[¹⁴C]-triolein absorption: a useful test in the diagnosis of malabsorption

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SUMMARY The [¹⁴C]-triolein absorption test has been assessed using a preprepared fat meal in a procedure that can be completed within a working day on an inpatient or outpatient basis. An initial study showed complete discrimination between 30 control subjects and 13 patients with raised faecal fat excretion. A prospective evaluation in 57 patients under investigation for gastrointestinal disease showed a sensitivity of 85% and specificity of 93% (100% if two obese subjects are excluded). The test discriminates satisfactorily between patients with and without fat malabsorption and makes the measurement of faecal fat unnecessary in most circumstances.

For many years, the measurement of faecal fat has been the gold standard used for assessing malabsorption.¹ There are, however, many difficulties associated with the collection of specimens and results may be misleading.² In addition the test is unpleasant for the patient, and for nursing and laboratory staff, and in order to carry out the collection under controlled conditions, the patient should be hospitalised.

Alternative methods of assessing fat absorption include the measurement of plasma turbidity after a standard meal, the microscopic examination of stool for fat globules, and the measurement of breath ¹⁴CO₂ after a dose of carbonyl-labelled triglyceride added to a fatty meal. Studies have been done using [¹⁴C]-triolein, [¹⁴C]-tripalmitin and [¹⁴C]-triolein,³ but the use of [¹⁴C]-triolein has been the most successful.⁴

In this study the triolein absorption test was carried out using a preprepared palatable fat meal in patients known to have fat malabsorption and in normal subjects. Subsequently a prospective study was carried out on patients being investigated for diarrhoea. The ranges obtained from the initial study were used to determine whether or not fat malabsorption was present. The results were then evaluated against the final diagnosis.

Methods

EXPERIMENTAL DESIGN AND SUBJECTS

The study which was approved by the Southern Derbyshire Health Authority Ethical Committee was conducted in two parts: (a) an initial comparison of 30 control subjects (group 1) with 13 subjects (age range 21-73 years, median 57) known to have a raised faecal fat (group 2). Of the subjects in group 1 (age range 22-88 years, median 32), 25 were laboratory staff and five were patients without evidence of gastrointestinal disease and with normal faecal fat (<18 mmol/24 h, <5 g/24 h). The objective of this part of the study was to establish a reference range for the procedure. (b) A prospective evaluation of the test in 57 subjects under investigation for possible gastrointestinal disease. They were assigned to either group 3 or group 4 on the basis of the diagnosis made subsequently on clinical, histological, radiological and laboratory grounds. Thirty one patients (age 17-78 years, median 45) assigned to group 3 were considered not to have a disease likely to cause fat malabsorption at the time of the test (Table 1). Group 4 consisted of 26 patients (age range 19-84 years, median 48) considered to have a disease likely to cause fat malabsorption (Table 2).

The 10 day rule was observed in women of child-bearing age and the test was not done in subjects under 16 years of age.

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PROCEDURE

After an overnight fast a basal breath sample was collected (see below). The patient then ingested a lemon mousse containing a standard amount of fat (Table 3) and 185 kBq (5 μ Ci) [¹⁴C]-triolein (Amersham International). The [¹⁴C]-triolein supplied as a solution in toluene was dispensed on to a stainless steel teaspoon and the toluene evaporated in a stream of cool air. After eating the mousse, using the teaspoon, the subject drank a glass of water to remove any radioactivity remaining in the mouth. Smoking, eating or drinking (except water) were not permitted for the first four hours of the test and the subject was encouraged to rest for the duration of the investigation. One hour after ingestion of the fat meal and at hourly intervals thereafter, breath samples were collected. After the fourth hourly collection, a light lunch was permitted together with tea or coffee. Breath samples were collected for a further three hours. The height and weight of the patients were noted.

ANALYTICAL PROCEDURES

Faecal fat was determined on a three day collection using the method of van de Kamer,⁵ modified by using tetramethylammonium hydroxide for the titration.

Expired CO₂ was collected into a trapping agent consisting of 2 ml hyamine hydroxide (2 mol/l), 2 ml ethanol and 50 μ l ethanolic thymolphthalein solution (100 mg/l). This volume trapped 2 mmol CO₂ and saturation was indicated by a change in colour of the thymolphthalein from blue to colourless. The apparatus for collecting the breath samples consisted of a plastic tube (disposable) inserted in a cork which was lodged in the end of a 10 ml plastic syringe barrel. A plastic tube (disposable) was fixed to the bottom end of the barrel and placed in the trapping solution in a scintillation vial. The importance of collecting end expiratory gas rather than tidal gas was explained to the patient. They were required to breathe out normally before blowing end expiratory gas into the vial. Careful supervision ensured the collection of satisfactory breath samples. Subsequently 10 ml of scintillation fluid (Instagel, Packard) was added to the vials which were left overnight to allow chemiluminescence to subside before counting for 20 minutes.

CALCULATIONS

Results were expressed as follows: (a) peak counts per minute (cpm – that is, breath sample with highest cpm) corrected to a body surface area of 1.73 m²; (b) peak CO₂ excretion/h expressed as % of the dose (based on peak cpm), calculated as:

$$\frac{\text{peak cpm} \times 9 \times \text{body wt (kg)} \times 100}{2 \times 1.11 \times 10^7}$$

Table 1 *Diagnosis of 31 patients assigned to group 3*

<i>Group 3 Diagnosis</i>	<i>n</i>	<i>Abnormal peak % dose (n)</i>
Infective diarrhoea resolved, unknown cause	1	–
Obese, no cause found for diarrhoea	4	2
Celiac, on GFD, with biopsy response	2	–
Reformed alcoholic	1	–
Ulcerative colitis malnutrition	1	–
Cryptosporidiosis	1	–
Colitis, unknown cause, resolved	1	–
Diverticular disease of colon	2	–
Irritable bowel syndrome	15	–
Rectal polyp	1	–
Normal (sibling has coeliac disease)	1	–
GI bleed, ?Mallory Weiss tear	1	–

Table 2 *Diagnosis of 26 patients assigned to group 4*

<i>Group 4 Diagnosis</i>	<i>n</i>	<i>Normal peak % dose (n)</i>
Postgastrectomy	1	1
Pernicious anaemia with evidence of malabsorption	1	–
Pancreatic insufficiency, diverticular disease	1	–
Diabetes, chronic pancreatitis	1	–
Obstructive jaundice	1	–
Cystic fibrosis	1	–
Shwachman-Diamond	1	–
Malabsorption, cause unknown	1	–
Celiac, not on GFD	9	–
Blind loop syndrome	1	–
Crohn's with small bowel involvement	3	–
Metastatic carcinoma tumour with steatorrhoea	1	–
Ileal resection	2	1
Ca colon, involving 50 cm term. ileum	1	–
Segmental ileitis, diverticular disease of colon	1	1

Table 3 *Preparation of mousse for fat absorption test*

<i>Recipe for Mousse (10 portions)</i>	
100 g sugar	
2–3 lemons – grated rind and strained juice	
400 ml double cream	
30 g ground gelatine	
2 egg whites	
300 ml water	
<i>Method</i>	
1 Dissolve gelatine in some of measured water	
2 Add remaining water to lemon juice	
3 Dissolve sugar in lemon juice, add gelatine and lemon rind. Cool	
4 Fold in slightly whipped cream	
5 Beat whites of egg until stiff	
6 Fold into mixture	
7 Divide into 10 equal portions	
8 Label FAT TEST and date, FREEZE	
<i>Analysis per portion</i>	
Carbohydrate	: 10 g
Fat	: 19.3 g
Protein	: 1.4 g

Fig. 1 Comparison of results in 30 control subjects (group 1) and 13 patients with steatorrhoea (group 2).

This formula is derived as follows: (i) it is assumed that normal CO_2 production is 9 mol/kg/h,⁶ (ii) each vial traps 2 mmol CO_2 , therefore the activity of

$^{14}\text{CO}_2/\text{mmol CO}_2 = \frac{\text{cpm}}{2}$ thus the amount of $^{14}\text{CO}_2$

produced = $\frac{\text{cpm}}{2} \times 9 \times \text{body wt (kg)}$, (iii) the dose of

triolein (185 kBq) = 1.11×10^7 cpm; (c) CO_2 excretion/3 h expressed as % of the dose (based on the sum of the 4th, 5th, and 6th hourly collections), calculated as:

$$\frac{\text{cumulative cpm} \times 9 \times \text{body wt (kg)} \times 3 \times 100}{2 \times 3 \times 1.11 \times 10^7}$$

The derivation of the formula is similar to that described in (b) above.

Results

COMPARISON OF SUBJECTS WITH STEATORRHOEA WITH CONTROL SUBJECTS

Excellent discrimination was shown (Fig. 1) between the 13 patients with steatorrhoea (group 2) and 30 control subjects (group 1). On the basis of these results the lower limits of normal fat absorption were

defined as 980 cpm, 2.8% and 6.9% for the three ways of expressing the results (Table 4).

RESULTS OF THE TEST IN PATIENTS

INVESTIGATED FOR POSSIBLE MALABSORPTION

The result of the [^{14}C]-triolein absorption test, as peak CO_2 excretion/h expressed as percentage of the dose, for both groups of patients is shown in Figure 2. An abnormal result was observed in two of the 31 patients in group 3. A normal result was observed in three of the 26 patients in group 4.

COMPARISON OF FAECAL FAT AND [^{14}C]-TRIOLEIN ABSORPTION TEST

Faecal fat was measured in 25 of the 100 subjects included in groups 1–4; the comparative data are shown in Figure 3. The 16 subjects in whom both tests

Table 4 Results for the initial comparative study. Data from group 1 expressed as mean $\pm$ 2SD to indicate the lower limit of normal. Data from group 2 are expressed as the mean $\pm$ 2SD

	Group 1 $\bar{x} \pm 2SD$	Group 2 $\bar{x} \pm 2SD$	<i>p</i>
Peak cpm	980	950	<0.0005
Peak CO_2/h	2.80	2.70	<0.0005
$\text{CO}_2/3\text{h}$	6.91	6.93	<0.0005

Fig. 2 Comparison of results of the test in 57 subjects under investigation for gastrointestinal disease (see text for description of groups 3 and 4).

were abnormal were from groups 2 and 4. The five subjects in whom both tests were normal were from groups 1 and 3. The subject with a normal faecal fat and low peak percentage dose/h (2.0%) was one of the obese subjects in group 3. The patient with a raised faecal fat and borderline peak percentage dose/h (2.8%) and the two patients with raised faecal fat and normal peak percentage dose/h (4.5 and 4.8%) are referred to in the Discussion.

Discussion

Newcomer *et al*⁴ showed that the [¹⁴C]-triolein absorption test with measurement of expired ¹⁴CO₂ was capable of discriminating between normal subjects and patients with steatorrhoea. A subsequent study² indicated that this procedure was the most satisfactory of the alternatives to the measurement of 72 h faecal fat and drew attention to the variability of bowel habit as demonstrated by the wide range of recovery of radio-opaque markers over the three day period. A 60 g fat load with breath collection over nine hours was used by these authors.

The study reported here was carried out to assess the value of the [¹⁴C]-triolein test as an alternative to the measurement of the three day faecal fat as a screening test for malabsorption. A smaller fat load

(19.3 g) was used to enable the procedure to be completed in a working day. In view of its unreliability, the faecal fat test is an unsatisfactory yardstick against which to compare the [¹⁴C]-triolein test. We therefore chose to evaluate the procedure against the diagnosis established subsequently on the basis of clinical, histological, radiological, and laboratory investigations.

Previous studies have used a variety of fat loads and meals as shown in Table 5. From a comparison of 25 and 50 g fat loads it was shown that the peak excretion of ¹⁴CO₂ occurred later with the larger fat load (seven, eight or nine hours), so that the test is less convenient in routine use.⁴ In our experience, the use of fatty emulsions is unpleasant for the subject and may cause nausea and delayed gastric emptying. Carbohydrate was included in order to make the mousse palatable. Peak ¹⁴CO₂ excretion occurred earlier in our subjects without fat malabsorption (groups 1 and 3) than in controls from a study⁴ using 25 g fat as a fatty emulsion (Table 6). The fat meal proposed in this study, presented as a lemon mousse, is well accepted by patients and enables the procedure to be completed satisfactorily between 9 00 am and 4 30 pm.

Table 5 Comparison of fat loads, [¹⁴C]-triolein activity and meals used in ¹⁴C-triolein absorption tests

Reference	Fat load and activity of [¹⁴ C]-triolein	Fat meal
2	60 g fat; 370 kBq	100 ml milk, 50 g full cream dried milk, 100 g ice-cream, 75 ml double cream and 1–2 g instant coffee powder with slice of toast or gluten free bread, lightly spread with butter.
4	25 g fat; 185 kBq	30 ml lipomul (20 g fat), 5 g triolein.
7	42 g fat; 74 kBq	Standardised breakfast; 42 g fat, 35 g carbohydrate and 26 g protein.
8	30 g fat; 185 kBq	1 ml olive oil, 10 ml arachidis oil, 2 slices toast with margarine, cheese and cup of tea.
9	32 g fat; 65 kBq	100 ml Intralipid
10	– 185 kBq	5 ml olive oil and 2 egg yolks
This study	19.3 g fat; 185 kBq	Lemon mousse, 19.3 g fat, 10 g carbohydrate and 1.4 g protein.

Table 6 Time of peak ¹⁴CO₂ excretion (up to six hours) in subjects without fat malabsorption

	Collection time (h)			
	3	4	5	6
This study (n=61)	0	24%	44%	32%
Newcomer <i>et al</i> 1979 (n=36)	0	6%	25%	69%

Fig. 3 Comparison of the triolein absorption test and faecal fat excretion in 25 subjects. The interrupted lines indicate the upper limit of normal faecal fat excretion and the lower limit of normal fat absorption by the [^{14}C]-triolein absorption test.

The results (Fig. 1) showed excellent discrimination between control subjects and patients with steatorrhoea for all three ways of expressing the data. The peak $^{14}\text{CO}_2$ excretion/h as a percentage of the dose, has been used by several groups of workers with a lower limit of normal of about 3.5%. The lower limit of normal in this study was slightly lower (2.8%) which may be due to the carbohydrate present in the meal inhibiting fatty acid oxidation.⁴ A lower limit of normal of 1.5% was seen after a meal containing 42 g fat, 35 g carbohydrate, and 26 g protein.⁷ Another group included two slices of toast with their fat load, however, and reported a lower limit of normal of 3.5%,⁸ although most of their subjects did not reach a peak $^{14}\text{CO}_2$ excretion until at least six hours. The fat load of 19.3 g, used in the present study led to peak $^{14}\text{CO}_2$ excretion within six hours in 97% of subjects without fat malabsorption.

Of the patients with fat malabsorption (groups 2 and 4), 29% showed a peak excretion later than six hours and peak excretion in general occurred later in these groups, although there were two patients who showed an early peak at three hours (Table 7).

Of the 31 patients assigned to group 3 – that is, those believed unlikely to have fat malabsorption, two had a peak percentage dose/h less than 2.8 (2.6 and 2.0%), and therefore consistent with fat malabsorption. Both were obese (>25% above their standard weight for height). Obesity is known to lead to falsely low results⁴ and recalculation of these patients' results on the basis of the ideal weight gives normal results. One patient assigned to group 3 (coeliac disease on a gluten free diet) had a borderline peak percentage dose/h of 2.8 and was shown subsequently to have a faecal fat of 34 mmol/24 h (Fig. 3). Thus of the 31 patients assigned to this

Table 7 Comparison of time of peak $^{14}\text{CO}_2$ excretion in subjects with and without fat malabsorption

		Time of peak $^{14}\text{CO}_2$ excretion (h)				
		3	4	5	6	7
Groups 1 and 3	n (%)	0 (0)	15 (25)	28 (46)	16 (26)	2 (3)
Groups 2 and 4	n (%)	2 (5)	1 (3)	11 (28)	14 (36)	11 (28)

group, the test correctly identified 28 as normal with respect to fat malabsorption (two were obese), and in one (incorrectly allocated to group 3), the test gave a false (but borderline) negative result, on the basis of a comparison with faecal fat. Twenty six patients were assigned to group 4 – that is, they were considered to have a disease likely to cause fat malabsorption, of whom 23 had an abnormal breath test. Two of the three patients in this group with normal breath tests (4.5%, 4.8%) had unequivocally raised faecal fat excretion (73 and 49 mmol/24 h, Fig. 3). The first underwent a gastroenterostomy in 1945 for peptic ulcer. In 1966 a diagnosis of pancreatitis was made and in 1967 a cholecystectomy was carried out. Symptomatic improvement was observed on treatment with pancreatic supplements. The second patient had diverticular disease of the colon and segmental ileitis with persistent hypokalaemia. The final patient in this group (peak % dose/h=3.6%) also had longstanding steatorrhoea after ileal resection for Crohn's disease. It is possible that both of these patients had bile acid malabsorption secondary to ileal disease leading to progressive depletion of their bile salt pool so that fat absorption from a meal early in the day was normal but over a 24 hour period fat absorption was significantly impaired.

The test thus accurately identified 23 patients as having fat malabsorption with three false negative results.

The sensitivity and specificity of the test were calculated from the prospective study and are shown in Table 8. If the two obese subjects are excluded then there were no false positive results so that the specificity of the test was 100%.

The predictive value of a positive test was 92% (100% if the two obese subjects are excluded). The predictive value of a negative test was 88%.

Table 8 Sensitivity and specificity of the $[^{14}\text{C}]$ -triolein absorption test

Breath test	Disease likely to cause fat malabsorption	
	Present	Absent
Normal	4	28
Abnormal	23	2 (obese)
Sens/spec	85%	93%

Although the sensitivity of the test in this series was only 85%, this is in part due to difficulties of a study of this nature. One of the four subjects with a normal breath test and disease likely to cause fat malabsorption had coeliac disease, but was on a gluten free diet. We have interpreted her peak percentage dose of 2.8 as normal; in practice, however, this value at the bottom of the reference range together with the clinical details would suggest that fat absorption may be low. Other tests, for example, those using carbohydrate permeability markers^{11,12} may provide a better guide to compliance than the assessment of fat absorption. Similarly, in the two patients with known ileal disease/resection other tests may have been more appropriate, although further study of the test in patients with ileal disease is indicated.

By combining data from earlier studies^{2,4,8} with this study, the predictive value of a positive test is 89% (excluding obese subjects) and the predictive value of a negative test is 97%. These results suggest that measurement of peak breath $^{14}\text{CO}_2$ provides a useful method for evaluating fat malabsorption and that the additional analytical work involved in the measurement of serum ^{14}C -fatty acids⁷ is unnecessary.

The results from both the initial study and the prospective evaluation indicate that the test satisfactorily discriminates between patients with and without fat malabsorption and in most circumstances makes the measurement of faecal fat unnecessary. By using this test as a screening procedure in patients with suspected malabsorption, subsequent investigation can be directed towards those conditions likely to be associated with either normal or impaired fat absorption. Although the radiation dose is less than the exposure from a chest radiograph,¹³ the test should not be used in children or during pregnancy. The accuracy of the test may be decreased in obesity, diabetes, and thyroid or pulmonary disease which alter the metabolism of absorbed $[^{14}\text{C}]$ -triolein or the excretion of breath $^{14}\text{CO}_2$.

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