Detection of intestinal fat malabsorption due to impaired lipolysis by the $^{13}$C-mixed triglyceride breath test in rats

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Abstract

**Background & Aim:** The $^{13}$C-mixed triglyceride ($^{13}$C-MTG) breath test has become popular for the detection of impaired intestinal lipolysis as a cause for fat malabsorption. However, the diagnostic value has been questioned because the relation between the extent of fat malabsorption and the corresponding result of the $^{13}$C-MTG breath test has not been established. We characterized the $^{13}$C-MTG breath test in rats with variable degrees of fat malabsorption, achieved by feeding the lipase inhibitor orlistat. **Methods:** Rats were fed high fat chow (35 en% fat) to which orlistat was added in amounts of 0, 50, 200, and 800 mg kg$^{-1}$ chow for 5 days. Breath $^{13}$CO$_2$ recovery was determined for 6 h after oral administration of $^{13}$C-MTG (13 mg kg$^{-1}$ BW). Total dietary fat absorption was measured by means of a 3-day fecal fat balance. **Results:** Upon orlistat administration, total fat absorption decreased in a dose-dependent way from 80.2 ± 2.2% to 32.8 ± 3.7% (mean ± SEM; 0 mg and 800 mg orlistat kg$^{-1}$ chow, respectively; $P<0.001$). Correspondingly, breath $^{13}$CO$_2$ recovery from $^{13}$C-MTG at 6 h decreased from 84.5 ± 7.8% to 42.0 ± 1.5% of the dose ($P<0.001$). The 6-h recovery of breath $^{13}$CO$_2$ appeared highly correlated with total fat absorption for the different dosages of orlistat ($r=0.88$, $P<0.001$). However, in rats with fat absorption higher than 70%, the coefficient of variation of cumulative breath $^{13}$CO$_2$ excretion was large (15%) compared with that of fat absorption (5%). **Conclusion:** The $^{13}$C-MTG breath test correlates significantly with the extent of fat malabsorption in a rat model of impaired intestinal lipolysis. However, the considerable interindividually variation of the $^{13}$C-MTG breath test does not support its application for diagnostic purposes in individual patients.
Introduction

Reduced secretion of pancreatic lipase into the intestine is a common feature of pancreatic insufficiency. This condition may lead to fat malabsorption due to incomplete intestinal hydrolysis of dietary triacylglycerols [1,2]. Intestinal fat malabsorption in patients can be quantified by means of a fat balance, but this method does not discriminate between the potential causes, such as impaired intestinal lipolysis, disturbed intestinal solubilization of long-chain fatty acids, or decreased chylomicron formation. Measurement of maximal pancreatic lipase output by means of an invasive, marker-corrected perfusion technique is considered to be the gold standard for pancreatic insufficiency tests [3,4]. A non-invasive test has been described to characterize pancreatic insufficiency in a functional way. In this test, a $^{13}$C-labeled mixed triglyceride ($^{13}$C-MTG; 1,3-distearoyl, 2[carboxyl-$^{13}$C]octanoyl glycerol) is orally ingested and the amount of $^{13}$C in expired air is determined [5]. $^{13}$C-MTG contains a $^{13}$C-labeled medium-chain fatty acid (octanoic acid) at its sn-2 position, and long-chain fatty acids (stearic acid) at the sn-1 and sn-3 positions of the glycerol backbone. The two stearoyl acylchains have to be hydrolyzed by the pancreatic enzyme lipase before $^{13}$C-octanoate can be absorbed, either in the form of a free fatty acid or as a mono-acylglycerol [6]. After its absorption, octanoate is rapidly oxidized [6,7]. Thus, the principle of the $^{13}$C-MTG test is based on lipolysis-dependent $^{13}$CO$_2$ excretion via the breath.

Since the original description of the $^{13}$C-MTG breath test, the test has become popular in clinical practice [5,8-11]. However, widely variable results have been obtained in children [9], healthy adults [12], and in cystic fibrosis patients with or without pancreatic enzyme replacement therapy [10,11]. The reason for this variability has not been elucidated: in fact, quantitative relationship between the extent of fat malabsorption due to impaired lipolysis and the corresponding result of the $^{13}$C-MTG breath test has never been demonstrated in humans or in defined animal models.

A reliable way to decrease the lipolysis activity dose-dependently is with the use of orlistat, an inhibitor of pancreatic lipase [13,14]. Orlistat, the chemically synthesized derivative of the natural product lipstatin, is a selective and potent inhibitor of lipases, among which, pancreatic lipase [15-23]. Orlistat inactivates pancreatic lipase by reacting covalently with serine (Ser-152) in the active site of the catalytic domain [24,25].

In the present study we aimed to determine the relationship between the extent of fat malabsorption and the results of the $^{13}$C-MTG breath test in a defined controlled animal model. We applied the dietary supplementation of orlistat as a reproducible inducer of various degrees of fat malabsorption in rats, in analogy to previous studies in mice and humans [26-28]. To ensure that orlistat-induced fat malabsorption was exclusively due to impaired lipolysis, we performed control experiments in which the absorption of the fatty acid [1-$^{13}$C]palmitic acid was determined, a substrate independent of lipolysis.
Materials and Methods

Rats

Male Wistar rats (Harlan, Zeist, The Netherlands), weighing approximately 400 g, were housed in an environmentally controlled facility with diurnal light cycling and free access to tap water and chow. Experimental protocols were approved by the Ethical Committee for Animal Experiments, Faculty of Medical Sciences, University of Groningen.

Materials

The mixed triglyceride (1,3-distearoyl, 2[1-13C]octanoyl glycerol) was purchased from Euriso-Top (Saint Aubin Cedex, France) and was 99% 13C-enriched. In previous articles [5,10,12], the breath test performed with the use of this compound has been denominated as the mixed-triglyceride breath test or as the 13C-MTG breath test. For reasons of consistency, we adhere to this nomenclature. [1-13C]palmitic acid was purchased from Isotec Inc. (Matheson, USA) and was 99% 13C-enriched. Orlistat (previously known as tetrahydrolipstatin, THL, Ro 18-0647) is a synthetic product and was kindly provided by Hoffmann-La Roche (Basel, Switzerland).

Study protocol

13C-MTG breath test. Rats were fed ground high-fat chow (35 en% fat; 4.538 kcal kg\(^{-1}\) food; fatty acid composition measured by GC analysis: C8-C12, 4.4 mol%; C16:0, 28.5%; C18:0, 3.9%; C18:1n-9, 33.2%; C18:2n-6, 29.3%; C18:3n-3, 0.2%) (Hope Farms BV, Woerden, The Netherlands) mixed with water (3:2, w/w) to form a homogenous paste. After 2 weeks on the diet, rats were divided into a control group (no orlistat added to the diet) and 3 orlistat groups (50, 200 or 800 mg orlistat per kg chow). There were 4 rats in each experimental group. Orlistat was ground together with the high-fat chow and mixed with water. Administration of orlistat started 2 days prior to the fat balance experiments. Food intake was recorded and feces was collected for 3 days, in order to perform a fat balance. Feces was stored at -20°C prior to analysis. After the fat balance, rats were fasted overnight. The following morning they were placed in an airtight container (volume ~ 4.5 L) through which CO\(_2\)-free air was passed at a continuous flow of 750 mL min\(^{-1}\). The air leaving the metabolic cage was partly diverted (50 mL min\(^{-1}\)) to a CO\(_2\) monitor (Capnograph IV, Gould Medical BV, Bilthoven, The Netherlands) for measuring percentage of total CO\(_2\) in the breath, and to 10 mL test tubes (Exetainers; Labco Limited, High Wycombe, United Kingdom) for collection of breath samples. The rats were placed in the container at least 30 min before administration of the test meal containing the label, to have the rats adapted to the cage and to obtain background breath samples. The test meal consisted of 13C-MTG (13 mg kg\(^{-1}\) body weight) mixed with high fat chow (6 g kg\(^{-1}\) body weight), orlistat and water. All rats ingested the test meal within 5 min. After ingestion of the test meal, 1-min breath samples were collected in duplicates at 30-min intervals for a period of 6 hours.

[1-13C]palmitic acid test. After 1 week on high fat chow, rats were equipped with permanent catheters in jugular vein, and duodenum as described by Kuipers et al. [29]. This experimental model allows to obtain multiple blood samples in unanesthetized rats without the
The $^{13}$C-MTG breath test in rats fed orlistat

interference of stress or restraint. Animals were allowed to recover from surgery for 6 days and were subsequently divided into 2 groups: 1 control group receiving no orlistat and an experimental group receiving 200 mg orlistat per kg chow. On day 7, 1.67 mL liquid fat kg$^{-1}$ body weight was slowly administered as a bolus via the duodenal catheter. The fat bolus was composed of olive oil (25% v/v; fatty acid composition: C16:0, 14%; C18:1n-9, 79%; C18:2n-6, 8%) and medium-chain triglyceride oil (75% v/v; composed of extracted coconut oil and synthetic triacylglycerols; fatty acid composition: C6:0, 2% max.; C8:0, 50-65% max.; C10:0, 30-45%; C12:0, 3% max.) and contained 33 mg kg$^{-1}$ body weight [$^{1-13}$C]palmitic acid and 0.47 mg kg$^{-1}$ body weight orlistat for the experimental group. The fat bolus represented approximately 15% of the daily fat intake. Blood samples (0.2 mL) were taken from the jugular cannula at baseline, 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, 5000 rpm, 4°C) and stored at -20°C until further analysis. Feces was collected in 24-h fractions starting 1 day before administration of the label and ending 2 days afterwards. Feces samples were stored at -20°C prior to analysis. Food intake was determined for 3 days.

Analytical techniques

Breath sample analysis. $^{13}$C-enrichment in aliquots of breath samples was determined by means of continuous flow isotope ratio mass spectrometry (Finnigan Breath MAT, Finnigan MAT GmbH, Bremen, Germany). The $^{13}$C-abundance of breath CO$_2$ was expressed as the difference per mil from the reference standard Pee Dee Belemnite limestone ($\delta^{13}$C$_{PDB}$, ‰). The proportion of $^{13}$C-label excreted in breath CO$_2$ was expressed as the percentage of administered $^{13}$C-label recovered per hour (% $^{13}$C dose h$^{-1}$), and as the cumulative percentage of administered $^{13}$C-label recovered over the 6-h study period (cum % $^{13}$C).

Plasma fats. Total plasma fats (triacylglycerols, phospholipids, etc.) were extracted, hydrolyzed and methylated according to Lepage and Roy [30]. Resulting fatty acid methyl esters were analyzed by gas chromatography to measure the total amount of palmitic acid and by gas chromatography combustion isotope ratio mass spectrometry to measure the $^{13}$C-enrichment of palmitic acid, as detailed below. The concentration of $^{13}$C-palmitic acid in plasma was expressed as the percentage of the dose administered per liter plasma (% dose/L).

Rat chow and fecal fats. Rat chow and feces were freeze-dried and mechanically homogenized, after which aliquots were extracted, hydrolyzed and methylated according to the method of Lepage and Roy [30]. Resulting fatty acid methyl esters were analyzed by gas chromatography to allow calculation of total fat intake, total fecal fat excretion, and total palmitic acid concentration in food and feces. Total fecal fat excretion of rats was expressed as g fat day$^{-1}$ and percentage of total fat absorption was calculated from the daily fat intake and the daily fecal fat excretion and expressed as a percentage of the daily fat intake.

$$\text{Total fat absorption} = \frac{\text{Fat intake (g day}^{-1}\text{)} - \text{Fecal fat excretion (g day}^{-1}\text{)}}{\text{Fat intake (g day}^{-1}\text{)}} \times 100\%$$

A similar calculation was performed to measure the absorption of [1-$^{13}$C]palmitic acid. Values were expressed as percentage of the dose administered (% dose).
Gas liquid chromatography. Fatty acid methyl esters were separated and quantified by gas liquid chromatography on a Hewlett Packard gas chromatograph Model 6890 equipped with a CP-SIL 88 capillary column (50 m x 0.32 mm; Chrompack, Middelburg, The Netherlands) and an FID detector. The gas chromatograph oven was programmed from an initial temperature of 150°C to 240°C in 2 temperature steps (150°C held 5 min; 150-200°C, ramp 3°C min⁻¹, held 1 min; 200-240°C, ramp 20°C min⁻¹, held 10 min). Quantification of the fatty acid methyl esters was done by adding heptadecanoic acid (C17:0) as internal standard.

Gas chromatography combustion isotope ratio mass spectrometry. ¹³C-enrichment of the palmitic acid methyl esters was determined on a gas chromatography combustion isotope ratio mass spectrometer (Delta S/GC Finnigan MAT, Bremen, Germany). Separation of the methyl esters was achieved on a CP-SIL 88 capillary column (Chrompack; 50 m x 0.32 mm). The gas chromatograph oven was programmed from an initial temperature of 80°C to 225°C in 3 temperature steps (80°C held 1 min; 80-150°C, ramp 30°C min⁻¹; 150-190°C, ramp 5°C min⁻¹; 190-225°C, ramp 10°C min⁻¹, held 5 min).

Calculations and statistics
The experimental data are reported as means ± SEM. Differences between sample means were calculated with the use of Student t-test or ANOVA followed by post-hoc analysis (Student-Newman-Keuls). For correlating two variables, regression lines were fitted by the method of least squares and expressed as the Pearson correlation coefficient r. Differences between means were considered statistically significant at the level of P<0.05. Analysis was performed using SPSS for Windows software (SPSS, Chicago, IL, USA).

Table 2.1 Nutritional data and breath ¹³CO₂ data obtained from control and orlistat-fed rats during ¹³C-MTG experiments (mean ± SEM).

<table>
<thead>
<tr>
<th>Orlistat mg kg⁻¹ chow</th>
<th>Fat intake (g day⁻¹)</th>
<th>Fecal fat (g day⁻¹)</th>
<th>Fat uptake (g day⁻¹)</th>
<th>Fat absorption (% intake)</th>
<th>Cum breath ¹³C (% dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.7 ± 0.2ᵃ</td>
<td>0.5 ± 0.1ᵃ</td>
<td>2.1 ± 0.1ᵃ</td>
<td>80.2 ± 2.2ᵃ</td>
<td>84.5 ± 7.8ᵃ</td>
</tr>
<tr>
<td>50</td>
<td>2.1 ± 0.2ᵇ</td>
<td>0.3 ± 0.0ᵃ</td>
<td>1.8 ± 0.2ᵃ</td>
<td>85.2 ± 0.8ᵃ</td>
<td>82.0 ± 4.9ᵃ</td>
</tr>
<tr>
<td>200</td>
<td>2.9 ± 0.1ᵇ</td>
<td>1.2 ± 0.1ᵇ</td>
<td>1.7 ± 0.1ᵇ</td>
<td>59.2 ± 2.1ᵇ</td>
<td>58.5 ± 5.3ᵇ</td>
</tr>
<tr>
<td>800</td>
<td>3.0 ± 0.2ᶜ</td>
<td>2.0 ± 0.2ᶜ</td>
<td>1.0 ± 0.1ᵇ</td>
<td>32.8 ± 3.7ᶜ</td>
<td>42.0 ± 1.5ᶜ</td>
</tr>
</tbody>
</table>

Unlike letters indicate a significant difference (P<0.05).

Results

¹³C-MTG test

Fecal fat balance. Nutritional data of the control and orlistat-fed rats are shown in Table 2.1. Rats fed 50 mg orlistat kg⁻¹ chow showed significantly lower food intake than the other groups. Administration of 50 mg orlistat kg⁻¹ chow did not lead to a change in fecal fat excretion. Fecal fat excretion in rats fed 200 and 800 mg orlistat kg⁻¹ chow, however, was significantly increased when compared with rats fed 0 or 50 mg orlistat kg⁻¹. In addition, fecal fat excretion in rats fed 800 mg orlistat kg⁻¹ chow was significantly higher compared with rats
fed 200 mg orlistat kg\(^{-1}\) chow. Net fat uptake, defined as fat intake minus fecal fat excretion, was significantly lower in rats fed 800 mg orlistat kg\(^{-1}\) chow than in the other groups. Percentage of total fat absorption was significantly decreased in the groups fed 200 and 800 mg orlistat kg\(^{-1}\) chow when compared with rats fed 0 or 50 mg orlistat kg\(^{-1}\). In addition, percentage of total fat absorption in rats fed 800 mg orlistat kg\(^{-1}\) chow was significantly lower compared with rats fed 200 mg orlistat kg\(^{-1}\) chow.

**Breath \(^{13}\)CO\(_2\) excretion measurements.** As shown in Figure 2.1A, the \(^{13}\)C excretion rate in breath after ingestion of \(^{13}\)C-MTG increased rapidly and reached a maximum value of approximately 16% dose/h at 4 h, in rats fed 0 or 50 mg orlistat kg\(^{-1}\). No difference in breath \(^{13}\)C expiration was observed between rats fed 0 or 50 mg orlistat kg\(^{-1}\). The \(^{13}\)C expiration rate was markedly different in the groups fed 200 and 800 mg orlistat kg\(^{-1}\) chow (Figure 2.1A). The \(^{13}\)C expiration rates rose more slowly and did not reach the high levels observed in the other two groups. The 6-h cumulative \(^{13}\)CO\(_2\) excretion data are summarized in Figure 2.1B and Table 2.1. The 6-h cumulative \(^{13}\)CO\(_2\) excretion, expressed as a percentage of the dose administered, was significantly lower when rats were fed 200 and 800 mg orlistat kg\(^{-1}\) chow compared with rats fed 0 and 50 mg orlistat kg\(^{-1}\) chow. In addition, the 6-h cumulative \(^{13}\)CO\(_2\) excretion of rats were fed 800 mg orlistat kg\(^{-1}\) chow was significantly reduced when compared with rats were fed 200 mg orlistat kg\(^{-1}\) chow.

**Figure 2.1** Time courses for (A) the excretion rates (% dose h\(^{-1}\)) and (B) the cumulative \(^{13}\)CO\(_2\) excretion (% cum) in breath (mean ± SEM) over the 6-h study period following oral ingestion of \(^{13}\)C-MTG (13 mg kg\(^{-1}\) body weight) to control rats and rats fed varying amounts of orlistat: 0 mg (■), 50 mg (●), 200 mg (□), and 800 mg (◇) orlistat kg\(^{-1}\) chow. Unlike letters indicate a significant difference (P<0.05).

**Relationship between total fat absorption and breath \(^{13}\)CO\(_2\) excretion.** If the result of the \(^{13}\)C-MTG breath test is exclusively determined by intestinal lipase activity, total fat absorption would be expected to correlate with recovery of \(^{13}\)CO\(_2\) in the breath after \(^{13}\)C-MTG ingestion in these experiments. Corresponding to the literature [31-33], the relationship between fat excretion and cumulative breath \(^{13}\)CO\(_2\) excretion was considered to be exponential. A significant correlation was indeed observed between the percentage of total fat absorption and 6-h cumulative \(^{13}\)CO\(_2\) expiration (r=0.88, P<0.001; Figure 2.2). However, as can be derived from individual data in Figure 2.2, the interindividual variation between
recovery of $^{13}$CO$_2$ excretion was large. Especially the individual $^{13}$C-results in rats with a dietary fat absorption higher than 60% showed strong overlap. In these rats, the coefficient of variation for percentage of total dietary fat absorption was only 5%, whereas coefficient of variation for cumulative breath $^{13}$CO$_2$ excretion was 15%.

![Figure 2.2](image)

**Figure 2.2** Relationship between the percentage of total fat absorption and breath $^{13}$CO$_2$ excretion after oral administration of $^{13}$C-MTG (13 mg kg$^{-1}$ body weight) in rats fed varying amounts of orlistat ($r=0.88, P<0.001$); 0 mg (■), 50 mg (●), 200 mg (□), and 800 mg (□) orlistat kg$^{-1}$ chow.

### $^{13}$C-palmitic acid test

Data of the $^{13}$C-palmitic acid experiment are shown in Table 2.2. No significant difference in mean fat intake was observed between control rats and rats fed 200 mg orlistat kg$^{-1}$ chow ($P=0.36$). Orlistat-fed rats excreted significantly more fat into the feces when compared with control rats ($P<0.01$). The percentage of total fat absorption was significantly decreased in orlistat-fed rats when compared with controls (46.7 ± 5.4% and 74.6 ± 1.3%, respectively, $P<0.01$).

<table>
<thead>
<tr>
<th>Orlistat mg kg$^{-1}$ chow</th>
<th>Fat intake (g day$^{-1}$)</th>
<th>Fecal fat (g day$^{-1}$)</th>
<th>Fat absorption (% intake)</th>
<th>$^{13}$C16:0 absorption (% dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.2 ± 0.2</td>
<td>0.6 ± 0.0</td>
<td>74.6 ± 0.3</td>
<td>83.7 ± 2.0</td>
</tr>
<tr>
<td>200</td>
<td>2.5 ± 0.2</td>
<td>1.3 ± 0.2*</td>
<td>46.7 ± 5.4**</td>
<td>87.0 ± 1.0</td>
</tr>
</tbody>
</table>

A symbol indicates a significant difference from the control group (0 mg orlistat kg$^{-1}$ chow). * $P<0.05$; ** $P<0.01$.

The amount of $^{13}$C-palmitic acid excreted into the feces was calculated for the 48-h period following administration of [1-$^{13}$C]palmitic acid. No significant difference in absorption of [1-$^{13}$C]palmitic acid over the 48-h period studied was observed between control and orlistat-fed rats ($P=0.71$, Table II), demonstrating that administration of orlistat does not affect the absorption of [1-$^{13}$C]palmitic acid. This is supported by the fact that $^{13}$C-palmitic acid concentrations in plasma after intraduodenal administration of [1-$^{13}$C]palmitic acid were similar in control and orlistat-fed rats (Figure 2.3). The data of the [1-$^{13}$C]palmitic acid experiment indicate that fat malabsorption in orlistat treated rats is solely due to impaired lipolysis.
Discussion

We investigated the potency of the \(^{13}\text{C}\)-MTG breath test to quantify fat malabsorption due to impaired lipolysis in rats fed different dosages of orlistat. After \(^{13}\text{C}\)-MTG ingestion, a significant correlation was observed between 6-h recovery of \(^{13}\text{CO}_2\) in breath and percentage of total fat absorption as shown in Figure 2.2. Two interesting observations arise from this figure. Firstly, rats fed 200 and 800 mg orlistat kg\(^{-1}\) chow have fat malabsorption to an extent that, if seen in patients, would coincide with steatorrhoea or bulky amounts of fat in the feces. Especially in these rats, the relation between breath \(^{13}\text{CO}_2\) recovery and fat absorption is strong. Apparently, under these conditions, the \(^{13}\text{C}\)-MTG breath test is a powerful analytical technique for the detection of fat malabsorption. Clinical studies in humans indeed have shown that the sensitivity and specificity of the \(^{13}\text{C}\)-MTG breath test to detect severe pancreatic insufficiency are high [5]. Secondly, from a clinical point of view, rats with fat absorption higher than 70% are a very interesting group. The extent of fat malabsorption in these animals reflects, in a sense, the distinction that has to be made between healthy subjects and patients whose fat malabsorption may easily be missed by examining the amounts of feces. In these rats, at a rather narrow range of fat absorption, the \(^{13}\text{CO}_2\) response after ingestion of \(^{13}\text{C}\)-MTG varies considerably (Figure 2.2, Table 2.1). These results indicate that, even in a homogeneous group of rats with the same genetic background and diet, a considerable variation exists under controlled circumstances.

Widely variable results with the \(^{13}\text{C}\)-MTG breath test have also been obtained in healthy children [9], healthy adults [12], and in cystic fibrosis patients with pancreatic enzyme replacement therapy [10,11]. So far, this variation has been blamed on large intra- and interindividual variation caused by differences in, e.g., gastric emptying, hepatic clearance and metabolism, endogenous \(^{13}\text{CO}_2\) production or pulmonary excretion [34-37]. The present data indicate that the high variability of the \(^{13}\text{CO}_2\) response is a rather intrinsic property of the \(^{13}\text{C}\)-MTG breath test, for which no optimal standardization seems possible at this moment.
Therefore, we propose that the $^{13}$C-MTG breath test is useful for the detection of severe fat malabsorption due to low lipase activity in groups of patients. However, the large variation in the $^{13}$CO$_2$ response at a mild degree of fat malabsorption limits the diagnostic possibilities of the $^{13}$C-MTG breath test in humans [10,11].

No data concerning the $^{13}$C-MTG breath test in rats have been published so far. If we compare the present results on the $^{13}$C-MTG breath test in control rats with data obtained in healthy humans, the 6-h cumulative percentage of breath $^{13}$CO$_2$ appears to be much higher in rats: 85% compared with 30% in humans [5,10,12]. We speculate that the extended fasting period of our rats directs the absorbed $^{13}$C-octanoic acid directly into the oxidation pathway. However, it can not be excluded that part of the difference is based on species specificity.

In vitro studies with orlistat have shown that orlistat is insoluble in aqueous buffers, very poorly soluble in micellar lipid phases, but exhibits good solubility in emulsified lipids [13,38-40]. Therefore, in studies of fat absorption in mice, rats, and humans, inhibition by orlistat was mainly determined by the concentration of the drug in the lipid phase [26,27,40]. In contrast, when the dose of orlistat was not pre-dissolved in the dietary fat, but simply admixed to the diet or administered as suspension or in capsules in a meal-contingent manner, the inhibitory effect on fat absorption was reduced to a variable extent [26,27]. Therefore, a meaningful comparison between our results on inhibition of fat absorption by orlistat and previously published studies is only possible if the experimental design and the mode of drug administration are taken into account. Since in the present study, orlistat was admixed to the diet, the major part of it was likely dissolved in the target dietary fat upon preparation and mixing of the diet. Except for the 50 mg kg$^{-1}$ experiment, the effect of orlistat on fat absorption was dose-dependent up to the largest dose tested. Whether with dose escalation the effect could be intensified or would level out is unknown. Previously, a similar dose-response relationship has been described in mice to which orlistat was administered either dissolved in the fat component of the meal or administered as suspension immediately after the meal [26]. In these mice, excretion of fat in the feces increased exponentially when orlistat dose was increased, until a plateau of 80% of the ingested amount [26]. The orlistat dose required for half maximal elimination of fat (ID$_{50}$) reported for mice was approximately 3.3 mg orlistat per g of fat ingested [26]. In our study, the ID$_{50}$ for rats was approximately 500 mg orlistat per kg chow, corresponding to roughly 2 mg orlistat per g of dietary fat. Thus, despite the different design of our study, the potency of orlistat expressed as dose per nutritional fat ingested was rather similar, indicating that the mode of action of orlistat in our study was very efficient.

To investigate whether the orlistat-induced fat malabsorption was not partially due to other intestinal effects of orlistat resulting in fat malabsorption, control experiments with [1-$^{13}$C]palmitic acid were performed. The [1-$^{13}$C]palmitic acid absorption test detects fat malabsorption due to impaired intestinal uptake of long-chain fatty acids [41]. If fat malabsorption in rats fed with orlistat were not solely due to the inhibition of intestinal lipolysis, an impaired absorption of intraduodenal administered [1-$^{13}$C]palmitic acid would be expected. However, fecal $^{13}$C-palmitic acid excretion and plasma $^{13}$C-palmitic acid concentrations were not affected at a dosage of 200 mg orlistat kg$^{-1}$ chow, despite significantly reduced absorption of dietary fats. These data indicate that the orlistat-fed rat model indeed is
specific for impaired lipolysis as cause of fat malabsorption, as has been shown before [26,42-45]. In addition, these data show that in this rat model lipolytic and non-lipolytic processes regarding fat malabsorption can be dissected and measured separately with the use of different stable isotope tests.

In summary, dietary orlistat administration to rats provides a model for fat malabsorption, specifically due to impaired intestinal lipolysis. The $^{13}$C-MTG breath test in this animal model correlates significantly with the extent of induced fat malabsorption. However, variation in $^{13}$CO$_2$ results between individual rats was large, especially in rats with dietary fat absorption higher than 70%. The present data do not support the application of the $^{13}$C-MTG breath test for diagnostic purposes in individual patients.

References

The $^{13}$C-MTG breath test in rats fed orlistat


