Gastrointestinal handling and metabolic disposal of ^{13}C-labelled tripalmitin during rehabilitation from childhood malnutrition

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We investigated the gastrointestinal handling and post-absorptive metabolic handling of [1,1,1-^{13}C]tripalmitin and [1-^{13}C]glycocholate during recovery from severe childhood malnutrition. Eight children were studied on three occasions: at admission (phase 1), during rapid catch-up growth (phase 2) and when weight-for-height had reached 90 % of the reference (phase 3). Breath samples were obtained over a 24 h period and stools were collected over 3 d following the administration of each tracer. At admission, the lipid content of stool expressed as a percentage of ingested lipid was 6 (range 0·7–28·9) but less variation was shown between children at phase 2 (3·3 (range 0·9–4·1)) and phase 3 (1·4 (range 0·4–2·5)). The excretion of ^{13}C in stool varied markedly between children at admission (11·1 (SD 5·4) % administered dose) and during rehabilitation (phase 2, 15·4 (SD 16·5) % administered dose; phase 3, 6·2 (SD 10·2) % administered dose). About 5 % of the absorbed label was recovered on breath at each stage (% absorbed dose; phase 1, 5·1 (SD 6·0); phase 2, 5·2 (SD 3·1); phase 3, 6·4 (SD 6·6)). None of the children exhibited significant bile salt malabsorption as a consequence of small intestinal overgrowth. Of the ^{13}C measured in stool, more label was recovered in fatty acids than triacylglycerols during each of the three phases and this was interpreted to reflect a failure to absorb the products of digestion. The results show that not all the children had problems associated with the digestion and absorption of ^{13}C-labelled tripalmitin in severe malnutrition and during recovery, which was not reflected in gross lipid balance across the gastrointestinal tract. Absorbed lipid was more likely to be deposited as adipose tissue than to satisfy the immediate needs for energy.

Lipids: Malnutrition: Gastrointestinal tract: Metabolism

Children with severe malnutrition have extensive perturbations in lipid metabolism, as shown by marked loss of subcutaneous adipose tissue and profound fatty infiltration of the liver (Waterlow, 1948; Schneider & Viteri, 1974). However, there is only limited information on the physiological and metabolic mechanisms that underlie the gastrointestinal handling and further metabolic disposal of the triacylglycerols (TAG) that make up dietary lipid, either in the acutely malnourished state or during the period of rapid catch-up growth. The rate at which the weight deficit can be repleted during catch-up growth is directly related to the dietary energy consumed (Ashworth, 1969); this repletion might readily be achieved by fortifying the diet with lipid. It is likely that, at each stage of recovery, the amount and pattern of dietary fatty acids play an important role in the outcome. However, at present there is no consensus on the most appropriate fatty acid profile from one stage to another.

Malnourished children frequently suffer chronic diarrhoea, which may sometimes be identified as steatorrhoea. Balance studies in which either the total lipid content of stool (Holemans & Lambrechts, 1955; Gomez *et al.* 1956; Robinson *et al.* 1957; Dutra de Oliveira & Rolando, 1964) or the concentrations of individual fatty acids in stool (Underwood *et al.* 1967) have been used to characterise how dietary lipid is handled by the gastrointestinal tract. Using these approaches it is not possible to determine with confidence whether the lipid measured in stool is derived directly from the diet, or carries a contribution from endogenous sources, such as biliary and other secretions,

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desquamated cells, or even the products of bacterial metabolism. In malnutrition, although stool lipid losses have been loosely characterised as malabsorption, there is indirect evidence for a contribution from impaired digestion, bacterial overgrowth of the small intestine (Lifshitz *et al.* 1970), excessive bile salt deconjugation, impaired resorption of bile salts and an increase in endogenous losses (Watson *et al.* 1977; Jackson & Golden, 1978; Durie *et al.* 1985), but these factors have not been directly related to the handling of lipid. Once absorbed, the hepatic partitioning of lipid between oxidation, export and other pathways appears to be impaired (Tanner, 1990), but the mechanistic basis of the problem remains unresolved.

Further studies are needed to define more precisely the gastrointestinal handling and metabolic disposal of dietary TAG in malnutrition and during rehabilitation. One approach is to employ orally administered substrates labelled with [13]C. We have previously determined the fate of label from orally administered ^{13}C-labelled tripalmitin (TP), given as an emulsion in healthy children and patients with cystic fibrosis, by determining the proportion of label excreted in stool and in breath as [13]CO_2 (Murphy *et al.* 1998). Patients with cystic fibrosis excreted more of the label in stool when compared with healthy children; this differences could have been the result of poor digestion of labelled TAG, which limits subsequent absorption (maldigestion). Alternatively, or in addition, the labelled products of digestion could have been malabsorbed because of a failure in the absorptive capacity of the gastrointestinal tract.

Intestinal malabsorption may also occur as the result of bile salt malabsorption and small bowel overgrowth. In a previous study (Schoeller *et al.* 1981) bile salts labelled with ^{13}C were used to examine bile salt malabsorption and small bowel overgrowth in children with suspected malabsorption. The approach was based on the principle that, if anaerobic bacteria have colonised the small intestine, bacterial cleavage of the amide bond between the labelled glycine and cholate moiety would be cleaved and the glycine metabolised to [13]CO_2 which is excreted on breath and/or that excess ^{13}C in stool is indicative of bile acid malabsorption.

In the present study we report the gastrointestinal and post-absorptive handling of ^{13}C-labelled TP and ^{13}C-labelled glycocholate (GCA) in severely malnourished children and during rehabilitation. The purpose of the study was to determine the extent to which the treatment for severe childhood malnutrition may influence: (1) the total amount of ^{13}C label excreted in stool, and whether gastrointestinal problems were associated with poor hydrolysis of TAG and/or absorption; (2) the proportion of absorbed labelled palmitic acid that is oxidised over 24 h; (3) bile salt malabsorption and small bowel overgrowth.

Materials and methods

Subjects

The Ethical Committee of the University Hospital of the West Indies gave approval for the study to be carried out.

Eight Jamaican children (four girls, four boys) aged 7–23 months were recruited into the study after informed consent was received from their parents. The main criterion for selection and inclusion in the study was severe malnutrition according to the Wellcome classification (Wellcome Trust Working Party, 1970), i.e. less than 80 % weight-for-age and the presence or absence of oedema. Exclusion criteria were evidence of other obvious pathology, such as renal disease, heart disease, sickle cell disease or infection with HIV. All clinical decisions regarding care and treatment were taken by the attending physicians. On admission, the children were started immediately on a milk-based diet based on a commercial infant feed (Nan; Nestle, Vevey, Switzerland) with the addition of maize oil to provide about 417 kJ/kg per d, 1·4 g protein/kg per d and 7 g lipid/kg per d. During rapid catch-up growth, the children were offered a milk-based formula that was made energy dense by the addition of coconut oil, and provided 625–750 kJ/kg per d, approximately 3 g protein/kg per d and 10 g lipid/kg per d.

Study design

Each child was studied on three occasions: within 48 h of admission, when acutely malnourished (phase 1); during rehabilitation, at the time when the child was gaining weight rapidly and had corrected 50 % of the weight deficit (phase 2); at late catch-up, when the child had reached at least 90 % of the expected weight-for-height (phase 3). Each study phase lasted for a period of 9 d, and on each occasion the children first received [1,1,1-^{13}C]TP, 20 mg/kg body weight (99 atom % excess (APE); Masstrace, Woburn, MA, USA), followed by a 3 d stool collection, a wash-out period of 3 d, and then [1-^{13}C]GCA (as a Na salt), 10 mg/kg body weight (99 APE; Masstrace), followed by a stool collection for 3 d. On the day before the administration of the ^{13}C-labelled compounds, breath and stool samples were collected to determine baseline abundance of [13]C.

The [1,1,1-^{13}C]TP was made up as an emulsion in a portion of the formula being consumed by the child, using sonication to incorporate the label. This emulsion was consumed as a single bolus by the subject. The [1-^{13}C]GCA was solubilised in 5 ml water and given as a single dose immediately before the child was fed. For the duration of the study each child received at intervals of 2–3 h the same standardised feed from the same batch of commercial formula in order to minimise any possible differences due to changes in the background isotopic abundance of the diet.

Following administration of the label, breath samples were collected, using a face mask, every 0·5 h for 6 h, then at 8 h, 10 h and 24 h. Samples of breath were transferred in duplicate into evacuated gas sample containers (Exetainers; Isochem, Finchampstead, Berks., UK) for analysis. The rate of CO_2 production by the subject was determined by indirect calorimetry (GEM; Europa Scientific Ltd, Crewe, Cheshire, UK) for a period of 15 min immediately before and after a feed. The individual rate of CO_2 production was used to determine the rate of ^{13}C recovery from the enrichment of [13]CO_2 on breath. It was possible to carry out measurements in four of the eight children. For those

children in whom it was not possible to measure the rate of CO_2 production, an average value of 6·4 ml/min per kg body weight was used; this value was derived from published values (Dane *et al.* 1985; Piedboeuf *et al.* 1991; Pierro *et al.* 1994). All stools passed over a 72 h period were collected, using diaper liners and stool collecting bags, and frozen immediately at $-20°C$.

Breath and stool analyses

The methodology for processing stools has been described previously (Murphy *et al.* 1995). The abundance of ^{13}C in stool and as $^{13}CO_2$ on breath was analysed by continuous-flow isotope-ratio MS (ANCA-*NT* GSL; Europa Scientific Ltd). Total lipid was measured in samples of stool collected during the ^{13}C-labelled TP studies by a modification of the method of Folch *et al.* (1957) with previous acidification. The TAG and fatty acid fractions extracted only from those stools with the highest level of ^{13}C enrichment were separated by TLC. The ^{13}C enrichment in total lipid and within the TAG and fatty acid fractions were analysed by continuous-flow isotope-ratio MS to determine the extent to which the label appeared unhydrolysed in stool, as TAG as the result of maldigestion, or appeared hydrolysed, as fatty acid as the result of malabsorption.

The $^{13}C{:}^{12}C$ of the samples and of the reference was used to calculate the $\delta^{13}C‰$ of the sample applying the following equation:

$$\delta^{13}C‰ = [(^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{standard})/^{13}C/^{12}C_{standard}] \times 10^3,$$

where the international standard is the Pee Dee Belemnite standard. The measured $\delta^{13}C‰$ was transformed to atom % (AP) with the formula:

$$AP = \frac{100}{\dfrac{1}{(\delta^{13}C_{sample}/1000 + 1)R} + 1},$$

where R is the $^{13}C{:}^{12}C$ of the international standard (R = 0·0112372) and $\delta^{13}C_{sample}$ is the $\delta^{13}C$ value of the sample.

The calculated AP were converted to APE by subtracting the average background ^{13}C adundance (+2 SD) from the ^{13}C enrichment of the samples containing the label. The proportion of the orally administered ^{13}C label which was recovered in total stool, as stool lipid, TAG and fatty acid was calculated from APE expressed as a percentage of label administered. The proportion of ^{13}C label excreted on breath as $^{13}CO_2$ over the study period was calculated by area under the curve of ^{13}C recovery on breath *v.* time from the APE expressed as a percentage of absorbed label.

Presentation of results and statistical analysis

The results are reported as means and standard deviations. The data were analysed by ANOVA and differences between means were considered significant with $P < 0.05$. The *post hoc* Bonferroni adjustment was made to the significance levels because of repeated comparisons. Statistical analyses were performed using SPSS for Windows (version 9.0, SPSS Inc. Chicago, IL, USA).

Results

Subject details

The physical characteristics of the eight subjects who entered into the study are presented in Table 1. Based on the Wellcome classification (Wellcome Trust Working Party, 1970), three subjects were diagnosed as suffering from marasmus, one from undernutrition, two from kwashiorkor and two from marasmic kwashiorkor. All subjects had been treated with broad-spectrum antibiotics (on the assumption that all severely malnourished patients will have at least one focus of infection, even though this might not be clinically obvious), which included metronidazole for presumptive small bowel overgrowth. The subjects were studied within 2 d of admission (phase 1) except for one subject, who was studied 6 d after admission, and all the studies were completed within 2 months of hospitalisation. At admission the subjects were stunted with height-for-age being 87 % of the reference (Hamill *et al.* 1979). On average the children gained 1 cm by phase 3, and the degree of stunting was not changed in relation to their age (NS). The body weight of the subjects increased between each successive phase. There was an increase in weight of 0·9 kg from phase 1 to phase 2 and 0·9 kg from phase 2 to phase 3, whether expressed as an absolute increase (NS), or in relation to the age (weight-for-age; NS) or to the height of the subjects (weight-for-height; $P < 0.01$). For each phase the rate of weight gain was

Table 1. The physical characteristics of the subjects during the three study phases of rehabilitation from severe malnutrition†

(Mean values and standard deviations for eight children)

	Phase 1		Phase 2		Phase 3	
	Mean	SD	Mean	SD	Mean	SD
Weight (kg)	5·47	1·46	6·38	1·87	7·26	1·81
Height (m)	0·64	0·07	0·64	0·07	0·66	0·06
Height-for-age (%)	86·5	7·7	86·4	6·0	87·1	6·5
Weight-for-age (%)	63·9	7·3	67·8	8·9	73·4	9·2
Weight-for-height (%)	75·9	14·1	90·1	10·4	98·8**	9·5
Rate of weight gain (g/kg per d)	1·17	6·73	11·77**	5·28	9·83*	6·02

Mean values were significantly different from those at phase 1: *$P < 0.05$, **$P < 0.01$.
† Phase 1, on admission to hospital of malnourished children; phase 2, during rapid catch-up growth; phase 3, when weight-for-height had reached 90 % of the reference value.

Table 2. Metabolisable energy, lipid, protein and carbohydrate consumed by the subjects during each study phase of rehabilitation from severe malnutrition†‡

(Mean values and standard deviations for eight children)

	Phase 1		Phase 2		Phase 3	
	Mean	SD	Mean	SD	Mean	SD
Metabolisable energy kJ/kg per d	451	90	634	148	651*	186
Protein consumed (g/kg per d)	1·44	0·54	3·25***	0·70	3·0***	0·65
Carbohydrate consumed (g/kg per d)	7·41	3·18	15·32***	3·32	13·46**	3·13
Lipid consumed (g/kg per d)	7·35	0·64	8·73	2·36	9·79	2·84

Mean values were significantly different from those at phase 1: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
† Phase 1, on admission to hospital of malnourished children; phase 2, during rapid catch-up growth; phase 3, when weight-for-height had reached 90 % of the reference value.
‡ For details of subjects, see p. 706 and Table 1.

calculated over the 9 d period by linear regression analysis. During phase 1 the rate of weight gain was approximately 1 g/kg per d. The rate of weight gain was ten times greater during phase 2 ($P < 0.01$) and phase 3 ($P < 0.05$) compared with phase 1. There was no difference in the rate of weight gain between phase 2 and phase 3 (NS). The metabolisable energy, protein, carbohydrate and lipid consumed at each phase are shown in Table 2. The metabolisable energy consumption was significantly different between phase 1 and phase 3 ($P < 0.05$), but not different between phases 2 and 3 or phases 1 and 2, which reflected an increased consumption of protein and carbohydrate during rehabilitation. The consumption of protein and carbohydrate was significantly increased at phase 2 compared with phase 1 ($P < 0.001$). Similarly, there was a significant increase in protein ($P < 0.001$) and carbohydrate ($P < 0.01$) at phase 3 compared with phase 1, but no difference was observed between phases 2 and 3. The amount of lipid consumed was not different amongst the three phases.

Baseline abundance of ^{13}C in stool and breath

On the day before the ^{13}C-labelled TP and GCA trials, background abundance of ^{13}C was determined in a stool sample and samples of breath collected over 24 h (Table 3). The limits of detection (equivalent to) for excess ^{13}C in stool and breath derived from baseline specimens collected from all the patients were no greater than 0·0029 and 0·0033 APE respectively. The variation in background ^{13}C abundance for baseline samples of stool before the phase 1

trial was taken to reflect the consumption before admission of foods such as cane sugar or maize products, which are naturally enriched with ^{13}C. The same batch of infant formula was used as the main constituent of the diets consumed throughout the study period, and this batch had a relatively low natural ^{13}C abundance of 1·0860 (SD 0·0003) atom %, which was reflected in the lower background ^{13}C abundance in the breath and stool during rehabilitation compared with admission. A small increase in the background abundance of ^{13}C in stool and breath in some children for the GCA study at admission may have reflected the true variability in relation to diet and metabolic state, or might have been the result of a carry-over effect of ^{13}C label from the preceding ^{13}C-labelled TP study. The variation between children in ^{13}C excretion measured in baseline breath samples before the studies at each phase was small for both the ^{13}C-labelled TP and GCA studies. We conclude that the variation in background ^{13}C:^{12}C was small and that any increase in the excretion of ^{13}C label on breath or stool was a direct consequence of the ^{13}C-labelled substrate.

Excretion of lipid in stool

All the collections of stool were complete, other than the loss of a single stool sample from one subject during phases 2 and 3. During phase 1, the average lipid in stool was 2·4 (SD 3·6; range 0·4–11·2) g/d, or 5·9 (SD 9·4; range 0·7–28·9) % of the dietary lipid. During phase 2, the average lipid in stool was 1·7 (SD 0·9; range 0·4–2·9) g/d, or 3·3 (SD 2·4; range 0·9–4·1) % of the dietary lipid and was not

Table 3. Abundance of ^{13}C (atom %) in stool and breath samples collected at baseline before the ^{13}C-labelled tripalmitin (TP) and glycocholate (GCA) trials at each study phase in children during rehabilitation from severe malnutrition†‡

(Mean values and standard deviations for eight children)

	Phase 1				Phase 2				Phase 3			
	TP		GCA		TP		GCA		TP		GCA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline stool ^{13}C	1·0876	0·0023	1·0881	0·0029	1·0832***	0·0004	1·0834**	0·00207	1·0839***	0·0003	1·0832***	0·0020
Baseline breath ^{13}C	1·0901	0·0033	1·0895	0·0025	1·0880	0·0022	1·0871	0·0016	1·0868	0·0016	1·0865*	0·0023

Mean values were significantly different from those at phase 1: *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$.
† Phase 1, on admission to hospital of malnourished children; phase 2, during rapid catch-up growth; phase 3, when weight-for-height had reached 90 % of the reference value.
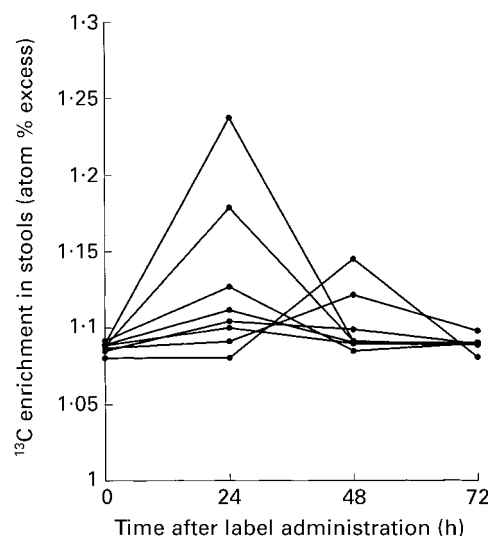‡ For details of subjects and experimental procedures, see p. 706 and Table 1.

Fig. 1. Time course for the excretion of ^{13}C in stool during phase 1 (at admission to hospital) following oral administration of [^{13}C]-labelled tripalmitin in eight severely malnourished children. For details of subjects and procedures, see p. 706 and Table 1.

significantly different from that for phase 1. During phase 3, the average lipid in stool was 0·9 (SD 0·6; range 0·2–2·4) g/d, or 1·4 (SD 0·7; range 0·4–2·5) % of the dietary lipid. Less than half the lipid was excreted in stool during phases 2 and 3 when compared with phase 1, although the differences were not statistically significant.

[^{13}C]Tripalmitin: excretion of ^{13}C in stool

Following a single oral dose of [1,1,1-^{13}C]TP in phase 1, the individual stools with the greatest ^{13}C enrichment were passed within 2 d of the study period (Fig. 1). The average peak enrichment of ^{13}C label in stool was 1·1405 (SD 0·0465) APE and by the third day levels of ^{13}C enrichment in individual stools were not different from baseline. During phases 2 and 3, both the time course and the peak

enrichments (phase 2, 1·1508 (SD 0·0777) APE; phase 3, 1·1306 (SD 0·0306) APE) were not different from phase 1. Using GC-isotope-ratio-MS methodology as described previously (Stolinski *et al.* 1997), palmitic acid represented the major fatty acid in those stools with the highest enrichment of ^{13}C. This finding would indicate that the ^{13}C label was restricted to the fatty acid species consumed by the children, i.e. as palmitic acid that had been given as a TAG. The total excretion of ^{13}C in stool as a percentage of the administered dose was not different between phase 1 (11·1 (SD 10·2) %), phase 2 (15·4 (SD 16·5) %) and phase 3 (6·2 (SD 10·2) %), with a wide range observed amongst children (phase 1, 1·3–26·4 %; phase 2, 2·9–44·0 %; phase 3, 0–30·6 %), (Table 4). When all study periods were considered together, there was a weak association between total lipid and the amount of ^{13}C label in stool (R 0·48; $P < 0.05$).

In Fig. 2, the proportion of ^{13}C in the TAG and fatty acid fractions of those stools with the greatest enrichment (as % administered dose) is shown for each phase. For TAG, ^{13}C label was recovered as TAG in three patients at phase 1 (0·7 (SD 1·6; range 0–4·7) %), with even less label appearing as TAG in stool during phase 2, apart from one child who excreted 7 % of the label in stool (0·9 (SD 2·8; range 0–7·0) %; NS), or phase 3 (0·02 (SD 0·05; range 0–0·18) %, NS). For fatty acids, the excretion of ^{13}C label in stool at phase 1 was 6·0 (SD 7·3; range 0–23·0) %) and declined during rehabilitation in phase 2 (4·8 (SD 3·7; range 2·5–11·8) %; NS) and phase 3 (3·3 (SD 3·8; range 0–11·8) %; NS). During phase 1, on average nine times as much appeared as fatty acid compared with TAG (NS). Almost all the label was in the form of fatty acid rather than TAG during phase 2 ($P < 0.001$) and phase 3 ($P < 0.05$).

1,1,1-^{13}Ctripalmitin: breath $^{13}CO_2$ excretion measurements

The recovery of ^{13}C label on breath as $^{13}CO_2$ was expressed as a percentage of absorbed label (dose administered − label recovered in stool), in order to take

Table 4. Excretion of ^{13}C in stool (as a percentage of the dose administered) and on breath as $^{13}CO_2$ (as a percentage of the dose absorbed), following the oral administration of [^{13}C]-labelled tripalmitin, during the three study phases in children during rehabilitation from severe malnutrition*†

(Individual values, mean values and standard deviations)

Subjects	Stool ^{13}C			Breath $^{13}CO_2$		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
1	4·1	44·0	6·9	1·3	3·9	21·2
2	26·4	13·1	30·6	0	8·2	2·4
3	24·7	37·8	6·6	4·0	4·9	1·9
4	1·3	4·4	0	3·7	10·7	6·95
5	17·3	0	0	2·8	1·7	4·2
6	6·7	7·9	1·95	0	6·3	3·7
7	4·1	13·2	1·1	13·8	1·9	1·4
8	4·0	2·9	2·3	15·2	4·0	9·8
Mean	11·1	15·4	6·2	5·1	5·2	6·4
SD	5·4	16·5	10·2	6·0	3·1	6·6

* Phase 1, on admission to hospital of malnourished children; phase 2, during rapid catch-up growth; phase 3, when weight-for-weight had reached 90 % of the reference value.
† For details of subjects and experimental procedures, see p. 706 and Table 1.
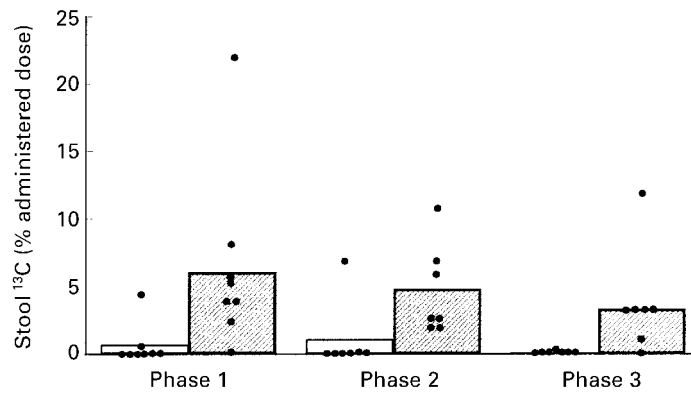
Fig. 2. The amount of ^{13}C within the triacylglycerol (□) and fatty acid (▨) fractions of stool during the three study phases following oral administration of [^{13}C]-labelled tripalmitin in eight severely malnourished children and during rehabilitation. Mean and individual values are shown. Phase 1, on admission to hospital; phase 2, during rapid catch-up growth; phase 3, when weight-for-height had reached 90 % of the reference value. For details of subjects and procedures, see p. 706 and Table 1.

into account differential losses of ^{13}C label within stool, in individual children and at different stages (Table 4). The time course and magnitude of excretion of ^{13}C label in breath over 24 h were similar between the phases (Fig. 3). On all occasions the excretion of label on breath as ^{13}CO$_2$ achieved maximum enrichment between 2 and 5 h following the administration of the label. There was a return to background levels of abundance by 24 h in almost all children. At phase 1, the enrichment of ^{13}C in breath at peak excretion was 1·0958 (SD 0·004) APE and was not different to phase 2 (1·0932 (SD 0·0054) APE) and phase 3

(1·0925 (SD 0·0046) APE). When expressed as area under the curve in phase 1, the percentage of absorbed ^{13}C label excreted on breath was 5·1 (SD 6·0; range 0–15·2), not different from phase 2 (5·2 (SD 4·5; range 1·7–10·7), or phase 3 (6·4 (SD 6·6; range 1·4–21·2)).

[1-^{13}C] glycocholate

Following the administration of [1-^{13}C]GCA in phase 1, there was a small increase in ^{13}C enrichment in stool from baseline within 24 h for three of the eight patients, ranging
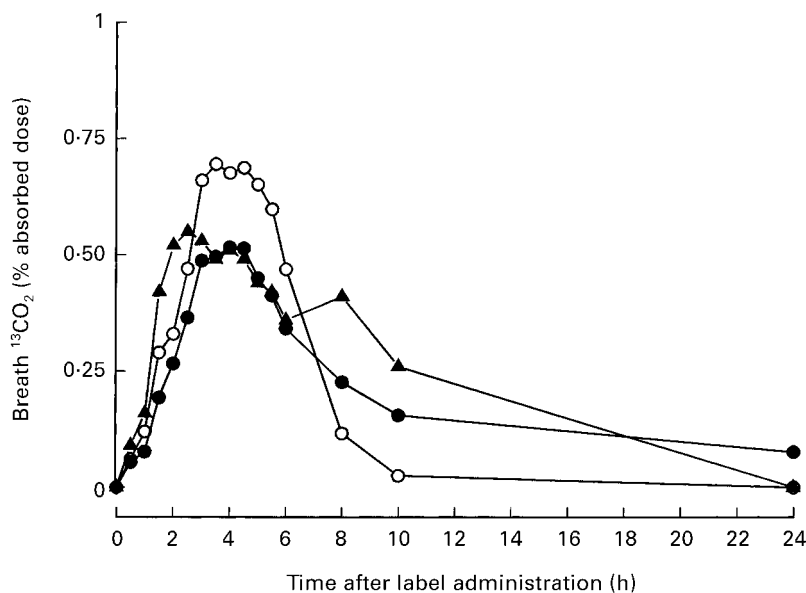

Fig. 3. Time course for the excretion of ^{13}C in breath as ^{13}CO$_2$ over 24 h following oral administration of ^{13}C-labelled tripalmitin in eight severely malnourished children and during rehabilitation. Mean values are shown. Phase 1 (●) on admission to hospital; phase 2 (○), during rapid catch-up growth; phase 3 (▲), when weight-for-height had reached 90 % of the reference value. For details of subjects and procedures, see p. 706 and Table 1.

from 1·0886 to 1·0916 APE, equivalent to <4 % of the administered dose. In one subject, enrichment of ^{13}C on breath was 1·0931 APE, equivalent to 6·2 % of the administered dose at phase 1. At phase 2, less of the ^{13}C label was excreted on breath (1·0917 APE), 2·5 % of the administered dose, and this amount was not different from the natural abundance of ^{13}C at phase 3 (1·0902 APE). These results demonstrate that the administered [1-^{13}C]GCA was not malabsorbed or further degraded to any substantial extent. The data are taken to mean that none of the children exhibited significant bile salt deconjugation as a consequence of small intestinal overgrowth.

Discussion

Gastrointestinal handling

In the present study we have examined the extent to which the treatment for severe malnutrition may influence the gastrointestinal handling of dietary lipid in children. By collecting stool, we have been able to determine the balance of labelled lipid across the gastrointestinal tract. By following the fate of label from orally administered ^{13}C-labelled TP we have examined the digestion and absorption and further oxidation of palmitic acid contained in TP, and from orally administered ^{13}C-labelled GCA we have been able to assess the extent to which bile salt deconjugation might contribute to any impairment of digestion or absorption.

Generally, for this group of children, the lipid content of stool was not greatly abnormal in malnutrition or during recovery, at about 5 g/d. However, a single value gives no sense of the capacity of the bowel to handle lipid in relation to the magnitude of the dietary intake, or the age and size of the subject. Expressing lipid in stool as a percentage of lipid intake in terms of lipid balance showed that lipid excreted in stool was about 6 % that of the lipid consumed in malnutrition, but varied between 1 and 29 %. During rehabilitation the values decreased to 3 and 1 % of the lipid consumed at phases 2 and 3 respectively, with less variation observed between children. In normal healthy children, aged between 6–11 years, we have found that stool lipid varies between 1 and 4 % of the lipid consumed (Murphy *et al.* 1991). The results of the present study reinforce the experience of other researchers that, in severely malnourished children, the lipid content of stools may be very variable.

Studies of stool lipid balance are based on the assumption that lipid measured in stool is dietary in origin, rather than the result of endogenous losses into the bowel, and no allowance is made for any colonic bacterial metabolism that may exert an influence on the lumen contents. One reason for giving ^{13}C-labelled palmitic acid as a labelled TAG was to probe the fate of luminal lipid. Palmitic acid was chosen as it is one of the principal fatty acids in the milk formula consumed by the children, accounting for about 15 % of the total fatty acid. This is the first occasion that ^{13}C-labelled TAG has been used to examine the gastrointestinal handling and metabolic disposal of dietary lipid throughout rehabilitation from childhood malnutrition. We have previously shown, in

healthy children, that on average 6 % of the administered ^{13}C label appeared in the stool after orally administering ^{13}C-labelled TP (Murphy *et al.* 1998). In contrast to lipid balance, within the group there was substantially greater recovery of ^{13}C in stool following the ingestion of ^{13}C-labelled TP, with recoveries in excess of 5 % in four children during phase 1, four children during phase 2 and three children during phase 3. During malnutrition the range for ^{13}C in stool (1–26 % of the administered dose) was identical to the range in values reported for lipid in stool (equivalent to 1–29 % of the lipid ingested). However, there was more variation between individuals in the excretion of label in stool during rehabilitation compared with the range in values for lipid balance. Overall, the ^{13}C content of stool tended to decrease as rehabilitation progressed. Based on these data, it would appear that ^{13}C-labelled TP may effectively trace the gastrointestinal handling of palmitic acid within dietary TAG as it moves through the bowel during malnutrition, but this does not mean that it traces total lipid ingested if different fatty acids are handed differently. During rehabilitation the variation observed might be attributed in part to the nature of the predominant lipids consumed in the diet in comparison with the labelled TAG. Lipid balance studies have shown that unsaturated fat is better digested and absorbed than long-chain saturated fat during treatment for malnutrition (Underwood *et al.* 1967). Using ^{13}C-labelled fatty acids, chain length and degree of unsaturation have been shown to influence the handling of dietary lipid in healthy women (Jones *et al.* 1999). Further studies are needed to explore directly the extent to which the gastrointestinal handling of other labelled fatty acids found in those oils (i.e. maize and coconut oils) consumed by the subject during treatment compares with the saturated long-chain fatty acid, palmitic acid examined in the present study.

Following ingestion of ^{13}C-labelled TAG, the appearance of ^{13}C label in stool as either TAG or fatty acid provides an indication of the extent to which ^{13}C-labelled TAG has been digested and absorbed. If labelled material is not digested, then ^{13}C is recovered in stool in the TAG fraction, whereas recovery of ^{13}C in the fatty acid fraction indicates that luminal hydrolysis has taken place, but the products of digestion have not been absorbed. This is the first report in which the extent of maldigestion and malabsorption of a ^{13}C-labelled TAG has been examined by this approach. At each stage of recovery there was more ^{13}C recovered in fatty acid than in TAG, although there was marked variability amongst children. These results would suggest that in general the gastrointestinal dysfunction observed was associated with the failure to absorb the products of digestion rather than an impaired ability to hydrolyse lipid. If there was a marked impairment of lipase activity, we would be most likely to attribute the limitation to a degree of pancreatic insufficiency. However, as it was not possible to rule out an important contribution from other lipases at different levels of the bowel, for example bacterial metabolism in the colon (Segal *et al.* 1990), we cannot conclude that there was no impairment of pancreatic function. In addition, there could have been continued TAG hydrolysis in stool after defaecation, although attempts

were made to limit further hydrolysis by immediately freezing samples and maintaining storage conditions at $-20°C$. We also determined the extent of bile salt malabsorption by measuring the excretion of ^{13}C label in stool following oral administration of [1-^{13}C]GCA. The recovery of ^{13}C label in stool was less than 1 % of the dose, except for one subject, in whom the recovery was about 4 %. From these data we conclude that there was no evidence of bile salt deconjugation.

Metabolic disposal

Once absorbed, the partitioning of the labelled fatty acids towards either oxidation or retention within the body was determined by measuring the recovery of ^{13}C label in breath as $^{13}CO_2$. The greatest recovery of label was on average about 15 % of the dose absorbed, and on average about 5 % of the absorbed dose was oxidised at each stage of rehabilitation.

There are not many reports with which to compare these results. In healthy children in the UK, following orally administered ^{13}C-labelled TP, the recovery of ^{13}C label on breath was on average 33 %, ranging from 15 to 43 % of the absorbed dose (Murphy *et al.* 1998), much greater than that for the malnourished children at any stage of recovery. In another study, a small percentage of the label (about 5 % of the administered dose) was recovered in breath following orally administered ^{13}C-labelled oleic acid (given as Hiolein®) in children with kwashiorkor (Iputo *et al.* 1998). This value is similar to that observed for the oxidation of labelled palmitic acid in the present study. Taking the two findings together would indicate that, in severe malnutrition and during rehabilitation, these ingested fatty acids are less likely to be partitioned towards oxidation and be retained in the body. The reduced oxidation of labelled fatty acids in malnourished children may reflect energy intakes in excess of requirements during treatment to promote catch-up growth and overcome an assumed reduced availability. However, there have been no published studies using labelled fatty acids to determine whether excess intakes would suppress exogenous lipid oxidation in either children or adults.

One assumption that underlies this conclusion is that any ^{13}C generated from the oxidation of labelled fatty acids will leave the body as $^{13}CO_2$. If there was any other major fate for the label, then there could be a substantial underestimation of the extent to which the labelled fatty acids had been oxidised. Previous studies have indicated that some of the $^{13}CO_2$ generated may be retained within the body or excreted in urine or through the skin or in stool. The extent to which $^{13}CO_2$ may enter these other routes of disposal remains unresolved, with estimates ranging from 10 to 50 % in normal adults (Irving *et al.* 1983). There is less information on which to base judgements in children during recovery from severe malnutrition, although an average value of 54 % was reported for the recovery of infused bicarbonate in a group of children with kwashiorkor (Iputo *et al.* 1998). If this value were to be applied as a correction factor for the recovery of $^{13}CO_2$ on breath, in the present study the values for oxidation would increase from approximately 3–4·5 % to 5–7 % during the different

stages of recovery. Thus, even when allowance is made for a substantial underestimate of the proportion of absorbed lipid that is oxidised, the overall interpretation is not altered, that most of the lipid ingested was retained in the body over the time course of the study.

In conclusion, the results of the present work indicate that during malnutrition not all the children had problems associated with the handling of dietary lipid and ^{13}C-labelled TP. There were some children with a greater degree of impairment, which was not reflected in gross lipid balance. In these children there was some improvement in the digestion and absorption of ^{13}C-labelled TP during rehabilitation. Poor gastrointestinal handling of ^{13}C-labelled TP was more likely to be associated with a failure to absorb the products of digestion. The absorbed lipid was more likely to be deposited as adipose tissue than used to satisfy the immediate needs for energy. The extent to which different fatty acids in the diet might be handled differently, both within the gastrointestinal tract and in further metabolism, needs to be determined, as there could be clinical advantages in reviewing the type of lipid used to increase the energy density of feeds during treatment.

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