Detection of impaired intestinal absorption of long-chain fatty acids: validation studies of a novel test in a rat model of fat malabsorption[1–3]

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ABSTRACT

Background: Classic fat balance studies detect fat malabsorption but do not discriminate between the potential causes of malabsorption, such as impaired intestinal lipolysis or reduced uptake of fatty acids.

Objective: We aimed to validate a novel test for the specific, sensitive detection of impaired intestinal uptake of long-chain unesterified fatty acids in an appropriate rat model of fat malabsorption.

Design: The absorption and appearance in plasma of [^{13}C]palmitic acid were determined in control rats and in rats with fat malabsorption due either to chronic bile deficiency (permanent bile diversion) or to oral administration of the lipase inhibitor orlistat (200 mg/kg diet). [^{13}C]Palmitic acid results were compared with the percentage absorption of ingested dietary fat determined by fat balance.

Results: Between 1 and 6 h after intraduodenal administration, plasma [^{13}C]palmitate concentrations in control rats were 4–10-fold higher than in bile-deficient rats ($P < 0.05$) but were not significantly different between orlistat-supplemented rats and their controls. In control and bile-deficient rats, plasma [^{13}C]palmitate concentrations allowed complete discrimination between normal (>92%) and reduced (<92%) fat absorption, whereas the percentage absorption of [^{13}C]palmitate over 48 h appeared to be highly correlated with the percentage absorption of ingested dietary fat ($r = 0.89$, $P < 0.001$).

Conclusions: The [^{13}C]palmitic acid absorption test detects impaired intestinal absorption of long-chain fatty acids selectively and sensitively in a rat model of fat malabsorption due to bile deficiency. Our data strongly support the use of the [^{13}C]palmitic acid absorption test for the diagnosis of clinical fat malabsorption syndromes. *Am J Clin Nutr* 2000;72:174–80.

KEY WORDS Isotope labeling, stable isotopes, absorption, dietary fat metabolism, malabsorption syndromes, bile, enterohepatic circulation, bile salts, rats, bile deficiency

INTRODUCTION

In Western diets, triacylglycerols composed of long-chain fatty acids constitute 92–96% of dietary fat (1). The absorption of these fats involves digestion, ie, lipolysis by lipolytic enzymes and the subsequent absorption of the hydrolyzed fatty acids and monoacylglycerols (referred to in the following as intestinal uptake) (1, 2). Clinical fat malabsorption syndromes are due to maldigestion (impaired lipolysis), as in pancreatic insufficiency (3); to impaired uptake of fatty acids, as in cholestatic liver disease (4); or to both, as in cystic fibrosis (5, 6). Intestinal fat absorption in patients is routinely analyzed by means of a fat balance study, which requires detailed analysis of daily fat intake and complete recovery of feces for 72 h. However, the balance method does not identify whether maldigestion or impaired intestinal uptake causes the fat malabsorption. Discrimination between the potential causes of fat malabsorption would make it possible to target subsequent diagnostic strategies and to evaluate the effects of specific therapeutic approaches.

Several ^{13}C-labeled fats have been used successfully in the specific detection of impaired lipolysis (6–10). Attempts to develop a specific test for the detection of impaired uptake of long-chain (unesterified) fatty acids have been less successful, possibly because of methodologic and analytic limitations. So far, the use of ^{13}C-labeled fats in the development of a fat malabsorption test has been limited to approaches involving label detection in breath and feces (11–14). The excretion rate of ^{13}C in the form of exhaled $^{13}CO_2$, however, does not necessarily reflect quantitative differences in the absorption of the ^{13}C-labeled parent compound because it is influenced by variations in postabsorptive metabolism (15, 16). In addition, test durations of up to 6 h, during which the patients were required to fast, were needed in these studies (11, 13).

The recent availability of gas chromatography–combustion isotope ratio mass spectrometry (GC-CIRMS) allows for the accurate determination of ^{13}C enrichments of plasma fatty acids (17). To develop a specific and sensitive diagnostic test to detect

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impaired intestinal absorption of long-chain fatty acids, we reasoned that the determination of plasma concentrations of absorbed ^{13}C-labeled fats, as a reflection of the efficiency of their absorption, would offer theoretic advantages over breath $^{13}CO_2$ analysis. The various steps involved in postabsorptive metabolism of the tracer before exhalation would have less of an effect on plasma ^{13}C-labeled fatty acid concentrations than on $^{13}CO_2$ expiration rates (12), and the required test duration could be reduced.

In the present study, we aimed to validate a novel test, tentatively called the [^{13}C]palmitic acid absorption test, in a rat model of fat malabsorption due to bile deficiency. In bile-deficient conditions, fat malabsorption appears to be due mainly to impaired solubilization and subsequent translocation of long-chain fatty acids (1, 18). We determined whether plasma concentrations of [^{13}C]palmitic acid could sensitively and specifically detect fat malabsorption due to bile deficiency.

MATERIALS AND METHODS

Animals and ^{13}C-labeled substrate

Male Wistar rats (Harlan, Zeist, Netherlands) weighing 300–400 g were housed in an environmentally controlled facility with diurnal light cycling and free access to food and tap water (and additional 0.9% NaCl solution, wt:vol, in the case of bile-deficient rats). The diet fed to the rats receiving orlistat and their controls (high-fat diet, *see* below) was mixed with water to form a homogeneous paste to which the animals had ad libidum access. Experimental protocols were approved by the Ethical Committee for Animal Experiments, Faculty of Medical Sciences, University of Groningen. [1-^{13}C]Palmitic acid was purchased from Isotec Inc (Matheson, Miamisburg, OH) and was 99% ^{13}C enriched.

Study protocol

For the bile-deficiency experiments, rats were assigned either to a standard low-fat diet (14% of energy as fat; 19.124 kJ/kg food; fatty acid composition measured by gas chromatographic analysis: 0.9 mol% 8:0–12:0, 25.2% 16:0, 5.5% 18:0, 30.3% 18:1n−9, 33.9% 18:2n−6, and 3.6% 18:3n−3) or to a high-fat diet (35% of energy as fat; 18.969 kJ/kg food; fatty acid composition measured by gas chromatographic analysis: 4.4 mol% 8:0–12:0, 28.5% 16:0, 3.9% 18:0, 33.2% 18:1n−9, 29.3% 18:2n−6, and 0.2% 18:3n−3). Both diets were from Hope Farms BV, Woerden, Netherlands. The protein and carbohydrate contents of the high-fat diet were 9% and 22% lower, respectively, than those of the low-fat diet. After 1 wk, the rats were equipped with permanent catheters in the jugular vein, bile duct (bile-deficient rats and their controls only), and duodenum as described previously (19). One day after surgery, catheters in the bile duct and duodenum either were connected at the time of surgery to restore enterohepatic circulation (control rats) or were chronically interrupted (bile-deficient rats). The animals were allowed to recover from surgery for 6 d.

On day 7, 1.67 mL fat/kg body wt was slowly administered as a bolus via the duodenal catheter. The fat bolus was composed of long-chain fatty acids in the form of olive oil (25% by vol), accounting for ≈25% (low-fat diet) or 15% (high-fat diet) of daily long-chain fat intake. The fatty acid composition of the olive oil was 14% 16:0, 79% 18:1n−9, and 8% 18:2n−6. Medium-chain triacylglycerol (75% by vol) was included in the fat bolus to make up a volume that could be administered reproducibly. The medium-chain triacylglycerol was composed of extracted coconut oil and synthetic triacylglycerols (fatty acid composition: 2 mol% 6:0, 50–65% 8:0, 30–45% 10:0, and 3% 12:0). [1-^{13}C]Palmitic acid was included in the bolus (33 mg/kg body wt) and constituted ≈2% of the bolus by weight. Blood samples (0.2 mL) were taken from the jugular cannula at baseline and 1, 2, 3, 4, 5, 6, and 24 h after administration of the label and were collected into tubes containing heparin. Plasma was separated by centrifugation (10 min, 900 × *g*, 4°C) and stored at −20°C until analyzed further. Feces was collected in 24-h fractions starting 1 d before administration of the fat bolus and ending 2 d afterward. Feces samples were stored at −20°C until analyzed. Food intake was determined for 3 d by weighing the animals' food containers daily.

For the orlistat experiments, the rats were randomly divided into 2 groups: a control group receiving no orlistat and an experimental group receiving 200 mg orlistat/kg diet. Orlistat was ground together with the high-fat diet and mixed with water (3:2, by wt) to form a homogeneous paste. Administration of orlistat started on day 4 after surgery, whereas the 72-h fat balance study started on day 6. On day 7, a fat bolus containing [1-^{13}C]palmitic acid was administered via the duodenal catheter by using the dosage and methods described above for bile-deficient rats and their controls, including blood sampling and feces collection.

Analytic techniques

Plasma lipids

Total (esterified and free) plasma fatty acids were extracted, hydrolyzed, and methylated according to the methods of Lepage and Roy (20). Resulting fatty acid methyl esters were analyzed by gas chromatography to measure the total amount of palmitic acid and by GC-CIRMS to measure the ^{13}C enrichment of palmitic acid. The concentration of [^{13}C]palmitic acid in plasma was expressed as a percentage of the dose administered per liter of plasma.

Rat diet and fecal fat

Feces was freeze-dried and mechanically homogenized. Aliquots of rat diet and freeze-dried feces were extracted, hydrolyzed, and methylated (20). Resulting fatty acid methyl esters were analyzed by gas chromatography to calculate total fat intake, total fecal fat excretion, and total palmitic acid concentration in food and feces. Fatty acid methyl esters were analyzed by GC-CIRMS to calculate the ^{13}C enrichment of palmitic acid. Total fecal fat excretion of the rats was expressed as grams of fat per day, and percentage total fat absorbed was calculated from the daily fat intake and the daily fecal fat excretion and expressed as a percentage of the daily fat intake.

$$\text{Percentage total fat absorbed} = \frac{\text{Fat intake (g/d)} - \text{fecal fat output (g/d)}}{\text{Fat intake (g/d)}} \times 100\% \quad (1)$$

A similar calculation was performed to measure the absorption of [1-^{13}C]palmitic acid, which was determined from the intake and excretion of [^{13}C]palmitic acid. Values were expressed as the percentage of the dose administered.

Gas-liquid chromatography

Fatty acid methyl esters were separated and quantified by gas-liquid chromatography with a Hewlett-Packard gas chromatograph (model 5880; Palo Alto, CA) equipped with a CP-SIL 88

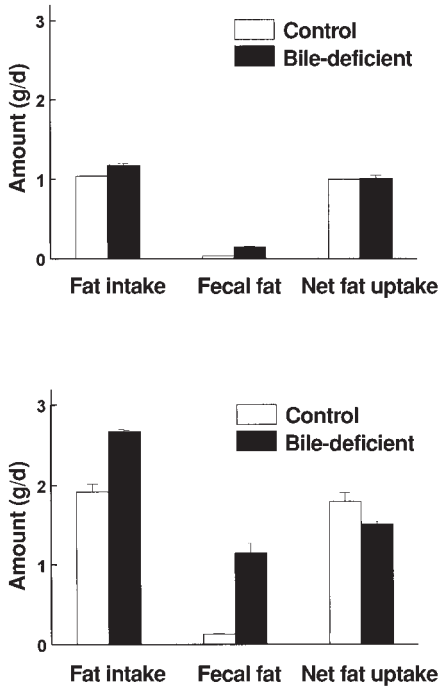

FIGURE 1. Mean (±SEM) total fat intake, fecal fat excretion, and net fat uptake (total fat intake minus fecal fat excretion) in control rats and in rats with intestinal bile deficiency due to permanent bile diversion consuming a low-fat (top: 14% of energy; $n = 9$ control rats, $n = 11$ bile-deficient rats) or a high-fat (bottom: 35% of energy; $n = 9$ control rats, $n = 6$ bile-deficient rats) diet. Values were obtained from 72-h fat balance studies. Fat contents of food and feces were measured by gas chromatographic analysis of fatty acid methyl esters. For fat intake and fecal fat there was a significant main effect of bile status and diet ($P < 0.001$) but no significant interaction of bile status and diet. For net fat uptake, there were no significant effects of bile status, diet, or their interaction.

capillary column (50 m × 0.32 mm; Chrompack, Middelburg, Netherlands) and a fast ionization detector (21, 22). The gas chromatograph oven was programmed from an initial temperature of 150 °C to 240 °C in 2 temperature steps: 150 °C for 5 min; 150–200 °C, ramp 3 °C/min for 1 min; and 200–240 °C, ramp 20 °C/min for 10 min. The fatty acid methyl esters were quantified in reference to heptadecanoic acid added as an internal standard.

Gas chromatography–combustion isotope ratio mass spectrometry

^{13}C Enrichment of the palmitic acid methyl esters was determined with a GC-CIRMS apparatus (Delta S/GC; Finnigan MAT, Bremen, Germany) (23, 24). Separation of the methyl esters was achieved on a CP-SIL 88 capillary column as described above (Chrompack). The gas chromatograph oven was programmed from an initial temperature of 80 °C to 225 °C in 3 temperature steps: 80 °C for 1 min; 80–150 °C, ramp 30 °C/min; 150–190 °C, ramp 5 °C/min; and 190–225 °C, ramp 10 °C/min for 5 min.

Calculations and statistics

The experimental data are reported as means ± SEMs. The significance of differences was calculated by using two-factor analysis of variance (ANOVA) and, when there was no significant interaction, main effects of bile status and diet were reported. To correlate 2 variables, linear regression lines were fitted by the method of least squares and expressed as the Pearson correlation coefficient. Statistical significance was considered at the level of $P < 0.05$. The statistical analyses were performed by using SPSS (version 6.0; SPSS Inc, Chicago).

RESULTS

Fecal fat balance

Bile diversion was associated with an increased intake of food with both the low-fat diet (18.9 ± 0.7 and 21.2 ± 0.7 g/d for controls compared with bile-deficient rats, respectively; $P < 0.05$) and the high-fat diet (15.7 ± 0.7 and 21.9 ± 1.6 g/d for controls compared with bile-deficient rats, respectively; $P < 0.01$). With ANOVA, the interaction term between bile status (control, intact enterohepatic circulation, or bile deficiency) and diet (low fat or high fat) was not significant. The fat balance data of control and bile-deficient rats comsuming the low-fat and high-fat diets are shown in **Figure 1**. Because of their greater food intake, the bile-deficient rats had a significantly greater dietary fat intake than did the control rats. With both diets, bile-deficient rats excreted significantly more fat into feces than did control rats ($P < 0.001$). However, the net fat intake, calculated as the amount of fat ingested minus the amount recovered in feces, did not differ significantly between the bile-deficient rats and their respective controls. As can be derived from Figure 1, increased food intake led to a proportionate increase in fat intake, which compensated for the increased fecal loss. Nevertheless, bile diversion significantly impaired the efficiency of dietary fat absorption (**Figure 2**); the percentage total fat absorbed in bile-deficient rats was only 87.2 ± 0.9% from the low-fat diet and 53.9 ± 3.9% from the high-fat diet ($P < 0.001$ compared with control rats). The interaction term between bile status (control, intact enterohepatic circulation, or bile deficiency) and diet (low fat or high fat) was not significant.

Excretion of [^{13}C]palmitic acid into feces

The percentage absorption of [1-^{13}C]palmitic acid in relation to that of dietary fat, as calculated from 48-h and 72-h fat balance measurements, is shown in **Figure 3**. A linear relation was observed between the percentage fat absorbed, as measured by fat balance, and the absorption of [1-^{13}C]palmitic acid, indicating that the absorption of the labeled palmitic acid was quantita-

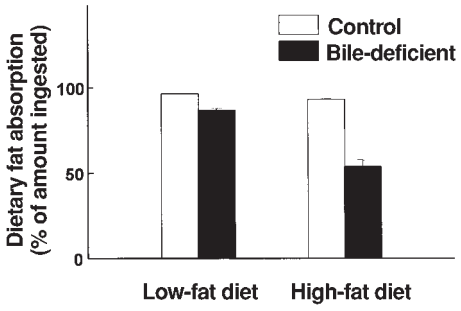

FIGURE 2. Mean (±SEM) dietary fat absorption efficiency in control rats and rats with intestinal bile deficiency due to permanent bile diversion consuming a low-fat (14% of energy; $n = 9$ control rats, $n = 11$ bile-deficient rats) or a high-fat (35% of energy; $n = 9$ control rats, $n = 6$ bile-deficient rats) diet. Values were obtained from 72-h fat balance studies (Figure 1). There was a significant effect of bile status ($P < 0.001$) but no significant effects of diet or the interaction of diet and bile status.

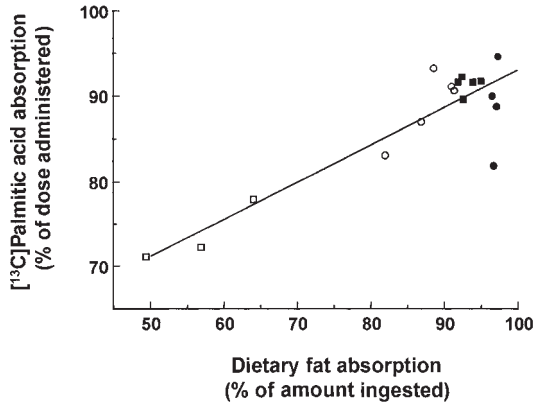

FIGURE 3. Correlation between the efficiency of absorption of dietary fat and that of [1-^{13}C]palmitic acid (33 mg/kg body wt intraduodenal). Efficiency of absorption was determined on the basis of a 48-h ([^{13}C]palmitic acid) or 72-h (dietary fat) balance study in which fatty acids were measured in diet and feces by gas chromatography and gas chromatography–combustion isotope ratio mass spectrometry. Each symbol represents the values obtained from one rat: low-fat diet in control rats (●; n = 4), low-fat diet in bile-deficient rats (□; n = 5), high-fat diet in control rats (■; n = 5), and high-fat diet in bile-deficient rats (○; n = 3). The line is characterized by the following equation: $y = 0.44x + 49$; $r = 0.89$, $P < 0.001$.

tively related to that of ingested dietary fat in a linear fashion. It is apparent that the observed relation was particularly pronounced under conditions of considerably hampered absorption.

Plasma [^{13}C]palmitic acid concentrations

[^{13}C]Palmitic acid concentrations in plasma, reflecting the uptake of long-chain fatty acids, are shown in **Figure 4**. Plasma concentrations of [^{13}C]palmitic acid increased within 1 h of intraduodenal administration of [1-^{13}C]palmitic acid to control rats given the low-fat diet, reaching a maximum value of 0.058 ± 0.021% of dose/L plasma 2 h after bolus administration (Figure 4, top). In rats with bile deficiency, plasma [^{13}C]palmitic acid concentrations were significantly lower than in control rats, and no plateau had been reached 6 h after [1-^{13}C]palmitic acid administration. The plasma [^{13}C]palmitic acid concentrations of rats given the high-fat diet increased within 1 h after administration and reached a maximum value of 0.055 ± 0.007% of dose/L plasma at 3 h (Figure 4, bottom). ANOVA indicated that there was a significant effect of biliary status on plasma [^{13}C]palmitic acid concentrations (control compared with bile-deficient rats).

Relation between fecal fat balance and plasma [^{13}C]palmitic acid concentrations

The ability of the [^{13}C]palmitic acid absorption test to detect impaired fat absorption, assuming (arbitrarily) 92% as the lower limit for physiologic fat absorption efficiency, is shown in **Figure 5**. At 1, 2, and 3 h after label administration, plasma [^{13}C]palmitic acid concentrations clearly differed between control and bile-deficient rats; in fact, there was no overlap. Plasma [^{13}C]palmitic acid concentrations at 3 h appeared to be the most discriminative: they were ≥4-fold higher in control than in bile-deficient rats, irrespective of the fat content of the diet. When any value between 0.005% and 0.010% of administered dose/L plasma is used as the lower limit for normal plasma values, the test has a sensitivity and specificity of 100% under the conditions we used.

[^{13}C]Palmitic acid absorption test in rats with impaired lipolysis

The results of the [^{13}C]palmitic acid absorption test in rats with fat malabsorption due to impaired lipolysis are shown in **Figure 6**. Fat absorption was 37% lower in rats fed orlistat than in control rats (46.7 ± 5.4% and 74.6 ± 1.3%, respectively; n = 4). Despite the significant degree of fat malabsorption, however, plasma [^{13}C]palmitic acid concentrations were not significantly different between control and orlistat-treated rats at 1, 2, 3, or 4 h after administration (Figure 6), or at 5, 6, or 24 h after administration (data not shown), indicating that fat malabsorption due to impaired lipolysis did not affect the results of the [^{13}C]palmitic acid absorption test.

DISCUSSION

We investigated the ability of the [^{13}C]palmitic acid absorption

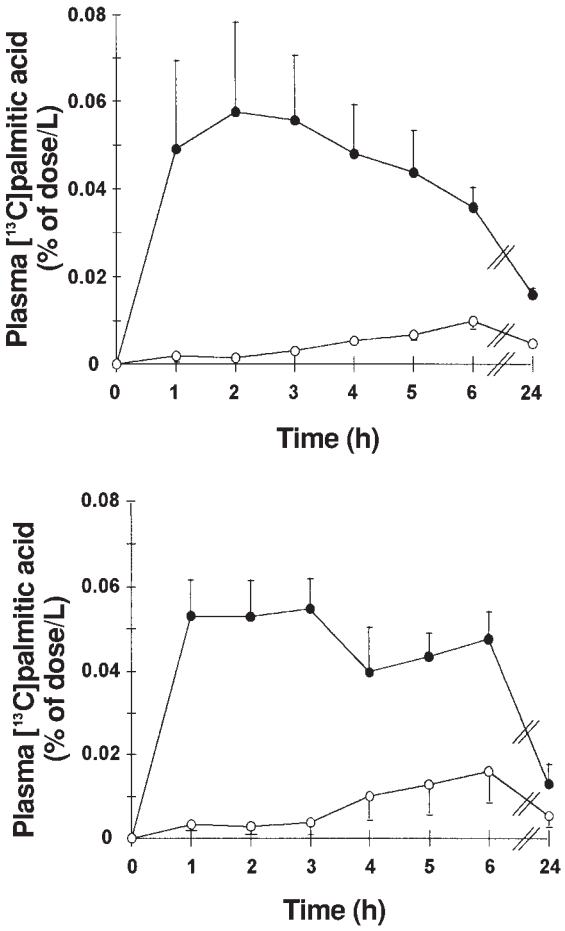

FIGURE 4. Mean (±SEM) plasma concentrations of [^{13}C]palmitic acid in control rats (●) and in rats with intestinal bile deficiency due to permanent bile diversion (○) consuming a low-fat (top: 14% of energy; n = 4 control rats, n = 5 bile-deficient rats) or high-fat (bottom: 35% of energy; n = 5 control rats, n = 3 bile-deficient rats) diet after intraduodenal administration of [1-^{13}C]palmitic acid (33 mg/kg body wt). Bile-deficient rats had significantly lower plasma concentrations than did control rats, $P < 0.01$ (ANOVA). There was no significant interaction of diet with bile status.

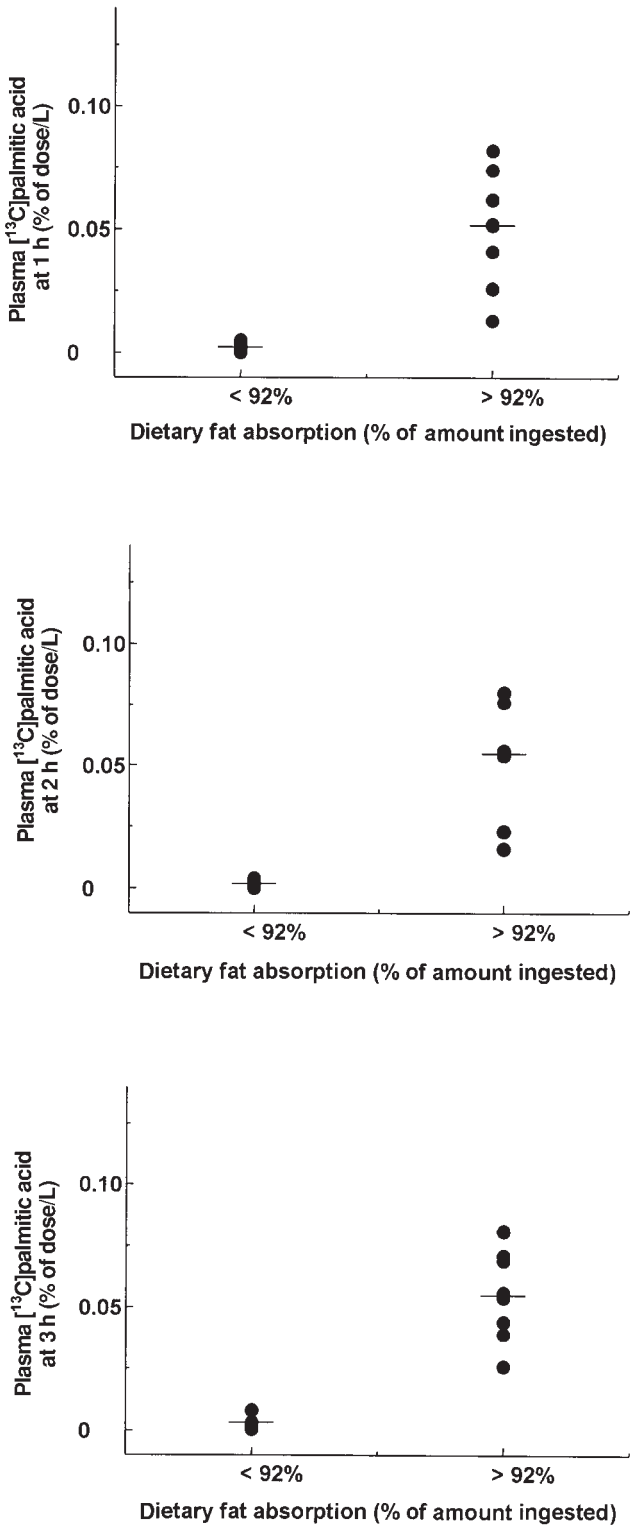

FIGURE 5. Plasma concentrations of [^{13}C]palmitic acid at 1, 2, and 3 h after intraduodenal administration of [1-^{13}C]palmitic acid (33 mg/kg body wt) in rats with fat absorption below (n = 8) or above (n = 9) 92% of the amount ingested, as determined by a 72-h fat balance study. Results for control rats and rats with intestinal bile deficiency due to permanent bile diversion consuming a low-fat (14% of energy) or a high-fat (35% of energy) diet were combined. Each symbol represents the data obtained from one rat. The horizontal lines represent the mean plasma concentrations of [^{13}C]palmitic acid in each group.

test to sensitively and specifically detect fat malabsorption due to impaired uptake of long-chain fatty acids. As an experimental model, we used freely moving, unanesthetized rats with intestinal bile deficiency due to diversion of their bile for ≥1 wk. Enterohepatic circulation of bile components was manipulated successfully in other rat studies of fat absorption (25–28). Under the condition of permanent bile diversion, fat malabsorption appears to be due mainly to impaired solubilization and subsequent translocation of long-chain fatty acids (1, 18). Our results indicate that plasma concentrations of [^{13}C]palmitic acid allow the sensitive discrimination between rats with unaffected fat absorption and bile-deficient rats with fat malabsorption. Control studies in orlistat-treated rats, which have a drug-induced inhibition of intestinal lipolysis, clearly indicated that the [^{13}C]palmitic acid absorption test was not affected by fat malabsorption due to impaired lipolysis (maldigestion), thus showing our test's specificity.

Total dietary fat absorption was examined in chronically bile-deficient rats fed a standard low-fat diet and a high-fat diet. In control rats, the absorption of dietary fat was highly efficient (>94%), independent of the dietary fat content, and in the same range as was observed in healthy humans (1, 18). Intestinal bile deficiency due to permanent bile diversion significantly reduced the efficiency of dietary fat absorption, but bile-deficient rats still absorbed 87% of their dietary fat when fed a low-fat diet. In humans as well, the efficiency of fat absorption seems to be relatively maintained with bile diversion (13). With a high-fat diet, bile-deficient rats absorbed only 54% of their dietary fat, indicating that the compensatory mechanisms that are apparently active when a low-fat diet is fed have a limited capacity. A thorough review of potentially available compensatory mechanisms during bile deficiency is beyond the scope of this article. Yet, note in Figure 1 that increasing the dietary intake (and, indirectly, the amount of fat) helped to maintain net fat uptake compared with control conditions. In addition, more distal segments of the small intestine may be recruited for fat absorption under bile-deficient conditions (29) and the fraction of fat transported via the portal vein may be increased (30).

Plasma [^{13}C]palmitic acid concentrations clearly differentiated between control rats and chronically bile-deficient rats within hours after administration of [1-^{13}C]palmitic acid. As can be derived from Figure 5, the [^{13}C]palmitic acid absorption test, as applied in this experimental model, allowed 100%-sensitive discrimination between the efficiency of fat absorption in individual rats with efficiencies > or <92% within hours after administration of the labeled parent compound. The present approach of measuring the plasma concentrations of [^{13}C]palmitic acid after enteral administration of the label had not been used thus far. Watkins et al (11) described lower 6-h cumulative excretion of $^{13}CO_2$ after oral administration of [^{13}C]palmitic acid in children with cholestasis or mucosal disease than in healthy control subjects or patients with pancreatic insufficiency. Our results indicate that the use of plasma concentrations of a stable isotope–labeled lipid can greatly decrease the duration of such a test without impeding its sensitivity. Our choice of [^{13}C]palmitic acid as a lipid tracer does not seem to be critical for the observed effects because we recently found that plasma concentrations of [^{13}C]linoleic acid differed significantly between control and bile-deficient rats within hours after the intraduodenal administration of this lipid tracer as well (31).

There was no significant effect of the dietary fat content on plasma concentrations of [^{13}C]palmitic acid (Figure 4). The apparent independence of the [^{13}C]palmitic acid absorption test

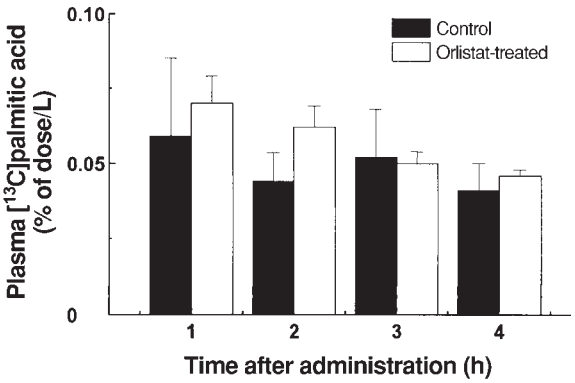

FIGURE 6. Mean (±SEM) plasma concentrations of [^{13}C]palmitic acid in rats fed a high-fat (35% of energy) diet with or without orlistat at indicated time points after the intraduodenal administration of [1-^{13}C]palmitic acid (33 mg/kg body wt). Dietary fat absorption (by 48-h fat balance) was reduced by 37% in orlistat-treated rats (n = 4) compared with control rats (n = 4) (P < 0.01). There were no significant differences in plasma [^{13}C]palmitic acid concentrations between the 2 groups at any time point.

from dietary fat intake is another feature that adds to the suitability of the test. However, it should also be clearly stated that the plasma concentrations of [^{13}C]palmitic acid do not provide quantitative information on the amount of dietary lipid actually taken up by the intestine or on the percentage of dietary fat absorbed. Net fat uptake (Figure 1) and the percentage of dietary fat absorbed (bile-deficient rats, Figure 2) were clearly different between the groups fed a low-fat and high-fat diet. The lack of correlation between [^{13}C]palmitic acid concentrations and either net fat uptake or percentage of dietary fat absorbed is not likely to be due to the fact that the administered quantity of [1-^{13}C]palmitic acid behaves completely differently from the bulk of dietary fat, given the positive, linear correlation between the absorption of dietary fat and that of [1-^{13}C]palmitic acid (Figure 3). We speculate that the lack of quantitative correlation between dietary fat absorption and plasma concentrations of [^{13}C]palmitic acid may be related to differences between the administration of the labeled fatty acid (unesterified fatty acid dispersed in oil administered into the duodenum during the light phase of the day-night cycle) and that of the dietary fat (mainly esterified in triacylglycerols and ingested in the form of diet components during the dark phase of the day-night cycle).

In addition to high sensitivity and ease of use, a novel test for the reliable evaluation of intestinal uptake of fatty acids should also be specific, ie, its results should not be affected by other causes of fat malabsorption. Clinically, the most relevant alternative cause of fat malabsorption is maldigestion or impaired lipolysis. Control experiments in which we administered tri-[1-^{13}C]palmitin intraduodenally to control and bile-deficient rats produced the same results as those obtained with [^{13}C]palmitic acid, indicating that lipolysis in the bile-deficient rats was not affected (data not shown). The results in orlistat-treated rats indicated that significant impairment of fat absorption due to the lipase inhibitor orlistat did not affect the appearance of [^{13}C]palmitic acid in plasma (Figure 6). Orlistat was used by us and by others previously to generate fat malabsorption in experimental animals (10, 32, 33). The results of the orlistat experiments point out the specificity of the [^{13}C]palmitic acid absorption test in selectively detecting impaired intestinal uptake of long-chain fatty acids independent of maldigestion of dietary lipids.

As stated above, a [^{13}C]palmitic acid absorption test based on plasma measurements of ^{13}C has not been used previously in humans. Recently, however, we reported a study that proves, in our opinion, the validity of this concept (6). In pediatric cystic fibrosis patients receiving pancreatic enzyme supplementation, the appearance of [^{13}C]linoleic acid in plasma after oral administration correlated significantly with the percentage of dietary fat absorbed, as determined by 72-h fat balance (r = 0.88, P < 0.001). The results of that study indicate that the fat malabsorption in pediatric cystic fibrosis patients, despite enzyme replacement therapy, is due to impaired absorption of long-chain fatty acids (6).

In summary, we showed in a rat model of fat malabsorption due to bile deficiency that the [^{13}C]palmitic acid absorption test, which is based on the quantitation of plasma [^{13}C]palmitic acid concentrations, is sensitive enough to allow complete discrimination between the percentage of dietary fat absorbed when it is > or <92% (control and bile-deficient rats, respectively), independent of the dietary fat content. We also showed that the test is specific, in the sense that the results are not affected by a significant degree of fat malabsorption due to impaired lipolysis. The present observations highlight the ability of the [^{13}C]palmitic acid absorption test to sensitively detect impairments in intestinal absorption of long-chain fatty acids in humans.

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