CHAPTER 4

The $^{13}$C-palmitic acid test with plasma sampling detects fat malabsorption in bile-diverted rats

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CHAPTER 4

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Abstract

**Background & Aims:** The fecal fat balance does not discriminate between the distinct intestinal processes, such as lipolysis, solubilization, chylomicron formation, as causes of fat malabsorption. In the present study we characterized a rat model with fat malabsorption due to bile diversion and we investigated the diagnostic potency of the [$^{1-13}$C]palmitic acid test. **Methods:** Bile-diverted and control rats were fed standard (14 en% fat) or high-fat chow (35 en% fat) for 2 weeks. After intraduodenal administration of [$^{1-13}$C]palmitic acid (33 mg kg$^{-1}$ BW) blood samples were taken for measurements of $^{13}$C-palmitate concentrations. Food intake was quantified and feces was collected for balance studies. Intestinal histology was determined. **Results:** Total fat absorption was highly efficient in control rats (mean ± SEM: standard chow 96.7 ± 0.2%; high-fat chow 93.2 ± 0.4%), but was significantly decreased in bile-diverted rats (standard chow 87.2 ± 0.9%, *P*<0.001; high-fat chow 53.9 ± 3.9%, *P*<0.001). Plasma $^{13}$C-palmitate concentrations allowed discrimination between normal (>91%) and decreased fat absorption due to bile diversion. **Conclusion:** The [$^{1-13}$C]palmitic acid absorption test detects fat malabsorption due to bile diversion in rats. Application of this test in clinical states with fat malabsorption may allow design of specific treatment strategies.
Introduction

Adequate absorption of dietary fats by the intestine is required for supply of energy, membrane constituents, and precursors for the formation of hormones and inflammatory mediators [1,2]. In Western diets, triacylglycerols composed of long-chain fatty acids constitute 92 to 96% of dietary fats [1]. The absorption of these fats involves several specific processes. Firstly, lipolysis, by lipolytic enzymes originating predominantly from the pancreas, leads to hydrolysis of triacylglycerols into fatty acids and 2-monoacylglycerols. Secondly, during the process of solubilization, mixed micelles are formed consisting of bile acids, phospholipids and cholesterol [3]. The mixed micelles are thought to act as the physiological transport vehicles of lipolytic products from dietary fats in the intestinal lumen. Finally, the fatty acids and monoacylglycerols are translocated across the intestinal epithelium, converted back to triacylglycerols, assembled into chylomicrons and secreted into the lymph [1,4,5].

The efficiency of intestinal fat absorption in patients is routinely determined by means of a fat balance, requiring detailed analysis of daily fat intake and the complete recovery of feces for 72 h. However, in the case of fat malabsorption, this method does not discriminate between the potential causes, such as impaired intestinal lipolysis, disturbed intestinal solubilization of long-chain fatty acids or chylomicron formation. Stable isotope techniques have been introduced in the development of novel diagnostic strategies. Several $^{13}$-labeled fats, such as tri-$[1-^{13}]$octanoin and 1,3-distearoyl, 2-$[1-^{13}]$octanoyl glycerol, have been successfully applied for the rather specific detection of impaired lipolysis [6-8]. Attempts to develop a specific test for the detection of impaired solubilization have been less successful. Watkins et al. [9] administered $[1-^{13}]$palmitic acid, tri-$[1-^{13}]$octanoin or tri-$[1-^{13}]$olein to patients with impaired lipolysis, bile salt deficiency or mucosal disease and measured breath $^{13}$CO$_2$ excretion.

So far, the use of $^{13}$-labeled fats for quantitative studies on defective fat absorption has been limited to breath and feces analysis [9-11]. 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, e.g., due to variations in the post-absorptive metabolism [12,13]. The availability of gas chromatography isotope ratio mass spectrometry allows for accurate determination of $^{13}$C enrichments in plasma fatty acids [14]. The determination of plasma concentrations of absorbed $^{13}$C-labeled fats as a measure of their absorption offers a theoretical advantage over breath $^{13}$CO$_2$ analysis, since numerous steps are involved in the (post-absorptive) metabolism of the tracer prior to exhalation of $^{13}$CO$_2$ [15].

In order to determine the potency of a novel test, the availability of an animal model is almost essential. Manipulation of the enterohepatic circulation of bile components has been repeatedly and successfully used in rat studies on fat (mal)absorption and metabolism [16-19]. During bile diversion, no bile components are available in the intestine and fat malabsorption appears to be mainly due to impaired solubilization of long-chain fatty acids [20]. However, recent observations have suggested that bile is also essential for efficient chylomicron formation [21-23]. Bile-diverted rats regain normal feeding behavior and normal growth curves within several days after surgical interruption of the enterohepatic circulation [24].
In the present study, firstly, we aimed to characterize the rat model of fat malabsorption due to bile diversion. For this purpose, total fat absorption and [1-\(^{13}\)C]palmitic acid absorption was determined in rats that received either standard (14 en% fat) or high-fat chow (35 en% fat). Secondly, we investigated the potency of the [1-\(^{13}\)C]palmitic acid test with plasma \(^{13}\)C-palmitic acid concentrations to detect fat malabsorption due to bile diversion. Control experiments, in which tri-[1-\(^{13}\)C]palmitoylglycerol was administered, were performed to check whether lipolysis is unaffected in our animal model.

Materials and Methods

Animals

Male Wistar rats (Harlan, Zeist, The Netherlands), weighing 300 to 400 g, were housed in an environmentally controlled facility with diurnal light cycling and free access to food and tap water (and additional saline, 0.9% NaCl w/v, in the case of bile-diverted rats). Experimental protocols were approved by the Ethical Committee for Animal Experiments, Faculty of Medical Sciences, University of Groningen.

\(^{13}\)C-labeled substrates

[1-\(^{13}\)C]-labeled palmitic acid was purchased from Isotec Inc. (Matheson, USA) and was 99% \(^{13}\)C-enriched. Tri-[1-\(^{13}\)C]palmitoylglycerol was purchased from ICN Biomedicals Inc. (Cambridge, United Kingdom) and was 99% \(^{13}\)C-enriched.

Study protocol

Rats were assigned to either standard chow (14 en% fat; 4.575 kcal kg\(^{-1}\) food; fatty acid composition measured by GC analysis: C8-C12, 0.9 mol%; C16:0, 25.2%; C18:0, 5.5%; C18:1n-9, 30.3%; C18:2n-6, 33.9%; C18:3n-3, 3.6%) or to 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). After 1 week, rats were equipped with permanent catheters in jugular vein, bile duct and duodenum as described by Kuipers et al. [24]. This experimental model allows for physiological studies in unanesthetized rats with long term bile diversion without the interference of stress or restraint. One day after surgery, catheters in bile duct and duodenum were either connected at time of surgery to restore the enterohepatic circulation (control rats) or catheters were chronically interrupted (bile-diverted rats). Animals were allowed to recover from surgery for 6 days.

On day 7, 1.67 mL 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 mol%; 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 mol%; C8:0, 50-65% max.; C10:0, 30-45%; C12:0, 3% max.) and contained either 33 mg kg\(^{-1}\) body weight [1-\(^{13}\)C]palmitic acid or 33 mg kg\(^{-1}\) body weight tri-[1-\(^{13}\)C]palmitoylglycerol. The fat bolus represented approximately 25 and 15% of the daily fat intake in the standard and
the high-fat group, respectively. 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, 2000 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 fat bolus and ending 2 days afterwards. Feces samples were stored at -20°C prior to analysis. Food intake was determined for 3 days by daily weighing of the food container.

**Analytical techniques**

*Plasma fats.* Total plasma fats (triacylglycerols, phospholipids, etc.) were extracted, hydrolyzed and methylated according to Lepage and Roy [25]. 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. The concentration of $^{13}$C-palmitic acid in plasma was expressed as the percentage of the dose administered per liter plasma (% dose L$^{-1}$).

*Rat chow and fecal fats.* Feces was freeze-dried and mechanically homogenized. Aliquots of rat chow and freeze-dried feces were extracted, hydrolyzed and methylated [25]. 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 gas chromatography combustion isotope ratio mass spectrometry to calculate the $^{13}$C-enrichment of palmitic acid. 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.

$$\text{Percentage of total fat absorption} = \frac{\text{Fat intake (g day}^{-1}) - \text{Fecal fat output (g day}^{-1})}{\text{Fat intake (g day}^{-1})} \times 100\%$$

A similar calculation was performed to measure the absorption of [1-$^{13}$C]palmitic acid and tri-[1-$^{13}$C]palmitoylglcerol. The absorption of the label was determined from the intake and excretion of $^{13}$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 5880 equipped with a CP-SIL 88 capillary column (Chrompack; 50 m x 0.32 mm) and an FID detector [26,27]. 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 achieved by adding heptadecanoic acid (C17:0) as internal standard.

*Gas chromatography combustion isotope ratio mass spectrometry.* $^{13}$C-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) [28]. Separation of the methyl esters was achieved on a CP-SIL 88 capillary column (Chrompack; 50 m x 0.32
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. Significance of differences was calculated with the use of the two-tailed Student’s t-test for unpaired data. For correlating two variables, linear 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 \( P<0.05 \).

Results

Fecal fat balance
In Table 4.1 nutritional data of control and bile-diverted rats on standard chow (14 en% fat) and high-fat chow (35 en% fat) are shown.

Standard chow (14 en% fat). Mean food intake, and thus fat intake, over the 3-day period was significantly increased in bile-diverted rats on standard chow when compared to control rats (\( P<0.05 \)). Bile-diverted rats excreted significantly more fat into the feces when compared to control rats (\( P<0.001 \)). Although the percentage of total fat absorption was lower in bile-diverted rats when compared to control rats (87.2 ± 0.9% vs. 96.7 ± 0.2%, respectively, \( P<0.001 \)), net fat uptake in both control and bile-diverted rats was similar (\( P=0.91 \)).

High-fat chow (35 en% fat). Both food and fat intake were significantly increased in bile-diverted rats when compared to control rats (\( P<0.01 \)). Similarly, fecal fat excretion was significantly increased in bile-diverted rats compared to control rats (\( P<0.001 \)). Again, there was no significant difference in the net fat uptake of either control and bile-diverted rats (\( P=0.10 \)), although percentage of total fat absorption was considerably decreased in bile-diverted rats when compared to control rats (53.9 ± 3.9% vs. 93.2 ± 0.4%, respectively, \( P<0.001 \)).

<table>
<thead>
<tr>
<th>Table 4.1</th>
<th>Nutritional data (mean ± SEM) of control and bile-diverted rats on standard chow (14 en% fat) and high-fat chow (35 en% fat).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
<td>n</td>
</tr>
<tr>
<td>Standard chow</td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>9</td>
</tr>
<tr>
<td>Bile-diverted</td>
<td>11</td>
</tr>
<tr>
<td>High-fat chow</td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>9</td>
</tr>
<tr>
<td>Bile-diverted</td>
<td>6</td>
</tr>
</tbody>
</table>

Symbols indicate significant difference within the same dietary group: #, \( P<0.05 \); §, \( P<0.01 \); *, \( P<0.001 \).
The $^{13}$C-palmitic acid test in bile-diverted rats

Figure 4.1  Correlation between total fat absorption and the absorption of $[1-^{13}]$C-palmitic acid (33 mg kg$^{-1}$ body weight). Results of both control and bile-diverted rats, standard (14 en% fat) and high-fat chow (35 en% fat) are combined. Equation of the line: $y=0.44x+49; r=0.89, P<0.001$.

Excretion of $^{13}$C-palmitic acid into feces

$[1-^{13}]$C-palmitic acid experiments. Table 4.2 shows the percentage absorption of $[1-^{13}]$C-palmitic acid, assessed by fecal $^{13}$C-palmitic acid concentration. 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. The highest levels of $^{13}$C-palmitic acid in the feces were observed in the first 24 h after bolus administration, which accounted for 81% of the amount of label excreted in 48 h in the case of control rats. $^{13}$C-palmitic acid excretion in bile diverted rats was significantly retarded with 62% excreted in the first 24 h ($P<0.005$). Control and bile-diverted rats on standard chow absorbed similar amounts of $[1-^{13}]$C-palmitic acid over the 48-h period studied ($P=0.95$). In bile-diverted rats on high-fat chow, the apparent absorption of $[1-^{13}]$C-palmitic acid was significantly lower than in their control counterparts ($P<0.001$).

Table 4.2  Absorption of $[1-^{13}]$C-palmitic acid (33 mg kg$^{-1}$ body weight) by control and bile-diverted rats on standard chow (14 en% fat) and high-fat chow (35 en% fat).

<table>
<thead>
<tr>
<th>Chow Category</th>
<th>n</th>
<th>$[1-^{13}]$C-palmitic acid absorption (% dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>4</td>
<td>88.8 ± 2.6</td>
</tr>
<tr>
<td>Bile-diverted</td>
<td>5</td>
<td>89.0 ± 1.8</td>
</tr>
<tr>
<td>High-fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control rats</td>
<td>5</td>
<td>91.3 ± 0.5</td>
</tr>
<tr>
<td>Bile-diverted</td>
<td>3</td>
<td>73.7 ± 2.1*</td>
</tr>
</tbody>
</table>

* indicates a significant difference within the same dietary group ($P<0.001$).

Relationship between fecal fat balance and absorption of $[1-^{13}]$C-palmitic acid. To compare whether $[1-^{13}]$C-palmitic acid was handled by the intestine similarly as dietary fats, the relationship between the percentage of total fat absorption and absorption of $[1-^{13}]$C-palmitic acid after 48 h was determined. Figure 4.1 shows a linear relationship between the absorption
of total fat and the absorption of [1-\(^{13}\!)\)C\)palmitic acid (r=0.89, \(P<0.001\); equation of the line: \(y = 0.44 \times + 49\)).

**Tri-[1-\(^{13}\!)\)C\)palmitoylglycerol experiments.** In control experiments with tri-[1-\(^{13}\!)\)C\)palmitoylglycerol we verified if lipolysis in bile-diverted rats was not impaired. In bile-diverted rats on standard chow, the absorption of tri-[1-\(^{13}\!)\)C\)palmitoylglycerol was virtually identical to that of [1-\(^{13}\!)\)C\)palmitic acid (88.3 ± 1.7\% and 89.0 ± 1.8\%, respectively). In bile-diverted rats on high fat chow, again, the absorption of tri-[1-\(^{13}\!)\)C\)palmitoylglycerol was not significantly lower than the absorption of [1-\(^{13}\!)\)C\)palmitic acid (80.8 ± 5.1\% and 93.6 ± 6.4\%, respectively). These results indicate that lipolysis per se is not impaired in the bile-diverted rat.

**Plasma \(^{13}\!)\)C-palmitic acid concentrations**

**[1-\(^{13}\!)\)C\)palmitic acid experiments.** Figure 4.2A shows the time course patterns of \(^{13}\!)\)C-palmitic acid appearance in plasma after intraduodenal administration of [1-\(^{13}\!)\)C\)palmitic acid to rats on standard chow (14 en\% fat). After administration of [1-\(^{13}\!)\)C\)palmitic acid to control rats, plasma \(^{13}\!)\)C-palmitic acid concentrations increased within 1 h, reaching a maximum value of 58 ± 21\% dose L\(^{-1}\) plasma at 2 h after bolus administration (Figure 4.2A). Upon bile diversion, plasma \(^{13}\!)\)C-palmitic acid concentrations were significantly lower than in controls \((P<0.05)\). A maximum value of 10 ± 2\% dose L\(^{-1}\) plasma was obtained at 6 h (Figure 4.2A). Figure 4.2B shows the time course patterns of \(^{13}\!)\)C-palmitic acid appearance in plasma after intraduodenal administration of [1-\(^{13}\!)\)C\)palmitic acid to rats on high-fat chow (35 en\% fat). After administration of [1-\(^{13}\!)\)C\)palmitic acid to control rats on high-fat chow, plasma \(^{13}\!)\)C-palmitic acid concentrations increased within 1 h after administration of the bolus and reached a maximum value of 55 ± 7\% dose L\(^{-1}\) plasma at 3 h (Figure 4.2B). Upon bile diversion, plasma \(^{13}\!)\)C-palmitic acid concentrations were significantly lower \((P<0.05)\) when compared to the values obtained in the controls rats (Figure 4.2B) and a maximum value of 16 ± 6\% dose L\(^{-1}\) plasma was obtained after 6 h.

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*Figure 4.2 Time courses of \(^{13}\!)\)C-palmitic acid concentration in plasma of rats fed (A) standard chow (14 en\% fat) and (B) high-fat chow (35 en\% fat) after intraduodenal administration of [1-\(^{13}\!)\)C\)palmitic acid (33 mg kg\(^{-1}\) body weight). (○) Control rats, (○) bile-diverted rats. Symbols indicate significant difference between control and bile-diverted rats after [1-\(^{13}\!)\)C\)palmitic acid administration (* \(P<0.05\), # \(P<0.01\)).*
**The $^{13}$C-palmitic acid test in bile-diverted rats**

**Relationship between fecal fat balance and plasma $^{13}$C-palmitic acid concentrations.**

To compare the results of the 3-day fecal fat balance with the results of the $^{13}$C-palmitic acid test, total fat absorption was related to plasma $^{13}$C-palmitic acid concentrations at 1 h, 2 h, and 3 h (Figure 4.3) after administration of $[1^{-13}]$C-palmitic acid. Already at 1 h and 2 h after label administration, a clear distinction between control and bile-diverted rats was observed with respect to plasma $^{13}$C-palmitic acid concentrations. Yet, plasma $^{13}$C-palmitic acid concentrations at 3 h were most discriminative. Plasma $^{13}$C-palmitic acid concentrations at 3 h were at least 3-fold lower in bile-diverted rats compared to controls, irrespective of the type of chow. If we would regard 91% as the lower limit for normal fat absorption and 10 to 20% dose $L^{-1}$ plasma as the lower limit for normal plasma values, the test has a sensitivity and specificity of 100%, under the conditions employed.

![Figure 4.3](image-url)  
**Figure 4.3** Correlation between the results of the 72-h fecal fat balance and plasma $^{13}$C-palmitic acid concentrations at (A) 1 h, (B) 2 h, and (C) 3 h after intraduodenal administration of $[1^{-13}]$C-palmitic acid ($33 \text{ mg kg}^{-1} \text{ body weight}$). Results of all experimental groups are combined: control rats, standard chow (○); control rats, high-fat chow (●); bile-diverted rats, standard chow (□); bile-diverted rats, high-fat chow (△).

**Tri-$[1^{-13}]$C-palmitoylglycerol experiments.** After administration of tri-$[1^{-13}]$C-palmitoylglycerol to control rats and bile-diverted rats on standard chow, the $^{13}$C-palmitic acid concentrations in plasma were not significantly different when compared with administration of $[1^{-13}]$C-palmitic acid. Maximum values of $^{13}$C-palmitic acid concentration in control and bile-diverted rats were $35 \pm 5\%$ dose $L^{-1}$ plasma and $12 \pm 1\%$ dose $L^{-1}$ plasma, respectively. When the rats were fed high-fat chow, again, similar results were obtained. Maximum values of $^{13}$C-palmitic acid concentration in control and bile-diverted rats were $50 \pm$
20% dose L\textsuperscript{-1}\ plasma and 6 ± 4% dose L\textsuperscript{-1}\ plasma, respectively. These results are in accordance with results on fecal excretion of \textsuperscript{13}C-palmitic acid reported above, and further underline that lipolysis is not impaired in the bile-diverted rat.

**Discussion**

In the present study we characterized a rat model with a defined cause of fat malabsorption due to bile diversion and we investigated the potency of the [1-\textsuperscript{13}C]palmitic acid test to detect this fat malabsorption. As no bile is available in the intestinal tract of bile-diverted rats, this animal model is useful for determining the contribution of bile (components) to the process of intestinal fat absorption. Although the role of bile for efficient fat absorption is well-established, its quantitative importance has been a matter of debate and is likely dependent on the amount and composition of dietary fats [16-18].

Total dietary fat absorption was examined in chronically bile-diverted rats on standard chow (14 en% fat) and on high-fat chow (35 en% fat). In control rats on either standard or high-fat chow, the absorption of dietary fats was very efficient and varied from 92% to 97%. This percentage fat absorption is in accordance to that found in healthy humans [20]. Total fat absorption was significantly decreased in bile-diverted rats. Bile-diverted rats on standard chow still absorbed 87% of their dietary fats. An explanation could be the formation of liquid crystalline vesicles in the intestinal lumen, as proposed by Carey et al. [20]. They suggested that liquid crystalline vesicles are formed, when the amount of fat in the aqueous intestinal phase is relatively high compared to the amount of bile. These vesicles may play an important role in in the uptake of fats by enterocytes in certain disease states [29]. However, bile-diverted rats on high-fat chow absorbed only 54% of their dietary fats, indicating that at relatively high dietary fat intake, surface increase and formation of vesicles are not sufficient to restore fat absorption completely.

Yet, in spite of the decrease in percentage of fat absorption in bile-diverted rats, the rats managed to maintain a similar net fat uptake by increasing their food intake. This effect was observed on either of the two diets. It has been observed previously that after bile diversion rats increase their food intake in order to maintain an adequate energy balance [24]. It is intriguing to speculate which physiological stimulus mediates the adaptation of food intake. A possible candidate is apolipoprotein A-IV. Synthesis of apo A-IV is stimulated upon transport of absorbed lipid via chylomicrons in lymph [30-32]. Evidence of decreased chylomicron assembly due to interference of biliary phospholipid availability by manipulation with cholestyramine or dietary zinc was presented previously [21,22]. Impaired chylomicron assembly in bile-diverted rats would decrease concentrations of apo A-IV in plasma, resulting in enhanced food intake [33].

Using this animal model, we found that at 1 h, 2 h, and 3 h after administration of [1-\textsuperscript{13}C]palmitic acid, plasma \textsuperscript{13}C-palmitic acid concentrations clearly differentiated between control rats and chronically bile-diverted rats (Figure 4.3). Using 10% dose L\textsuperscript{-1}\ plasma as the lower limit of normal plasma values and 91% as the lower limit of normal fat absorption, the test had a sensitivity and specificity of 100% under the test conditions used. The results were
The $^{13}$C-palmitic acid test in bile-diverted rats

essentially similar on standard and high-fat chow, emphasizing the potency of the $^{13}$C-palmitic acid absorption test. It is interesting to hypothesize on the low plasma $^{13}$C-palmitic acid concentrations in bile-diverted rats when compared to control rats. It is not likely that the whole effect is solely due to impaired solubilization, because fat absorption in bile-diverted rats on standard chow is still rather efficient. However, the effect of diminished solubilization could be enhanced by the absence of stimulatory effects of biliary phospholipids on assembly of intestinal chylomicrons [21-23]. If chylomicron assembly is impaired, $^{13}$C-palmitic acid will appear in plasma to a lesser extent and on a slower time scale.

Time course patterns of plasma $^{13}$C-palmitic acid concentrations in control and bile-diverted rats were considerably different (Figure 4.2). In control rats plasma $^{13}$C-palmitic acid concentrations increased rapidly and peak values were observed after 1 to 3 h. In bile-diverted rats plasma $^{13}$C-palmitic acid concentrations continuously increased up to 5 or 6 h but did not reach the high values obtained in the control rats. Brand & Morgan [34] showed that fat absorption occurs largely from the upper small intestine in control rats, whereas, in the absence of bile lower small intestine is also involved. Presumably, the absorptive reserve of the distal small intestine is called upon in the case of bile diversion and much of the fat which failed to enter the proximal intestinal mucosa is absorbed more distally [35]. The delayed time course patterns of plasma $^{13}$C-palmitate concentrations upon bile diversion could also be due to decreased intestinal motility. In support of this explanation, fecal $^{13}$C-palmitate excretion was significantly retarded in bile-diverted rats compared to controls in the first 24 h after administration of [1-$^{13}$C]palmitate. A cyclic pattern of motor activity known as the migrating motor complex occurs in dogs, humans, and most other mammals during fasting [36-38]. Feeding interrupts the migrating motor complex and induces a different pattern of intermittent contractile activity. However, the migrating motor complex activity does not seem to be affected by bile diversion in rats [34] and dogs [39].

The fat bolus containing the [1-$^{13}$C]palmitic acid was administered to the rats intraduodenally. Although oral administration would have been more physiological, intraduodenal administration has the benefit that the results are not influenced by gastric emptying. Intra-individual and inter-individual variation of gastric emptying have been shown to vary widely in humans [40,41] and would probably affect the outcomes of the study. This is also a likely explanation for the high specificity and sensitivity of the test in our experiments. An adventitious circumstance is that experiments run for a shorter period when the bolus is administered intraduodenally, as gastric emptying of oils delays the process of fat absorption with approximately 2 to 3 h [42,43].

In order to determine if stable isotopically-labeled fats are handled within the body similar as those of dietary origin, the percentage of total fat absorption was compared to the absorption of [1-$^{13}$C]palmitic acid. Under physiological circumstances, palmitic acid is consumed in the diet in the form of mixed triacylglycerols predominantly esterified at the $sn$-1 and $sn$-2 positions [44]. Hydrolysis of dietary triacylglycerols in the intestinal lumen by pancreatic lipase and other enzymes, such as carboxyl ester hydrolase, results in the generation of 2-monoacylglycerols and fatty acids. After interaction with bile components and the formation of mixed micelles, the hydrolyzed fats can be absorbed. The absorption of dietary fats was significantly correlated with the absorption of [1-$^{13}$C]palmitic acid, assessed by fecal
13C-palmitic acid concentration (Figure 4.1). Thus, it seems that the fate of [1-13C]palmitic acid with respect to absorption reflects the fate of total mass of dietary fats under the experimental circumstances of this study. It has to be noted that the line of correlation does not cut the origin of the plot, and that the slope of the line is smaller than unity. Apparently, [1-13C]palmitic acid is preferentially absorbed when compared to dietary fats, which may be due to the fact that only tracer amounts of [1-13C]palmitic acid were administered to the rats in a specific, soluble form.

Absorption of tri-[1-13C]palmitoylglycerol and plasma 13C-palmitic acid concentrations were determined to ascertain that lipolysis was not affected in the bile-diverted rat model. In case of impaired lipolysis, tri-[1-13C]palmitoylglycerol could only be hydrolyzed partially, resulting in decreased plasma 13C-palmitic acid concentrations when compared to administration of [1-13C]palmitic acid. In addition, fecal 13C-palmitic acid concentrations would be expected to be increased in analogy to studies involving drug-induced impairment in lipolysis [45]. In both control and bile-diverted rats on standard chow, the plasma 13C-palmitic acid concentration curves after administration of either [1-13C]palmitic acid or tri-[1-13C]palmitoylglycerol were not significantly different (Figure 4.2). After administration of tri-[1-13C]palmitoylglycerol, fecal excretion of 13C-palmitic acid was not significantly increased compared with administration of [1-13C]palmitic acid. Based on these observations, we conclude that lipolysis is not a rate-limiting step in this experimental model, in accordance to previous observations [18,29]. Hamilton et al. [18] reported that in bile-diverted rats absorption of free fatty acids was equal to the absorption of triacylglycerols with respect to both palmitic acid and stearic acid. Similarly, Porter et al. [29] reported that lipolysis was unimpaired in bile fistula man.

In summary, we show in a rat model of fat malabsorption due to bile deficiency that percentage of dietary fat absorption depends on the presence of bile in the intestinal lumen. With the use of this rat model, the [1-13C]palmitic acid absorption test, based on the quantification of plasma 13C-palmitic acid concentrations, was sensitive enough to discriminate between total fat absorption above 91% (control rats) and below 91% (bile-diverted rats). These observations underline the potency of the 13C-palmitic acid absorption test in combination with the technique of gas chromatography combustion isotope ratio mass spectrometry to detect disorders in intestinal solubilization. Application of the test in clinical absorption studies may allow a differentiated diagnosis and subsequent specific treatment.

References

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