CHAPTER 3

The $^{13}$C-mixed triglyceride breath test in healthy adults: determinants of the $^{13}$CO$_2$ response

M. Kalivianakis, H.J. Verkade, F. Stellaard, M. van der Werf, H. Elzinga, R.J. Vonk

*Eur J Clin Invest (1997) 27, 434-442*
CHAPTER 3

The $^{13}$C-mixed triglyceride breath test in healthy adults: determinants of the $^{13}$CO$_2$ response

Abstract

**Background & Aim:** Defects in lipolysis due to pancreatic insufficiency can be diagnosed by the mixed triglyceride $^{13}$CO$_2$ breath test. However, the effects of various test conditions on the $^{13}$CO$_2$ response have only partially been elucidated. **Methods:** In healthy adults we performed the $^{13}$CO$_2$ mixed triglyceride breath test and we compared a) the inter- and intra-individual variation in the $^{13}$CO$_2$ response, b) the effect of two different test meals, c) the effect of an additional meal during the test, and d) the effect of physical exercise during the test. **Results:** Upon repeating the test in the same individual (test meal cream), repeatability coefficients were large, either with respect to time to maximum $^{13}$C excretion rate (3.8 h), maximum $^{13}$C excretion rate (4.9% $^{13}$C dose/h), or cumulative recovery of $^{13}$C over the 9-h study period (22.7% $^{13}$C dose). The cumulative $^{13}$C expiration over 9 h obtained with the test meal composed of cream was quantitatively similar to that obtained with bread and butter: 42.2 ± 8.4%, and 47.7 ± 6.3%, respectively. Fasting for 9 h during the test resulted in similar $^{13}$C expiration rates and cumulative $^{13}$C expiration (43.4% ± 7.2%), when compared to consumption of an additional meal at 3 h after the start of the test (38.3 ± 5.3%). The $^{13}$CO$_2$ response increased in 5 out of 7 subjects, but decreased in the other 2, when moderate exercise was performed (bicycle ergometer, 50W for 5 h). **Conclusion:** Repeatability of the MTG test in healthy adults is low. The present results indicate that a solid and a liquid test meal, containing similar amount of fats, give similar cumulative $^{13}$CO$_2$ responses, and that stringent prolonged fasting during the test is unnecessary. Standardization of physical activity seems preferable, since unequivocal effects of moderate exercise on the $^{13}$CO$_2$ response were observed in the individuals studied.
Introduction

A common feature of pancreatic insufficiency is a reduced output of pancreatic lipase. This condition may lead to lipid malabsorption due to reduced intestinal hydrolysis of triacylglycerols [1,2]. Measurement of maximal pancreatic lipase output by means of an invasive, marker-corrected perfusion technique is considered to be the gold standard of pancreatic insufficiency tests [3,4]. A non-invasive test has recently been described in which a $^{13}$C-labeled mixed triglyceride (MTG) was ingested together with a test meal, after which the amount of $^{13}$C in expired air was determined [5]. The MTG used is 1,3-distearoyl, 2[carboxyl-$^{13}$C]octanoyl glycerol. This molecule contains a $^{13}$C-labeled medium-chain fatty acid (octanoic acid) at the sn-2 position, and long-chain fatty acids (stearic acid) at the sn-1 and sn-3 positions of the glycerol backbone of the triacylglycerol [5]. The two stearoyl chains have to be hydrolyzed by lipolytic enzymes in the intestine (mainly of pancreatic origin) before $[^{13}$C]octanoate can be absorbed, either in the form of a free fatty acid or of a monoacylglycerol [6]. It has been known that, after absorption, octanoate is rapidly oxidized to a considerable extent [6,7]. Thus, the principle of the MTG test is based on lipolysis-dependent $^{13}$CO$_2$ excretion via the breath. The applicability of the MTG test in pancreatic insufficiency has been demonstrated in adults [5,8,9], and preliminary data on the potential applicability in children are available [10].

A general problem of breath tests using labeled lipids for the diagnosis of pancreatic insufficiency or fat malabsorption in general, is a relatively poor sensitivity and specificity, probably due to the numerous steps involved in the metabolism of the tracer compound [11]. Differences in gastric emptying, solubilization by bile acids, mucosal absorption, hepatic clearance and metabolism, endogenous CO$_2$ production and pulmonary excretion may obscure the relationship between the quantity of label expired and the aim of the study, for example hydrolysis in the intestine [12-15]. Up to now, none of these breath tests has been clinically validated in different disease states. Presently, the discriminating test parameters are only poorly defined, which limits the application of these tests in clinical studies.

The aim of the present study was to further characterize the MTG breath test and to identify some factors apart from pancreatic insufficiency that may influence the quantitative recovery of $^{13}$CO$_2$ in breath. We examined the variation of the $^{13}$CO$_2$ response within and between healthy human adults, in which no rate-limiting variation in pancreatic exocrine function was expected. In addition, we evaluated various determinants of the $^{13}$CO$_2$ response:

1. The test meal. A variety of test meals has been described for breath tests with a diversity of substrates [5,14,16,17]. So far, no standardized test meal for clinical purpose of these breath tests has been proposed. A disadvantage of a test meal such as bread and butter for children could be the extended time it would take for consumption, and the risk of not consuming it quantitatively. Furthermore, such a test meal is not applicable to small infants. Therefore, we examined whether the $^{13}$CO$_2$ response of a liquid test meal is similar to the $^{13}$CO$_2$ response of the mentioned solid test meal.
2. The fasting condition during the test. Since it may be cumbersome to keep patients, in particular infants, fasted for at least six hours, we determined to what extent consumption of an extra meal during the test influenced the $^{13}$CO$_2$ response.

3. Physical activity. It is known that physical activity considerably affects the production rate of CO$_2$ and nutrient oxidation [18-20], however it is not established to what extent it influences the results of the $^{13}$C-MTG test.

**Materials and methods**

**Subjects**

The studies were conducted with four male and seven female volunteers with a mean age of 23 ± 1 (SEM) years and a mean body mass index of 20.9 ± 0.3 kg/m$^2$. The volunteers were healthy according to medical histories and did not have symptoms of lipid malabsorption, such as diarrhea or gastrointestinal complaints. Informed consent was obtained, and the study protocol was approved by the Medical Ethics Committee of the University Hospital Groningen.

$^{13}$C-labeled substrate

MTG (mixed triglyceride, 1,3-distearoyl, 2[carboxyl-$^{13}$C]octanoyl glycerol) was purchased from the Belgian Institute of Isotopes (IRE, Fleurus, Belgium) and from Euriso-Top, Saint Aubin Cedex, France, and was 99% $^{13}$C-enriched. The chemical purity exceeded 98%. Both the isotopic and chemical purity were checked by NMR.

**Study protocol**

The subjects were instructed to avoid consumption of naturally $^{13}$C-enriched foods (e.g. corn or corn products, pineapple, cane sugar) for at least two days prior to the study. After an overnight fast (approximately 10 hours), each subject consumed a test meal consisting of either 75 ml cream or 2 slices of bread and 25 g butter, each mixed with $^{13}$C labeled MTG (4 mg/kg body weight). Breath samples were collected in duplicates before consumption of the test meal to provide a value of baseline $^{13}$C-excretion in expired CO$_2$, and were subsequently collected at 30-min intervals for a period of 9 hours after the ingestion of the test meal. Unless stated otherwise, all experiments were performed under standard conditions, which implied that: 1. the test meal consisted of 75 ml cream, 2. no additional food or liquids were permitted during the 9-h period except for water, tea and coffee without sugar and milk, and 3. the subjects only performed light office tasks during the tests.

**Analytical techniques**

Breath was collected by expiration via a straw into a 10 ml tube (Exetainers; Labco Limited, High Wycombe, United Kingdom), from which aliquots were taken to determine $^{13}$C-enrichment by means of continuous flow isotope ratio mass spectrometry (Finnigan Tracer 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 Belemnitite limestone
The $^{13}$C-MTG breath test in healthy adults

($\delta^{13}$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, and as the percentage of administered $^{13}$C-label recovered over the 9-h study period.

Mean values of whole body CO$_2$ excretion were measured by indirect calorimetry (Oxycon, model ox-4, Dräger, Breda, The Netherlands) at 3 separate periods of 5 minutes during the 9-h study period.

**Intra- and inter-individual variation**

The intra-individual variation was examined in eleven subjects by repeating the study under identical (standard) conditions within four weeks after the first test. The individual repeatability of the test was examined with the use of repeatability coefficients according to Bland and Altman [21]. Coefficients of repeatability are based on the mean of the differences between repeated measurements on a series of subjects and the standard deviation of the differences. The definition of a repeatability coefficient adopted by the British Standards Institution is the expectation that 95% of differences is within two standard deviations of the mean difference [22]. Repeatability coefficients were calculated with respect to three parameters: time to maximum $^{13}$C excretion rate, $t_{max}$, maximum $^{13}$C excretion rate, and cumulative recovery of $^{13}$C after 9 h [23].

**Influence of two different test meals on $^{13}$CO$_2$ expiration**

In six subjects the influence of two different easily applicable test meals on the $^{13}$CO$_2$ response in breath after oral ingestion of MTG was examined: 1. 75 ml cream (1040 kJ, 26 g fat, 2 g carbohydrate, 2 g protein), and 2. two slices of bread with 25 g butter (1550 kJ, 22 g fat, 32 g carbohydrate, 6 g protein). In addition, the influence of either test meal in itself on $^{13}$C-enrichment of breath was examined in each subject, by repeating the test with a test meal to which no $^{13}$C-MTG was added.

**Prolonged fasting during the test versus consumption of an additional meal**

In seven subjects the effect of prolonged fasting on the $^{13}$C expiration rate was examined. Fasting represented the standard condition mentioned above, whereas in the non-fasting condition an additional meal was consumed at 3 hours after the start of the experiment. The additional meal consisted of 2 slices of bread and 30 g strawberry jam (960 kJ, 2 g fat, 49 g carbohydrate, 6 g protein). The experiments were performed with the initial test meal (time 0 h) consisting of 75 ml cream. In five subjects a control study was performed, in which the $^{13}$C-labeled MTG was omitted from the test meal.

**Influence of physical exercise on $^{13}$CO$_2$ expiration**

In seven subjects, the influence of physical exercise on the $^{13}$CO$_2$ response was investigated. The results from the tests done under standard conditions were compared to those obtained during moderate exercise on a bicycle ergometer. The physical exercise started at 10 minutes before the consumption of the test meal (75 ml cream). The energy performance was 50 watt for 5 hours, which represents an intensity of approximately 25 to 35% of the subjects’ maximal aerobic capacity ($V_{O_2 max}$). Drinking of water was allowed ad libitum during the
bicycle test for the whole 9-h period. The last 4 hours of the experiment (time 5-9 h) the subjects were in rest. Five subjects underwent a control study during which the influence of the test meal and exercise as mentioned above was examined under background conditions, i.e. without the addition of the label.

**Statistical methods**
The experimental data are reported as means ± SEM. Statistical comparisons between the data were performed with the use of the two-tailed non-parametric Wilcoxon signed-rank test for pairs. Differences between means were considered statistically significant at the level of $P<0.05$. For statistical analysis three different characteristics of the $^{13}$CO$_2$ expiration were analyzed according to Matthews et al. [23]: 1. the time to the maximum $^{13}$C excretion rate, $t_{\text{max}}$, 2. the maximum $^{13}$C excretion rate (expressed as % $^{13}$C dose per hour), and 3. the recovery of $^{13}$C over the 9-h study period (expressed as % cumulative $^{13}$C excreted).

**Results**

**Background variation of $^{13}$CO$_2$ expiration after an unlabeled test meal**
The background variation of $^{13}$C in breath was examined in several subjects by performing the test without administration of the label under 4 different experimental conditions: standard condition, test meal consisting of bread and butter, additional meal at 3 h after the start of the test, and moderate exercise during the test for 5 hours. There was no detectable change in the average expiration of $^{13}$CO$_2$ over the 9-h study period, in any of the experimental settings. The inter-individual background $^{13}$C-variation in breath CO$_2$ at the various time points was small (average SEM 0.12‰), as was the intra-individual variation (average SEM 0.21‰, data not shown).

**Intra- and inter-individual variation**
The baseline $^{13}$C-abundance in breath prior to consumption of the test meal was -26.2 ± 0.2‰ in test 1. The standard error of the analysis at this enrichment level was 0.03‰ (n=10). After ingestion of the $^{13}$C-MTG containing test meal at time 0, different time-course patterns were observed for the excretion of $^{13}$C-label in breath over the 9-h study period, with maximum excretion rates varying between 3 and 8 h after administration of the $^{13}$C-labeled test meal (Figure 3.1, closed squares). At the maximum excretion rate, the enrichment of $^{13}$C in breath was -21.6 ± 0.5‰ and at the end of the 9-h study period, the enrichment of $^{13}$C in breath had not yet returned to the level of baseline $^{13}$C-abundance (-24.6 ± 0.3‰). When expressed as a proportion of administered $^{13}$C, the peak excretion rate of label in breath in the first test was 9.3 ± 1.3% $^{13}$C per hour of the administered dose, varying between 5.0 and 16.3%. Over the 9-h study period the excretion of $^{13}$C in breath was 40.3 ± 5.0% of that administered, ranging between 16.7 and 66.4% (Table 3.1; the subject numbers in Table 3.1 are corresponding to the subject numbers in the figures).
On repeating the test under identical circumstances, the time-course patterns of most individuals appeared rather similar to the first test with maximum excretion rates varying between 3 and 9 h after administration of the $^{13}$C-labeled test meal (Figure 3.1, open squares). However, in one subject (Figure 3.1, subject 1) a strikingly different time course of label expiration was observed when compared to the first test: $t_{\text{max}}$ of 3 h in the first study and a $t_{\text{max}}$ probably after 9 h. The mean results on $^{13}$CO$_2$ expiration were not significantly different when compared to the first test: the baseline $^{13}$C-abundance was $-26.0 \pm 0.1\%e$, the enrichment of $^{13}$C in breath at peak excretion was $-21.7 \pm 0.5\%e$, and the enrichment of $^{13}$C in breath at the end of the 9-h study period was, again, not yet at baseline $^{13}$C-abundance ($-24.8 \pm 0.6\%e$). When expressed as a proportion of administered $^{13}$C, the peak excretion rate of label was 8.3 ± 1.0\%, ranging from 4.5 to 14.5\%, and excretion of label in breath over the 9-h study period was 33.2 ± 3.6\%, ranging from 18.8 to 48.3\% (Table 3.1).

For the 3 parameters studied, the repeatability coefficients [21] of time to maximum $^{13}$C excretion $t_{\text{max}}$, maximum $^{13}$C excretion rate, and cumulative recovery of $^{13}$C after 9 h were 3.8 h, 4.9\% $^{13}$C dose/h, and 22.7\% $^{13}$C cumulative excreted, respectively (Figure 3.2). Thus, for example, since the repeatability coefficient of the cumulative percentage $^{13}$C excreted is 22.7\%, the cumulative percentage $^{13}$C excreted in a second experiment performed under identical circumstances may be 22.7\% above or below the cumulative percentage $^{13}$C excreted.
in the first experiment. This lack of repeatability is much less obvious when only Figure 3.1 is considered.

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**Figure 3.2** Repeatability of test results of test 1 and test 2 calculated according to Bland and Altman [21], in which (A) the time to the maximum $^{13}$C excretion rate $t_{max}$, (B) the maximum $^{13}$C excretion rate (expressed as % $^{13}$C dose per hour), and (C) the recovery of $^{13}$C over the 9-h study period (expressed as % cumulative $^{13}$C excreted), were determined after oral ingestion of MTG (4 mg per kg body weight).

**Influence of two different test meals on $^{13}$CO$_2$ expiration**

Time courses for the excretion rate of $^{13}$C in breath for the subjects ingesting the two different test meals (cream versus bread and butter) are shown in Figure 3.3. The maximum $^{13}$C excretion rate occurred at similar time points ($t_{max}$ 5.0 ± 0.6 h and 4.8 ± 0.5 h, for the cream test meal and the bread-and-butter test meal, respectively), and values were not significantly different from each other: 10.3 ± 2.2% and 9.7 ± 1.2% $^{13}$C dose/hour, respectively ($P=0.84$). The 9-h cumulative $^{13}$C expiration amounted to 42.3 ± 8.4% (ranging from 16.7 to 66.4%) for the cream test meal and to 47.7 ± 6.3% (ranging from 34.5 to 78.0%) for the bread-and-butter test meal ($P=0.69$) (Table 3.1).

In order to compare our data with those of Vantrappen et al. [5], who performed the MTG test for 6 hours, we also calculated the cumulative $^{13}$C excretion over a 6-h period. For the cream test meal, this value was 28.4 ± 7.5% (n=6, ranging from 2.9 to 47.6%), and for the bread-and-butter test meal 32.8 ± 5.1% (n=6, ranging from 22.0 to 55.5%). These recoveries during 6 h after label administration were comparable to those described by Vantrappen et al.
The $^{13}$C-MTG breath test in healthy adults

[5], who obtained a recovery of $33.5 \pm 1.4\%$ (n=25, ranging from 23 to 52%). Yet, the inter-individual variation between our data appeared considerably larger.

Table 3.1  
Cumulative 9-h $^{13}$CO$_2$ excretion in breath after oral ingestion of MTG (4 mg per kg body weight) in healthy adults for the 4 different experimental conditions mentioned in the text: a) standard experimental condition (test 1 and test 2), b) the test meal consisted of 2 slices of bread and 25 g butter, c) three hours after the start of the test an additional meal was ingested consisting of 2 slices of bread and 30 g of strawberry jam, and d) during the first 5 h of the test subjects were bicycling at 50 watt. The subject numbers are corresponding to the subject numbers mentioned in the figures.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Standard condition</th>
<th>Test meal</th>
<th>Additional meal at 3 h</th>
<th>Bicycling for 5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>66.4</td>
<td>43.5</td>
<td>49.5</td>
<td>31.6</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>61.3</td>
<td>78.0</td>
<td>41.3</td>
<td>85.3</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>25.1</td>
<td>43.5</td>
<td>26.3</td>
<td>44.6</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>31.5</td>
<td>40.3</td>
<td>27.0</td>
<td>25.3</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>16.7</td>
<td>44.5</td>
<td>19.2</td>
<td>35.3</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>52.7</td>
<td>43.5</td>
<td>45.1</td>
<td>49.8</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>50.0</td>
<td>45.1</td>
<td>55.0</td>
<td>73.2</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>50.4</td>
<td>43.5</td>
<td>59.2</td>
<td>56.6</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>29.9</td>
<td>43.5</td>
<td>34.5</td>
<td>35.3</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>36.1</td>
<td>43.5</td>
<td>45.1</td>
<td>49.8</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>23.0</td>
<td>43.5</td>
<td>34.5</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Figure 3.3  
The influence of two different test meals on the excretion rate of $^{13}$C in breath after oral ingestion of MTG (4 mg per kg body weight) in 6 healthy adults. ( ) Test meal consisting of 75 ml cream; (–) test meal consisting of 2 slices of bread and 25 g butter.

Prolonged fasting during the test versus consumption of an additional meal

Figure 3.4 shows the $^{13}$C expiration rate data, comparing the fasting and non-fasting experimental condition. In the fasting condition, the maximum $^{13}$C excretion rates occurred at $t_{\text{max}} 4.7 \pm 0.6$ h, and, after consumption of an additional (low fat) meal at time point 3 h, at $t_{\text{max}}$
4.4 ± 0.4 h, and did not differ between the two groups: 10.7 ± 1.9% and 9.4 ± 1.4% 13C dose/hour, respectively (P=0.38). The 9-h cumulative 13C expiration amounted to 43.4 ± 7.2% (ranging from 16.7 to 66.4%) for the fasting condition and to 38.3 ± 5.3% (ranging from 19.2 to 55.0%) for the non-fasting condition (not significantly different, P=0.38) (Table 3.1). The consumption of an additional low-fat meal at 3 h after the start of the experiment neither altered the form nor the height of the mean curve for all subjects.

Figure 3.4  The influence of the consumption of an additional meal 3 h after the start of the experiment on the excretion rate of 13C in breath after oral ingestion of MTG (4 mg per kg body weight) in 7 healthy adults. ( ) Prolonged fasting during the test; ( ) Consumption of an additional carbohydrate rich meal 3 hours after the start of the experiment.

Influence of physical exercise on 13CO2 expiration

The effects of physical exercise on the 13C excretion were not similar in the seven subjects studied (Figure 3.5). In five of the seven subjects, physical exercise during the test induced an increase in the maximal 13C excretion rate and cumulative 13C excretion when compared to the test under standard conditions (i.e. resting during the entire experiment). However, one of the seven subjects responded in the opposite direction (Figure 3.5, Table 3.1; subject 1), as no increase of the peak excretion rate was observed after physical exercise, and an actual decrease in the cumulative 13C excretion was noticed. The remaining subject (Figure 3.5, Table 3.1; subject 4) showed a small increase in the maximal 13C excretion rate, but still the cumulative 13C excretion was decreased. The peak excretion of 13C-label in breath during the rest session in the subjects occurred at t_{max} 5.2 ± 0.7 h after administration of the 13C-labeled test meal and amounted to 9.8 ± 1.7% 13C dose per hour (n=7). The total excretion of 13C over the 9-h study period was 43.1 ± 7.1%. Bicycling for 5 h at moderate intensity decreased the time to maximum 13C excretion (t_{max} 3.9 ± 0.9 h), however, this effect was not significant (P=0.22). Furthermore, the maximum 13C excretion rate in the seven subjects was significantly increased (16.3 ± 2.6% versus 9.8 ± 1.7%, P=0.03), although total excretion of 13C over the 9-h study period was not significantly increased (50.3 ± 8.5% versus 43.1 ± 7.1%, P=0.47).
The $^{13}$C-MTG breath test in healthy adults

Discussion

The present investigations in healthy adults were designed to further characterize the determinants of the $^{13}$C-MTG test in healthy volunteers. Intra- and inter-individual variations were examined by repeating the study in the same individuals on a separate occasion. We also determined to which extent the $^{13}$CO$_2$ response was influenced by different test meals, by prolonged fasting, and by physical exercise.

The variability of measurements in different subjects (i.e. the inter-individual variation) is usually considerably larger than the variability between measurements on the same subject (i.e. the intra-individual variation) [24]. Obviously, both kinds of variability are important to assess the potential applicability of a diagnostic test. Only in one recent study the repeatability of breath tests was investigated based on the ingestion of $^{13}$C-labeled lipid, involving [1-$^{13}$C]palmitic acid in healthy volunteers [17]. Also in this study a poor repeatability was reported. No information is available on the repeatability of the MTG test under physiological or pathological conditions. We examined the repeatability of the MTG test by determining coefficients of repeatability [21]. The calculated repeatability coefficients from data of 11 healthy adults for the 3 parameters studied are considerable (see Results section). The values of the repeatability coefficients reflect the range of outcomes in which a repeated test in the same individual will result with a 95% likelihood. Thus, for example, since the repeatability coefficient of the cumulative percentage $^{13}$C excreted is 22.7%, the cumulative percentage $^{13}$C excreted in a second experiment is predicted to be (with a 95% likelihood) 22.7% above or below the cumulative percentage $^{13}$C excreted in the first experiment. These large repeatability coefficients are predominantly due to a considerable intra-individual variation. The acceptability of a certain diagnostic test is based on the actual values obtained and their repeatability coefficients in patients, compared to healthy controls. At present, only
information on the mean values of \(^{13}\)CO\(_2\) expiration of pancreatic insufficiency patients is available from the literature. It remains to be established whether the means and repeatability coefficients of diseased individuals allow a clear discrimination from unaffected individuals using the MTG-test.

Various test meals have been described for the use in breath tests with a diversity of lipid substrates [5,14,16,17]. So far, no standardized test meal for clinical purpose of these breath tests has been proposed. Theoretically, however, the test meal might influence the time course of the \(^{13}\)CO\(_2\) response, for example by affecting the rate of gastric emptying [25-29]. In our present study a liquid and a solid test meal with a comparable amount of fat were applied to investigate the effect of different test meals on the \(^{13}\)CO\(_2\) response. The cumulative percentage \(^{13}\)C of the dose excreted was comparable for the two test meals. This particular feature is valuable for the potential application of the MTG test in children, since a liquid test meal is more convenient to administer.

The originally described MTG breath test [5] lasted for 6 hours. Cumulative excretion might be the most discriminative parameter when MTG is used as a clinical test. However, also the cumulative excretion of \(^{13}\)CO\(_2\) depends on the end point (e.g. 6, 9 or more hours), which has not yet been defined by validation studies. In order to evaluate possible diagnostic advantages we extended the collection period of breath sampling up to 9 hours. In subjects, who reached their maximum excretion rate before 6 h, an increase in oxidation by approximately 50% was observed when the cumulative percentage \(^{13}\)C excreted after 9 hours was compared to that after 6 hours. However, in 2 subjects (Figure 3.1, subjects 5 and 8), the cumulative percentage \(^{13}\)C excreted after 9 hours was more than twice the amount excreted after 6 hours. It is tempting to speculate that these particular subjects have a decreased gastric emptying rate, compared to the others. If so, especially subjects with slow gastric emptying will be diagnosed incorrectly when the time period of the breath test is not sufficiently long.

The originally described MTG-test [5] involved prolonged fasting for 6 hours. This feature of the test would probably limit its applicability in (very young) pediatric patients. To allow eating during the test would alleviate the applicability. However, the results of the \(^{13}\)CO\(_2\) response should then not be influenced by eating. Present data indicate that the consumption of an additional low-fat meal 3 hours after the start of the experiment does neither change the form of the \(^{13}\)C expiration curve nor the height of the curve. These observations suggest that eating an additional meal during the test does not influence the results, which is in agreement with the findings of Schwabe et al. [7]. They concluded that oral administration of glucose did not seem to inhibit \(^{14}\)C-labeled octanoic acid oxidation [7].

In five out of seven individuals studied, the performance of physical exercise during the test increased the cumulative recovery of \(^{13}\)C in breath, when compared to performance of the test under resting conditions. Exercise enhances the oxidation of carbohydrates and lipids, which is associated with a higher CO\(_2\) and \(^{13}\)CO\(_2\) response. Available data suggest that gastric emptying during exercise is subject to a number of factors including calorie count, meal osmolality, meal temperature and exercise conditions [30]. There are several indications suggesting that light to moderate exercise accelerates gastric emptying of either liquid and solid meals [30-33]. Intestinal absorption per se has not been evaluated in great detail during exercise, but probably changes little [34]. All together, this explains the results of the five
The $^{13}$C-MTG breath test in healthy adults

mentioned individuals quite well. Unexpectedly, two subjects responded differently. At present, no clear explanation could be obtained for the particular results in these two subjects; both were healthy according to medical histories and, like the others, showed no symptoms of lipid malabsorption, such as diarrhea or gastrointestinal complaints. This observation does underline, however, the importance of standardizing the resting conditions during the MTG breath test.

In summary, we have found that the repeatability of the mixed triglyceride test in healthy adults is low. The results also suggest that two distinct test meals (cream versus bread and butter) give a similar cumulative $^{13}$CO$_2$ response and that stringency on continuous fasting during the test is unnecessary, which is in favor of the applicability of the test in pediatric patients. Standardization of resting conditions still seems preferable. In future patient studies it remains to be established whether MTG-test results obtained under conditions of an impaired intestinal lipolysis allow the sensitive and specific discrimination of affected from unaffected individuals.

References


57