CLINICAL RESEARCH

Comparison of simple screening tests for fat malabsorption

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Abstract

Three tests were evaluated as screening procedures for fat malabsorption—namely, measurement of serum optical density, serum triglyceride concentration, and $^{14}\text{CO}_2$ breath excretion after the administration of a 60 g fat meal containing 10 μCi glycerol tri[1- ^{14}C]oleate. The results of these tests were compared with fat excreted in a three-day faecal collection after adjustment for completeness of collection as assessed by using non-absorbable radio-opaque markers. Fifty-two patients with various symptoms and eight normal subjects were studied. The maximum increase in serum optical density or triglyceride concentration above the fasting value discriminated poorly between subjects with normal and increased adjusted faecal fat excretion. In contrast, seven- or eight-hour cumulative $^{14}\text{CO}_2$ breath excretion provided good discrimination with only four (7%) false-positive and no false-negative results.

The simplicity and convenience of breath analysis make it an attractive alternative to analysis of faecal fat excretion in screening for fat malabsorption.

Introduction

Faecal fat analysis has widely recognised disadvantages including the unpleasantness of stool collection and analysis and uncertainty about the completeness of collection and dietary fat intake. Nevertheless, measuring the fat content of a three- to five-day stool collection remains the standard screening test for fat malabsorption. Alternative screening procedures include oral fat loading with subsequent measurement of serum lipid or absorbance changes^{1 2} and carbon-14-labelled-triglyceride loading with measurement of breath $^{14}\text{CO}_2$ excretion.^{3 4} Although

each procedure has been claimed to discriminate well between healthy subjects and patients with steatorrhoea, none has gained widespread acceptance.

These tests have not been compared with each other nor have they been evaluated against faecal fat assay using markers to check the completeness of stool collection. Accordingly, we re-evaluated these alternative procedures, comparing $^{14}\text{CO}_2$ breath excretion, serum triglyceride concentration, and serum optical density after an oral fat load containing ^{14}C triolein with a three-day faecal fat assay adjusted for recovery of non-absorbable markers.

Subjects and methods

SUBJECTS

Fifty-two unselected subjects (24 men and 28 women) aged 15-81 years (mean 51 years) with various gastrointestinal and non-gastrointestinal symptoms participated in the study. Their body weights ranged from 27 to 110 kg (mean 61 kg). Thirty-nine subjects were admitted for the entire study. Faecal collections were made at home in the remaining 13 subjects. The table shows the various diagnostic categories of the subjects.

Diagnostic grouping of patients in study

Group	Subgroup	No of patients
Small-bowel disease (n = 23)	Coeliac disease (treated)	7
	Coeliac disease (untreated)	2
	Postgastrectomy	6
	Crohn's disease	3
	Duodenal ulcer	3
	Terminal ileal resection	1
	Postvagotomy	1
Pancreatic disease (n = 5)	Previous acute pancreatitis	2
	Chronic pancreatitis	2
	Carcinoma of head of pancreas	1
Hepatic disease (n = 8)	Alcoholic liver disease	4
	Primary biliary cirrhosis	2
	Secondary biliary cirrhosis	1
	Chronic active hepatitis	1
Miscellaneous disorders (n = 16)	Polycystic kidney disease	1
	Dietary iron deficiency	1
	Folate deficiency (unknown cause)	1
	Growth failure	1
	Respiratory cachexia	1
	Colonic disease	3
	Irritable bowel syndrome	8

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Eight healthy laboratory staff (six men and two women) aged 33-54 years (mean 42 years), with body weights from 52 to 79 kg (mean 68 kg) and no gastrointestinal symptoms, were included in the study but did not make faecal collections. Faecal fat values were assumed to be normal.

All subjects gave their informed written consent to the study, which was approved by the hospital ethical committee.

PROTOCOL

At the start of each study a blood sample was taken from each subject after an overnight fast. Subjects blew into a scintillation phial containing hyamine hydroxide and ethanol, trapping 2 mmol of CO_2 . They were then given a 60 g fat meal to which had been added 10 μCi of glycerol tri[1- ^{14}C]oleate (Radiochemical Centre, Amersham). With the meal subjects were given a slice of toast or gluten-free bread (those with coeliac disease) lightly spread with butter. Blood samples were collected hourly from two to six hours and breath samples hourly from two to nine hours. In nine subjects breath samples were collected for only seven or eight hours. Serum was separated within 30 minutes of collection and stored at 4°C.

Subjects remained at rest during the test and were allowed one cup of water or black coffee without sucrose every two hours. Eight radio-opaque polyethylene pellets (Portex Ltd, Hythe, Kent) were given three times a day with each of the main meals for five days, and from days 3 to 5 faeces were collected. During the five-day period inpatients received a diet containing about 70 g fat a day, while subjects collecting faeces at home were instructed by the hospital dietitians to prepare food with a fat content of about 70 g per day.

The fat meal consisted of 100 ml milk (fat content 3.8 g); 50 g full-cream dried milk (fat content 13.2 g); 100 g ice cream (fat content 8.2 g); 75 ml double cream (fat content 36 g); and 1.2 g instant coffee powder. The meals were made up in bulk, portioned, and stored at -20°C until use.

METHODS

Faecal analysis—The weighed stool sample was radiographed and the number of pellets counted. The presence of 72 pellets was taken as 100% marker recovery. Faecal fat was estimated in the stool⁵ and the result adjusted for marker recovery.⁶

Serum optical density was measured within seven hours of collection and the maximum increase recorded at room temperature with a Perkin Elmer spectrophotometer at 620 nm using the fasting sample as blank.

Serum triglyceride concentrations were measured within four days of collection using an LKB reaction rate analyser.⁷ The maximum increase in triglyceride concentration above the fasting sample was recorded.

Breath $^{14}\text{CO}_2$ activity was measured in the hourly samples by liquid scintillation counting (Nuclear Enterprises, Edinburgh) and compared with a standard obtained by diluting the stock ^{14}C -triolein. Breath samples and the standard were counted under identical conditions. The hourly values were expressed⁸ as a percentage of the total radio-activity ingested per mmol $\text{CO}_2 \times 10^4$. Cumulative hourly values from zero to nine hours were recorded.

Results

Marker recovery—The mean faecal pellet recovery was 74% in subjects with an abnormal adjusted faecal fat excretion (range 14-112%) and 93% in subjects with normal adjusted faecal fat values (range 22-125%). Figure 1 shows the distribution of values.

Mean faecal weight for the three-day collection was 595 g in those with an abnormal adjusted faecal fat excretion (range 70-2090 g) and 445 g in those with a normal adjusted value (range 95-1500 g). Figure 1 shows the distribution of values.

Faecal fat—Figure 2 shows adjusted faecal fat values. Abnormal values (defined as greater than 18 mmol/24 h (5 g/24 h)) were detected in 32 subjects (62%). In seven subjects (13%) faecal fat excretion was normal but became abnormal after adjustment for marker recovery. In subjects with increased faecal fat excretion, values after adjustment always lay outside the normal range.

Serum optical density—Peak values occurred within the six-hour period of measurement in 55 subjects (92%). Peak optical density was 0.75 ± 0.31 (mean $\pm$ SD) in subjects with a normal adjusted faecal fat

excretion but 0.52 ± 0.32 in those with an abnormal adjusted faecal fat excretion. This difference in mean values was significant ($p < 0.01$), but discrimination between the two groups was nevertheless poor (fig 2).

Serum triglyceride concentrations—Peak values were observed within the six-hour period of measurement in 52 subjects (87%). The peak concentration was 1.66 ± 1.06 mmol/l (147 ± 94 mg/100 ml) in subjects with normal adjusted faecal fat excretions but 0.98 ± 0.74 mmol/l (87 ± 66 mg/100 ml) in subjects with abnormal adjusted faecal fat excretions. This difference in mean values was significant ($p < 0.01$), but discrimination between the two groups was again poor (fig 2). The correlation between peak triglyceride concentration and peak absorbance was good ($r = 0.78$, $p < 0.01$).

FIG 1—Recovery of faecal pellets and distribution of faecal weight based on three-day faecal collection. Horizontal lines indicate mean values.

● = Subjects with abnormal adjusted faecal fat excretion. ▲ = Subjects with normal adjusted faecal fat excretion. ■ = Subjects with normal adjusted faecal fat excretion and abnormal cumulative seven-hour excretion of $^{14}\text{CO}_2$.

Breath $^{14}\text{CO}_2$ activity is usually expressed as peak percentage $^{14}\text{CO}_2$ excretion per mmol CO_2 .³ In the present study peak values were achieved in only 15 subjects (25%) by seven hours and in 25 (42%) by nine hours. Figure 3 gives the cumulative excretion of $^{14}\text{CO}_2$ at six, seven, eight, and nine hours and shows that discrimination between subjects with normal and abnormal adjusted faecal fat excretions was good at seven and eight hours but less accurate at six and nine hours. The cumulative seven-hour breath $^{14}\text{CO}_2$ expressed as the percentage triolein dose $\times 10^4$ was used in subsequent expressions of the $^{14}\text{CO}_2$ data. The lower limit of normal was taken as $185 \times 10^{-4}\%$. No false-negative results were obtained, but four of the 60 subjects yielded false-positive values with respect to adjusted faecal fat values. One subject had coeliac disease, was receiving a gluten-free diet, and did not have diarrhoea. The second subject had macrocytic anaemia due to folate deficiency, and a repeat breath test confirmed the original low value; further investigation was refused by the patient. The third patient had inflammatory bowel disease; results of liver function tests were abnormal, and a liver biopsy specimen showed severe fatty changes. The fourth patient was diagnosed as having the irritable bowel syndrome, and a repeat breath test confirmed the original low value.

Discussion

The results we obtained using ^{14}C -triolein showed good discrimination between subjects with and without steatorrhoea as assessed by faecal fat analysis. In contrast, the discrimination

FIG 2—Cumulative seven-hour excretion of ¹⁴CO₂, peak optical density, peak triglyceride concentration, and unadjusted and adjusted faecal fat excretions in all subjects. (Symbols as in figure 1.)
Conversion: SI to traditional units—
Triglyceride: 1 mmol/l ≈ 88.6 mg/100 ml.
Faecal fat: 1 mmol/24 h ≈ 28.4 mg/24 h.

FIG 3—Cumulative six-, seven-, eight-, and nine-hour excretions of ¹⁴CO₂ in all subjects. (Symbols as in figure 1.)

obtained with serum triglyceride concentrations or absorbance measurements was considerably poorer.

Several groups have used oral fat tolerance tests to screen for steatorrhoea in adults. Most workers use butter with toast to provide the fat load, given either as a fixed quantity of lipid^{1 8} or as 0.5 or 1.0 g/kg body weight.^{2 9} Plasma lipid changes are usually measured by spectrophotometry or nephelometry. These tests have been claimed to discriminate well between subjects with and without fat malabsorption. In our experience (fig 2), however, the poor discrimination obtained with serum triglyceride concentrations or absorbance measurements invalidates their use as screening tests, although the conditions of testing differed from those used by other authors.

Most workers assessing ¹⁴CO₂ breath tests have used ¹⁴C-glycerol tripalmitate and in general claimed good discrimination between healthy subjects and patients with steatorrhoea.^{3 4 10-13} These workers gave the ¹⁴C-triglyceride with corn oil as either 1 g/kg body weight^{3 10-12} or 25 g per subject.^{4 13} By contrast two groups, one using a 12.5 g fat load of sour cream¹⁴ and a second 25 g corn oil¹⁵ but otherwise a similar technique, found poor discrimination.

The first use of ¹⁴C-triolein was reported in 1966 but in only a few patients.¹⁶ Newcomer *et al*⁴ compared ¹⁴CO₂ breath excretion after 5 μ Ci triolein and 5 μ Ci tripalmitin given sequentially to the same subjects together with 25 g corn oil. In their larger study they claimed good discrimination between healthy subjects and those with steatorrhoea with both triglycerides, although ¹⁴C-triolein was superior.

We chose a 60 g fat meal rather than a smaller fat load to increase the rise in maximum serum absorbance and triglyceride values. Differences in the timing of peak ¹⁴CO₂ excretion noted between this and previous studies probably relate to variations in the amount and composition of the inert fat load or in the

rate of gastric emptying. Increasing the corn-oil load from 25 to 50 g delays excretion of ^{14}C from ingested ^{14}C -triolein by several hours.⁴

Attempts to increase the discriminatory power of breath tests by adjusting ^{14}C excretion for body weight (PSW *et al*, unpublished observations) and basal metabolic rate¹⁷ have not proved successful. Obese subjects (body weight 25% in excess of standard weight), however, have reduced ^{14}C excretion after ^{14}C -tripalmitin.¹⁰ By contrast, ^{14}C excretion is enhanced in diabetes mellitus.¹⁵ The interpretation of the test may be in doubt in these disorders and in others affecting the distribution or metabolism of ^{14}C -triglyceride such as thyrotoxicosis, chronic respiratory disorders, and parenchymatous liver diseases, but evaluation remains to be carried out.

Non-absorbable radio-opaque pellets are simple and convenient to use and correlate closely with polyethylene glycol and chromic oxide as continuous faecal markers.⁶ Our finding of seven subjects in whom the breath-test values became consistent with the adjusted faecal fat excretion suggests that using markers is important in such evaluations. Nevertheless, in one subject with an obstructing carcinoma of the colon who was not included in the study no pellets were recovered despite passage of stool. Plain abdominal x-ray examination showed retention of all the pellets proximal to the obstructing lesion.

We conclude that measuring either maximal triglyceride concentration or absorbance after a 60 g fat meal does not discriminate satisfactorily between subjects with and without increased faecal fat excretion. Cumulative ^{14}C breath excretion after ingestion of 10 μCi ^{14}C -triolein (administered with the fat meal), however, provided good discrimination with no false-negative and only four apparent false-positive results.

^{14}C breath testing is simple to perform, reliable, and acceptable to patients and staff. These advantages make it an attractive alternative to stool collection in screening for fat malabsorption. While it may be premature to suggest that breath testing might replace faecal fat analysis completely, our results suggest that the finding of normal ^{14}C breath excretion is good evidence of normal fat absorption. Under these circumstances stool collection may be avoided.

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ONE HUNDRED YEARS AGO A memorandum has been drawn up by the Local Government Board, embodying the hygienic principles laid down by various English and foreign authorities as requisite to be observed in the establishment of a cemetery, to prevent it from becoming a source of nuisance and danger to the living. The dangers to public health, to which places of burial may give rise, are of two kinds, viz, the contamination of air by the gaseous and volatile, and of drinking water by the liquid and soluble, products of decomposition. Contamination of air may take place in several modes. The gases evolved from putrefying bodies may make their way to the surface through pores or fissures in the ground, or may pass into open graves dug in their neighbourhood. Or they may diffuse themselves laterally through the ground-air, and be drawn up into the interior of houses. Or noxious emanations may be given off from putrid drainage-water, whether baled out of graves and thrown upon the surface, or draining into open channels or watercourses. Thus nuisance and danger to health may be occasioned, not only to gravediggers and persons attending funerals, but also to the inhabitants of houses in the neighbourhood of the burial-ground. To obviate these risks, it is necessary that the number of decomposing bodies in a given portion of ground should not at any time be so great that the gaseous products cannot be oxidised into harmless substances in the interstices of the soil, or taken up by vegetation; that a sufficient depth of earth intervene between corpses and the surface; and that the soil be of a suitable nature and properly drained, the drainage-water being innocuously disposed of. Furthermore, since the atmospheric contamination which

has to be especially guarded against is that of the air in the interior and neighbourhood of human habitations and frequented places, it is necessary that the place of burial should be in an open situation and at a sufficient distance from dwellings, in order that any effluvia arising from it may be diluted by diffusion, or dispersed by the winds, so as not to find their way, in an injurious state of concentration, to places where they will be liable to be inhaled. Foul liquids from graves may enter and pollute a stream or wells in the vicinity of a graveyard, may be injured by percolation from it, and in either case, if the water be used for drinking, injury to health may be occasioned. The liability of wells to pollution obviously depends partly upon their proximity to it, and partly upon the configuration and geological structure of the ground. Thus an intervening impervious bed of clay will prevent foul matters from reaching a well, and filtration through a sufficient distance of porous aerated soil decomposes such matters into harmless inorganic substances, which are fixed by the soil or taken up by plants. It is necessary therefore, in order to obviate risk from this cause, that a cemetery should have a suitable soil and be properly drained; and that it should be at a sufficient distance from subterranean sources of water-supply; and in such a position with respect to them that the percolation of foul matters from one to the other may be impossible. The sanitary requirements for a cemetery indicated under the foregoing remarks may be summed up under four headings:—1 Suitable soil and proper elevation of site; 2 A suitable position, especially with respect to houses and sources of water-supply; 3 Sufficient space; 4 Proper regulation and management. (*British Medical Journal*, 1881.)