Abstract

Aim—To examine the gastrointestinal handling of [1-13C]palmitic acid given as the free acid by measuring the excretion of 13C label in stool in 16 healthy children and 11 patients with cystic fibrosis on their habitual enzyme replacement treatment.

Methods—After an overnight fast, each child ingested 10 mg/kg body weight [1-13C]palmitic acid with a standardised test meal of low natural 13C abundance. A stool sample was collected before the test and all stools were collected thereafter for a period of up to five days. The total enrichment of 13C in stool and the species bearing the 13C label was measured using isotope ratio mass spectrometry.

Results—The proportion of administered 13C excreted in stool was 24.0% (range 10.7–64.9%) in healthy children and only 4.4% (range 1.2–11.6%) in cystic fibrosis patients. The enrichment of 13C in stool was primarily restricted to the species consumed by the subjects (that is as palmitic acid).

Conclusion—There does not appear to be a specific defect in the absorption of [1-13C]palmitic acid in patients with cystic fibrosis. The reasons why cystic fibrosis patients appear to absorb more of this saturated fatty acid than healthy children is not clear and requires further investigation.

Keywords: fatty acids; absorption; stable isotopes; cystic fibrosis

Gastrointestinal handling of [1-13C]palmitic acid in healthy controls and patients with cystic fibrosis

Jane L Murphy, Amanda E Jones, Michael Stolinski, Stephen A Wootton

Institute of Human Nutrition, University of Southampton, Southampton

J L Murphy
A E Jones
M Stolinski
S A Wootton

Correspondence to: Dr S A Wootton, Clinical Nutrition and Metabolism Unit, Mailpoint 113, Level C West Wing, Southampton General Hospital, Tremona Road, Southampton SO16 6YD

Accepted 2 January 1997
The aim of the present study was to examine the gastrointestinal handling of [1-13C]palmitic
acid, was used as a vehicle to administer the [1-13C]palmitic acid. The cystic fibrosis
patients used the same amount of enzymes with the test meal as they would usually take
with a snack meal and ranged from 0–4566 IU lipase/kg body weight/day (mean 2330 IU lipase/kg body weight/day).

A stool sample was collected on the day before the labelled test meal to measure baseline 13C excretion. Thereafter, all stools passed were collected and processed individually for a period of up to five days.

**STOOL ANALYSES**

The methodology for collecting, processing stools, and measuring the enrichment of 13C in stool has been described previously. Enrichment of 13C in stool was analysed by continuous flow IRMS (ANCA-NT GSLe, Europa Scientific). Lipids were extracted from baseline and 13C enriched stools by modification of the method of Polch and coworkers,16 saponified, methylated, evaporated to dryness, and taken up in hexane for GC-IRMS analysis (Orchid Gas Chromatograph Interface Module and ANCA-NT Isotope Ratio Mass Spectrometer; Europa Scientific). The 13C enrichment of stool was expressed as the delta per mil relative to the reference standard Pee Dee Belemnite as defined by Craig (13C, ‰). The analytical precision for GC-IRMS analyses of unenriched and 13C enriched samples for each fatty acid in stool expressed as SD values ranged from 0.52‰ to 7.9‰. The proportion of administered 13C label in stool was calculated according to the formula presented by Schoeller and coworkers.17

**PRESENTATION OF RESULTS**

The results are reported as mean (SD). Statistical comparisons between the groups were performed using the unpaired Student’s t test. Differences between means were considered statistically significant where p<0.05. Associations between variables were tested by the Pearson product moment correlation coefficient (R).

**Results**

The excretion of total lipid in stool, as a gross measure of both maldigestion and malabsorption, of the cystic fibrosis patients on their habitual PERT was twice as great as that observed in the healthy children (5.8 (3.8) v 2.6 (1.6) g/day; p<0.01). In healthy children, 24.0 (14.5)% of the administered 13C label was excreted in the stool with a sixfold difference between subjects (range 10.7–64.9%; fig 1). In contrast, the patients with cystic fibrosis excreted significantly less 13C label in stool: only 4.4 (3.6)% of the administered 13C label appeared in stool (range 1.2–11.6%; p<0.0001). There was no relationship between the excretion of 13C label and total lipid in stool in either group (healthy children, R = −0.19, p>0.49; cystic fibrosis, R = −0.15, p>0.65).

Figure 2 shows a chromatogram obtained using GC-IRMS from a baseline and 13C enriched stool sample collected from one of the children with cystic fibrosis and is representative of the stool fatty acid chromatograms obtained from both groups of subjects. The percentage contribution of each fatty acid to total stool lipid was determined from the beam area within which the 13C enrichment was measured. In both groups more than 97% of the total fatty acids in stool were represented by just five fatty acids (palmitic acid, stearic acid, oleic acid, and two unidentified peaks). Of these, palmitic acid was the principal fatty acid in stool and accounted for between 36.2–45.8% of the total fatty acids in stool. All of the fatty acids in the baseline stool samples exhibited the same level of 13C enrichment (about −29.0%). The stools with the greatest 13C enrichment were usually obtained on the day after the test meal and the enrichment declined to baseline values by day 3 or 4 in all subjects. Only palmitic acid exhibited changes in 13C enrichment (to values in excess of +53% for the most enriched stool samples in every subject). No enrichment above baseline levels were observed in any of the other major fatty acids.

**Discussion**

The aim of the present study was to examine the gastrointestinal handling of [1-13C]palmitic
Gastrointestinal handling of [1-13C]palmitic acid in controls and cystic fibrosis

Acid, given orally as the free acid, in patients with cystic fibrosis and healthy children. This is the first report where the absorption of dietary fatty acids derived from lumenal hydrolysis of triglycerides has been directly measured in groups of patients with cystic fibrosis on habitual PERT and in healthy children. Almost a quarter of the [13C] label administered was excreted in stool in healthy children, while very little [13C] label appeared in the stools of patients with cystic fibrosis on their habitual PERT, even in those patients with the greatest stool lipid losses of 15 g/day. This would indicate that a greater proportion of the labelled palmitic acid was absorbed in these cystic fibrosis children than in the control children despite raised stool lipid losses. We have also demonstrated that palmitic acid represented the major stool fatty acid and in those stools with the highest enrichment of [13C], the [13C] label was primarily restricted to the species consumed by the subjects (that is as palmitic acid).

The differences observed in the excretion of [13C] label in stool between subjects, particularly in the healthy children, could be attributable to poor solubilisation of the fatty acid in micelles. A proportion of the free fatty acids may have formed insoluble soaps with divalent cations in the gastrointestinal tract and may have been excreted in stool.12 13 Differences between the gastrointestinal handling of [1-13C]palmitic acid and dietary lipid were reflected in the poor association observed between [13C] label and total lipid in stool implying that [1-13C]palmitic acid was not handled in the same way as triglycerides within the diet. It could be that the presentation of [1-13C]palmitic acid to the absorptive site may not be the same as palmitic acid normally hydrolysed from dietary triglycerides in the gastrointestinal tract.

The results from the present study do not support the existence of a malabsorptive defect of [1-13C]palmitic acid in this group of cystic fibrosis patients, if anything the absorption of palmitic acid was enhanced. It could be that differences in the intraluminal environment such as alterations in pHi in the cystic fibrosis patients may favour the formation of mixed micelles from free fatty acids from the sn-1 or sn-3 positions than the 2-monoglycerides. The form of the labelled fatty acid, palmitic acid, may have represented a larger proportion of absorbable lipid in cystic fibrosis patients compared with healthy children. Alternatively prolonged transit time may also increase the time in which the labelled fatty acid could be absorbed or that alterations in the integrity of the gut mucosa (for example unstirred water layer) may also increase the absorption of [1-13C]palmitic acid. While there are no direct comparable studies of fatty acid absorption, it is pertinent to note that a study by Frase and coworkers demonstrated increased jejunal glucose absorption and a reduction in the thickness of the unstirred water layer in patients with cystic fibrosis compared with healthy children.14 They proposed that this may be attributed to a reduction in the diffusion barriers at the level of the enterocyte caused by abnormal mucus. Whether this could account for the apparently greater absorption of palmitic acid (and possibly fatty acids in general) has not been tested.

In conclusion more of the administered [1-13C]palmitic acid was absorbed in cystic fibrosis patients compared with healthy children. This would suggest that there is no specific defect in the absorption of palmitic acid in patients with cystic fibrosis. The reason why cystic fibrosis patients appear to absorb more of the [1-13C]palmitic acid remains unclear. Further studies using [13C] labelled triglycerides are required to ascertain whether the labelled free fatty acid used in the present study is handled in the same way as the labelled fatty acid esterified into triglycerides.

We thank the children and parents whose cooperation was essential for the completion of this study and for the assistance from Mrs A Houndslow, Miss R Treener, Dr C J Rolles, Dr G Connell, Dr B Zorich, and Mrs J Gavin. The support of the Cystic Fibrosis Trust, Scientific Hospital Supplies UK Ltd, and the Wessex Medical Trust is gratefully acknowledged.