13C-methacetin breath test reproducibility study reveals persistent CYP1A2 stimulation on repeat examinations

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Abstract

AIM: To find the most reproducible quantitative parameter of a standard 13C-methacetin breath test (13C-MBT).

METHODS: Twenty healthy volunteers (10 female, 10 male) underwent the 13C-MBT after intake of 75 mg 13C-methacetin p.o. on three occasions. Short- and medium-term reproducibility was assessed with paired examinations taken at an interval of 2 and 18 d (medians), respectively.

RESULTS: The reproducibility of the 1-h cumulative 13C recovery (AUC0–60), characterized by a coefficient of variation of 10%, appeared to be considerably better than the reproducibility of the maximum momentary 13C recovery or the time of reaching it. Remarkably, as opposed to the short gap between consecutive examinations, the capacity of the liver to handle 13C-methacetin increased slightly but statistically significantly when a repeat dose was administered after two to three weeks. Regarding the AUC0–60, the magnitude of this fixed bias amounted to 7.5%. Neither the time gap between the repeat examinations nor the gender of the subjects affected the 13C-MBT reproducibility.

CONCLUSION: 13C-MBT is most reproducibly quantified by the cumulative 13C recovery, but the exactitude thereof may be modestly affected by persistent stimulation of CYP1A2 on repeat examinations.

INTRODUCTION

There is no surprise that non-invasive diagnostic procedures in medicine attract much attention from either side participating in the search of any cause of malfunctioning of the organism-patients would definitely prefer methods sparing them the necessity of encroaching the body integrity, like for example a liver biopsy, whereas physicians too would prefer to avoid inflicting pain on their patients. A brilliant idea of per oral administration of 13C-enriched substrates in order to get information on the metabolic efficiency or functional mass of the liver is a
practical answer to the requirement outlined above. Continuous research work has brought about considerable progress and nowadays it is possible to assess by means of $^{13}$C breath tests the microsomal, the cytosolic, and the mitochondrial function of the liver\textsuperscript{[1-4]}. A look at pertinent literature shows that during the past decade, from among the compounds applied for microsomal $^{13}$C breath tests, $^{13}$C-methacetin has steadily been making its way to be recognized as the most frequently used substrate. A number of features support its usefulness as a functional liver probe: a fast metabolism to acetaminophen and $^{13}$CO$_2$ by cytochrome P450 1A2 (CYP 1A2), safety at low doses applied for a breath test, and a low cost\textsuperscript{[1-4]}. Accordingly very promising results on its diagnostic usefulness were obtained in patients with chronic hepatitis C virus infection\textsuperscript{[5,6]}, primary biliary cirrhosis\textsuperscript{[7,8]}, non-alcoholic steatohepatitis\textsuperscript{[9]}, and various stages of liver cirrhosis\textsuperscript{[10-14]}, including those awaiting a liver transplantation\textsuperscript{[13]}. 

A vital asset of any measurement or diagnostic method used in medical practice is an ability to provide reproducible results. Quite surprisingly a search of data on the reproducibility of the $^{13}$C-methacetin breath test ($^{13}$C-MBT) revealed this item as being almost a completely blank research area. We decided therefore to search in this prospective study for a quantitative parameter which would offer the best reproducibility for a standard $^{13}$C-MBT.

**MATERIALS AND METHODS**

**Subjects**

Twenty healthy non-obese subjects, 10 female and 10 male were invited to enter the study; their mean age was 25 years (range 21-31 years), and their average body mass index was 22.38 ± 0.64 kg/m$^2$. During a screening interview the participants declared themselves as being almost a completely blank research area. We decided therefore to search in this prospective study for a quantitative parameter which would offer the best reproducibility for a standard $^{13}$C-MBT.

Every subject underwent three examination sessions, held on separate days. In half of the volunteers the two first sessions were taken 2-4 d apart, and the third one was pursued 2-3 wk later. In the other half of the subjects the schedule was inversed, i.e. the first two examination measurements were taken 2-3 wk apart, followed by a third one 2-4 d after the second one. The assignment of the order of intervals separating the sessions (short-long or long-short) was randomized. Accordingly, the assessment of the short-term reproducibility involved 20 pairs of examinations separated by a median 2 d break (range 2-4 d), whereas the medium-term reproducibility was evaluated on 20 pairs of the most distant examinations, i.e. taken at a median interval of 18 d (range 17-23 d).

The volunteers came to the laboratory in the morning, after a 12-h overnight fast and abstaining from cigarette smoking (if applicable). In the female volunteers the examinations were taken always within the same phase of their menstrual cycle. After a 15-min rest in a sitting position, necessary for stabilization of the metabolism, a basal sample of the exhaled air was collected. At the time point designated “0” the subjects took 75 mg $^{13}$C-methacetin (code INC590P, Euriso-Top SA, Saint-Aubin, France; according to the certificate of analysis, the manufacturer guarantees $\geq 99\%$ isotopic $^{13}$C atom enrichment determined by proton NMR) orally dissolved in 200 mL unsweetened black tea. Samples of expiratory air for $^{13}$C-breath test were collected at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 40, 50, 60, 75, 90, 105, 120, 150 and 180 min\textsuperscript{[20]}. The procedure of collecting the breath air was standardized: the subjects took in breath and held it for 10 s, then steadily blew the air into a special aluminium covered plastic bag of 1.1 L capacity (Fischer Analysen Instrumente GmbH, Leipzig, Germany) equipped with a mouthpiece and an unidirectional valve\textsuperscript{[21,22]}. The bag was closed with a plastic cork immediately at the end of the exhalation and stored for analysis. The subjects remained seated quietly and fasted throughout the whole period of the collection of the samples.

**Measurement of $^{13}$CO$_2$: and derivation of the breath test parameters**

Concentrations of $^{13}$CO$_2$: in the probes of the exhaled air were measured with the use of a non-dispersive isotope-selective infrared spectrometry apparatus (IRIS, manufactured by Wagner Analysen Technik Vertriebs GmbH, Hamburg, Germany; a model equipped with 16 ports for simultaneous mounting of bags with air samples was used). Following the procedures described previously\textsuperscript{[20,21,24]}, curves of the momentary and cumulative recovery of $^{13}$C in the exhaled air relative to the administered oral dose of $^{13}$C-methacetin were constructed within the time domain of 0 to 180 min. Subsequently the following parameters were derived: $D_{\text{max}}$ - the maximum momentary $^{13}$C recovery in expiratory air, $T_{\text{max}}$ - the time elapsing from the intake of the substrate until the occurrence of the $D_{\text{max}}$, and $AUC$ - the cumulative $^{13}$C recovery within...
Table 1  Reproducibility of the $^{13}$C-methacetin breath test

|                | $D_{\text{max}}$ | $T_{\text{max}}$ | AU$C_{0-60}$ |
|----------------|------------------|------------------|--------------|
| $S_{\text{term}}$ | 16.23%           | 30.57%           | 10.00%       |
| $M_{\text{term}}$ | 16.46%           | 32.49%           | 10.00%       |
| $S_{\text{term}}$ | 15.55% dose/h    | 16.86 min        | 5.84% dose   |
| $M_{\text{term}}$ | 16.80% dose/h    | 16.73 min        | 5.86% dose   |
| $S_{\text{term}}$ | N                | N                | N            |
| $M_{\text{term}}$ | N                | Y                | N            |
| $S_{\text{term}}$ | 3.80% dose/h     | 4.0 min          | 1.43% dose   |
| $M_{\text{term}}$ | 3.51% dose/h     | 3.8 min          | 1.26% dose   |

$T_{\text{max}}$: Time to reach the maximum momentary $^{13}$C elimination; $D_{\text{max}}$: Maximum momentary $^{13}$C elimination; AU$C_{0-60}$: 60-min cumulative $^{13}$C elimination in expiratory air, respectively; $S_{\text{term}}$: Short term reproducibility assessment on the basis of 20 pairs of $^{13}$C-methacetin breath tests separated by a median 2-d break; $M_{\text{term}}$: Medium-term reproducibility evaluation involved 20 pairs of examinations accomplished at a median interval of 18 d. CV$_{p}$: Coefficient of variation for paired examinations; RC: Repeatability coefficient. Delta$^{0.05}$: The least difference detectable at $P = 0.05$ level, two-tailed in the case of 20 paired examinations.

RESULTS

Similar basal concentrations of $^{13}$CO$_{2}$ in the expiratory air were observed on the three study days, amounting to -25.79‰ ± 0.25‰, -25.44‰ ± 0.21‰ and -25.59‰ ± 0.22‰. After oral intake of the $^{13}$C-methacetin solution an expected biphasic course of $^{13}$CO$_{2}$ content within the exhaled air was observed, characterized by a rapid rise followed by a less steep decline. R_ANOVA indicated that when referred to the basal situation, the rise in breath $^{13}$CO$_{2}$ was statistically significant between the 6th and the 150th minute (Figure 1).

Basic reproducibility assessment

As shown in Table 1, the $T_{\text{max}}$ exhibited rather an unsatisfactory reproducibility, whereas the $D_{\text{max}}$ displayed a fair reproducibility, supposedly sufficient from the point of view of diagnostic applications of the breath test. The reproducibility of the cumulative $^{13}$C recovery relative to the administered substrate dose was strongly dependent on the time span included for the calculation of this parameter, and improved rapidly with inclusion of the subsequent measurement points (Figure 2). The AU$C_{0-60}$ attained a CV$_{p}$ of 10%-identical in both the case of the...
short- or the medium-term reproducibility. The inclusion of data from beyond the 60 min did not bring about much refinement of the reproducibility of the AUC.

No statistically significant difference between the short- and medium-term reproducibility of the breath test parameters was found. Moreover, none of the parameters considered had a different short- or medium-term reproducibility in men and women.

Bland and Altman statistics of reproducibility

Bland and Altman plots pertaining to representative parameters of the $^{13}$C-MBT are provided in Figure 3. Bland and Altman statistics revealed no proportional bias in either $D_{\text{max}}$ or $\text{AUC}_{0-60}$ (Table 1). The same applied to the short-term reproducibility of $T_{\text{max}}$, whereas analysis of its medium-term reproducibility showed that the slope of the linear regression of the between-day differences on the corresponding means of the paired measurements was statistically significantly different from zero (Table 1).

The Bland and Altman statistic disclosed a fixed bias in the case of the medium-term reproducibility of either $D_{\text{max}}$ or $\text{AUC}_{0-60}$. It means that the mean differences between the paired measurements taken a median of 18 d apart differed statistically significantly from zero. A closer look at the pertinent differences indicated that the ability of the liver to handle $^{13}$C-methacetin increased when a repeat dose was administered after two to three weeks ($D_{\text{max}}$: $34.60\% \pm 2.75\%$ dose/h on the first administration vs $39.08\% \pm 2.77\%$ dose/h on the second administration, $P = 0.015$; $\text{AUC}_{0-60}$: $20.80\% \pm 0.99\%$ dose on the first administration vs $22.27\% \pm 0.98\%$ dose on the second administration, $P = 0.017$). No such effect was observed if two doses of $^{13}$C-methacetin were administered in close sequence ($D_{\text{max}}$: $34.26\% \pm 2.41\%$ dose/h on the first administration vs $34.91\% \pm 2.68\%$ dose/h on the second administration, not significant (NS); $\text{AUC}_{0-60}$: $21.16\% \pm 0.96\%$ dose on the first administration vs $21.03\% \pm 1.07\%$ dose on the second administration, NS]. A graphical representation of the phenomenon observed is provided in Figure 4.

DISCUSSION

Without any doubt provision of reproducible measurement results is a feature of paramount importance, expected to be assured by methods designed for research and/or clinical applications in medicine. Breath tests performed with the use of stable isotopes, such as $^{13}$C, cannot be considered an exception in this respect, especially because during the past decade their clinical use has been constantly growing. An increase in the number of relevant scientific papers indicates that, from among the tests dedicated to assess the activity of cytochrome P450, known as the microsomal breath tests, the $^{13}$C-MBT has lately taken the lead. Accordingly, Yaron Ilan in his very recent review provides evidence-based arguments that the $^{13}$C-MBT is a powerful tool to aid hepatologists in bedside decision making.

While searching the literature we came across only one study which was aimed at the evaluation of the reproducibility of the $^{13}$C-MBT. Petrolati et al performed repeat measurements separated by an interval of 12 wk in 10 healthy volunteers. This scarcity of data encouraged us to undertake a systematic, prospective study on the reproducibility of the quantitative measures of a standard $^{13}$C-MBT. Much effort was given in order to assure comparable conditions while performing the $^{13}$C-MBT. Accordingly, all the examinations were started at the same time in the morning, a 15-min rest always preceded the collection of the basal samples of expiratory air, the procedure of taking the breath samples was standardized, and comfortable surroundings were provided to the volunteers so that they could stay relaxed while maintaining a sitting position throughout the examination. Moreover, the female participants were always examined in the same phase of the menstrual cycle. While designing the study protocol we decided to adopt the currently accepted mode of administration of $^{13}$C-methacetin, namely a fixed oral dose of 75 mg. It should be noted, however, that formerly other body mass adjusted dosage regimens were applied, encompassing 5 mg/kg in the pioneer work by Krambiegel et al, then 2 mg/kg, as well as 1 mg/kg, and finally Ikura et al applied in infants 0.5 mg/kg $^{13}$C-methacetin.

Taking into account the investigative nature of our research, we generously took as many as 19 samples of breath air throughout 3 h. This approach appears to be precedent in nature, because for routine clinical use just a few measurement points are usually considered. Nine samples were collected in the study by Zipprich et al at (10, 20, 30, 40, 50, 60, 80, 100 and 120 min after application of the substrate), and twelve samples of the expiratory air—every 15 min for 3 h of observation —were taken by Ciccocepioppo et al. A team of German researchers from Bochum collected thirteen samples—at 5, 10, 15, 20,
One should mention that our frequent sampling of breath air (an aliquot was taken every 3 min during the first half hour) is similar to the new approach recently proposed by Lalazar et al. who introduced a “continuous” $^{13}$C-MBT wherein samples of breath air are taken every 2-3 min and analysed with a laser-based device.

Our intention was to determine the length of the $T_{\text{max}}$ with the greatest exactitude possible. Nevertheless, with the CV$_P$ between 30.6% and 32.5%, the reproducibility of this parameter appeared to be a bit disappointing. Apparently the $T_{\text{max}}$ may therefore not be the most useful parameter for the purposes of clinical decision making.

On the other hand, we established on the basis of the within-subject study protocol that the repeatability of the $T_{\text{max}}$ was sufficient to discern a difference in its length as small as 4 min ($P = 0.05$ level, two-tailed, $n = 20$).

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**Figure 3** Bland and Altman statistics (plot of differences between pairs vs their means) of the short- (filled diamonds) and medium-term (open rectangles) reproducibility of the $^{13}$C-methacetin breath test. $T_{\text{max}}$: Time to reach the maximum momentary $^{13}$C elimination (panel A); $D_{\text{max}}$: Maximum momentary $^{13}$C elimination (panel B); $\text{AUC}^{0-60}$: 60-min cumulative $^{13}$C elimination in expiratory air (panel C). On each panel the respective borders of the 95% confidence intervals are plotted.
Thus a convincing proof of reliability of this test has been obtained. Quite unexpectedly a thorough analysis of reproducibility, involving the application of the Bland and Altman statistic, disclosed the existence of a fixed bias in the case of the medium-term reproducibility of the 24h and the AUC0. It was found that those two parameters increased statistically significantly if the repeat examinations were separated by a two- to three-week gap, which was not the case when the results of examinations taken at a close sequence were compared. A similar trend can, however, be discerned when looking at the results obtained by Petrolati et al\(^{[13]}\) in their attempt to evaluate the reproducibility of the \(^{13}\)C-MBT. They found that the 45-min cumulative \(^{13}\)C recovery (AUC\(_{0-45}\)) in breath air after peroral intake of 75 mg \(^{13}\)C-methacetin amounted to 17.5% ± 2.8% dose and to 18.8% ± 4.3% dose on the first and the second occasion, respectively. Although the two means did not differ statistically significantly (\(P = 0.30\)), it is evident that the AUC\(_{0-45}\) determined on the second occasion increased by 7.4%. Quite a similar magnitude of increment was determined in our study-the AUC\(_{0-60}\) rose by 7.5% on the repeat measurement taken after two to three weeks (\(P = 0.017\)), whereas it remained unchanged with an average difference of -0.6% (NS) between measurements performed a median of 2 d apart. A delayed and persisting stimulation of a minor, but statistically significant, magnitude of the CYP1A2 capacity to metabolize methacetin would therefore be inferred. It remains unclear if the phenomenon disclosed would have a clinically relevant impact. It is rather obvious that not all patients with liver disease require performance of a series of \(^{13}\)C-MBTs, which most likely may pertain to those awaiting liver transplantsations\(^{[19]}\). At present one cannot predict whether a stimulation of CYP1A2 observed on repeated \(^{13}\)C-MBT in healthy volunteers will occur also in patients with major impairment of the liver functional reserve. Nevertheless the error in estimating the cumulative \(^{13}\)C recovery in breath air caused by a stimulation of CYP1A2 by \(^{13}\)C-methacetin seems to be quite small, not exceeding 8% according to our results, as well as those obtained by Petrolati et al\(^{[13]}\).

Summing up, the study renders evidence that the \(^{13}\)C-MBT provides satisfactorily reproducible results. Remarkably, the cumulative \(^{13}\)C recovery appear to be the most reproducible quantitative index of the test, the computation of which over the first hour following administration of the substrate offers a reasonable 10% coefficient of variation, regardless of the time span separating the repeat measurements. One should be aware that the exactitude of this parameter may be modestly affected by a persistent stimulation of CYP1A2 on repeat examinations.

**COMMENTS**

**Background**

Great progress has been made during the past two decades with respect to the diagnostic use of stable isotopes in hepatology - it is now possible to assess by means of \(^{13}\)C breath tests the microsomal, the cytosolic, or the mitochondrial function of the liver. From the point of view of a patient those tests are particularly attractive as in many instances they may result in avoidance of the inconvenience of a repeat liver biopsy.
Research frontiers

A crucial asset of any measurement or diagnostic method used in medical practice is provision of reproducible results. Stable isotope breath tests cannot be exempted from this scrutiny. Quite surprisingly, the reproducibility of one of the most popular among the 13C breath tests applied in hepatology, the 13C-methacetin breath test (13C-MBT) has not been sufficiently examined before.

Innovations and breakthroughs

This prospective study is the first one dedicated to thorough evaluation of the repeatability of the 13C-MBT. According to the study results, cumulative 13C recovery is the most reproducible quantitative index of the 13C-MBT, the computation of which over the first hour following administration of the substrate offers a 10% coefficient of variation, regardless of the time span separating the repeat measurements. An additional finding is that in the case of repeat examinations the exactitude of this parameter may be modestly affected by a persistent stimulation of CYPIA2 responsible for a fixed bias which amounted to 7.5%.

Applications

Promising results on the usefulness of the 13C-MBT to evaluate reliably and accurately the metabolic liver functional reserve were documented in patients with various stages of liver cirrhosis, including those awaiting a liver transplantation, as well as those with chronic hepatitis C virus infection, primary biliary cirrhosis, or non-alcoholic steatohepatitis.

Terminology

The breath tests dedicated to get information on the metabolic efficiency of the liver are based on an elegant but simple idea of monitoring the elimination of 13CO2 in expiratory air after per oral administration of 13C-enriched substrates. Depending on the chemical structure of such substrates, the microsomal, the cytosolic, or the mitochondrial function of the liver may be evaluated noninvasively.

Peer review

The study was conducted carefully and the paper is well written.

REFERENCES

1. Ilan Y. Review article: the assessment of liver function using breath tests. Aliment Pharmacol Ther 2007; 26: 1293-1302
2. Braden B, Lembcke B, Kuker W, Caspary WF. 13C-breath tests: current state of the art and future directions. Dig Liver Dis 2007; 39: 795-805
3. Modak AS. Stable isotope breath tests in clinical medicine: a review. J Breath Res 2007; 1: R1-R13
4. Armuza A, Candelli M, Zocco MA, Andreoli A, De Lorenzo A, Nista EC, Miele L, Cremonini F, Cazzato IA, Grieco A, Armuzzi A, Candelli M, Zocco MA, Andreoli A, De Lorenzone A, Nista EC, Miele L, Cremonini F, Cazzato IA, Grieco A, Candelli M, Zocco MA, Andreoli A, De Lorenzone A, Nista EC, Miele L, Cremonini F, Cazzato IA, Grieco A. Exact profiles of 13CO2 concentration and normal ALT. J Viral Hepat 2006; 13: 295-303
5. Braden B, Faust D, Sarrazin U, Zeuzem S, Dietrich CF, Caspary WF, Sarrazin C. 13C-methacetin breath test as liver function test in patients with chronic hepatitis C virus infection. Aliment Pharmacol Ther 2002; 16: 1977-1996
6. Lafazzi L, Macchi R. Hershovici T, Hadjaj T, Shubu M, Ohana H, Hemed N, Ilan Y. A continuous 13C methacetin breath test in noncirrhotic patients with chronic hepatitis C virus infection. J Viral Hepat 2008; 15: 716-728
7. Holtmeier J, Leuschner M, Schneider A, Leuschner U, Caspary WF, Braden B. 13C-methacetin and 13C-galactose breath tests can assess restricted liver function even in early stages of primary biliary cirrhosis. Scand J Gastroenterol 2006; 41: 1336-1341
8. Schneider A, Caspary WF, Saich R, Dietrich CF, Sarrazin C, Kuker W, Braden B. 13C-methacetin breath test shortened: 2-point-measurements after 15 minutes reliably indicate the presence of liver cirrhosis. J Clin Gastroenterol 2007; 41: 33-37
9. Portincasa P, Grattagliano I, Lauterburg BH, Palmieri VO, Palaciano G, Stellard F. Liver breath tests non-invasively predict higher stages of non-alcoholic steatohepatitis. Clin Sci (Lond) 2006; 111: 135-143
10. Matsumoto K, Suhio M, Liu M, Kawabe T, Shiratori Y, Okano K, Sugimoto T. 13C-methacetin breath test for evaluation of liver damage. Dig Dis Sci 1987; 32: 344-348
11. Klatt S, Taut C, Mayer D, Adler G, Beckh K. Evaluation of the 13C-methacetin breath test for quantitative liver function testing. Z Gastroenterol 1997; 35: 609-614
12. Pfaffenbach B, Götz O, Szymanski C, Hagemann D, Adamek RJ. [The 13C-methacetin breath test for quantitatively noninvasive liver function analysis with an isotope-specific nondispersive infrared spectrometer in liver cirrhosis]. Dtsch Med Wochenschr 1998; 123: 1467-1471
13. Zipprich A, Meiss F, Steudel N, Szegoleit U, Fleig WE, Kleber G. 13C-Methacetin metabolism in patients with cirrhosis: relation to disease severity, haemoglobin content and oxygen supply. Aliment Pharmacol Ther 2003; 17: 1559-1562
14. Festi D, Capodicasa S, Sandri L, Colaiocco-Ferrante L, Stanisic A, Vitacolonna E, Vestito A, Simonpi M, Mazzella G, Portincasa P, Roda E, Colechia A. Measurement of hepatic functional mass by means of 13C-methacetin and 13C-phe-noylalanine breath tests in chronic liver disease: comparison with Child-Pugh score and serum bile acid levels. World J Gastroenterol 2005; 11: 142-148
15. Petrolati A, Festi D, De Berardinis G, Colaiocco-Ferrante L, Di Paolo D, Tisone G, Angelico M. 13C-methacetin breath test for monitoring hepatic function in cirrhotic patients before and after liver transplantation. Aliment Pharmacol Ther 2003; 18: 785-790
16. The representatives of 61 States. Preamble to the Constitution of the World Health Organization. Proceeding of the International Health Conference; 1946 Jun 19-Jul 22. New York: Official Records of the World Health Organization, 1946: 100
17. Jonderko K, Kasicka-Jonderko A, Szykiewicz-Trepiak D, Błońska-Fajfrowska B. Feasibility of a breath test with a substrate of natural 13C-abundance and isotope-selective non-dispersive infrared spectroscopy: a preliminary study. J Gastroenterol Hepatol 2005; 20: 1228-1234
18. Kasicka-Jonderko A, Jonderko K, Kamińska M, Szymszal M, Długajczyk M, Bula M, Bielecka M, Błońska-Fajfrowska B. Breath 13CO2 profiles after intake of three naturally abundant in 13C foods rich in carbohydrates. Ann Acad Med Siles 2006; 60: 206-212. Available from: URL: http://journals.indexescopernicus.com/abstracted.php?cid=0676292
19. Jonderko K, Kasicka-Jonderko A, Kamińska M, Błońska-Fajfrowska B. A systematic study of a neutral meal suitable for subjects undergoing 13CO2 breath tests. Med Sci Monit 2008; 14: CR543-CR546
20. Kasicka-Jonderko A, Jonderko K, Chabior E, Błońska-Fajfrowska B. Exact profiles of 13CO2 recovery in breath air after per oral administration of [13C] methacetin in two groups of different ages. Isotopes Environ Health Stud 2008; 44: 295-303
21. Jonderko K, Duś Z, Szymszal M, Kasicka-Jonderko A, Błońska-Fajfrowska B. Normative values for the 13C-mixed triglyceride breath test in two age groups. Med Sci Monit 2009; 15: CR255-CR259
22. Jonderko K, Tisone G, Angelico M. A continuous 13C-methacetin breath test for noninvasive assessment of intrahepatic inflammation and fibrosis in patients with chronic HCV infection and normal ALT. J Viral Hepat 2008; 15: 716-728
23. Jonderko K, Spinková M, Kamińska M, Kasicka-Jonderko A, Błońska-Fajfrowska B. Ability to digest starchy assessed non-invasively with a 13C2O2 breath test - comparison of results obtained in two groups of different age. Med Sci Monit 2009; 15: CR128-CR133
24. Kasicka-Jonderko A, Jonderko K, Kamińska M, Błońska-Fajfrowska B. Interference of acute cigarette smoking with [13C]methacetin breath test. Isotopes Environ Health Stud 2011; 47: 34-41
25. Kasicka-Jonderko A, Jonderko K, Kamińska M, Bielecka M, Błońska-Fajfrowska B. 13C-alpha-Ketoisocaproic acid breath test revisited: an in-depth reproducibility study advocates an extended breath sampling period. Dig Dis Sci 2007; 52: 3481-3487
26. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1: 307-310

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26 Brennan P, Silman A. Statistical methods for assessing observer variability in clinical measures. BMJ 1992; 304: 1491-1494

27 Loo FD, Palmer DW, Soergel KH, Kalbfleisch JH, Wood CM. Gastric emptying in patients with diabetes mellitus. Gastroenterology 1984; 86: 485-494

28 Jonderko K. Short- and long-term reproducibility of radioisotopic examination of gastric emptying. Int J Rad Appl Instrum B 1990; 17: 297-301

29 Armitage P. Statistical methods in medical research. Oxford: Blackwell Scientific Publications, 1978

30 StatSoft. Electronic statistics textbook. Tulsa: StatSoft, 2007. Available from: URL: http://www.statsoft.com/textbook/stathome.html

31 Jonderko K, Gabriel-Jaśniok A, Szymszal M, Kasicka-Jonderko A, Błońska-Fajfrowska B. Unreliability of breath methane as a candidate indicator of functional bowel disorders. Gut Liver 2008; 2: 180-185

32 Krusiec-Swidergol B, Jonderko K. Multichannel electrogastrography under a magnifying glass—an in-depth study on reproducibility of fed state electrogastrograms. Neurogastroenterol Motil 2008; 20: 625-634

33 Jonderko K, Kasicka-Jonderko A, Krusiec-Swidergol B, Dzielicki M, Strój L, Doliński M, Doliński K, Błońska-Fajfrowska B. How reproducible is cutaneous electrogastrography? An in-depth evidence-based study. Neurogastroenterol Motil 2005; 17: 800-809

34 Kasicka-Jonderko A, Szymszal M, Jonderko K, Piekarska A, Błońska-Fajfrowska B. Reproducibility of liquid gastric emptying measurement with the use of an ultra low dose of 13C-Sodium acetate and isotope-selective nondispersive infrared spectrometry. Ann Acad Med Siles 2005; 59: 144-152. Available from: URL: http://tomeb.viamedica.pl/aams/pdf/2.pdf

35 Kasicka-Jonderko A, Kamieńska M, Jonderko K, Setera O, Błońska-Fajfrowska B. Short- and medium-term reproducibility of gastric emptying of a solid meal determined by a low dose of 13C-octanoic acid and nondispersive isotope-selective infrared spectrometry. World J Gastroenterol 2006; 12: 1243-1248

36 Ilan Y. A fourth dimension in decision making in hepatology. Hepatol Res 2010; 40: 1143-1154

37 Liu YX, Huang LY, Wu CR, Cui J. Measurement of liver function for patients with cirrhotosis by 13C-methacetin breath test compared with Child-Pugh score and routine liver function tests. Chin Med J (Engl) 2006; 119: 1563-1566

38 Lalazar G, Adar T, Ilan Y. Point-of-care continuous (13)C-methacetin breath test improves decision making in acute liver disease: results of a pilot clinical trial. World J Gastroenterol 2009; 15: 966-972

39 Krumbiegel P, Günther K, Faust H, Möbius G, Hirschberg K, Schneider G. Nuclear medicine liver function tests for pregnant women and children. I. Breath tests with 14C-methacetin and 13C-methacetin. Eur J Nucl Med 1985; 10: 129-133

40 Wutzke KD, Forberger A, Wigger M. Effect of alcohol consumption on the liver detoxication capacity as measured by [13C]methacetin- and [methyl-13C]methionine-breath tests. Isotopes Environ Health Stud 2008; 44: 219-226

41 Ciccocioppo R, Candelli M, Di Francesco D, Ciocca F, Taglieri G, Armuzzi A, Gasbarrini G, Gasbarrini A. Study of liver function in healthy elderly subjects using the 13C-methacetin breath test. Aliment Pharmacol Ther 2003; 17: 271-277

42 Ikura Y, Iwasaki A, Tsubaki T, Akasawa A, Onida T, Katsumun T, Miura K, Ebisawa M, Saito H, Koya N. Study of liver function in infants with atopic dermatitis using the 13C-methacetin breath test. Int Arch Allergy Immunol 1995; 107: 189-193

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