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Powell-Brett, Sarah; Hall, Lewis A; Roberts, Keith J

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A standardised nutritional drink as a test meal for the ^{13}C mixed triglyceride breath test for pancreatic exocrine insufficiency: A randomised, two-arm crossover comparative study

Sarah Powell-Brett | **Lewis A. Hall** | **Keith J. Roberts**

Department of Hepatobiliary, Pancreatic and Transplant Surgery, University Hospitals Birmingham, NHS Foundation Trust, Birmingham, UK

Correspondence
Sarah Powell-Brett, Department of Hepatobiliary, Pancreatic and Transplant Surgery, University Hospitals Birmingham, NHS Foundation Trust, 3rd floor, Nuffield House, Mendelsohn Way, Edgbaston, Birmingham B15 2GW, UK.
Email: Sarah.powell-brett1@nhs.net

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Abstract

Background: The ^{13}C mixed triglyceride breath test (^{13}C MTGT) is a diagnostic test for pancreatic exocrine insufficiency (PEI). It is poorly standardised with much heterogeneity of the test meal, the commonest being toast and butter. A standardised oral nutritional supplement that could be easily transported, stored and made up would be valuable for making this test accessible outside of specialist centres.

Methods: A prospective, randomised, two-arm crossover study of different test meals was carried out in 14 healthy controls. The ^{13}C MTGT was performed in identical conditions on two separate days. Two test meals were given in random order, either standard (toast and butter) or novel (oral nutritional supplement), with 250 mg of ^{13}C-labelled mixed triglyceride incorporated. Breath samples were taken postprandially to calculate cumulative percentage dose recovery (cPDR) of ^{13}C at 6 h.

Results: All 14 participants completed both arms of the study with no protocol deviations. The mean cPDR was 39.39% (standard deviation [SD] 5.19) for the standard test meal and 39.93% (SD 5.20) for the novel test meal. A one-way repeated measures analysis of variance (ANOVA) found no significant difference in cPDR between the two meals, $F(1, 13) = 0.18$, $p = 0.68$ (minimum detectable difference of 0.81 at 80% power).

Conclusion: This study demonstrates that a standardised oral nutritional supplement can be used without compromising ^{13}C recovery. Using this test meal provides a standardised dietary stimulus to the pancreas, avoiding possible variation in quantity of dietary components with other test meals. Further, the ease of use of this method would help establish the ^{13}C MTGT test more widely.

KEYWORDS
^{13}C breath test, exocrine pancreatic insufficiency, pancreatic function

Highlights

- The ^{13}C mixed triglyceride breath test (^{13}C MTGT) is a useful diagnostic test mainly used in specialist centres.
- Lack of a standardised protocol is a key limitation to more widespread use.
- The test meal in particular shows much heterogeneity across the literature.
- A standardised oral nutritional supplement could make the ^{13}C MTGT more versatile and robust.

- This two-way, randomised crossover trial showed no difference in cumulative percentage dose recovery (cPDR) with a standardised oral nutritional supplement in comparison to toast and butter.
- This novel test meal minimises potential for error, negates the need for any kitchen equipment and could make the ^{13}C MTGT more accessible for routine clinical use.
- Further validation studies in specific patient cohorts are needed.

BACKGROUND

Stable isotope ^{13}C breath tests are increasingly used as diagnostic tests in healthcare since their introduction in the 1970s.[1] ^{13}C is a stable, nonradioactive carbon isotope used as a tracer and is generally considered ethically acceptable in humans of any age. The ^{13}C mixed triglyceride breath test (^{13}C MTGT) is an accurate, safe and noninvasive diagnostic method for pancreatic exocrine insufficiency (PEI).[1] The test meal performs two functions: first as a medium for the ingestion of a tracer (^{13}C-labelled mixed triglyceride [^{13}C MTG]) and second as a stimulus for the pancreas to secrete pancreatic enzymes. Pancreatic lipases hydrolyse triacylglycerol releasing ^{13}C-labelled octanoate and monoacylglycerol, which are rapidly absorbed, transported to the liver where they are oxidised, generating $^{13}CO_2$, which is exhaled and sampled for $^{13}C/^{12}C$ ratio. Change relative to baseline values is used to calculate ^{13}C excretion. The increase in $^{13}CO_2$ concentration from baseline reflects pancreatic lipase secretion.

Many studies have sought to optimise the test meal used to carry the ^{13}C MTG tracer.[2–4] An ideal test meal should be standardisable, reproducible, acceptable to patients, contain 16–26 g of fat,[4,5] around 500 kcal (for maximum pancreatic stimulation),[6,7] be easy to prepare and have the ability to incorporate ^{13}C-labelled substrate without encountering losses in assembly or ingestion. Most recent studies use some variation on bread and butter with fluid to create a solid–liquid test meal with a specified amount of fat; however, the quantity of and medium for fat shows much variance. It would be preferable to move to a standardised oral nutritional supplement rather than a meal that requires a kitchen/equipment to create, making it easier to apply in all settings. A disadvantage of bread and butter for some people (children and elderly frail) is the extended time it could take for consumption and the risk of incomplete consumption. In addition, toast and butter is difficult to standardise and, in many environments, difficult to access (need for food standards storage, a fridge and a toaster). Studies on the physical properties of a meal have shown that a solid meal results in slower gastric emptying, and using homogenised meals results in a similar level of enzyme secretion but with an earlier postprandial peak.[6] The ^{13}C MTGT has previously been used without a solid component to good effect, using cream, oral nutritional supplements and even just milk (in infants).[3,8]

Our research group uses the ^{13}C MTGT as a reference test for PEI in several settings; outpatient clinic, ward based, research facilities and at home. Therefore, we explored whether the ^{13}C recovery using a set oral nutritional supplement (that does not require refrigeration or any reconstitution) was comparable to the ^{13}C recovery of the solid test meal described by Dominguez-Munoz et al. (the most recently investigated and most widely used test meal at present).

METHODS

This study is a prospective, randomised, two-arm crossover comparative study of two different test meals to be used with the ^{13}C MTGT. This study was performed at University Hospitals Birmingham NHS Foundation Trust; it was conducted according to the guidelines laid down in the Declaration of Helsinki, and all study procedures were approved by the West-Midlands – Black Country Research Ethics Committee. All subjects completed a written consent form.

Test meal development

Our test meal was designed based on the requirements described earlier and chosen from readily available oral nutritional supplements available to purchase online that could be used in any environment without the need for refrigeration, reconstitution or any kitchen equipment. Acceptability was ascertained through a local Patient and Public Involvement and Engagement group. ^{13}C MTG losses in the preparation and ingestion of the test meal are an important consideration. Pure ^{13}C MTG, the test meal with ^{13}C MTG added, and the test meal container post ingestion were weighed to ensure that this was not a concern using our novel test meal. The final test meal comprised a Fortisip 2Kcal and a Nutillis Complete Crème Level 3. The Level 3 component enables the ^{13}C MTG to be folded into the test meal (using the same spoon used for ingestion to minimise potential for losses) without it settling at the bottom or on the sides of the container. See Table 1 for macronutrient information; a suitable alternative to Nutillis

Complete Crème Level 3 has also been included in this table.

Inclusion and exclusion criteria

Subjects had to be over 18 and have capacity to consent; exclusion criteria comprised the following: pregnancy, any gastrointestinal (GI) disorders, recent unintentional weight loss, symptoms suggestive of exocrine insufficiency (as assessed by the PEI-Questionnaire), medications that may affect gastric emptying and previous GI operations (except for an appendicectomy).

Study protocol

Each subject was randomly assigned to receive either the novel or the standard test meal at their first attendance and then they repeated the test with an alternate meal at their second attendance. The second test day had to be at least 7 days after the first to allow for adequate ^{13}C washout. At the initial screening interview, subjects were given pretest instructions. They had to refrain from ingesting foods rich in ^{13}C (corn products, cane sugar, pineapple, kiwi, broccoli or sweet corn) for 48 h before the start of the test and they were asked to fast (except for small volumes of water) and refrain from smoking for 12 h before the test. Breath samples were collected by blowing through a straw into an inverted exetainer (Exetainer; Labco Limited) and immediately capping. Subjects were asked to attend 30 min before the test start time to allow heart rate and temperature to settle; baseline breath samples were taken and then the assigned test meal with 250 mg of ^{13}C MTG (weighed on a five-figure balance) carefully incorporated into it was ingested over a maximum of 5 min at around 8 a.m. Repeat samples were then taken every 30 min up to a total of 6 h. Patients with diabetes also had their blood glucose checked hourly. For the duration of the test, subjects had to remain sedentary and fasted. Small quantity of water was allowed for comfort and a short trip to the toilet at a gentle pace if needed. The $^{13}CO_2$ content of the breath samples was determined using gas chromatography isotope ratio mass spectrometry (IRMS). Enrichment of $^{13}CO_2$ in the posttest meal samples was calculated by subtracting the abundance of $^{13}CO_2$ in the baseline sample from that of each posttest meal sample. The PDR for ^{13}C for each sample was calculated using the equation shown in Figure 1. The cumulative PDR (cPDR) was calculated by adding individual PDR values averaged over the time interval.

Statistics

Data were summarised as either median and interquartile range (IQRs) for nonparametric continuous variables, mean and standard deviation (SD) for parametric continuous variables or by percentage for categorical variables. The Shapiro–Wilk test was used to test for normality. ^{13}C enrichment of exhaled breath was determined with an isotope ratio mass spectrometer (IRMS). The primary outcome was cPDR at 6 h. A one-way repeated analysis of variance (ANOVA) was run to look for a difference in cPDR between the two test meals. A one-sample mean *t*-test was used to determine minimal detectable difference. Stata 16.1 was used for all statistics.

RESULTS

A total of 14 healthy controls were recruited, all receiving both control and test meal at a median of 8 days apart (IQR 8–14.5) with no protocol deviations. cPDR was normally distributed (tested by Shapiro–Wilk test) with no significant outliers (see Box plots, Figure 3). The mean cPDR for toast and butter was 39.39% (SD 5.19) for the novel test meal 39.93% (SD 5.20). A one-way

TABLE 1 Novel test meal constituents.

Product			Fat (g)	Kcal	Protein	Carb	Sugars	Fibre
Fortisip 2Kcal	200 ml	Liquid	17.2	300	20	41	30.8	0
Nutillis Complete Crème Level 3	125 g	Mousse	11.8	306	12	36.4	14.8	3.2
Total			23.4	606	24	73.5	16.6	3.2
Nutillis Complete drink Level 3*	125 ml	Thick liquid	11.6	306	12	36.4	6.8	4.0

*Alternative to the Nutillis complete crème.

$$\text{PDR } h^{-1} = \frac{VCO^2\ (\text{mmol}.h^{-1}) \times \text{Breath } CO^2 \text{ enrichment (ppm } ^{13}C \text{ excess)} \times 100}{\text{mmol excess } ^{13}C \text{ in dose} \times 10^6}$$

FIGURE 1 Calculation for the percentage dose recovered (PDR) at each time point.

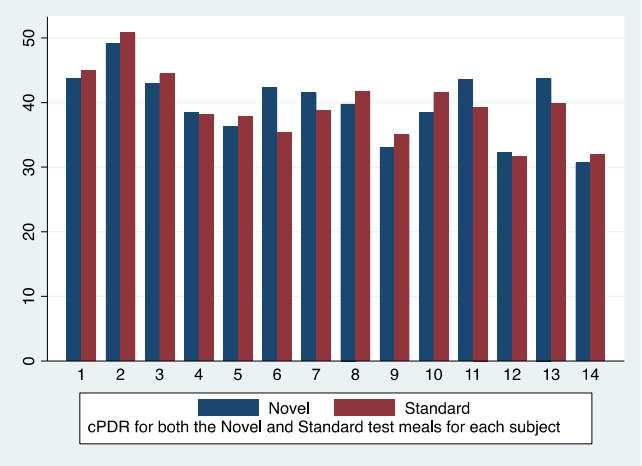

FIGURE 2 Cumulative percentage dose recovery (cPDR) of standard versus novel test meal in every subject.

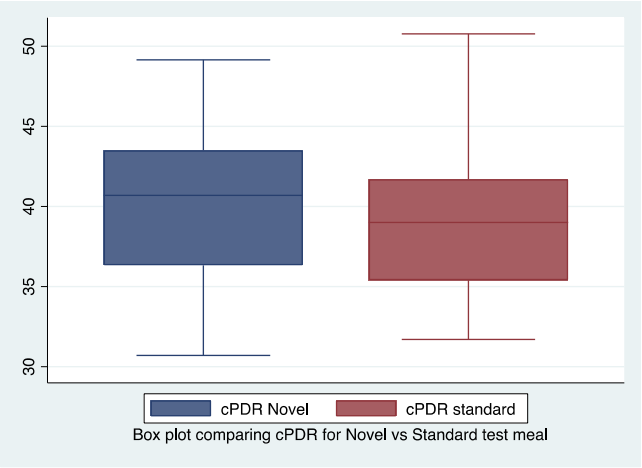

FIGURE 3 Box plot comparing cumulative percentage dose recovery (cPDR) for the novel versus the standard test meals.

repeated measures ANOVA found no significant difference in cPDR between the two meals, $F(1, 13) = 0.18$, $p = 0.68$. See Figure 2 for bar chart of all results and Figure 3 for a box plot of the novel versus the standard test meal. With a sample size of 14, there is a minimal detectable difference of 0.8 at 80% power. A one-sample mean *t*-test was used to determine minimal detectable difference (see Figures 2 and 3).

DISCUSSION

This study was a prospective, randomised, two-arm crossover comparative study of two different test meals to be used with the ^{13}C MTGT. For our sample of healthy controls, we have shown no difference in the cPDR of ^{13}C using a novel test meal of a standardised oral nutritional supplement in comparison to toast and butter. This is the latest in a series of studies to investigate the optimum test meal for the ^{13}C MTGT, but the first to validate a standardised oral nutritional supplement, that may permit (and encourage) use of the test in more settings.

At present, the test meal used to carry the ^{13}C MTG tracer and to stimulate pancreatic secretion is poorly standardised with a wide variety being described in published work. The original Vantrappen test meal comprised 100 g bread with 0.25 g/kg body mass of butter.[9] In an early validation study, Löser et al. used 100 g toast, 15 g butter and 10 g chocolate cream (Nutella).[10] Keller et al. modified this, increasing the fat content to 26 g with excellent sensitivity and specificity.[5] Bozek et al. examined four test meals: ^{13}C MTG alone; with a wheat roll; with a wheat roll and 10 g butter; and with a wheat roll and 30 g butter.[2] They found that the addition of unlabelled fat is essential for ^{13}C recovery and that a lower fat load resulted in earlier and greater overall ^{13}C recovery. The Dominguez-Munoz group performed a three-arm randomised crossover comparative study trialling 16, 32 and 48 g of fat; 16 g of fat resulted in the greatest area under the receiver operating characteristic curve but with insignificant differences between the three fat contents.[4] There are many other, some quite complex, versions of the test meal, most with a solid–liquid meal and a few with a homogenised or in a liquid form.[1–4,8,11]

The main limitation of this study is that our test subjects are healthy controls only. To be confident in the use of this novel test meal in a routine clinical setting, it should be validated in the target patient groups of pancreatic cancer, chronic pancreatitis and cystic fibrosis. Our group is currently undertaking a body of work investigating PEI diagnostics and has successfully used this test meal in over 80 recruits, including patients with pancreatic cancer and chronic pancreatitis, and the test meal has been well tolerated in this cohort. A further limitation is that this test meal is still not suitable for young children (under 6 years of age) and vegan/lactose-intolerant patients.

The ^{13}C MTGT is a safe, noninvasive diagnostic test for PEI that is at present limited in its use outside of specialist units as it is challenging to set up and sensitive to even small variation. Part of the problem is the lack of a robust, standardised protocol. Our group performed a systematic review and meta-analysis of the methodology and accuracy of the ^{13}C MTGT and found wide variation in several areas, most markedly the test meal constituents and the total testing timeframe.[12] We believe that creating a test meal that is easy to standardise, does not require kitchen equipment and can be used in any setting is an important step in making the ^{13}C MTGT more user friendly and easier to roll out in various hospital and even home settings. The authors do not suggest a universally standardised protocol here as there is further work to be done on validating both a standardised test meal in patient cohorts and a shorter testing timeframe first.

AUTHOR CONTRIBUTION

Sarah Powell-Brett designed the study, collected and analysed the data, prepared the manuscript and approved the final version. Lewis A. Hall collected and interpreted the data, prepared the manuscript and approved the final version. Keith J. Roberts conceived the idea for the study, prepared the manuscript and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

TRANSPARENCY DECLARATION

The lead author confirmsthat this manuscript is an honest, accurate, and transparent account ofthe study being reported. The reporting of this work is compliant withSTROBE guidelines. The lead author affirmns that no important aspectsof the study have been omitted and that any discrepancies from thestudy as planned (Approved by HRA and Health and Care Research-Wales 19/WM/0358) have been explained.

ORCID

Sarah Powell-Brett http://orcid.org/0000-0003-4488-6862
Lewis A. Hall http://orcid.org/0000-0002-5012-2108

PEER REVIEW

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AUTHOR BIOGRAPHIES

Sarah Powell-Brett is a West Midlands surgical trainee currently working as a research fellow at University Hospitals Birmingham undertaking a PhD in novel PEI diagnostics.

Lewis A. Hall is a West Midlands academic foundation doctor with an interest in surgery and research who undertook an intercalated degree focused on PEI diagnostics.

Keith J. Roberts is a consultant liver transplant and HPB surgeon at University Hospitals Birmingham, an honorary professor at University of Birmingham and the RCS surgical specialty lead for pancreatic cancer.

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