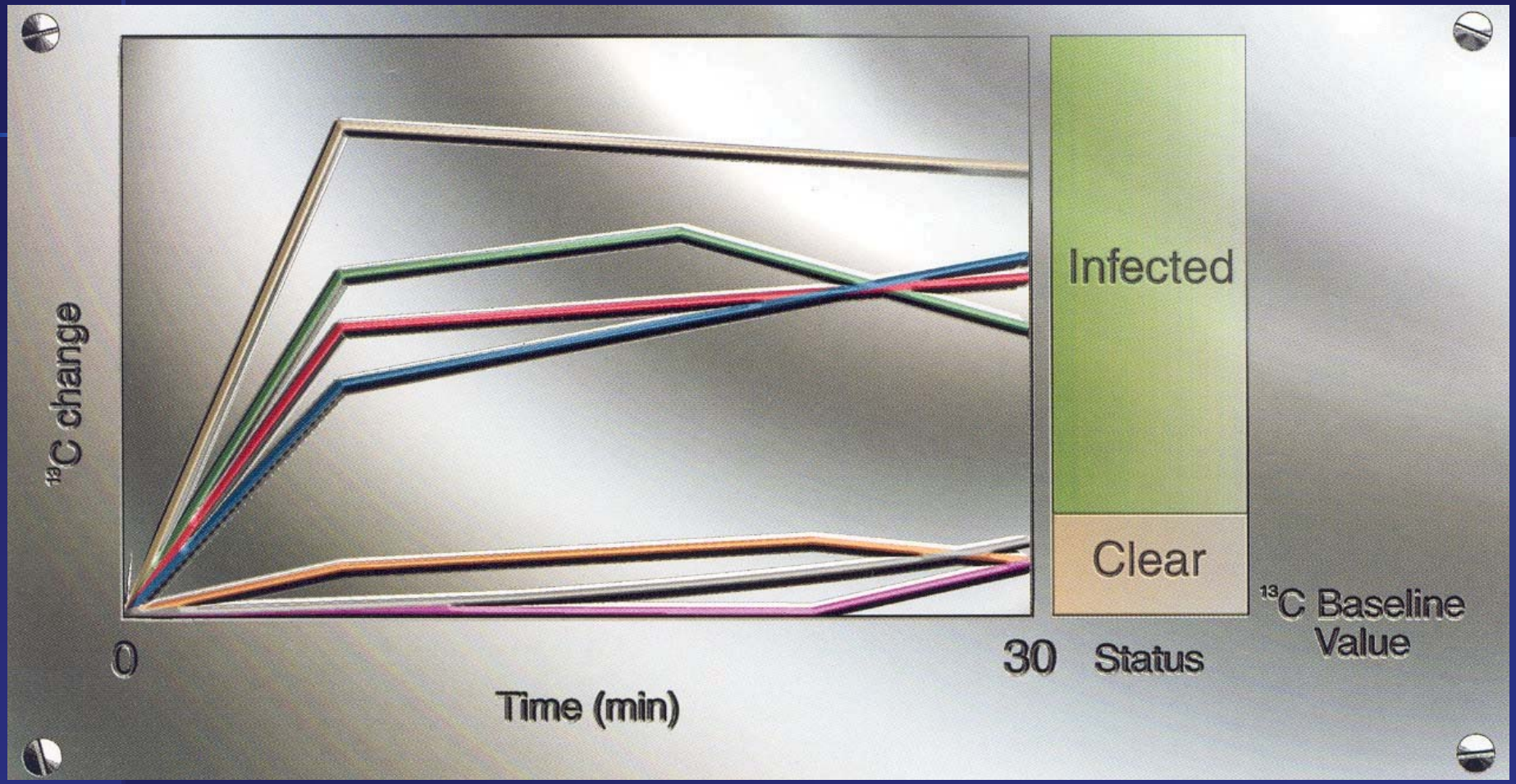
Helicobacter pylori diagnosis



Degradation pathway

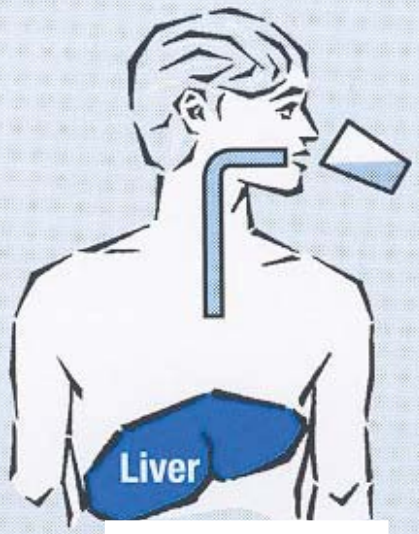


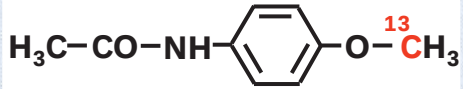
Interpretation



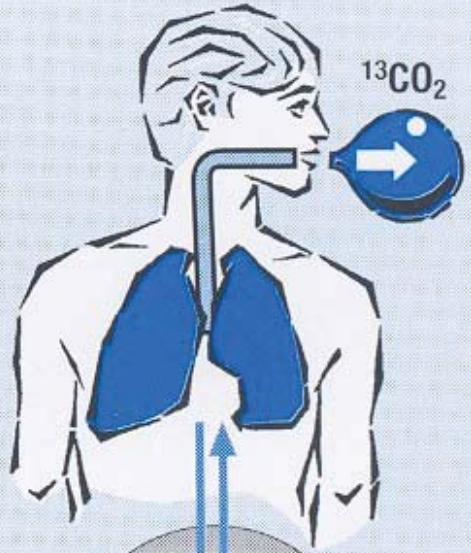
^{13}C-Methacetin and $^{13}C_2$-aminopyrine for liver function tests

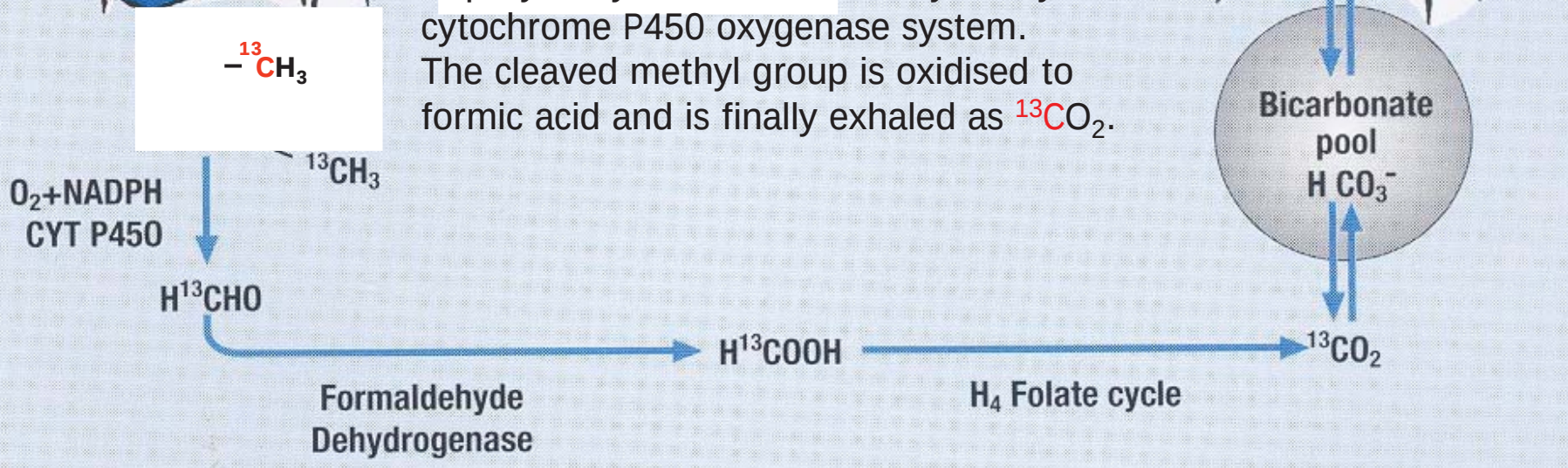
Basis of the [^{13}C]methacetin breath test



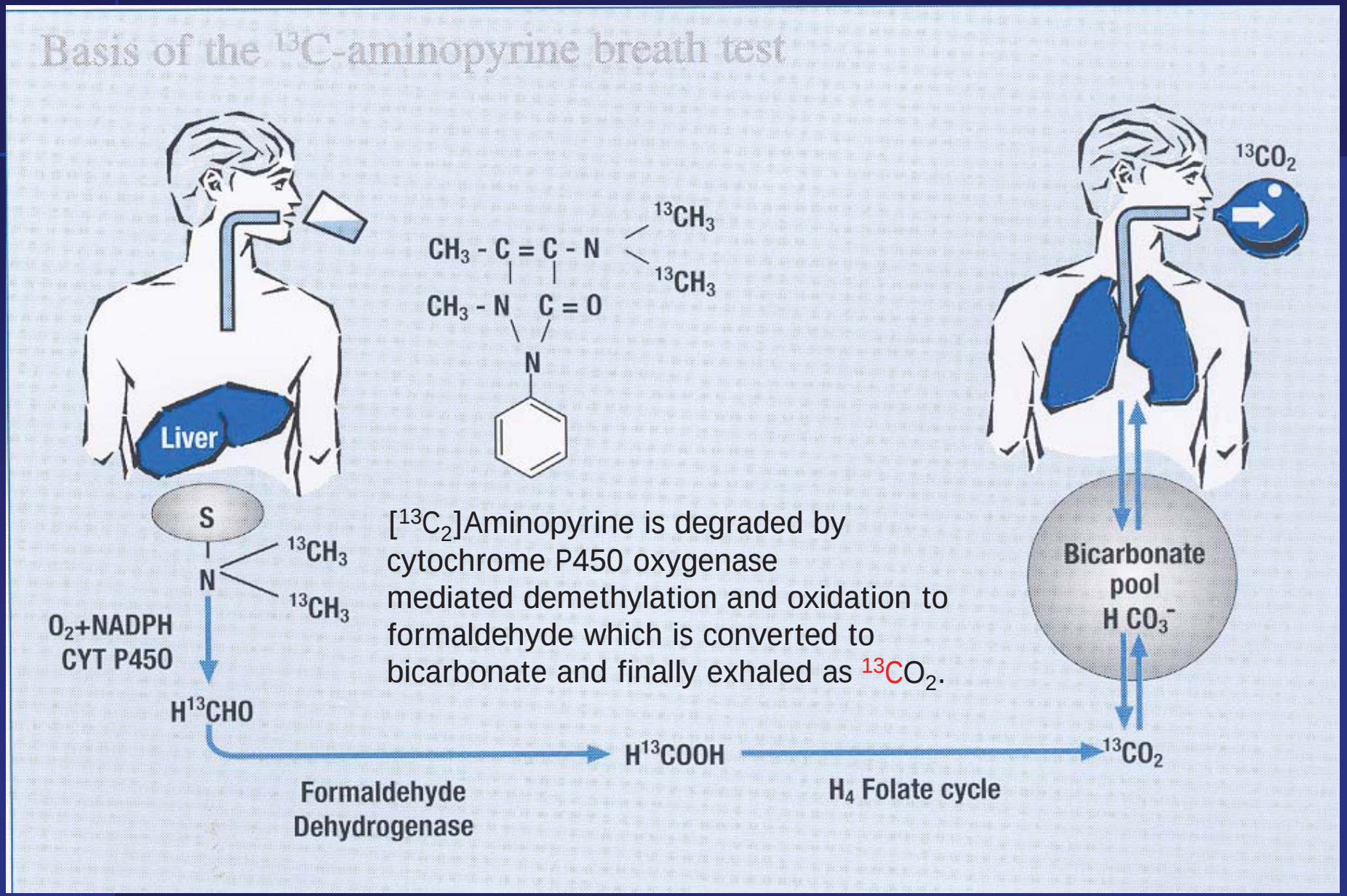


The microsomial dealcylation of [^{13}C]methacetin (paracetamol derivative) to p-hydroxyacetanilide is catalysed by the cytochrome P450 oxygenase system. The cleaved methyl group is oxidised to formic acid and is finally exhaled as $^{13}CO_2$.



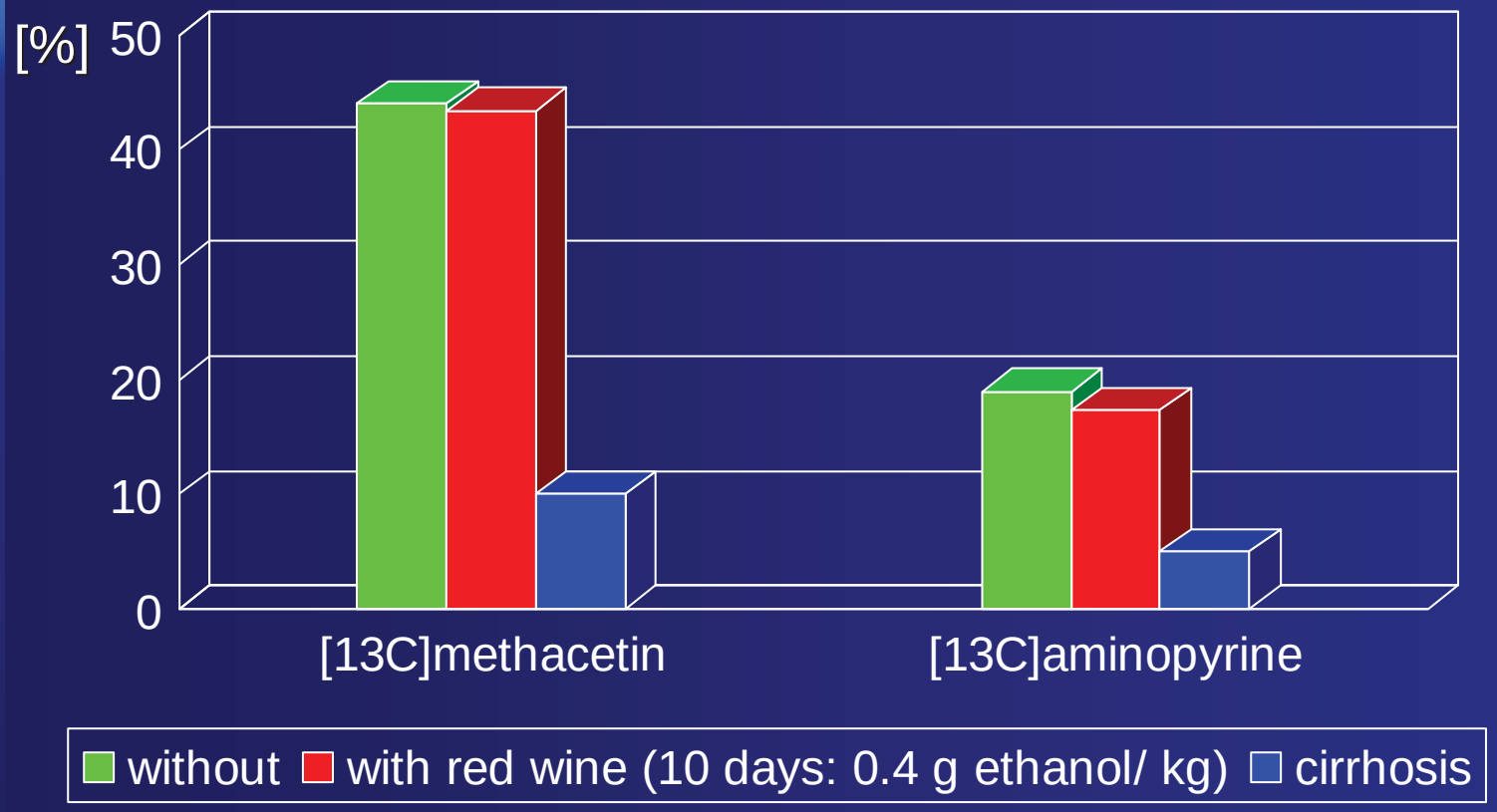


Degradation pathway



[$^{13}C_2$]Aminopyrine is degraded by cytochrome P450 oxygenase mediated demethylation and oxidation to formaldehyde which is converted to bicarbonate and finally exhaled as $^{13}CO_2$.

$^{13}CO_2$-exhalation without and after red wine drinking in healthy adults in striking comparison to subjects suffering from liver cirrhosis

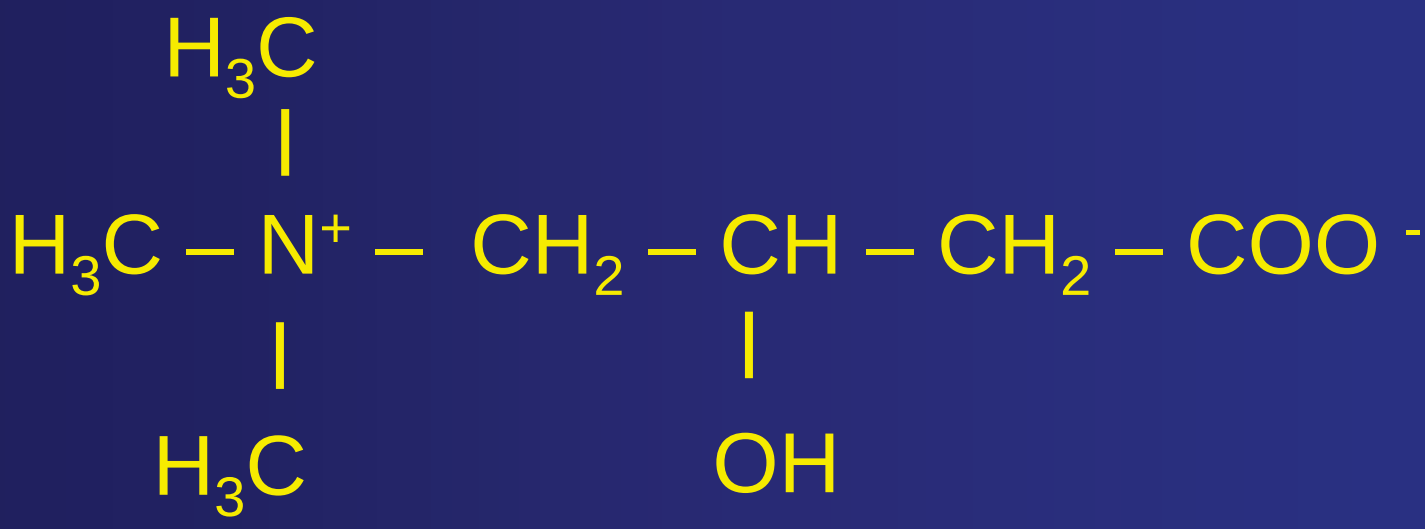


The impact of L-carnitine supplementation on fat oxidation and protein turnover

L-carnitine

- Point out: The primary function of L-carnitine is to „shuttle“ fatty acids into the mitochondria where they can be broken down by β-oxidation.

Chemical structure of L-carnitine



How L-Carnitine Helps During Exercise

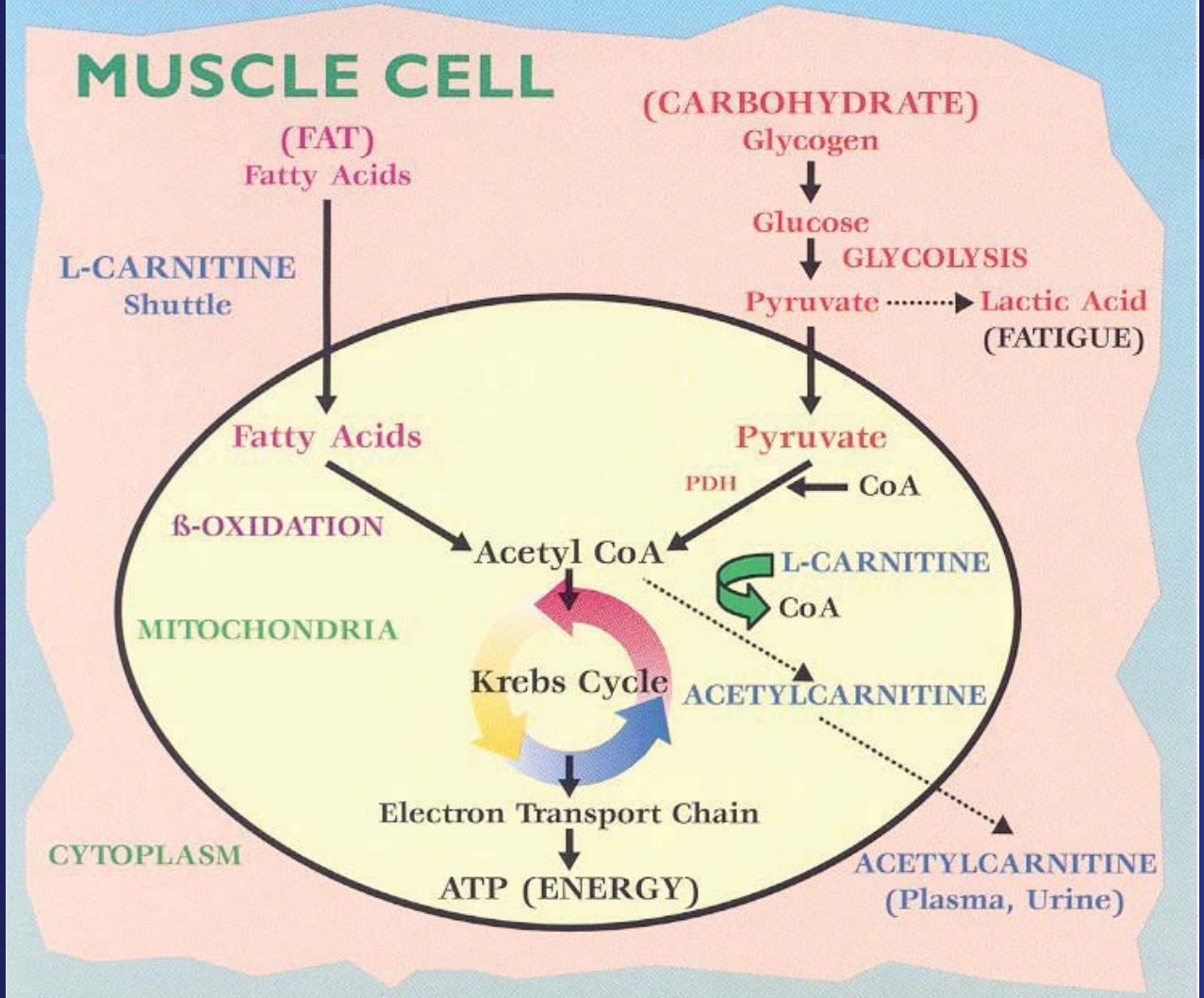


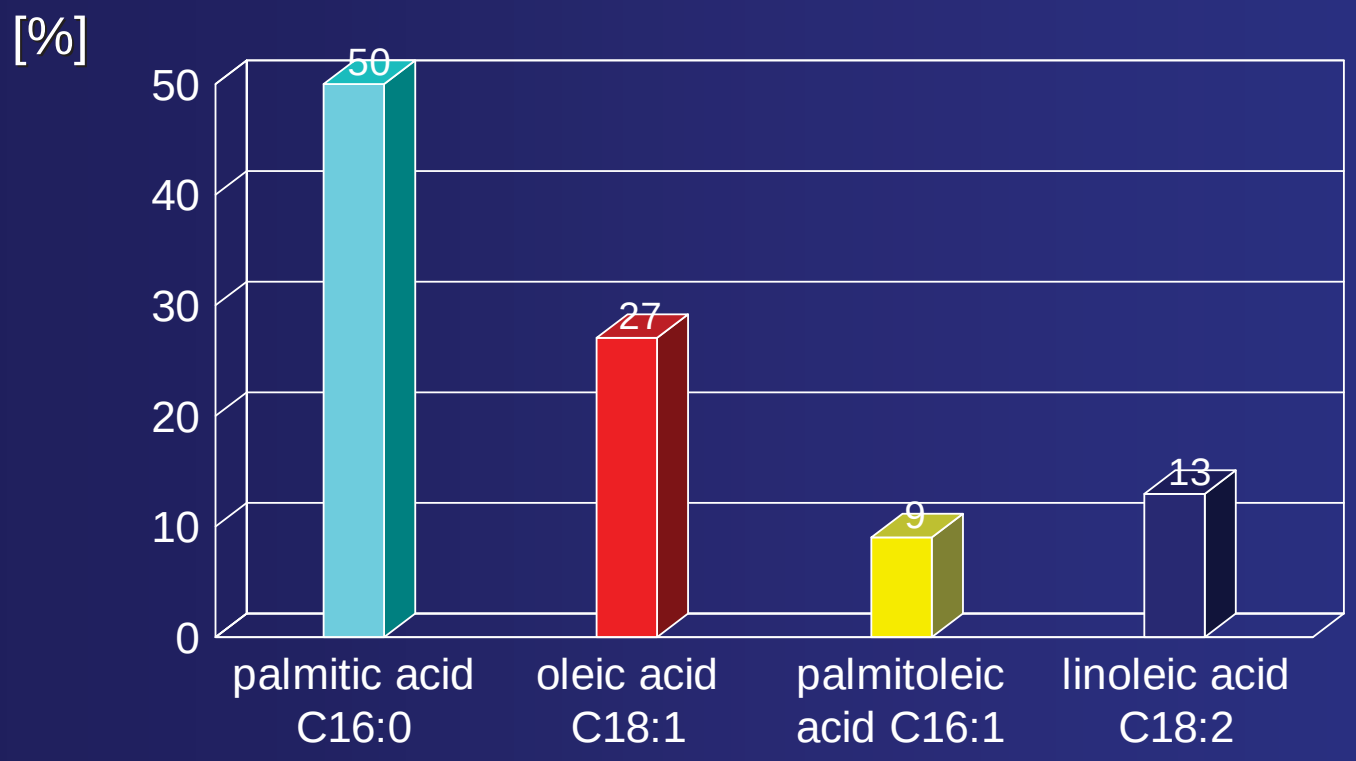
Figure 1: Proposed Mechanism of Action of L-Carnitine during Exercise.

Aim of the study

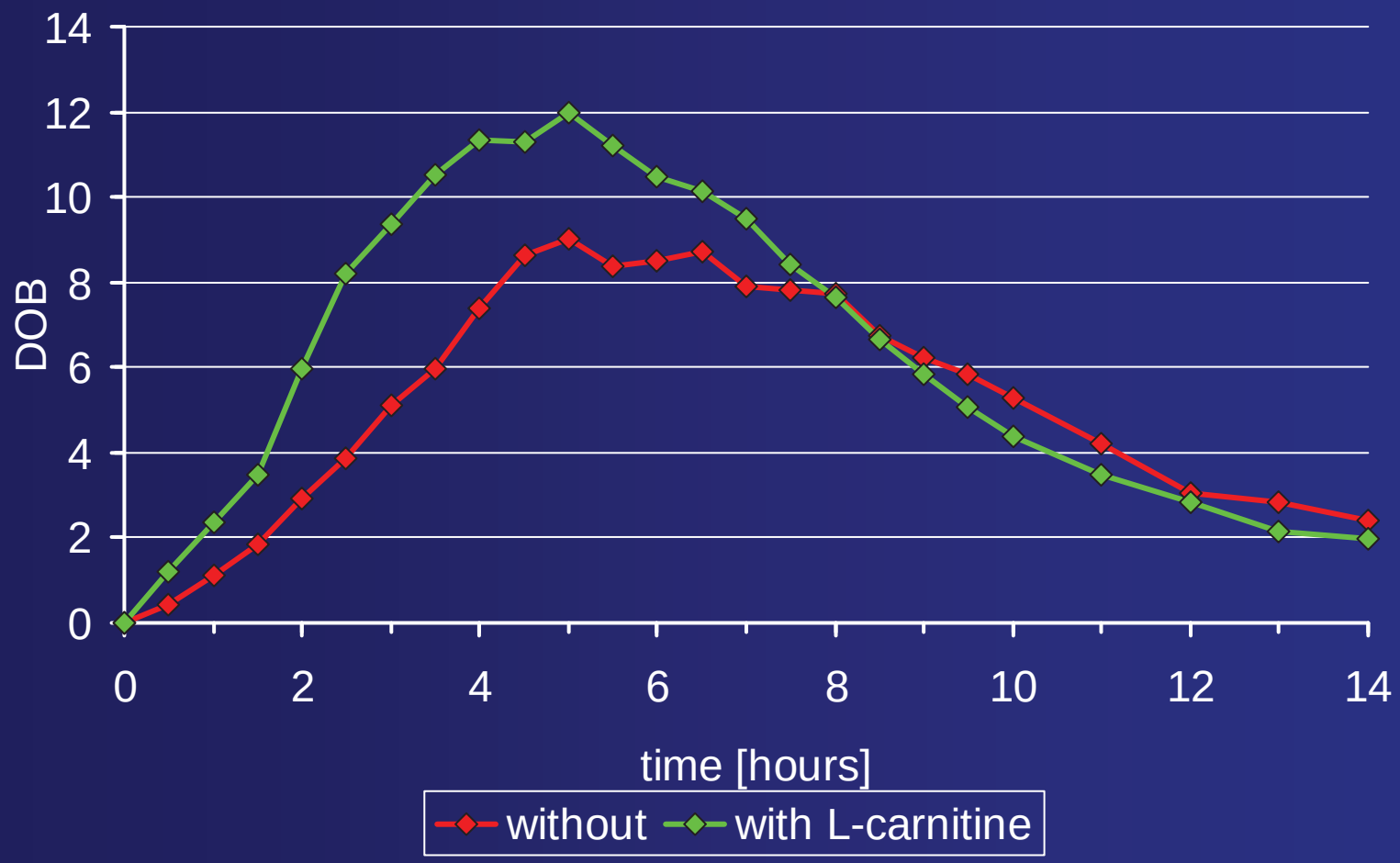
Evaluation of the metabolic effect of L-carnitine supplementation on

- whole-body protein turnover
  - [^{15}N]glycine, three-compartment model along with
- fat oxidation
  - [U-^{13}C]algae lipid mixture,
- in slightly obese subjects.

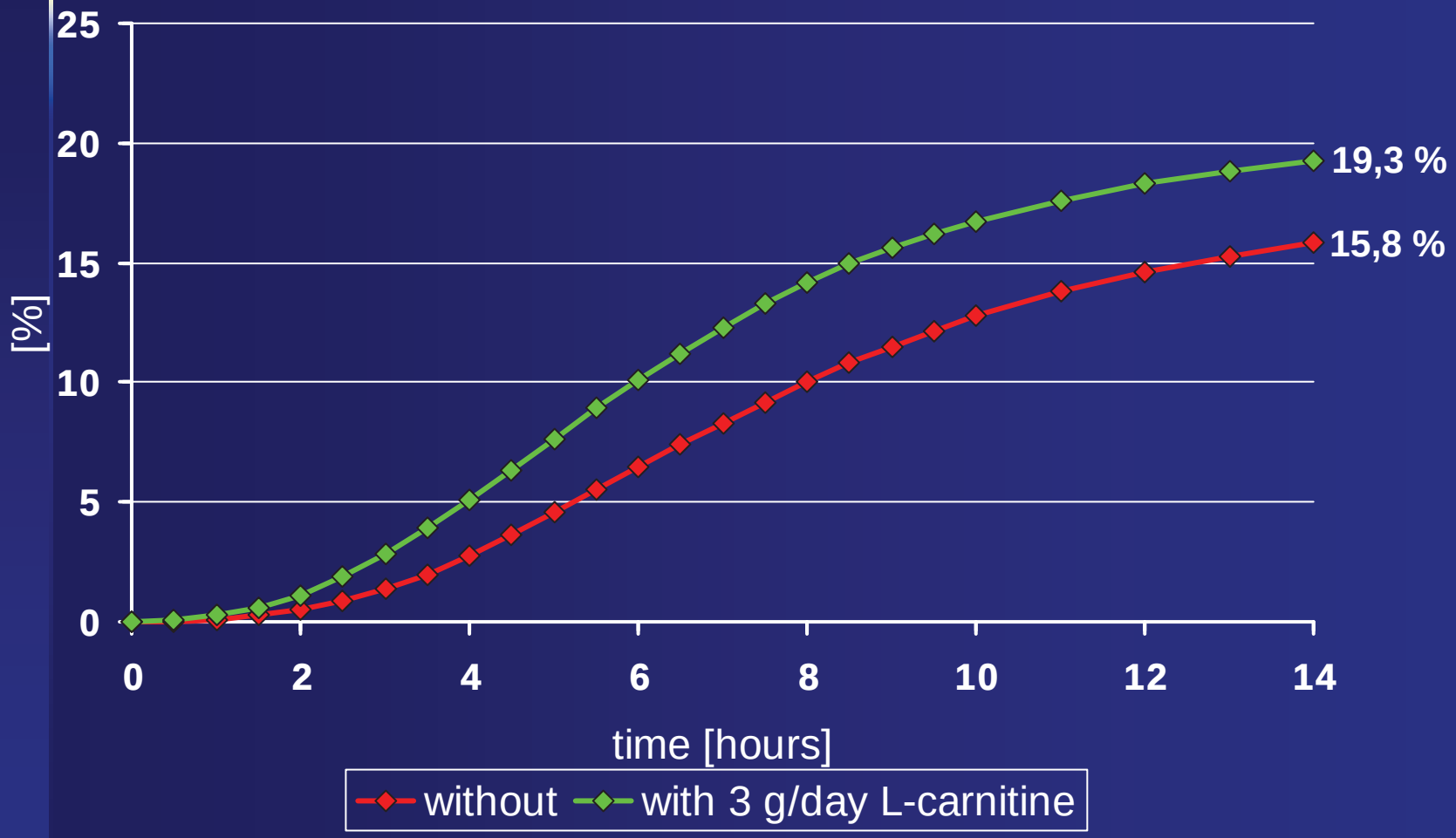
Composition of ^{13}C-algae fatty acid mixture
“Comparatively representative for fatty acid metabolism”



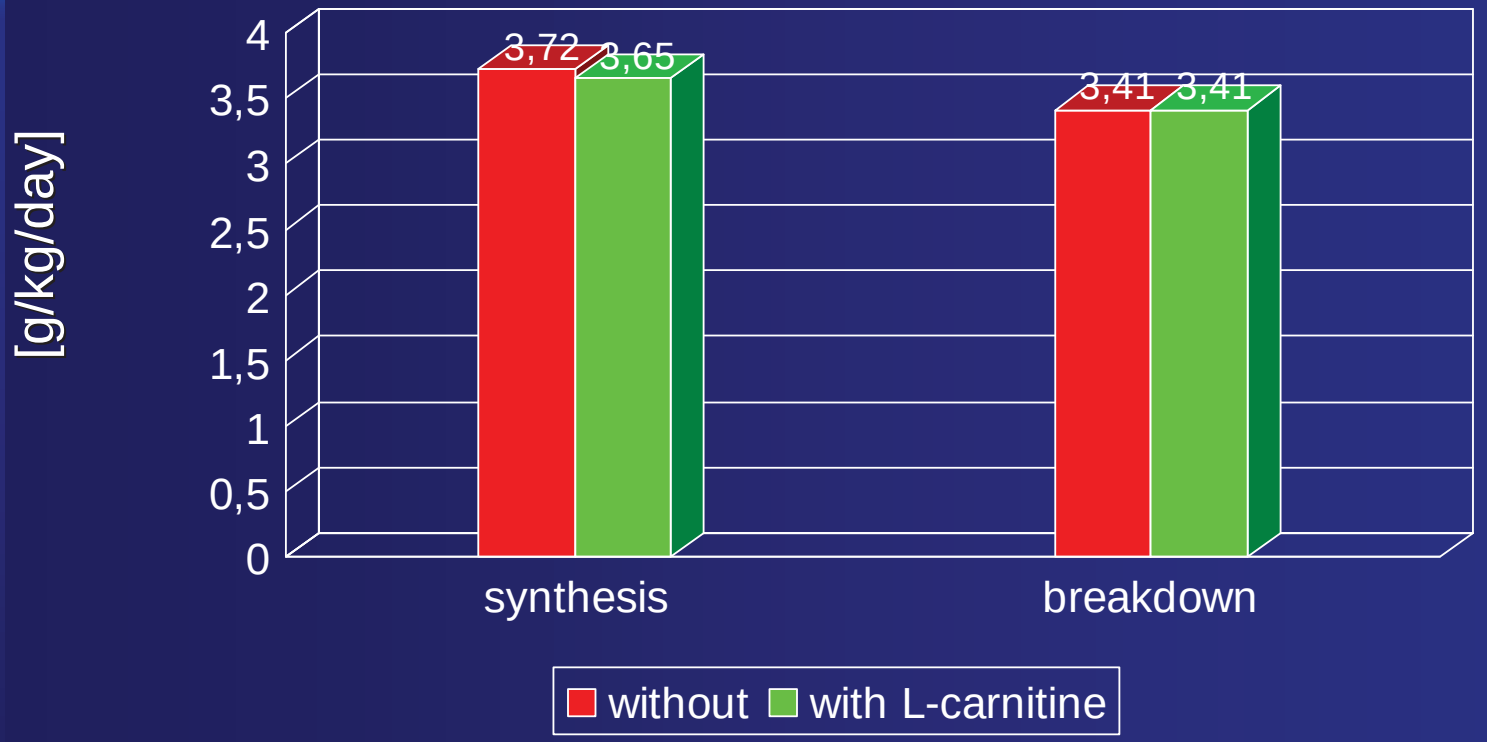
Results
$^{13}CO_2$-enrichmet



Cumulative $^{13}CO_2$-exhalation



Protein turnover rates



Interpretation, conclusion

- Anyway, it turned out that L-carnitine seemingly increased the fat oxidation in slightly obese subjects, whereas protein turnover remains unchanged.

Reprinted from
Vol 53 No 8
August 2004

ELSEVIER

Metabolism
Clinical and Experimental

The Effect of L-Carnitine on Fat Oxidation, Protein Turnover, and Body Composition in Slightly Overweight Subjects

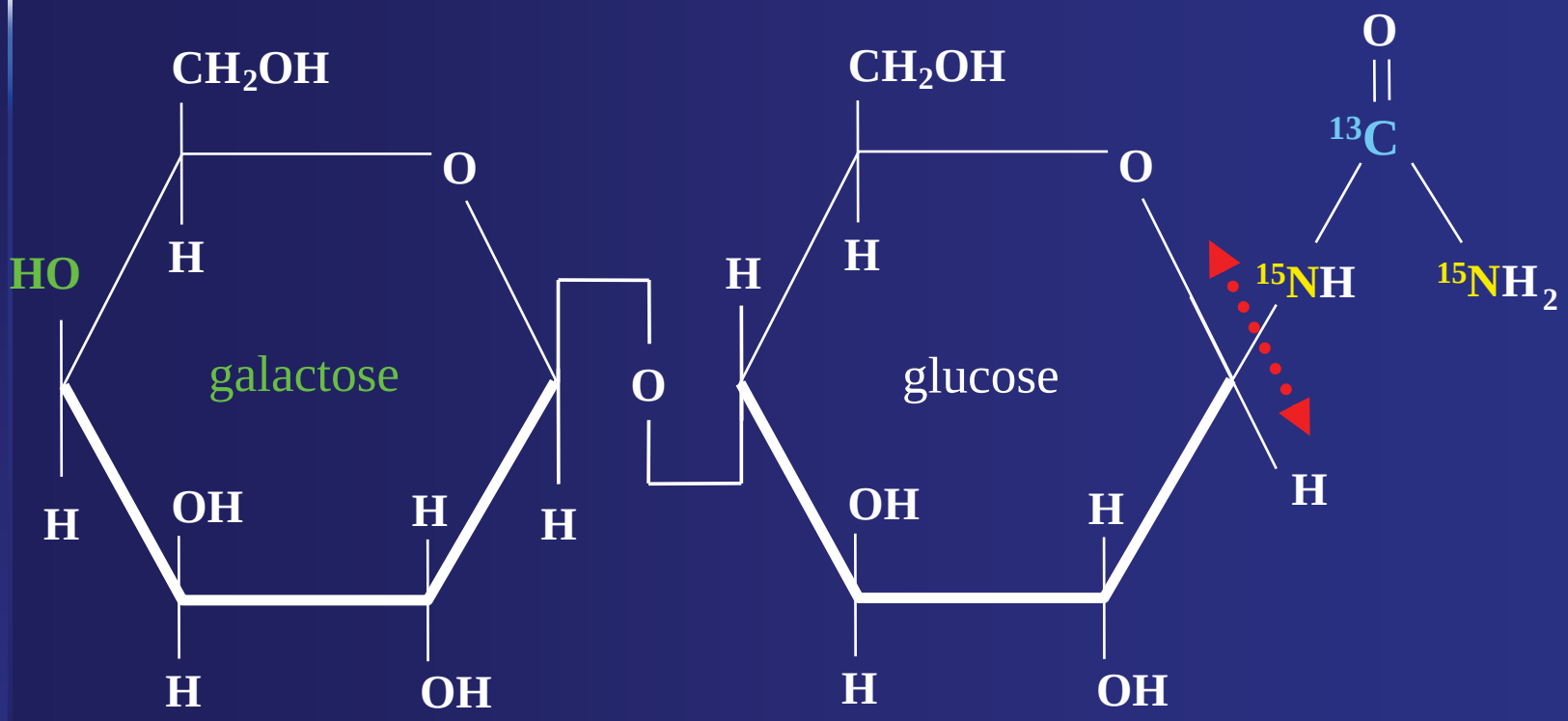
Klaus D. Wutzke, Henrik Lorenz

Glycosyl-[^{13}C]ureides for measuring the orocoecal transit time

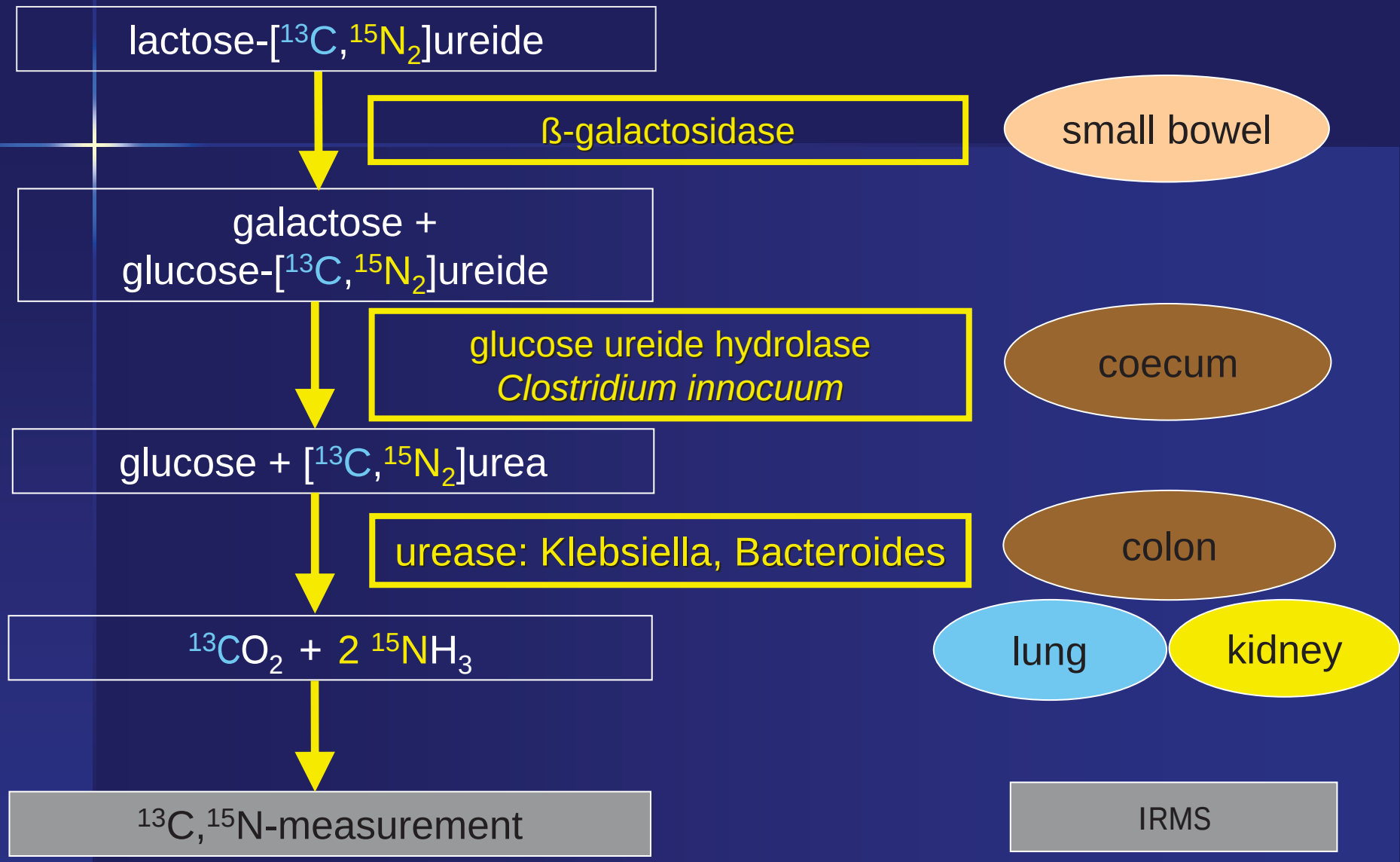
[^{13}C]-Sugar ureides

- Address: No enzymatic degradation of the sugar-urea bond in the small intestine alleged.
- *Clostridium innocuum* is accounted for the exclusive degradation.
- Ubiquitous present in the coecum (Mohr, BBA;1998).
- Launched in 1993 by Heine, AJG.
- Experienced with own laboratory synthesis: Lactose-[^{13}C]ureide: marker for measuring orocoecal transit time (OCTT). Credit: Wutzke, EJCN;1997, 2004, 2010.

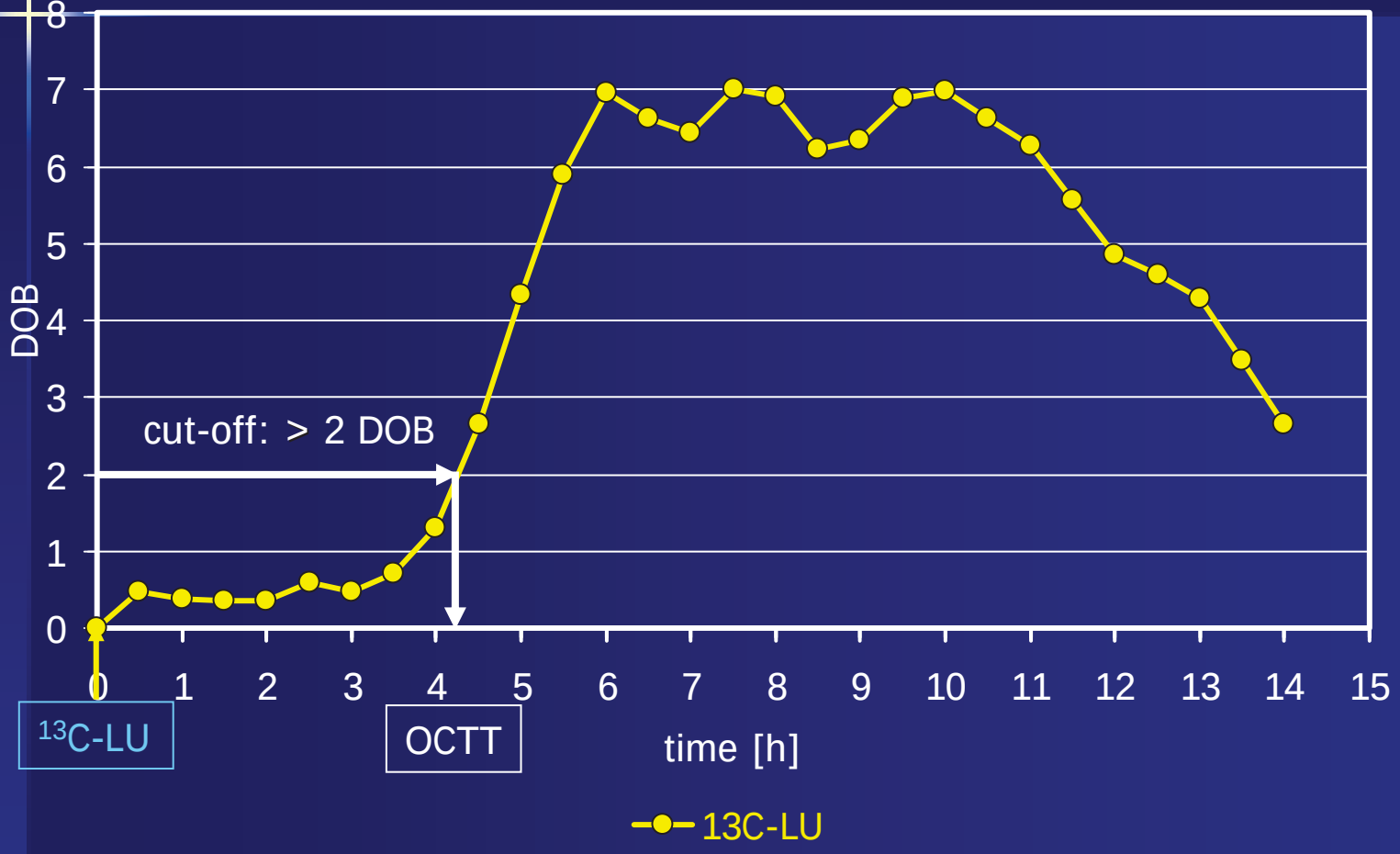
Lactose-[^{13}C,$^{15}N_2$]ureide (DLLU)



Gradual degradation of lactose-[^{13}C,$^{15}N_2$]ureide



Measurement of the orocoecal transit time with lactose-[^{13}C]ureide



European Journal of Clinical Nutrition (1997) 51, 11–19

Evaluation of oro-coecal transit time: a comparison of the lactose-[^{13}C, ^{15}N]ureide $^{13}CO_2$- and the lactulose H_2-breath test in humans

KD Wutzke, WE Heine, C Plath, P Leitzmann, M Radke, C Mohr, I Richter, HU Gülzow and D Hobusch

Children's Hospital, Department of Medicine, University of Rostock, PO Box 10 08 88, Rembrandtstr. 16/17, 18057 Rostock, Germany

Objective: The lactulose H_2-breath test is the most widely used non-invasive approach for evaluation of oro-coecal transit time (OCTT). In the present study, doubly-labelled lactose-[^{13}C, ^{15}N]ureide (DLLU) was synthesized to investigate the OCTT in comparison to the conventional lactulose H_2-breath test. Additionally, the bacterial breakdown rate (BBR) and rate of elimination and the metabolic pathways of the cleavage products of DLLU ($^{13}CO_2$, [^{15}N]urea, and $^{15}NH_3$) were investigated.

Design and subjects: In a first study, DLLU was administered as a single oral-pulse-labelling (dosage: one gram) either without and after pretreatment of five grams of unlabelled lactoseureide (LU) on the day prior to the study to twelve healthy adult volunteers after breakfast. Breath and urine were collected in one and two hour-intervals, respectively, over a one-day period. ^{13}C-enrichment in breath as well as ^{15}N-enrichment in urine fractions were measured by continuous flow-isotope ratio mass spectrometry (CF-IRMS). In a second study, lactulose was administered to the same subjects (dosage: ten grams). Breath was collected in quarter, half and one hour-intervals over a ten hour-period. Hydrogen concentration in breath was analysed using an electro-chemical detector.

Results: The comparison of the lactose-[^{13}C]ureide $^{13}CO_2$-breath test and the lactulose H_2-breath test showed that the mean increase of the ^{13}C-enrichment in CO_2 occurred 1.18 h later than the mean increase of H_2 in breath. The resulting OCTTs derived from the two methods were 3.02 ± 1.4 and 1.84 ± 0.5 h ($P < 0.05$) and the corresponding BBRs were 9.63 ± 3.4 and 6.07 ± 1.7 h ($P < 0.01$), respectively. The ^{15}N-enrichment of urinary urea and ammonia without and after pretreatment with LU started between two and three hours after DLLU-administration. The cumulative percentage urinary excretion of the ^{15}N- and ^{13}C-tracer was 29.9% and 13.6% respectively, and was slightly increased after LU-pretreatment to 32.1% and 14.6% of the dose administered. A total of 35.2% of the ^{13}C was found to be exhaled and remained approximately constant after LU-pretreatment (36.2%).

Conclusions: The use of the lactulose H_2-breath test for evaluation of the OCTT showed a statistically significant shortening of 1.18 h in comparison to the lactose-[^{13}C]ureide $^{13}CO_2$-breath test in healthy adults. The most important limitations of the lactulose H_2-breath test are its low specificity and sensitivity due to dose-dependent accelerations of OCTT, interfering H_2-rise from malabsorbed dietary fibre and H_2-non-producers. In contrast, our lactose-[^{13}C]ureide $^{13}CO_2$-breath test was confirmed to avoid these disadvantages and to yield reliable results. This test is recommended especially if higher sensitivity and specificity is required, if IRMS-technique is available and if lactulose H_2-tests lead to insufficient results.

Sponsorship: Deutsche Forschungsgemeinschaft (DFG).

Descriptors: $^{13}CO_2$-, H_2-breath tests, bacterial breakdown rate; doubly-labelled lactoseureide; lactulose; oro-coecal transit time

Introduction

The lactulose [4-(β-D-galactopyranosyl)-D-fructose] hydrogen (H_2)-breath test is a simple, inexpensive and non-invasive method widely used for quantifying the oro-coecal transit time (OCTT). After ingestion of lactulose, H_2 is produced during bacterial fermentation of the non-absorbable disaccharide lactulose in the human colon. Hydrogen is subsequently absorbed and either excreted in breath or metabolised by gut bacteria to CH_4, H_2S, and short chain fatty acids (Bond and Lewitt, 1978). The OCTT reflects arrival of the 'head' of the lactulose in the coecum and has been used as a marker of 'mouth to coecum transit time'.

Comparisons have been made between OCTTs reported in various lactulose H_2-breath test studies demonstrating the influences by several intestinal and other factors that cause a large variability of the values (King and Toskes, 1986; Jorge, Wexner and Ehrenpreis, 1994; Rumessen, Hamberg and Gudmand-Hoyer, 1989, 1990; Sarno *et al*, 1993; Stainforth and Rose, 1989; Wilberg, Pieramico and Malfertheiner, 1990).

Other conventional methods for the determination of OCTT are based on radiographic and scintigraphic methods using ^{111}In-labelled plastic particles, [^{14}C]lactulose and [^{99m}Tc]diethylene triamine-pentaacetic acid (DTPA)

Correspondence: KD Wutzke, Ph.D.
Received 29 February 1996; revised 12 September 1996; accepted 20 September 1996

European Journal of Clinical Nutrition (2004) 58, 568–572

www.nature.com/ejcn

ORIGINAL COMMUNICATION

The use of ^{13}C-labelled glycosyl ureides for evaluation of orocaecal transit time

KD Wutzke[1]* and B Glasenapp[2]

[1]*Children's Hospital, Research Laboratory, University of Rostock, Rostock, Germany; and* [2]*Department of Anaesthesia, University of Rostock, Rostock, Germany*

Objective: In the present study, cellobiose-[^{13}C]ureide and glucose-[^{13}C]ureide were synthesized and tested as alternative substrates for noninvasive evaluation of the orocaecal transit time (OCTT).

Design: Experimental study.

Intervention: In total, 1 g cellobiose-[^{13}C]ureide was administered together with a continental breakfast either without or after predosing of 5×1 g unlabelled cellobiose ureide on the day prior to study commencement. After 2 weeks, the same subjects ingested glucose-[^{13}C]ureide (dosage: 0.57 g) either without or after predosing of the respective unlabelled ureide under identical conditions. Expired air samples were taken over 10 h. $^{13}CO_2$-enrichment was measured by isotope ratio mass spectrometry (PDZ Europa, Sandbach, UK). The OCTT was calculated from the interval between ^{13}C-ureide administration and the detection of a significant and sustained ^{13}C-rise of 2 delta over baseline in breath.

Setting: University of Rostock, Children's Hospital, Research Laboratory.

Subjects: Eight healthy adults aged 22–55 y.

Results: After application of cellobiose-[^{13}C]ureide and glucose-[^{13}C]ureide OCTTs of 401 and 415 min, respectively, were measured. The predosing resulted in higher and steeper ^{13}C-enrichments and caused a significant shortening of OCTTs of 265 and 287 min, respectively ($P = 0.012$ and 0.017).

Conclusions: The onset of $^{13}CO_2$-enrichment reflected the degradation of glycosyl-[^{13}C]ureides by glucose ureide hydrolase. The predosing with unlabelled ureides prior to pulse labelling with cellobiose-[^{13}C]ureide and glucose-[^{13}C]ureide (the latter is the key substance of the enzymatic sugar-ureide degradation) led to an induction of enzyme activity and resulted in a more precise and similar estimation of the OCTT when using both ^{13}C-labelled ureides.

European Journal of Clinical Nutrition (2004) **58,** 568–572. doi:10.1038/sj.ejcn.1601846

Keywords: ^{13}C-labelled glycosyl ureides; enzyme induction; glucose ureide hydrolase; orocaecal transit time

Introduction

When quantifying the orocaecal transit time (OCTT), the lactulose hydrogen (H_2) breath test is the most widespread conventional method.

Comparisons among OCTTs that have been determined in various lactulose H_2-breath test studies have shown the influence of several intestinal and dose-dependent factors that can cause a large variability of the values (King & Toskes, 1986; Rumessen *et al*, 1990; Wilberg *et al*, 1990; Sarno *et al*, 1993). The development of noninvasive measuring methods of OCTTs is of great interest, with respect to the most important limitation of lactulose H_2-breath tests: transit disorders can occur especially after administration of high lactulose dosages (Sarno *et al*, 1993).

^{13}C-labelled glycosyl ureides formed under acidic conditions from reducing sugars and urea were found to be suitable for measurement of OCTTs (Heine *et al*, 1995). The molecular bond between the carbohydrate moieties and ^{13}C-urea has been shown to resist enzymatic degradation in the gastrointestinal tract, but to be split exclusively by *Clostridium innocuum* (Mohr *et al*, 1999). This property makes them suitable as markers for OCTT. In our investigations, lactose-[^{13}C]ureide was confirmed to yield reliable results and to avoid the disadvantages of lactulose H_2-breath tests (Wutzke *et al*, 1997).

In continuation of these investigations, we synthesized glucose-[^{13}C]ureide (^{13}C-GU), and a lactose-[^{13}C]ureide-related disaccharide ureide: cellobiose-[^{13}C]ureide (^{13}C-CU).

*Correspondence: KD Wutzke, University of Rostock, Children's Hospital, Research Laboratory, Rembrandtstrasse 16/17, D-18055 Rostock, Germany.
E-mail: klaus-dieter.wutzke@med.uni-rostock.de
Guarantor: KD Wutzke.
Contributors: BG was the principal investigator and doctoral candidate of KDW.
Received 17 February 2003; revised 3 June 2003; accepted 30 June 2003

Utilisation of doubly stable isotope labelled *Lactobacillus johnsonii* yoghurt in humans

- In the face of *Lactobacillus johnsonii* (La1), a probiotic lactobacillus strain of human origin, is evidently able to adhere to the intestinal mucosa.
- One of the important properties of probiotics is the ability to survive the dangerous journey through the intestine.
- For evaluation of this issue (phenomenon), La1 was used for doubly labelling with ^{15}N and ^{13}C to follow the metabolic fate of orally administrated doubly stable isotope ^{13}C-,^{15}N-labelled La1 in humans.

Aim of the study

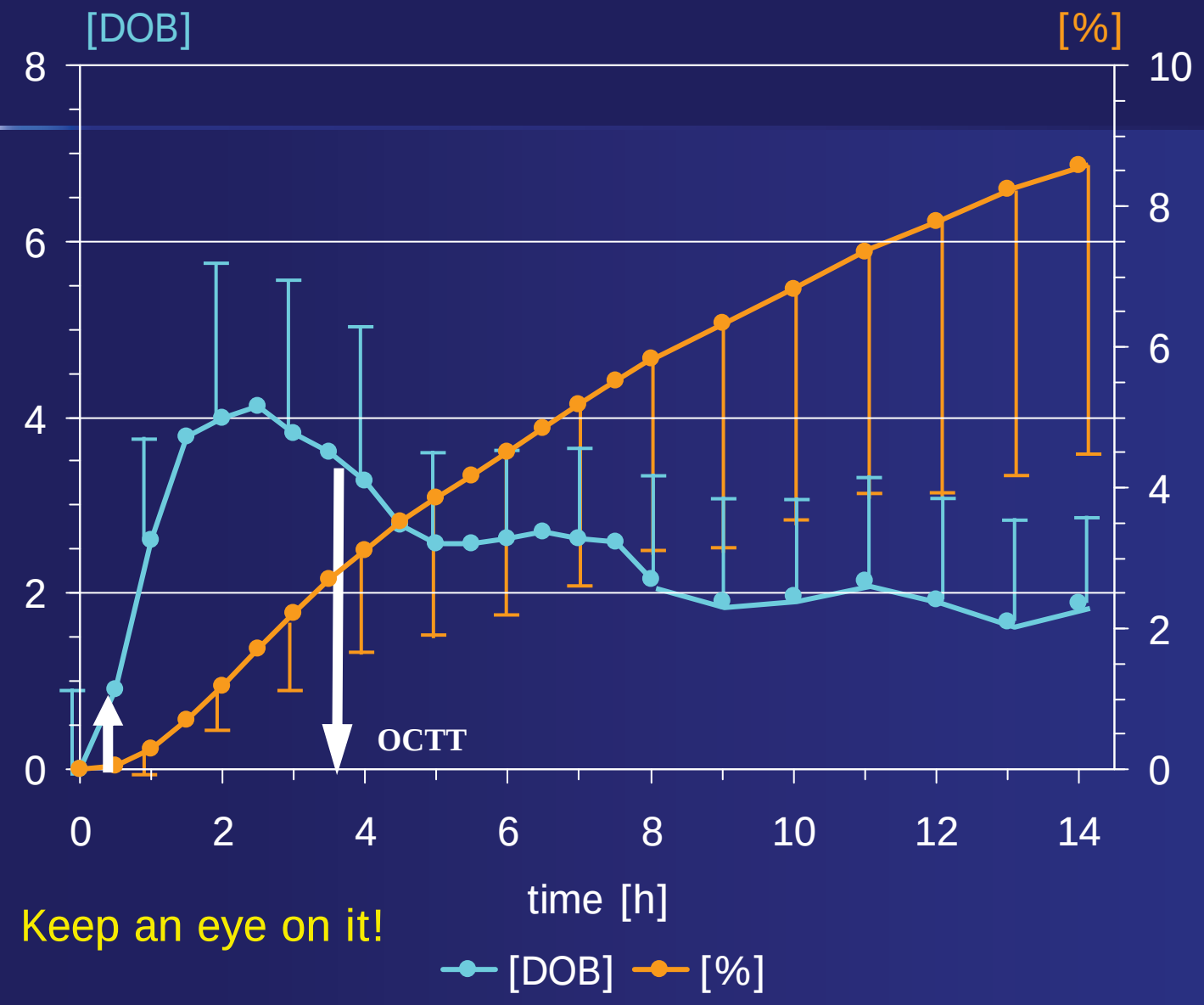
- Investigation of the metabolic fate of doubly ^{13}C-,^{15}N-labelled *Lactobacillus johnsonii*
  - the $^{13}CO_2$-exhalation,
  - the urinary and faecal ^{13}C- and ^{15}N-excretion, respectively,
  - and the corresponding isotopic enrichment of specific blood plasma fractions.

Material and methods

- Universal labelling of La1 with ^{13}C and ^{15}N
- by fermentation (Biostad, Braun, Melsungen, Germany) in a medium containing
  - [U-^{15}N]yeast extract (prior labelled by [^{15}N]H_4Cl) and
  - [U-$^{13}C_6$]glucose (Campro Scientific, Berlin)

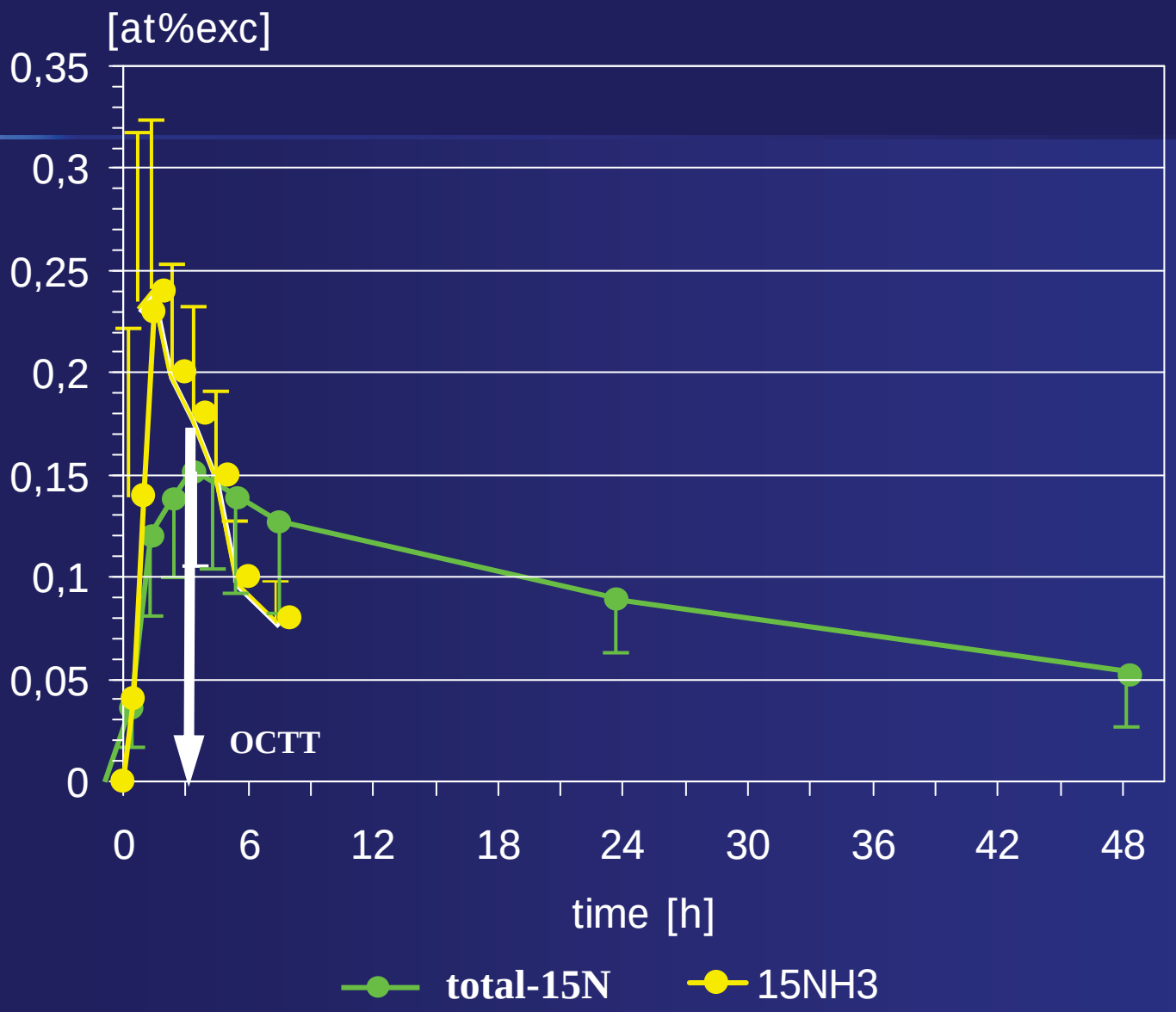
Fermenter Biostad, B. Braun, Melsungen

$^{13}CO_2$-Enrichment and cumulative percentage exhalation

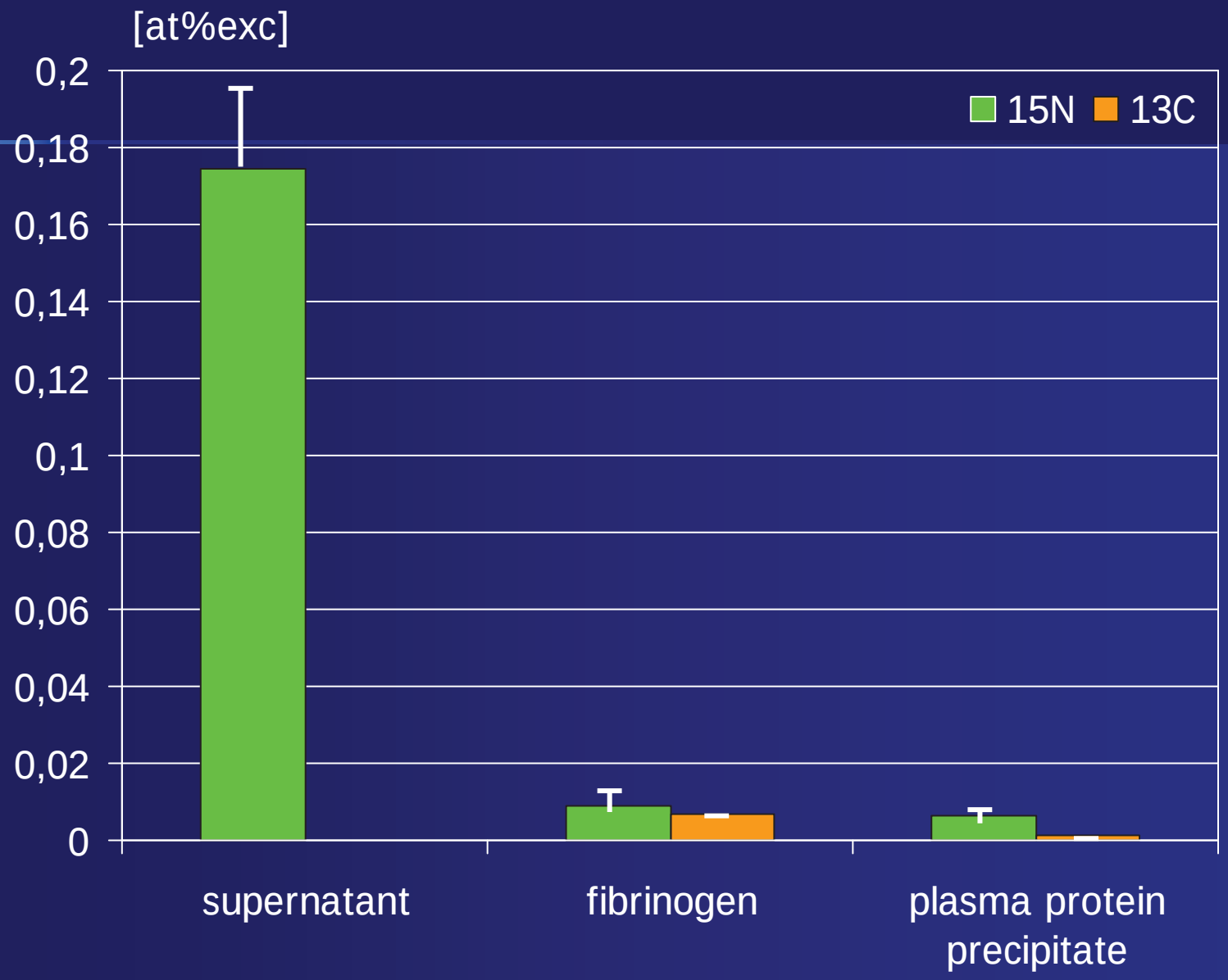


Keep an eye on it!

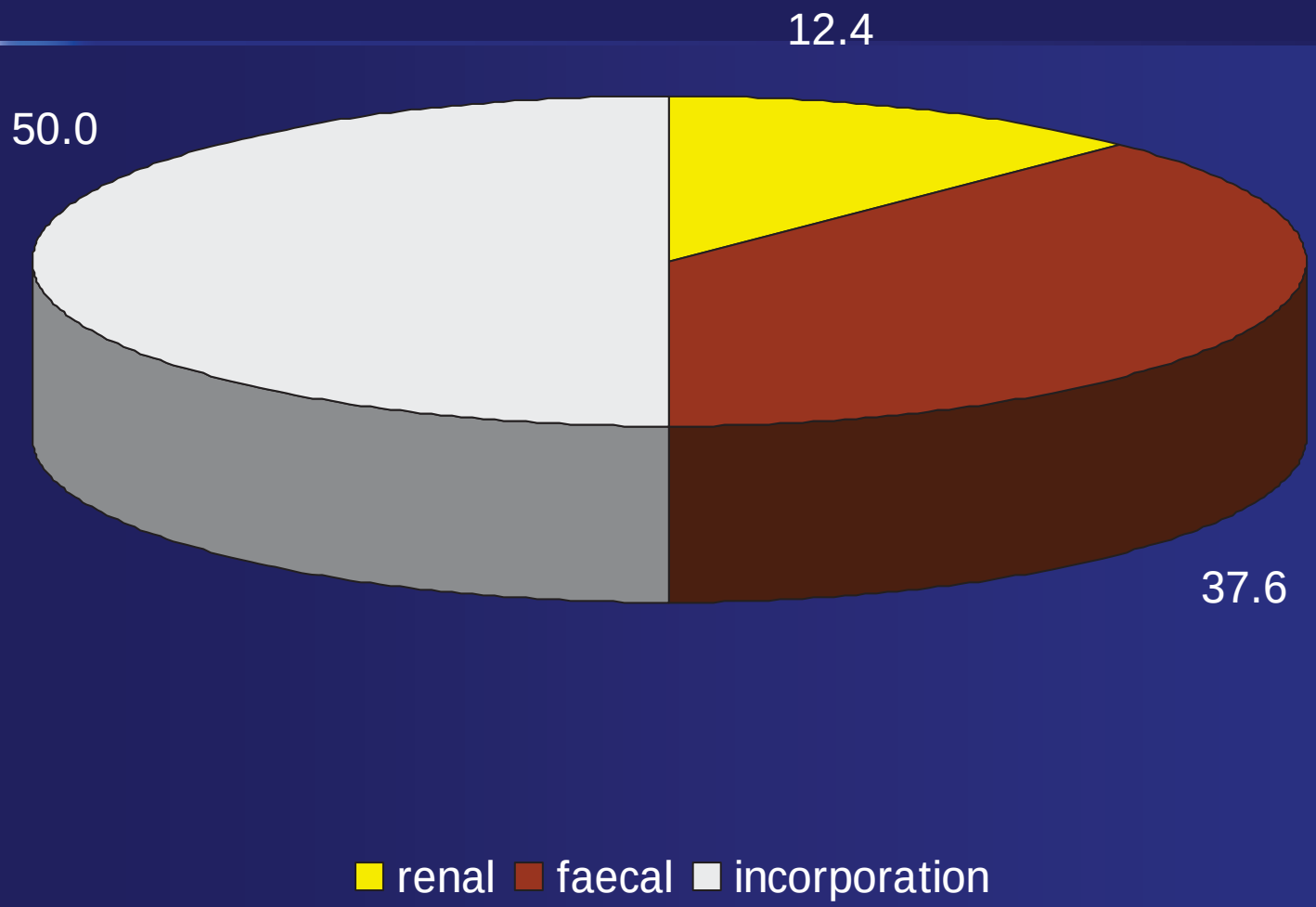
Urinary total and ammonia ^{15}N-enrichment



Isotope incorporation in different fractions of the blood



Percentage total ^{15}N-excretion and ^{15}N-incorporation



Percentage total ^{13}C-excretion and ^{13}C-incorporation