^{14}C-TRIPALMITIN BREATH TEST AS A DIAGNOSTIC AID FOR FAT MALABSORPTION DUE TO PANCREATIC INSUFFICIENCY

I-Wen Chen, Kambiz Azmudeh*, Alastair M. Connell, and Eugene L. Saenger

University of Cincinnati, College of Medicine, Cincinnati, Ohio

Specific radioactivity of expired $^{14}CO_2$ after ingestion of a test meal containing 5 μCi of ^{14}C-labeled tripalmitin was measured at 1-hr intervals for 8 hr and also at 24 hr. Two or more days later, the test was repeated with ten tablets of Viokase (containng 650 FIP units of lipase per tablet) given immediately after the ^{14}C-test meal. A mean peak specific radioactivity of expired $^{14}CO_2$ ($^{14}CO_2$ value, expressed as percent dose per millimole $\times$ 10^4) without Viokase was 53.3 $\pm$ 3.3 (1 s.e.m.) for ten normal subjects and 25.8 $\pm$ 5.1 for 18 malabsorption patients. The test was repeated with Viokase in seven fat malabsorption patients with pancreatic involvement and fat malabsorption patients with no evidence of pancreatic involvement. The parameters examined were the absolute increase in the peak $^{14}CO_2$ value due to Viokase administration and the ratio of post-Viokase to pre-Viokase peak $^{14}CO_2$ values. Both parameters for the group with pancreatic involvement (the increase of 27.9 $\pm$ 3.4 and the ratio of 6.1 $\pm$ 1.7) were significantly greater than those for the group with no evidence of pancreatic involvement (5.2 $\pm$ 1.4 and 1.3 $\pm$ 0.1) ($p < 0.001$ and $p < 0.05$). In addition, six patients with diabetes mellitus (five with and one without steatorrhea) were studied. There was a delay in the appearance of $^{14}CO_2$ in breath and their mean peak $^{14}CO_2$ value was 53.1 $\pm$ 6.7. When the test was repeated with Viokase in three of the diabetic patients with steatorrhea, the delayed $^{14}CO_2$ expiration was restored to normal. The results suggest that the $^{14}CO_2$ values without and with Viokase administration are of value in differential diagnosis of pancreatic malabsorption in patients without diabetes.

The use of ^{14}C-labeled fat in estimation of fat absorption was originally developed by Schwabe, et al (*1*). Since then the method was modified by other investigators whereby specific radioactivity of expired $^{14}CO_2$ was measured instead of counting total radioactivity in the breath after ingestion of ^{14}C-labeled triglyceride (*2–5*). Blomstrand, et al studied $^{14}CO_2$ expiratory pattern in one patient with total pancreatectomy after feeding ^{14}C-labeled fats and found that only 7% of total administered radioactivity was recovered as $^{14}CO_2$ in 24-hr breath with glyceryl tripalmitate-1-^{14}C whereas the recovery was 62% with oleic acid-1-^{14}C (*6*). When ^{14}C-triglyceride was administered together with pancreatic enzymes, the recovery increased to 33%. They concluded that measurement of $^{14}CO_2$ in expired air after ingestion of ^{14}C-labeled long-chain fatty acids as triglycerides and free acids could be used for assessment of pancreatic function and evaluation of substitution therapy. The ^{14}C-tripalmitin breath test was also used in our laboratory to study fat absorption in more than 60 patients and ten normal volunteers and was found to be a simple and reliable test for fat malabsorption (*7*). We further investigated the possible use of this test in conjunction with Viokase administration for differentiating pancreatic fat malabsorption from other types of fat malabsorption.

MATERIALS AND METHODS

Subjects. The subjects studied in this report were ten normal volunteers who were free of any apparent disease and 18 patients. The patients were carefully

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For reprints contact: I. W. Chen, Radioisotope Laboratory, Cincinnati General Hospital, Cincinnati, Ohio 45229.

* Present address: 39 Spruce Terrace, Wayne, N.J. 04470.

TABLE 1. RESULTS OF BREATH TESTS: MALABSORPTION PATIENTS WITH PANCREATIC INVOLVEMENT

Patient	Age/sex	Diagnosis	Fecal fat (gm/day)	Peak $^{14}CO_2$ values: Pre-Viokase	Post-Viokase	Post-pre Viokase	Ratio†
CS	53/M	Pancreatic carcinoma with 95% pancreatomy	15.3	3.4 (7)*	45.5 (5)	42.1	13.4
MB	35/M	Chronic pancreatitis with pancreatic insufficiency	10.2	4.7 (5)	28.8 (5)	24.1	6.1
DB	46/M	Chronic pancreatitis with pancreatic insufficiency	11.0	5.7 (3)	35.3 (8)	29.6	6.2
AD	45/F	Chronic pancreatitis with pancreatic insufficiency	13.0	26.8 (8)	50.5 (8)	23.7	1.9
CP	38/M	Chronic pancreatitis with pancreatic insufficiency	—	28.6 (8)	65.7 (6)	37.1	2.3
CB	55/F	Chronic pancreatitis with pancreatic insufficiency	—	27.6 (8)	43.0 (7)	15.4	1.5
HC	55/F	Chronic pancreatitis with pancreatic insufficiency	29.9	2.2 (7)	24.2 (8)	23.0	11.0
Mean ± 1 s.e.m.						27.9 ± 3.4	6.1 ± 1.7

* Numbers in parentheses show the time to reach maximum activity (hours) after the ^{14}C-test meal was ingested.
† Ratio of post- to pre-Viokase peak $^{14}CO_2$ value.

examined and followed by one of us during the study period and were divided into three groups.

1. Seven fat malabsorption patients with pancreatic involvement who were either clinically diagnosed as typical chronic pancreatitis with pancreatic insufficiency or pancreatic carcinoma with pancreatectomy (Table 1).
2. Five fat malabsorption patients without clinical evidence of pancreatic involvement (no history of alcoholism and biliary tract diseases) who had either abnormal fecal fat excretion or abnormal peak $^{14}CO_2$ value (Table 2).
3. Six diabetic patients, five with and one without steatorrhea (by fecal analysis).

Methods. Procedures for measurement of ^{14}C fat absorption were similar to those of Kaihara and Wagner (*3*). After overnight fasting, patients were asked to ingest a test meal containing 5 μCi of ^{14}C-labeled fat at around 8 am and eight breath samples were collected at 1-hr intervals. A 24-hr sample was also collected whenever possible. The test meal was prepared by dissolving 5 μCi of tripalmitin-carboxyl-^{14}C (New England Nuclear Corp.) in 0.5 ml of peanut oil; this solution was then mixed with 35–40 ml of Lipomul (Upjohn, containing about 25 gm of corn oil) and administered orally. When the test result suggested malabsorption, the test was repeated 2 or more days later. In the second test ten tablets of Viokase (VioBin Corp. containing 650 FIP units of lipase per tablet, also containing protease, nuclease, and amylase) were given orally immediately after the ^{14}C-test meal.

TABLE 2. RESULTS OF BREATH TESTS: MALABSORPTION PATIENTS WITHOUT EVIDENCE OF PANCREATIC INVOLVEMENT

Patient	Age/sex	Diagnosis	Fecal fat (gm/day)	Peak $^{14}CO_2$ Values: Pre-Viokase	Post-Viokase	Post-pre Viokase	Ratio†
ES	59/M	Postgastrectomy syndrome	12.0	32.1 (5)*	35.4 (6)	3.4	1.1
DS	46/M	Chronic abdominal pain (unknown etiology)	—	25.4 (7)	32.0 (8)	6.6	1.3
DS	50/M	Chronic diarrhea (unknown etiology)	—	27.6 (3)	36.3 (6)	8.7	1.3
AK	74/M	Nontropical sprue	—	24.2 (8)	25.1 (7)	0.9	1.0
MD	33/F	Postgastrectomy syndrome	11.2	9.5 (3)	15.7 (4)	6.2	1.6
Mean ± 1 s.e.m.						5.2 ± 1.4	1.3 ± 0.1

* Numbers in parentheses show the time to reach maximum activity (hours) after the ^{14}C-test meal was ingested.
† Ratio of post- to pre-Viokase peak $^{14}CO_2$ value.

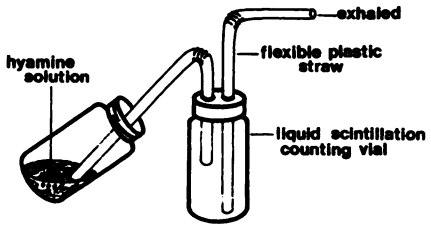

FIG. 1. Disposable device for collection of breath sample.

A simple and disposable device as shown in Fig. 1 was used in the collection of the carbon dioxide of breath. The subjects exhaled normally into a liquid scintillation vial containing 2 ml of standardized hyamine–ethanal solution (*3*) through a trap made of a liquid scintillation vial and two plastic flexible straws. The trap was used solely for preventing the subject from accidentally sucking the alkaline solution into the mouth. With normal breathing, decoloration of hyamine solution containing phenolphthalein took place within about 1–2 min. Most samples thus collected gave clear solution after mixing with scintillation fluid; rarely, slightly turbid samples were encountered but the turbidity due to the presence of moisture did not sufficiently quench the radioactive counts as confirmed by the use of the internal standard method. The amount of CO_2 collected in each vial was calculated from the volume and normality of hyamine solution used. The normality was determined by titrating the hyamine solution with 0.1 *N* hydrochloric acid. The radioactive carbon dioxide expired was expressed as a percent of the administered radioactivity per millimole of carbon dioxide $\times 10^4$: the term "$^{14}CO_2$ value" is used in this paper to denote this value.

Fecal fat in 72-hr stool collection was also measured (*8*) in 13 of 18 patients studied. Patients were directed to consume about 100 gm of fat daily during the period of stool collection. Daily fat excretion levels greater than 6.0 gm were considered to represent a malabsorption syndrome.

RESULTS

Figure 2 shows time courses of radioactive carbon dioxide expired by normal and malabsorption subjects. The peak $^{14}CO_2$ value was observed at 7 hr in both normal and malabsorption subjects. The mean peak $^{14}CO_2$ value of 53.3 ± 3.3 (1 s.e.m.) for normal subjects is significantly higher than that of 25.8 ± 5.1 for malabsorption patients. The peak $^{14}CO_2$ values for normal volunteers ranged from 33.8 to 83.8. We arbitrarily selected the peak $^{14}CO_2$ value of 38.0 as the lower normal limit since the numbers of false-positive (10%) and false-negative (15%) are the least at this level (*7*).

Two parameters were used to compare pancreatic malabsorption and malabsorption resulting from other causes: they were the absolute increase in the peak $^{14}CO_2$ value due to Viokase administration and the ratio of post-Viokase to pre-Viokase peak $^{14}CO_2$ values. The results are summarized in Tables 1 and 2. Table 1 shows results obtained from seven malabsorption patients with pancreatic involvement. Fecal fat was greater than 10 gm/day in all patients except two in whom no stool collection could be made. Their pre-Viokase peak $^{14}CO_2$ values are all abnormally low. With Viokase administration, the peak $^{14}CO_2$ value increased significantly as compared with the pre-Viokase value (paired Student's t-test, $p < 0.001$) (*9*). The mean increase in the peak $^{14}CO_2$ value due to Viokase administration was 27.9 ± 3.4, representing a mean increase of 6.1-fold. For patients with various malabsorption syndromes but without clinical evidence of pancreatic involvement, the mean increase was 5.2 ± 1.4 (Table 2). This increase was also statistically significant (paired Student's t-test, $p < 0.05$) but was significantly smaller than that observed in the group of patients with pancreatic involvement (standard Student's t-test, $p < 0.001$). The mean ratios of post- to pre-Viokase values for these two groups of patients were also statistically different (standard Student's t-test) with $p < 0.05$.

Figure 3 summarizes the results of breath test obtained from three diabetic patients with possible pancreatic insufficiency. All three patients had abnormal fecal fat excretion. The mean $^{14}CO_2$ value at 8 hr was within the normal range. Perhaps the most striking feature of the $^{14}CO_2$ expiration pattern of diabetic patients was the considerable delay in

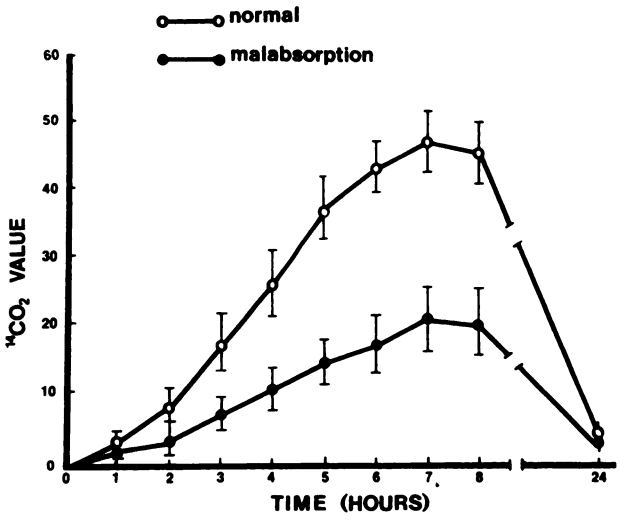

FIG. 2. Time courses of mean $^{14}CO_2$ value (% administered radioactivity per millimole of expired $CO_2 \times 10^4$) by ten normal subjects and 18 fat malabsorption patients. Vertical bars represent 1 s.e.m.

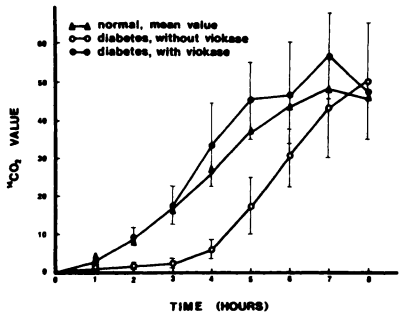

FIG. 3. Time courses of mean $^{14}CO_2$ value (% administered radioactivity per millimole of expired $CO_2 \times 10^4$) by three diabetic patients with fat malabsorption. Vertical bars represent 1 s.e.m.

the appearance of radioactivity in breath as compared with healthy controls. This difference was especially distinctive at 3 hr when the average $^{14}CO_2$ value for diabetic patients was only 3.9 as compared with 18.0 for the control group. With Viokase administration, this delayed pattern became normal in all three diabetic patients with fat malabsorption. The breath test without Viokase administration was also carried out in three other diabetic patients; one uncomplicated diabetes (normal fecal fat) and two with steatorrhea (by fecal analysis). All of them also exhibited the delayed $^{14}CO_2$ expiration pattern and the normal peak $^{14}CO_2$ values.

DISCUSSION

Results of our studies show that a good separation between the pancreatic and nonpancreatic fat malabsorption can be obtained by measurement of the peak $^{14}CO_2$ values with and without Viokase administration. However, the post-Viokase values for three pancreatic malabsorption patients (MB, DB, and HC in Table 1) were still within the abnormal range of 28.8, 35.3, and 24.2 even though the increases due to Viokase administration were 6.1-, 6.2-, and 11.0-fold, respectively, suggesting that other forms of fat malabsorption may exist or that Viokase given to these three patients was only partly effective.

The average increase in the post-Viokase value of nonpancreatic malabsorption is much smaller but is nevertheless statistically significant. The increase in the post-Viokase peak value of about the same magnitude was also observed in one patient without malabsorption. This increase in the post-Viokase value in the patients without clinical evidence of pancreatic insufficiency could not be explained at present. However, this degree of increase is small as compared with that observed in patients with pancreatic malabsorption and a clear separation could be made between these two groups of malabsorption patients by this parameter.

The delayed $^{14}CO_2$ expiration observed in diabetic patients (Fig. 3) may be partially explained by delayed emptying of the stomach frequently observed in patients with diabetes mellitus (*10*). Restoration of the delayed $^{14}CO_2$ expiration to normal by Viokase administration tends to suggest that pancreatic insufficiency may also play a role in the delayed appearance of $^{14}CO_2$ in the breath. All three patients were clinically suspicious of chronic pancreatitis and their fecal fat excretion was abnormally high. It is uncertain at present whether this shift in the $^{14}CO_2$ expiration pattern by Viokase is indicative of pancreatic insufficiency. However, it is certain that the peak $^{14}CO_2$ value obtained by the breath test is of no diagnostic use for steatorrhea in diabetes because four of five diabetic patients with steatorrhea had normal peak $^{14}CO_2$ values. The low peak $^{14}CO_2$ value (24.1) observed in the other patient (one of the three shown in Fig. 3) was obtained at 8 hr. Unfortunately, we were not able to collect his breath specimens at later hours and therefore we could not be sure it was the peak $^{14}CO_2$ value. The normal peak $^{14}CO_2$ values observed in diabetic patients with fat malabsorption could be the result of increased fat metabolism in diabetes (*11*)

Results of our studies suggest that the breath test used in conjunction with Viokase may serve as a valuable aid for differential diagnosis of fat malabsorption due to pancreatic insufficiency. With the simple and disposable device adapted in our laboratory for breath collection, the test can be carried out easily and rapidly with relatively low cost in any laboratory equipped with a liquid scintillation counter.

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