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Sensitivity and specificity of an abbreviated ^{13}C-mixed triglyceride breath test for measurement of pancreatic exocrine function

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Abstract

Background: A modified ^{13}C-mixed triglyceride breath test (^{13}C -MTGT) detects moderate pancreatic exocrine insufficiency noninvasively and reliably, but it requires prolonged breath sampling (6 hours (hr)).

Objective: We aimed to investigate whether ^{13}C -MTGT can be abbreviated, to optimize clinical usability.

Methods: We analyzed the ^{13}C-MTGT of 200 consecutive patients, retrospectively. Cumulative 1–5 hr ^{13}C-exhalation values were compared with the standard parameter (6-hr cumulative ^{13}C-exhalation). We determined the sensitivity and specificity of shortened breath sampling periods, by comparison with the normal values from 10 healthy volunteers, whom also underwent a secretin test to quantitate pancreatic secretion. Moreover, we evaluated the influence of gastric emptying (GE), using a ^{13}C-octanoic acid breath test in a subset ($N = 117$).

Results: The 1–5 hr cumulative ^{13}C-exhalation tests correlated highly and significantly with the standard parameter ($p < 0.0001$). Sensitivity for detection of impaired lipolysis was high (≥77%), but the specificity was low (≥38%) for the early measurements. Both parameters were high after 4 hrs (88% and 94%, respectively) and 5 hrs (98% and 91%, respectively). Multivariate linear correlation analysis confirmed that GE strongly influenced early postprandial ^{13}C-exhalation during the ^{13}C-MTGT.

Conclusion: Shortening of the ^{13}C -MTGT from 6 to 4 hrs of duration was associated with similar diagnostic accuracy, yet increased clinical usability. The influence of GE on early postprandial results of the ^{13}C-MTGT precluded further abbreviation of the test.

Keywords

Breath test, diagnostic testing, gastric emptying, lipolysis, mixed triglyceride breath test, optimization, pancreatic disease, pancreatic function

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Introduction

At present, clinicians almost exclusively use the fecal elastase-1 test for measurement of pancreatic exocrine function, although this test has limited sensitivity for mild-to-moderate pancreatic exocrine insufficiency, and despite its limited specificity in patients with diarrhea.[1,2] The lack of clinically available alternatives prompted creation of a research project supported by the United European Gastroenterology (UEG) National Societies LINK Award Programme, Harmonising Diagnosis and Therapy of Pancreatitis across Europe (HaPanEU), which explicitly aims to standardize the use of diagnostic criteria and test tools for pancreatic exocrine insufficiency. In particular, this research project aims to define the optimal way to perform and analyze alternative pancreatic function tests, such as secretin-enhanced magnetic resonance cholangiopancreatography (MRCP) and ^{13}C-breath tests, under clinical conditions.

We have shown previously that a modified version of the ^{13}C-mixed triglyceride breath test (^{13}C-MTGT) detects moderate pancreatic exocrine insufficiency non-invasively and reliably.[3] Compared with other versions of the ^{13}C-MTGT, our modified test uses higher lipid

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loads, in order to exceed lipolytic capacity in individuals with moderately decreased enzyme secretion and employs strict limitation of physical activity, to reduce the variability of endogenous carbon dioxide production. The ^{13}C-MTGT is generally based on the principle that intestinal triglyceride absorption requires prior hydrolysis by pancreatic lipase to produce free fatty acids and mono-acyl-glycerol. These metabolites are absorbed and transported to the liver. Hepatic metabolism subsequently leads to formation of $^{13}CO_2$ that is absorbed into the bloodstream, transported to the lung and exhaled. It has been shown that the increase in $^{13}CO_2$-concentration in breath correlates with pancreatic lipase secretion.[3–5]

The optimal parameter for evaluation of pancreatic exocrine function by ^{13}C-MTGT, reported by various groups including ours, is cumulative $^{13}CO_2$-exhalation (in a percentage of dose administered) over 5–8 hours (hrs)[3–6]; thus, although the ^{13}C-MTGT is a rather convenient, noninvasive indirect pancreatic function test, its long period of breath sampling and patient immobilization is a major drawback for clinical application. Therefore, as part of the UEG National Societies' LINK Award Programme HaPanEU, we aimed to study whether it is possible to shorten the ^{13}C-MTGT for clinical purposes. Because digestion of dietary lipids by pancreatic lipase cannot occur before the meal has entered the duodenum, we additionally aimed to investigate whether gastric emptying velocity influences the results of the ^{13}C-MTGT.

To achieve these goals, we retrospectively analyzed 200 consecutive ^{13}C-MTGT performed at our institution and we compared the cumulative ^{13}C-exhalation over 1- to 5-hr periods with the standard parameter, 6 hr of cumulative ^{13}C-exhalation. These patients' data were compared to those of healthy volunteers, in whom pancreatic exocrine function was quantified using both the secretin-test as the 'gold standard' for pancreatic function testing and the ^{13}C-MTGT. Furthermore, in a subset of 117 patients whom also received a ^{13}C-octanoic acid breath test (^{13}C-OAT) for measurement of gastric emptying velocity, we tested the influence of gastric emptying on the results of the ^{13}C-MTGT test.

Materials and methods

Participants

We identified and retrospectively analyzed data from 200 consecutive patients whom underwent a ^{13}C-MTGT at our institution for clinical reasons, between January 2010 and February 2011. These patients had presented with symptoms suggestive of pancreatic disease and/or pancreatic exocrine insufficiency, such as upper abdominal pain, bloating, diarrhea/steatorrhea and weight loss.

Data were compared to those of 10 healthy subjects (mean age 28 ± 1 years, mean body mass index (BMI) of 23.1 ± 0.8 kg/m^2 (six were women)), in whom a secretin test was performed. Such direct tests using hormone stimulation are the most sensitive and specific tests for assessing the pancreatic exocrine reserve; and therefore, are accepted as reference standards.[7,8] All of our healthy volunteers also received a ^{13}C-MTGT and a gastric emptying test, ^{13}C-OAT. The data from these subjects have been partially published before.[3]

Ethics

The study protocol for evaluation of pancreatic exocrine function in healthy volunteers was approved by the local ethics committee (Ethik-Kommission der Ärztekammer Hamburg, reference number 1822) and all subjects gave written informed consent prior to any study-related procedures.

Secretin test, a direct pancreatic function test

The tip of a double lumen Lagerlöf tube was placed into the distal duodenum, for constant collection of gastric and duodenal juice on ice, during a 30 min basal period and then during 60 min with receipt of an intravenous infusion of 1U/kg*hr secretin (Secrelux®, Goldham Bioglan Pharma GmbH, Zusmarshausen, Germany). This duodenal juice was fractionated in 10-min intervals and we performed analyses of pH, volume, bicarbonate and enzyme outputs, as described previously.[9–12]

^{13}C-mixed triglyceride breath test, an indirect test of pancreatic function

All the subjects evaluated in this study received a standardized test meal,[3] consisting of two slices of white bread, 20 g butter and 30 g chocolate cream (Nutella®, Ferrero Rocher, Germany). The latter was carefully mixed with 250 mg ^{13}C-MTG (2-octanoyl(1-13C)-1,3 distearoyl glycerol, Euriso-top, Saarbrücken, Germany, catalog number INC650P), a triglyceride with ^{13}C-octanoic acid labeled with one ^{13}C-atom bound to the Sn-2 position and unmarked long-chain fatty acids bound to the Sn-1 and Sn-3 positions. The meal was ingested within 10 min, together with 200 ml of water. Its total caloric value was 420 kcal (1770 kJ). All subjects were instructed to remain seated during study procedures, and we collected breath samples before ingestion of the test meal and every 30 minutes, for the 6 hrs thereafter. We determined the $^{13}CO_2/^{12}CO_2$ isotope ratio in the subject's breath by

using isotope-selective non-dispersive infrared spectrometry (IRIS®, Kibion/Wagner Analysen Technik, Bremen, Germany).[13] Our results were analyzed as delta values and expressed as cumulative ^{13}C-exhalation, in the percentages of dose recovered over the 1–6 hr intervals. Our tests were performed by three nurses highly experienced in pancreatic function testing and finally, evaluated by a gastroenterologist.

Gastric emptying breath test

A subset of 117 patients additionally underwent a ^{13}C-OAT for measurement of gastric emptying of solids.[14–16] Their test meal consisted of 200 ml orange juice, two slices of white bread, 10 g butter, 50 g ham and one scrambled egg, with the yolk doped with 91 mg ^{13}C-octanoic acid (Euriso-top, Saarbrücken, Germany) and was to be ingested within 10 min. We collected breath samples before the test meal and at 15-min intervals for 4 hrs, postprandially. Our subjects were instructed to remain seated throughout the test procedures. The $^{13}CO_2/^{12}CO_2$ isotope ratio in the subjects' breath was determined by using IRIS.[13] Results were analyzed as delta values and expressed as cumulative ^{13}C-exhalation in the percent of dose recovered over the 1–4 hr intervals.[16] We evaluated the half time of gastric emptying (T ½) and lag time (T lag), according to methods in Ghoos et al.[14] Tests were performed by three nurses whom were highly experienced in pancreatic function testing and finally, they were evaluated by a gastroenterologist. We excluded ^{13}C-OAT data from further analysis if the subjects had received a modified (vegetarian) test meal and/or if the time interval between both breath tests exceeded 4 weeks.

Definitions and statistical methods

We have shown before that cumulative ^{13}C-exhalation over 6 hrs (cut-off: 26.8% of the dose administered) is the best parameter for evaluation of the ^{13}C-MTGT and has an excellent sensitivity (100%) and specificity (92%) for detection of impaired lipase secretion in patients with pancreatic afflictions, when compared with the secretin test as the reference standard.[3] Thus, we used cumulative ^{13}C-exhalation over 6 hrs as the internal reference parameter for evaluating both sensitivity and specificity of shorter patient breath-sampling periods. We did this by testing for whether the established parameter and breath test data obtained over shorter periods of time gave compatible results, indicating either normal or reduced pancreatic exocrine function. Furthermore, we divided patients with decreased exocrine function, according to the standard parameter, as having exhalation that was: severely impaired (cumulative ^{13}C-exhalation over 6 hrs <8.9% of dose), moderately impaired (cumulative ^{13}C-exhalation over 6 hrs 8.9–17.9% of dose) or mildly impaired (cumulative ^{13}C-exhalation over 6 hrs that was 17.9% to <26.8% of the original dose given) pancreatic exocrine function. We determined the normal values for the abbreviated breath sampling periods from the data obtained in healthy volunteers ($n = 10$, defined as the mean value of healthy controls – SD).

We used 2-tailed Student's *t*-tests for paired and unpaired data, for statistical analyses and univariate or multivariate linear regression analyses, applying them as appropriate. For the association between cumulative ^{13}C-exhalation over 6 hrs and cumulative ^{13}C-exhalation over shorter periods of time, we additionally used the Deming regression (http://peltiertech.com/WordPress/deming-regression-utility/), as both parameters can be considered to be experimental. We expressed our study data as a mean ± SD, unless indicated otherwise. We performed statistical analyses using the JMP® version 6.0.3 (SAS Institute, Cary, NC, USA).

Results

Of the 200 patients investigated, 19 had incomplete data, so they were excluded from further analysis (Figure 1). Among the remaining 181 patients there were 116 women, the patients' mean age was 52.0 ± 17.7 years and their mean body mass index (BMI) was 23.4 ± 4.8 kg/m^2. Breath tests were generally tolerated well by all subjects and no relevant adverse events occurred. We compared the cumulative ^{13}C-exhalation (in percent of dose administered) over the 1- to 6-hr intervals with normal values in these subjects.

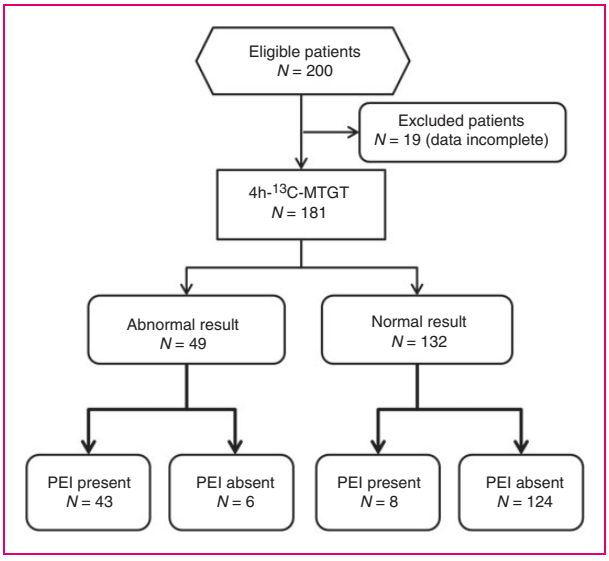

Figure 1. Patient flow.
^{13}C-MTGT: ^{13}C-mixed triglyceride breath test; PEI: pancreatic exocrine insufficiency.

Table 1. Normal values for the ^{13}C-exhalation over 1–5 h, as obtained in healthy volunteers, and the correlation in patients between cumulative ^{13}C-exhalation over 1–5 h and the standard 6-h parameter

	Normal value (% of dose)	Correlation coefficient	Sensitivity	Specificity
1 h	≥1.6%	0.66[a]	97%	38%
2 h	≥4.2%	0.82[a]	93%	71%
3 h	≥7.9%	0.89[a]	77%	88%
4 h	≥13.8%	0.94[a]	88%	94%
5 h	≥20.9%	0.98[a]	98%	91%

[a] $p < 0.0001$.
h: hours.

In 130 patients, our standard parameter for measurement of pancreatic exocrine function (that is, 6-hr cumulative ^{13}C-exhalation) was equal to or exceeded 26.8% of the dose administered and was therefore regarded as normal,[3] but 51 patients had pathologically decreased 6-h cumulative ^{13}C-exhalation that was compatible with impaired lipolysis and pancreatic exocrine insufficiency.

Cumulative ^{13}C-exhalation over 1–5 hrs correlated in a highly significant manner with the standard parameter, i.e. 6-hr cumulative ^{13}C-exhalation, as is shown in Table 1. Individual data for cumulative ^{13}C-exhalation over 4 and 5 hrs are presented in Figure 2. Conventional linear regression and Deming regression gave almost identical results for both correlation lines (4 h: linear: $y = 0.68x - 3.59$, $R = 0.94$ versus Deming: $y = 0.68x - 3.67$, $R = 0.97$; and 5 h: linear: $y = 0.86x - 2.22$, $R = 0.99$ versus Deming: $y = 0.86x - 2.23$, $R = 0.99$).

When taking normal values obtained in healthy volunteers into account (Table 1), sensitivity was already high for early postprandial measurements (1 and 2 hrs postprandially); however, specificity was low at these time points. Cumulative ^{13}C-exhalation over 4 hrs was the first measurement that showed satisfactory results for both sensitivity and specificity (Table 1 and Figure 2, cut off: 13.8% of ^{13}C-dose exhaled cumulatively). Importantly, all subjects with more than mildly impaired lipolysis according to the standard parameter were also detected using the 4 hr value, whereas 8 out of 30 patients with mildly impaired lipolysis had normal 4-hr ^{13}C-exhalation (Figure 3). Sensitivity and specificity of 5-hr cumulative ^{13}C-exhalation versus the standard parameter were high (Table 1 and Figure 2).

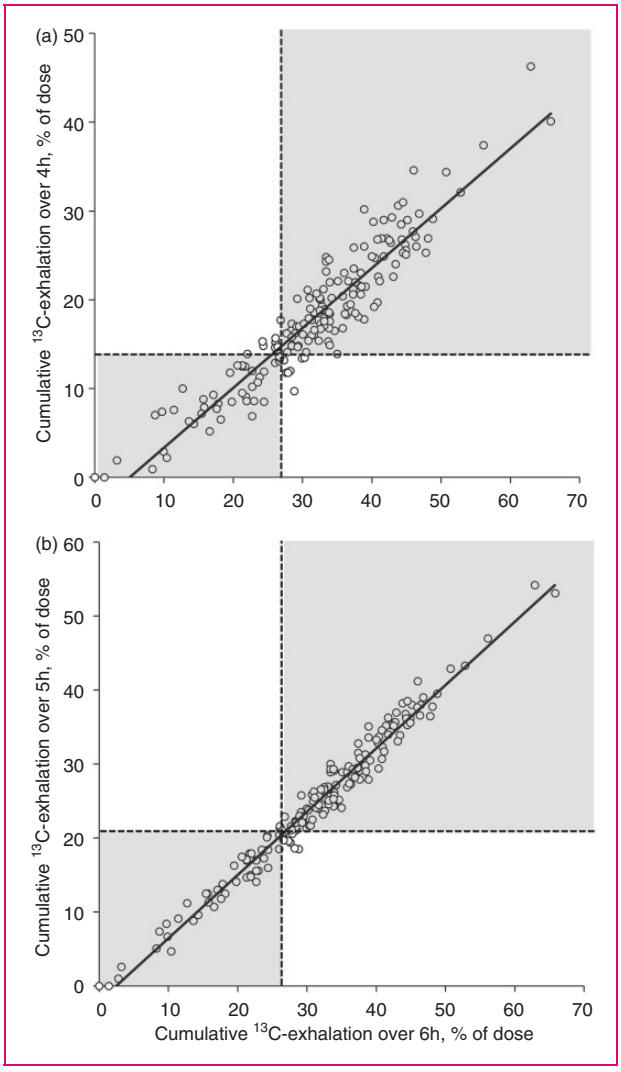

Figure 2. Cumulative ^{13}C-exhalation (in percent of dose) over (a) 4 hrs and (b) 5 hrs postprandially, in association with the standard parameter (cumulative ^{13}C-exhalation over 6 hrs). Investigations were performed in 181 patients whom received a ^{13}C-MTGT for clinical reasons. Cumulative ^{13}C-exhalation over 4 and 5 hrs correlated highly significantly with the standard parameter ($R = 0.94$ and $R = 0.98$, respectively; $p < 0.0001$). Dotted lines mark the lower level of normal; and thus, grey areas mark tests that give compatible results using the standard parameter and the abbreviated test.
hrs: hours; ^{13}C-MTGT: mixed triglyceride breath test.

Impact of gastric emptying on the ^{13}C-MTGT results

A total of 117 patients with evaluable data for the ^{13}C-MTGT also received a ^{13}C-OAT, for measurement of gastric emptying. Of these, 19 patients had to be excluded, because they received a different (vegetarian) test meal or because the time interval between both tests exceeded 4 weeks.

Among the remaining 98 patients, there were 64 women. The mean age of all of these patients was 52.0 ± 17.4 years and their mean BMI was 23.7 ± 4.8 kg/m^2. Of these, 68 patients had normal gastric emptying of solids, with a gastric emptying half

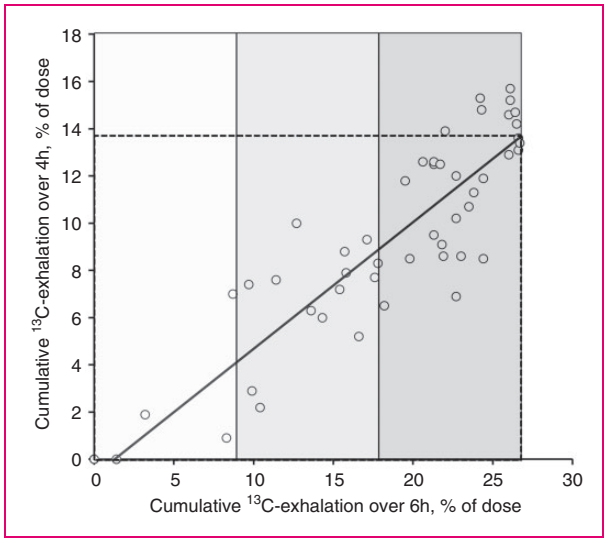

Figure 3. Cumulative ^{13}C-exhalation (in percent of dose) over 4 hrs postprandially, in association with cumulative ^{13}C-exhalation over 6 hrs in 51 subjects with impaired lipolysis, according to the standard parameter. The areas marked in different shades of grey represent severely, moderately and mildly impaired intestinal lipolysis. Broken lines give the lower level of normal for the standard parameter and the result of the abbreviated test. Remarkably, all subjects with more than mildly impaired lipolysis according to the standard parameter were also detected by the abbreviated test. Only 8 out of 30 subjects with mildly decreased cumulative ^{13}C-exhalation over 6 hrs had normal ^{13}C-exhalation over 4 hrs. hrs: hours.

time (t ½) <200 min[16,17]; whereas in 30 patients, the gastric emptying t ½ exceeded 200 min, indicating delayed gastric emptying. Seven out of these patients had impaired intestinal lipolysis, as their 6-h ^{13}C-exhalation was below 26.8% of the dose. Thus, in patients with delayed gastric emptying, the percentage of patients with pathological lipolysis, according to the standard parameter of the ^{13}C-MTGT, was not higher than in the total patient group (23% versus 28%).

Univariate linear regression analysis revealed that cumulative ^{13}C-exhalation rates over 1–4 hrs during the gastric emptying and the pancreatic function test were always significantly correlated ($n = 98$; $R \geq 0.238$; $p \leq 0.018$). Multivariate linear regression models confirmed there was a consistent association between ^{13}C-exhalation rates during both tests: Cumulative 1-hr to 4-hr ^{13}C-exhalation during the pancreatic function test was significantly predicted by cumulative ^{13}C-exhalation during the gastric emptying test, when adjusted for age, gender and BMI; however, the influence of gastric emptying on the 1-hr cumulative ^{13}C-exhalation during the ^{13}C-MTGT was considerably higher ($R^2 = 0.159$; $p < 0.001$) than on the 4-hr cumulative ^{13}C-exhalation ($R^2 = 0.073$; $p = 0.037$).

These findings indicate there was an association between gastric emptying velocity and the results of the pancreatic function test, particularly if evaluating early postprandial measurements of the ^{13}C-MTGT; however, cumulative 6-hr ^{13}C-exhalation during the ^{13}C-MTGT was similar, for patients with normal or delayed gastric emptying ($31.0 \pm 12.6\%$ versus $31.0 \pm 10.9\%$, $p > 0.5$). The same was true for cumulative 4-hr ^{13}C-recovery during the ^{13}C-MTGT ($17.3 \pm 8.6\%$ versus $16.6 \pm 7.8\%$; $p > 0.5$).

Discussion

Our data showed that in a large patient population, the standard parameter used for evaluation of our modified version of the ^{13}C-MTGT, that is cumulative ^{13}C-exhalation over 6 hrs following application of the test meal, correlates significantly with cumulative ^{13}C-exhalation over shorter periods of time (1–5 hrs, postprandially); however, early postprandial ^{13}C-exhalation rates (1–3 hrs) during the ^{13}C-MTGT appeared to be strongly influenced by gastric emptying; and thus, have insufficient specificity for detection of pancreatic exocrine insufficiency. By contrast, with cumulative 4-hr and 5-hr ^{13}C-exhalation rates, both sensitivity and specificity nearly reached or exceeded 90%. Importantly, even the 4-hr value detected all patients with moderate or severe exocrine insufficiency and more than two-thirds of the patients with mildly impaired intestinal lipolysis.

We had shown previously that in contrast to other noninvasive pancreatic function tests,[1] a modified version of the ^{13}C-MTGT detects moderate pancreatic exocrine insufficiency reliably.[3] The standard parameter for evaluation of pancreatic exocrine function using this test is the cumulative ^{13}C-exhalation over the 6 hrs following a test meal. To avoid an increase in overall CO_2-production that also influences $^{13}CO_2$-exhalation, subjects need to remain seated during the test procedures[18]; thus, although the ^{13}C-MTGT is a rather convenient, noninvasive indirect pancreatic function test, the long period of breath sampling and immobilization is a major drawback for clinical application, so shorter test versions would be highly desirable. The clinical need for such a test is underlined by the fact that current multinational research agendas explicitly aim to simplify the ^{13}C-MTGT and so this study has been performed as part of the HaPanEU project (Harmonizing diagnosis and therapy of pancreatitis across Europe).

The findings of our present study suggest that for clinical purposes the breath sampling period may be shortened to 4 hours. Comparison of the results of 181 ^{13}C-MTGT revealed that cumulative ^{13}C-exhalation over 4 hours postprandially had 88% sensitivity and 94% specificity for detection of pancreatic exocrine

insufficiency when compared to the standard parameter. Cumulative ^{13}C-exhalation over 5 hours gave almost identical results compared with the standard parameter (figure 2); however, it would only allow a minor abbreviation of the test. Moreover, even cumulative ^{13}C-exhalation over 4 hours detected all patients with moderate or severe exocrine insufficiency and more than 2/3 of the patients with mildly impaired intestinal lipolysis. Accordingly, an abbreviated version of the ^{13}C-MTGT with breath sampling over 4 instead of 6 hours appears to be sufficient for clinical purposes.

A further reduction of the breath sampling period would still be attractive and our data show indeed that even cumulative 1 h-^{13}C-exhalation does not only correlate tightly with the standard parameter ($R^2 = 0.66$, $p < 0.0001$) but also detects almost all patients with impaired ^{13}C-exhalation according to the standard parameter (97% sensitivity, Table 1). However, specificity of cumulative 1 h-^{13}C-exhalation was only 38%. Since digestion of dietary lipids by pancreatic lipase cannot occur before the meal has entered the duodenum, we tested the influence of gastric emptying on results of the ^{13}C-MTGT in a subset of 98 patients who received both, the ^{13}C-MTGT for measurement of pancreatic exocrine function and a ^{13}C-OAT for measurement of gastric emptying. Multivariate linear regression analyses revealed a significant association between the parameters of the ^{13}C-MTGT and gastric emptying velocity that was particularly strong early postprandially. Accordingly, the influence of gastric emptying on results of the ^{13}C-MTGT probably precludes a further abbreviation of the breath sampling period to less than 4 hours.

Whether the modified ^{13}C-MTGT may give false positive results in patients with lipid malabsorption due to other etiologies than pancreatic exocrine insufficiency[19] deserves further studies.

In conclusion, abbreviation of the breath sampling period to 4 hrs is possible for clinical investigation of pancreatic exocrine function when using our modified version of the ^{13}C-MTGT. The influence of gastric emptying on early postprandial results of the ^{13}C-MTGT precludes a further reduction of the breath sampling period. Importantly, the abbreviated test still detects all patients with moderate or severe exocrine insufficiency and more than two-thirds of the patients with mildly impaired intestinal lipolysis. Thus, in line with current multinational research agendas such as the HaPanEU project, we showed that the ^{13}C-MTGT can be abbreviated and still represents a reliable, noninvasive pancreatic function test.

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Conflict of interest

None declared.

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