

Exocrine pancreatic insufficiency: accuracy and clinical value of the uniformly labelled ^{13}C -Hiolein breath test

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Abstract

Background and Aims—The ^{13}C -Hiolein breath test (98% [^{13}C] labelled long chain triglyceride mixture (highly labelled triolein)) was evaluated as a non-invasive, non-radioactive test for exocrine pancreatic insufficiency. Accuracy and clinical validity were examined with reference to both the secretin pancreozymin test and faecal fat analysis.

Methods—A secretin pancreozymin test and faecal fat analysis were performed in 46 patients, 30 with exocrine pancreatic insufficiency and 16 with normal pancreatic function. In all of these patients and in seven healthy volunteers (controls), a ^{13}C -Hiolein breath test was performed using 2 mg/kg [^{13}C] labelled Hiolein with a standard rice snack (1.5 g/kg; 25% fat). $^{13}\text{CO}_2/^{12}\text{CO}_2$ enrichment in the exhaled breath was measured by isotope ratio mass spectrometry.

Results—In patients with pancreatic steatorrhoea the $^{13}\text{CO}_2$ response was below the 95% confidence interval of $^{13}\text{CO}_2$ exhalation in the controls. These responses were also diminished ($p < 0.001$) compared with patients with impaired lipase output but normal fat excretion and with disease as well as healthy controls. There was a linear correlation between stimulated lipase output and the ratio of lipase output/ $^{13}\text{CO}_2$ response ($r = 0.95$). Among the 40 patients in whom direct pancreatic function testing was clinically indicated, the sensitivity of the ^{13}C -Hiolein test for detecting steatorrhoea was 91.7%, with a specificity of 85.7%.

Conclusions—In patients with pancreatic disease the ^{13}C -Hiolein breath test reflects impaired lipase output and indicates decompensated lipolysis. The ^{13}C -Hiolein breath test is a convenient alternative to faecal fat analysis.

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Keywords: pancreas, steatorrhoea, breath tests, lipase, triolein, Hiolein, malabsorption.

An ideal non-invasive test for chronic pancreatitis should quantitatively measure exocrine pancreatic function, and should be sensitive enough to detect functional impairment of the pancreas in diseases other than chronic pancreatitis or in old age, even in

the absence of morphological alterations. At the same time, the test should be able to determine both the necessity and the success of supplementation with pancreatin. Obviously, no such test is currently available.¹

Current tests of pancreatic function are cumbersome, invasive, and expensive – for example, the Lundh and the secretin pancreozymin test, neither of which is as yet standardised – or time consuming and dependent on the patient's cooperation (for example, the fluorescein dilaurate test). Analysis of faecal chymotrypsin, elastase 1, or fat may be resented by both patients and technicians.²⁻⁴

Whereas the secretin pancreozymin test and the fluorescein dilaurate test correlate with the occurrence of pancreatic steatorrhoea,^{5,6} neither reflect lipolytic activity and lipolysis to an equal degree and the level of lipolysis in particular guides enzyme replacement therapy.

Therefore, most breath tests for the diagnosis of exocrine pancreatic insufficiency attempt to reflect both pancreatic lipolytic activity and impaired lipolysis or pancreatic steatorrhoea. On a 'no touch' principle of function testing and given that radioactivity is not applied, these breath tests for maldigestion of fat can be regarded as successful examples of patient oriented diagnostic procedures.

Breath tests for fat malabsorption using radioactive ^{14}C -triolein or a ^{14}C -labelled mixed triglyceride as the substrate for lipolysis have been shown to be valid and diagnostically useful methods.⁷⁻⁹ Using stable isotopes offers the advantage of being non-radioactive and developments were initiated in this respect to examine these techniques.¹⁰⁻¹⁴

This is to our knowledge the first study examining the accuracy and clinical value of the ^{13}C -Hiolein breath test using a 98% [^{13}C] labelled long chain triglyceride mixture (Table I; highly labelled triolein) in patients with exocrine pancreatic insufficiency, mostly due to chronic pancreatitis.

By contrast with the carboxy labelled ^{13}C -triolein as a substrate, ^{13}C -Hiolein is a mixture of different uniformly ^{13}C -labelled triglycerides. This uniform ^{13}C label facilitates monitoring of lipolysis by $^{13}\text{CO}_2/^{12}\text{CO}_2$ isotope ratio mass spectrometry as the signal is amplified according to the reiterative release of $^{13}\text{CO}_2$ during β -oxidation, thereby allowing the use of tracer doses.¹³

If the ^{13}C -Hiolein breath test permitted detection of impaired lipolytic activity in

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TABLE I Fatty acid composition of Hiolein

Fatty acid	Common name	Proportion (%)
18:1 ω-6	Oleic acid	50.6
16:0 ω-6	Palmitic acid	16.9
18:2 ω-6	Linoleic acid	19.6
18:3 ω-3	α-Linolenic acid	3.0
18:0 ω-6	Stearic acid	2.3
16:1 ω-9	Palmitoleic acid	1.8
16:2 ω-6	No common name	1.4
16:3 ω-3	No common name	1.2

Analysis of sample lot No 0242 used in this study.

patients with chronic pancreatitis, this would provide a clean and elegant method for diagnosing exocrine pancreatic insufficiency, and for monitoring the efficacy of pancreatic enzyme replacement therapy.

Our investigation was therefore carried out with a special emphasis on the value of the ¹³C-Hiolein breath test to measure lipolytic activity, referring to the secretin pancreozymin test as the gold standard,¹⁵ as well as pancreatic steatorrhoea, compared with faecal fat analysis,¹⁶⁻¹⁸ and hence encompassing all degrees of pancreatic dysfunction.

Methods

PATIENTS

Forty patients with suspected pancreatic disease and an additional six patients with intestinal causes of malabsorption (coeliac sprue, bacterial overgrowth) underwent a secretin pancreozymin test as an established part of our diagnostic routine function tests. In 16 patients, including all six with intestinal causes of malabsorption, exocrine pancreatic function was normal as determined by the secretin pancreozymin test.

Thirty patients were shown to have exocrine pancreatic insufficiency by an abnormal secretin pancreozymin test. In 22 of these patients, chronic pancreatitis was diagnosed by the clinical course and an abnormal secretin pancreozymin test in addition to an abnormal pancreatogram at endoscopic retrograde cholangiopancreatography (ERCP) or signs of chronic pancreatitis at ultrasonography or computed tomography (CT). Five patients with an abnormal secretin pancreozymin test had pancreatic carcinoma. One patient with exocrine insufficiency had a pancreas divisum and status after biliary pancreatitis, and in two patients the aetiology of the exocrine pancreatic insufficiency remained unexplained. Table II lists the characteristics of the patient groups.

TABLE II Characteristics of the patient groups

Group	n	Age (y) (mean (range))	Sex (M:F)	Faecal fat (g/day) (mean (SEM))	Lipase output (U/30 min) (mean (SEM))
I	12	57 (40-71)	10:2	31.1 (6.8)	4016 (1371)
II	18	49 (26-69)	15:3	3.3 (0.3)	40880 (4183)
III	10	44 (27-62)	8:2	4.1 (0.7)	147265 (22167)
IV	7	35 (30-48)	3:4	ND	ND
V	6	50 (29-73)	4:2	12.5 (1.4)	101761 (19183)

ND=Not determined.

Group I: pancreatic steatorrhoea

Twelve of the 30 patients with an abnormal secretin pancreozymin test had pancreatic steatorrhoea (faecal fat excretion >7 g/day; stimulated lipase output <10% of the normal mean (<12 000 U/30 min after cholecystokinin-pancreozymin (CCK-Pz)).

Ten of them had chronic pancreatitis (two with diabetes mellitus), two pancreatic carcinoma (a 5.5×9.8 cm mass of the pancreatic head (with lymph node involvement) in one, and an extensive carcinoma of the pancreatic body plus tail with involvement of the mesenteric artery and lymph nodes in the other patient).

Group II: exocrine pancreatic insufficiency without steatorrhoea

These 18 patients had exocrine pancreatic insufficiency (secretin pancreozymin test) without steatorrhoea. Twelve patients in this group had chronic pancreatitis, two had chronic pancreatitis with diabetes mellitus in addition, and three had pancreatic carcinoma (all three with a large tumour mass of the body of the pancreas with lymph node involvement, and in one additionally of the head of the pancreas). Exocrine insufficiency was unexplained in one patient.

Group III: patients with normal exocrine pancreatic function and without steatorrhoea

Among the 10 patients in this group the secretin pancreozymin test and faecal fat excretion were normal. Eight were considered as having functional abdominal pain, one patient had functional diarrhoea (mean stool weight 267 g/day), and one patient had morphological signs of chronic pancreatitis (ERCP and ultrasonography) without functional impairment (a patient with alcoholic pancreatitis showing pancreatic calcifications, diabetes mellitus (unrelated to pancreatitis), and cirrhosis of the liver).

Group IV: normal controls

This group consisted of seven healthy volunteers who underwent the Hiolein test for their own curiosity, to gain personal experience with the test, and for helping us with the study. In these subjects (colleagues and laboratory staff), a pancreatic function test and faecal fat analysis was not performed.

Group V: non-pancreatic steatorrhoea (disease controls)

Six patients with diarrhoea (mean stool weight 458 (SD 102) g/day) and steatorrhoea (range=9.5-18.6 g/day) of intestinal origin underwent a secretin pancreozymin test (with normal results) which excluded exocrine pancreatic insufficiency.

¹³C-HIOLEIN BREATH TEST

Hiolein (Martek Corporation, Columbia, USA) is an algal product with uniformly

^{13}C -labelled triglycerides (derived from $^{13}\text{CO}_2$ as the sole atmospheric carbon source for the assimilation process), which comprises a triglyceride mixture with naturally occurring long chain fatty acids (Table I). This substrate is 98% uniformly labelled [^{13}C] thereby facilitating the use of small tracer doses for generating $^{13}\text{CO}_2$ abundances in breath that are within the detectable range.¹³

Patients and controls received 2 mg 98% [^{13}C]-Hiolein/kg with a commercial rice snack as the sole meal in the morning after an overnight fast (Yinwa Kabuki rice snack, Gumport, Norderstedt, Germany (1.5 g/kg)). This snack (25% (w/w) fat, 37% (w/w) carbohydrates) does not change baseline $^{13}\text{CO}_2/^{12}\text{CO}_2$ abundance and additionally allows measurement of starch malassimilation indicated by increased breath hydrogen concentrations (rice starch breath hydrogen test) as described.¹⁹ However, the starch load was only 1/3 of that used by Kerlin *et al.*¹⁹ and (therefore) only four patients had a rise of H_2 concentrations >20 ppm after the rice starch meal. These data are not discussed as a measure of pancreatic function. A dose of 200 ml mineral water was taken with the test meal and additional aliquots were permitted five hours later. The next meal was permitted eight hours after the rice meal.

Basal breath samples were obtained before and at the end of the test meal, which was consumed within 10 minutes. Postprandial breath samples were collected at 30 minute intervals for eight hours and at 60 minute intervals from eight to 12 hours. A final sample was obtained 24 hours after the meal.

The study was performed in accordance with the guidelines of the Helsinki Declaration and the use of ^{13}C -Hiolein for purposes of the study was approved by the local ethics committee of the University of Frankfurt.

ISOTOPE RATIO MASS SPECTROMETRY

Enrichment of $^{13}\text{CO}_2/^{12}\text{CO}_2$ in breath was measured by continuous flow isotope ratio mass spectrometry using a Tracermass mass spectrometer (Europa Scientific Ltd, Crewe, England) and was expressed as δ values (referring to the international standard Pee Dee Belemnite) as described earlier.^{20, 21}

Results are presented as δ over baseline values (DOB) and cumulative recovery after 12 hours. Cumulative recovery of the tracer dose in breath was calculated by multiplying the integrated enrichment time curve by the normal endogenous CO_2 production²¹ assuming total CO_2 production to be 300 mmol/m² surface area/h. Body surface area was calculated according to Haycock *et al.*²²

SECRETIN PANCREOZYMIN TEST

The secretin pancreozymin test was performed after duodenal intubation with a Lagerlöf tube under fluoroscopic control as described previously.^{15, 23-25} Stimulation was carried out by intravenous injection of 1 U/kg synthetic secretin (Secretolin, Hoechst, Frankfurt, Germany)

and 1 U/kg CCK-Pz (Ferring Pharmaceuticals, Malmö, Sweden) 30 minutes later.

Duodenal aspirates were collected for 2×15 minutes (baseline), 2×15 minutes after secretolin, and 4×15 minutes after CCK-Pz with the subjects recumbent on their right side. This procedure overcomes the need of using a marker for recovery and acid contamination of duodenal aspirates is prevented by careful manual suction at the gastric port.²⁴

All fractions were collected on ice and analysed immediately after sampling. Volume, bicarbonate concentration, and the activities of amylase, lipase (Monotest kit, Boehringer, Mannheim, Germany, with triolein as substrate), and trypsin (test kit, Boehringer, Mannheim, Germany, with benzoyl-arginine-*p*-nitroanilide as substrate) were measured in each sample using established procedures.²⁵ In our laboratory, a lipase output <65 000 U/30 min after CCK-Pz is abnormal.

FAECAL FAT ANALYSIS

Faecal fat analysis was performed by the van de Kamer method¹⁶ or near infrared reflectance analysis, which give equivalent results in our laboratory.¹⁸ Stools were collected for 72 hours taking care that patients were on a diet containing about 100 g fat/day and that pancreatic enzymes were discontinued for ≥four days before stool collection. Daily stool output was weighed, homogenised, and analysed separately, and mean daily output was calculated from the three day period. A faecal fat excretion ≥7 g/day represents steatorrhoea, whereas stool weight >200 g/day is considered abnormal.⁴

CALCULATIONS AND STATISTICS

Results are presented as means (SEM). Statistical analysis (descriptive statistics, 95% confidence intervals (95% CIs), analysis of variance with Bonferroni multiple comparison correction, and curve fitting) was performed with the software program PRISM (version 1.0, GraphPad, San Diego, USA). A *p* value <0.05 was considered significant.

Results

The cumulative ^{13}C recovery 12 hours after the test meal displayed a linear and significant correlation with peak δ over baseline responses (DOB_{max}) according to the equation:

$$Y = 1.42 \pm 0.12 X + 9.13 \pm 3.50 \\ (r = 0.859; p < 0.0001; n = 50, \text{ Fig 1})$$

DOB_{max} -responses were not significantly different between healthy controls (53.9 (5.1) ‰; group IV), patients with normal pancreatic function without steatorrhoea (50.2 (3.0) ‰, group III), and patients with exocrine pancreatic insufficiency without steatorrhoea (51.0 (3.0) ‰, group II). The lower 95% CI of the control group was 41.4 ‰.

DOB_{max} responses in group I patients (pancreatic steatorrhoea), however, were significantly lower than in any other group

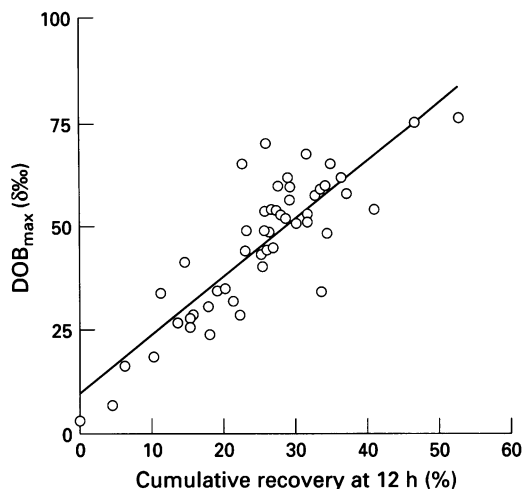


Figure 1: Correlation of the cumulative recovery at 12 hours after Hiolein ingestion (% of dose administered) with peak δ over baseline ¹³CO₂ responses (DOB_{max}, $\delta\%$).

tested ($p < 0.001$ compared with groups II, III, and IV; $p < 0.01$ compared with group V; Bonferroni multiple comparisons test).

Because of the multiple comparisons and the limited number of patients, the difference in

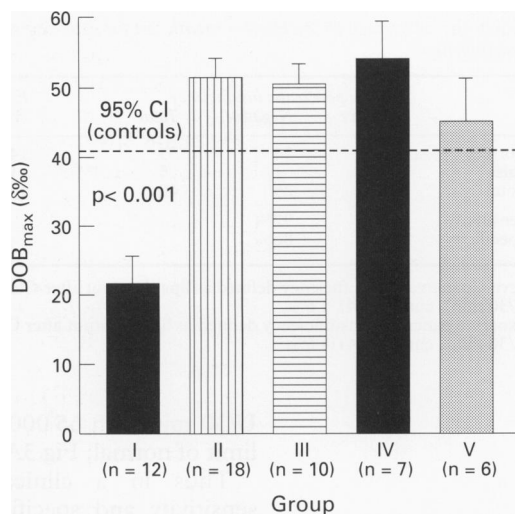


Figure 2: Peak δ over baseline responses (DOB_{max}, $\delta\%$) in patients with pancreatic steatorrhoea (group I), exocrine pancreatic insufficiency without steatorrhoea (group II), patients without exocrine pancreatic insufficiency and without steatorrhoea (group III), normal controls (group IV), and disease controls (non-pancreatic steatorrhoea, group V). DOB_{max} responses are significantly diminished in group I ($p < 0.001$ compared with any other group). The dotted line refers to the lower limit of the 95% CI of the controls (group IV). The reduction of DOB_{max} responses in group V is not significant (*v* controls).

DOB_{max} responses in the six patients with non-pancreatic steatorrhoea (44.9 (6.1) $\delta\%$) was not significant compared with the controls (Fig 2).

The relation of DOB_{max} responses to the duodenal lipase output after stimulation of the pancreas with CCK-Pz was assessed in the 46 patients who underwent a secretin-pancreozymin test (Fig 3A). With only one exception (carcinoma of the pancreas), all patients with a post-CCK-Pz lipase output $< 12\,000$ U/30 min ($< 10\%$ of the mean normal lipase output) had DOB_{max} responses < 41.4 $\delta\%$ (the lower 95% CI of the controls).

Of the other six patients with low DOB_{max} responses, two had exocrine pancreatic insufficiency without steatorrhoea (one of them with an abnormal starch H₂ breath test (40 ppm)), one patient (group III, also with a positive H₂ breath test (40 ppm)) had chronic pancreatitis coincident with liver cirrhosis and diabetes mellitus, and the remaining three patients belonged to group V (non-pancreatic steatorrhoea).

Among the 40 patients with suspected pancreatic disease (groups I–III) – that is, those in whom pancreatic function tests were clinically indicated – DOB_{max} responses were correctly positive (< 41.1 $\delta\%$) in 12 patients with a lipase output $< 12\,000$ U/30 min (11 of them with pancreatic steatorrhoea). One false negative DOB result was found in a patient with pancreatic carcinoma. False positive results (with reference to lipase) were obtained in three patients. Two of these patients (one with chronic pancreatitis and one with chronic pancreatitis combined with diabetes mellitus and cirrhosis of the liver) had an abnormal breath H₂ test indicating additional causes of malabsorption. Furthermore, the post-CCK-Pz lipase output was borderline in two of the three false positive tests (64 106 and 64 549

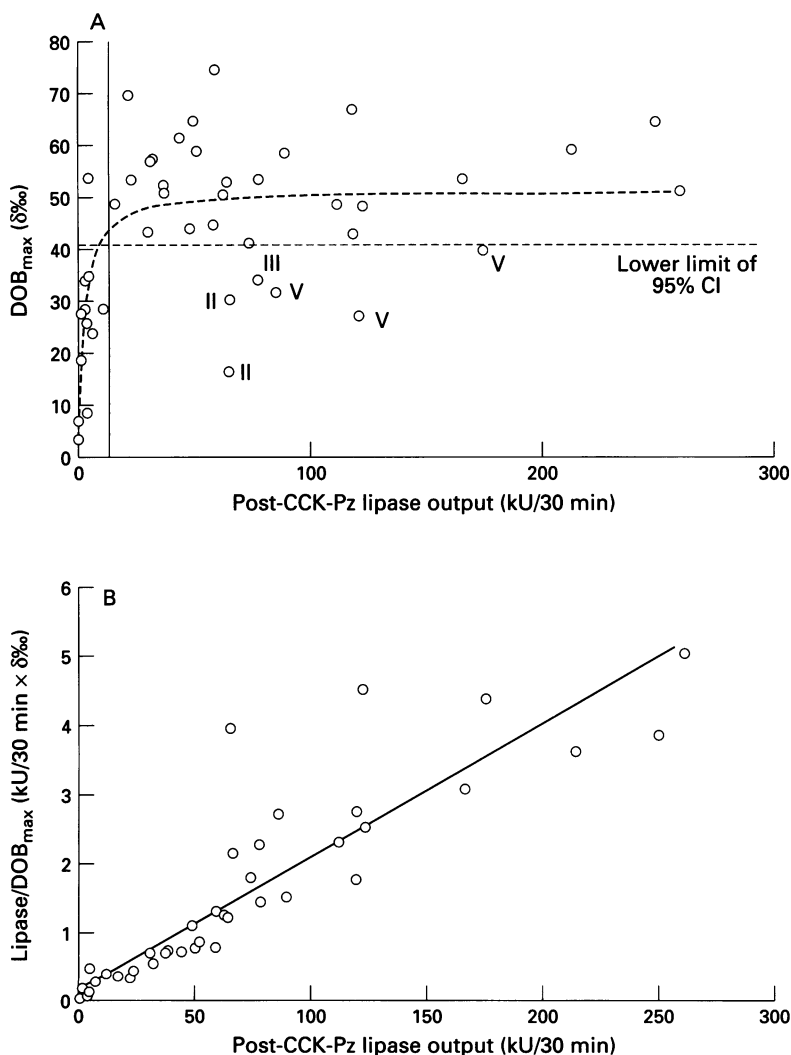


Figure 3: (A) Hyperbolic correlation of peak δ over baseline responses (DOB_{max}, $\delta\%$) with stimulated duodenal lipase output (30 minutes) after CCK-Pz. The horizontal dotted line indicates the lower limit of normal (95% CI used as the cut off), the vertical line indicates 10% residual lipase secretion (after 12 000 U/30 min). The respective groups are given for those patients with low DOB_{max} responses despite less severely reduced or normal lipase output. (B) Transformation of the data (stimulated duodenal lipase output/30 min/DOB_{max} *v* stimulated duodenal lipase output/30 min) shows linearity ($r = 0.95$), which corroborates the hyperbolic curve fitting in Fig 3A.

TABLE III Accuracy of the Hiolein breath test for detecting severe and exocrine pancreatic insufficiency

	Severe pancreatic insufficiency			Exocrine pancreatic insufficiency		
	Positive	Negative	Total	Positive	Negative	Total
Correct	11	24	35	14	9	23
False	4	1	5	1	16	17
Total	15	25	40	15	25	40
Sensitivity		92%			47%	
Specificity		86%			90%	

Severe pancreatic insufficiency defined as lipase output after CCK-Pz <10% of normal (12 000 U/30 min), cut off at 41.4 δ‰
 Exocrine pancreatic insufficiency defined as lipase output after CCK-Pz abnormal (<65 000 U/30 min), cut off at 41.4 δ‰.

U/30 min with 65 000 U/30 min as the lower limit of normal; Fig 3A).

Thus in a clinically relevant scenario, sensitivity and specificity of the ¹³C-Hiolein breath test for detecting pancreatic steatorrhoea were 91.7% and 85.7%, respectively (cut off at 41.4 δ‰ DOB_{max}, Table III).

The correlation of DOB_{max} versus lipase output after stimulation was characterised by a hyperbolic function ($Y=mX/(k+X)$, constants

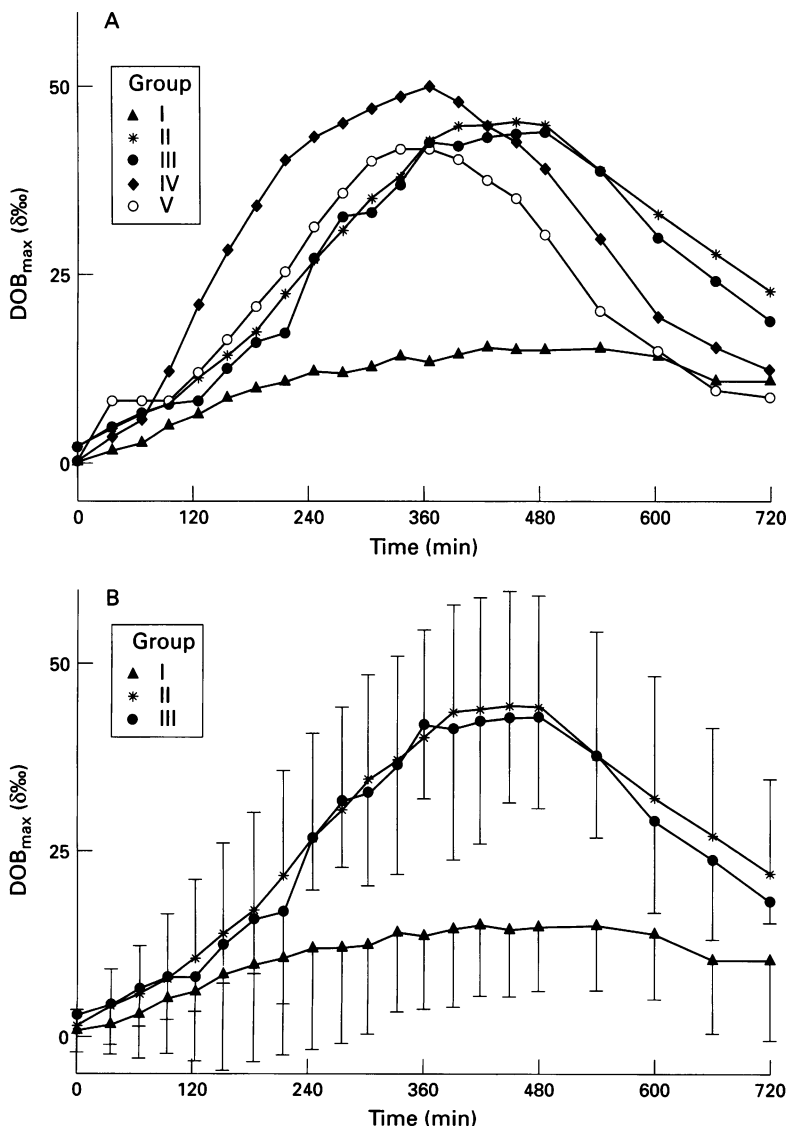


Figure 4: Time course of the ¹³CO₂ response after Hiolein ingestion. (A) Mean values, groups I–V. (I) Pancreatic steatorrhoea, (II) exocrine pancreatic insufficiency without steatorrhoea, (III) patients with normal pancreatic function, (IV) normal controls, (V) non-pancreatic steatorrhoea. (B) Mean (SD) values in patients with suspected (group III) and confirmed (groups I, II) pancreatic disease. Peak times are almost identical (see also Fig 5).

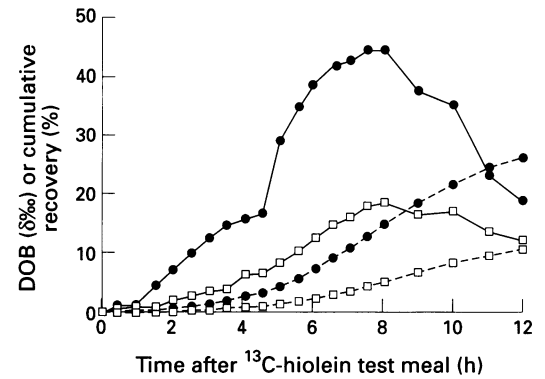


Figure 5: Effect of high dose pancreatin supplementation (Kreon micropellets, 80 000 U lipase given with the test meal) on the DOB responses (straight lines) and cumulative recovery of ¹³C (dotted lines) in one patient with pancreatic steatorrhoea. (□) Without pancreatin; (●) with pancreatin supplementation.

$m=51.34$ (2.64) (95% CI 46.01–56.67) and $k=2.30$ (0.89) (95% CI 0.51–4.09), $r=0.702$; Fig 3A).

Plotting lipase/DOB_{max} versus lipase output (Fig 3B) yielded a linear curve fit ($Y=0.021$ (95% CI 0.019–0.023) ± 0.001 X; $r=0.949$ ($p<0.0001$)).

Peak maxima of the DOB responses occurred six to nine hours after ingestion of the labelled test meal (Fig 4A, B), showing that a more feasible sampling period of eight hours would be sufficient. Although not significant, patients with malabsorption (group V) and the controls (group IV, comprising more females and younger subjects that the 'target' groups of patients with pancreatic disease) tended towards slightly earlier DOB_{max} responses.

However, testing the effect of high dose pancreatin supplementation (Kreon micropellets, Kali Chemie, Hannover, Germany; single dose of 80 000 U lipase with the test meal) in one patient of group I with a grossly abnormal DOB response (Fig 5), showed that pancreatin may restore the ¹³CO₂/¹²CO₂ response to normal but does not accelerate the time of peak occurrence (DOB_{max}).

Discussion

Breath tests are clinically feasible methods for assessing pancreatic dysfunction, because they do not require stool or urine collection (which is often incomplete) and because they are non-invasive. Compared with former breath tests using ¹⁴C labelled substrates (triglycerides), breath tests using the stable isotope ¹³C as a label have the additional advantage of being non-radioactive.

Another potential advantage of the [U-¹³C-] Hiolein breath test, which behaves like triolein¹³ is the use of only trace amounts of a nutritionally relevant substrate – that is, physiologically ingested long chain triglycerides (Table I).

Vantrappen *et al* have shown that another ¹³C-mixed triglyceride test may reflect duodenal lipase activity. This molecule bears the ¹³C label at the carboxy C atom of the medium chain fatty acid at C2 of the glycerol moiety (¹³C-octanoate). However, whereas

liberation of this substrate is dependent on duodenal lipolytic activity, and the use of octanoate ensures rapid oxidation, the test lipid applied is not a normal constituent of food.¹¹

Another substrate with rapid oxidation (peak maximum at 90–120 minutes) used for assessing pancreatic function by a breath test is ^{13}C -cholesteryl octanoate.^{9,26} This compound is not a substrate for classic pancreatic lipase activity but for cholesterol esterase. It is not hydrolysed by lingual or by gastric lipase. Therefore, monitoring of therapeutic lipase supplementation may give results that are different from a system which recognises these contributions (such as labelled triolein).

Evaluating the clinical validity and potential of the non-invasive and non-radioactive ^{13}C -Hiolein breath test, we have shown that DOB_{max} -responses of the breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio accurately reflect duodenal lipolytic activity after stimulation with CCK-Pz in patients with exocrine pancreatic insufficiency of different severities.

This correlation is not linear but hyperbolic, a finding that is confounded by the known correlation between lipase output after CCK-Pz and steatorrhoea⁵ as well as by results obtained with other breath tests for impaired lipolysis^{9,11} or impaired amylase output.¹⁴

Because pancreatic steatorrhoea is restricted to patients with severely impaired lipase output (<10% of normal – that is, <12 000 U/30 min), a significant reduction of DOB_{max} -responses was only found in patients with pancreatic steatorrhoea (Fig 2). The Hiolein breath test thus distinguished two groups of patients: those with a stimulated lipase output below the cut off at 10% of normal and patients with less (or no) functional impairment.

Focusing on diagnosing or monitoring pancreatic steatorrhoea, sensitivity (92%) and specificity (86%) of the $^{13}\text{CO}_2$ -Hiolein breath test were well acceptable with the diagnostic cut off at 41.4 ‰. These results are comparable with those obtained with radioactive triolein.⁷

However, the breath test did not detect mild or moderate forms of exocrine pancreatic dysfunction without steatorrhoea – that is, patients with a reduced duodenal lipase output after stimulation with CCK-Pz (12 000–65 000 U/30 min), resulting in an overall sensitivity of only 46.7% (specificity 90%) for diagnosing exocrine pancreatic insufficiency (lipase <65 000 U/30 min after CCK-Pz).

Because of the non-linear (hyperbolic) correlation between lipase output and the malassimilation of fat, this finding is not regarded as reflecting insensitivity of the new breath test. Rather it is the consequence of the physiological ('true') relation between lipase output and fat digestion.

Therefore, as with faecal fat analysis, we would not expect any fat absorption test principle to diagnose mild to moderate degrees of exocrine pancreatic insufficiency with clinically acceptable accuracy.^{7–9}

By contrast, more graded responses of lipolysis ($^{13}\text{CO}_2$ enrichment in the breath) to the pancreatic lipase output have been found

by Vantrappen *et al*¹¹ with the mixed triglyceride breath test resulting in a substantially better sensitivity (0.81) for the detection of pancreatic insufficiency. As this study used a similar amount of unlabelled fat, this implies that impaired lipolysis was detected at stages of exocrine pancreatic insufficiency when overall fat absorption was still complete. This test may therefore be more vulnerable regarding false positive results in cases of malabsorption.

Our data show that the ^{13}C -Hiolein breath test is a valid test for pancreatic steatorrhoea but does not reflect pancreatic function unless decompensation (steatorrhoea) occurs. Consequently, the clinical value of the Hiolein test is primarily targeted at diagnosing the patient who requires pancreatin therapy and to monitor the efficacy of enzyme supplementation (Fig 5), similar to faecal fat analysis.

Newer modifications of faecal fat analysis based on near infrared reflectance analysis allow a more convenient and acceptable quantitative analysis of pancreatic steatorrhoea than the van de Kamer method. Inadequate stool collection, however, can still render faecal fat analysis potentially misleading.^{17,18}

Therefore, a valid, clean, and feasible method both for detecting pancreatic steatorrhoea and monitoring enzyme replacement therapy may be advantageous in a clinical setting.

Because the fatty acids of Hiolein have their oxidation peak at six to nine hours, a second measurement eight hours after substrate ingestion may be adequate and sufficient.

Isotope ratio measurements without mass spectrometry (for example, non-dispersive isotope selective infrared analysis),²⁷ and established logistics for isotope ratio mass spectrometry on an outpatient base are recent important developments, which will help to promote stable isotope breath tests in gastroenterology as a routine clinical application. In malnourished paediatric patients – for example, in cystic fibrosis – the ^{13}C -Hiolein breath test may be used as an accurate and convenient measure of pancreatic steatorrhoea (Braden *et al*, unpublished observations).

Part of this work has been presented in abstract form at the 48th Annual Meeting of the Deutsche Gesellschaft für Verdauungs- und Stoffwechselkrankheiten, Frankfurt, September 1993 (*Z Gastroenterol* 1993; 31: 543 A).

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