ORIGINAL ARTICLE

Maldigestion and malabsorption of dietary lipid during severe childhood malnutrition

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Background: Diets rich in lipid are used to provide energy density in treating children with severe malnutrition, but the extent to which their digestion and absorption can cope with the load effectively is uncertain.
Aim: To determine the extent of impaired digestion or absorption, in three groups of eight malnourished children (aged 5–23 months) using isotopic probes of the predominant fatty acids in coconut and corn oil used to fortify the diet.
Methods: Each child received oral doses of one of three ^{13}C labelled triglycerides (trilaurin, triolein, or trilinolein). The recovery of ^{13}C label in stool either as triglyceride (TAG) or fatty acid (FA), was used to assess digestion and absorption. In a separate test, the recovery of label in stool following an oral dose of [^{13}C]-glycocholate was measured to assess bile salt malabsorption.
Results: The median recovery of label in stool was 9% (range 1–29%) of administered dose. Following treatment there was a reduction in stool ^{13}C excretion for the labelled TAG (<1%). In half the subjects, label was recovered as TAG in stool (median 0.6%, range 0–44%). Most label in stool was recovered as FA (median 30%, range 0–100%). Following [^{13}C]-glycocholate, label was recovered in excess in about one third of studies.
Conclusion: Abnormalities in the gastrointestinal handling of lipid were observed in over 50% of children with severe malnutrition, reflecting problems in absorption, although impaired solubilisation or hydrolysis could also be contributory factors. The underlying lesion improves as treatment progresses, leading to concomitant improvement in function.

The provision of adequate energy in the diet is important if young children are to satisfy the needs for growth during recovery from illness. An increase in the energy density of foods is often achieved by increasing the lipid content. However, in children with severe malnutrition, who are most in need of additional dietary energy, there are perturbations in lipid metabolism. Early studies suggest an increase in the lipid content of stool during the acute illness, and during early recovery.[1–3] Impairment in aspects of digestion or absorption have been considered as contributory factors. There is evidence to implicate pancreatic insufficiency,[4 5] bacterial overgrowth of the small intestine,[6] impaired reabsorption of bile salts, excessive bile salt deconjugation,[7 8] intestinal desquamation, and villous atrophy of the small intestine.[9]

The handling of different triglycerides (TAG) in the gastrointestinal tract is determined in part by the physicochemical properties of the individual constituent fatty acids (FA). No consideration has been given to this factor in the formulation of recovery diets. There is a need to know whether there is any clinical advantage in selecting one lipid source over another, especially during rapid catch up growth when a diet rich in lipid has been recommended to enable speedier recovery.[10]

By tracing the fate of individual dietary FA labelled with the stable isotope ^{13}C, it is possible to make a more direct assessment of the extent of maldigestion and malabsorption of individual FA consumed in the diet. We have used ^{13}C labelled FA to assess the handling of lipid by the gastrointestinal tract in children with severe malnutrition, and at different stages during treatment and rehabilitation. We differentiated between recovered label present in the parent TAG molecule or in the isolated FA following digestion to obtain a measure of the extent to which there might be impaired digestion, rather than impaired absorption. The predominant FA in coconut oil (48% lauric acid) and corn oil (31% oleic and 52% linoleic acid), used to fortify the diets of children with severe malnutrition, have been given as a ^{13}C labelled FA esterified into a TAG. We were particularly interested to know whether the medium chain FA, lauric acid was better handled than the others studied. As small bowel overgrowth is common in severe malnutrition, we used stable isotopic probes of bile salts to indicate the extent of bile salt malabsorption or deconjugation.[11]

MATERIALS AND METHODS

Patients

This study was carried out in infants and young Jamaican children who were admitted to the Tropical Metabolism Research Unit, University of the West Indies, Jamaica, for the treatment of severe malnutrition. Of the children receiving treatment on the unit, three groups of eight children aged 5–23 months were assigned to receive one of three [1,1,1–^{13}C]-labelled TAG (trilaurin, triolein, or trilinolein). The main criterion for selection and inclusion in the study was severe malnutrition according to the Wellcome Classification: less than 80% weight for age and kwashiorkor, marasmus, or marasmic-kwashiorkor.[12] Exclusion criteria were evidence of other obvious pathology, such as renal disease, heart disease, sickle cell disease, or infection with HIV. The children were managed according to a standardised protocol.[13] On admission, the children were started immediately on a milk based diet, based on a commercial infant formula (Nan, Nestlé, Switzerland) with the addition of corn oil to provide around 417 kJ/kg/day (100 kcal/kg/day) and 7 g/kg/day lipid. During rapid catch up growth, the children were offered a milk based formula that was made energy dense by the addition of corn

Abbreviations: FA, fatty acid; TAG, triglyceride

Table 1 Recovery of ^{13}C label in stool (% administered dose) in children during each of the three phases

	Phase 1	Phase 2	Phase 3
Trilaurin	8.4 (1.4–28.7)	3.5 (0–5.4)*	1.2 (0–5.1)*
Triolein	10.5 (0.5–27.5)	0.1 (0–3.7)*	0.5 (0–10.6)*
Trilinolein	7.2 (0.3–20.8)	1.7, 2.4, 11.8*	0.5 (0–4.7)*

Values are given either as median (ranges in parentheses) or individual values if label was detected in three or less children in the group.
*p<0.05 compared with phase 1.

or coconut oil, and provided 625–750 kJ/kg/day (150–180 kcal/kg/day) and approximately 10 g/kg/day lipid.

The Ethical Committee of the University Hospital of the West Indies gave approval for the study to be carried out, and the parent or legal guardian of each child provided informed consent.

Study design

Each child was studied on three occasions, within 48 hours 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), and at late catch up when the child had reached at least 90% of the expected weight for height (phase 3). Each phase lasted for a period of nine days, and on each occasion the child first received the [1,1,1–^{13}C]-labelled TAG, 20 mg/kg body weight (99 atom % excess, Masstrace, Woburn, USA), followed by a three day stool collection, a washout period of three days, and then [1–^{13}C]-glycocholate, 10 mg/kg body weight (99 atom % excess, Masstrace, Woburn, USA), followed by a stool collection for three days. On the day before the administration of either ^{13}C labelled compound, stool samples were collected to determine baseline abundance of ^{13}C.

The ^{13}C labelled TAG were made up as an emulsion in a portion of the formula being consumed as a single bolus by the child, using sonication to incorporate the label. The ^{13}C labelled glycocholate was solubilised in 5 ml water and given as a single dose immediately before the child was fed. Each child received the same standardised feed at 2–3 hourly intervals from the same batch of commercial formula in order to minimise any possible differences as a result of changes in the background isotopic abundance of the diet. Following administration of the label, all stools passed over the three day period were collected, using nappy liners and stool collecting bags, and frozen immediately at −20°C.

Stool analyses

The methodology for processing stools has been described previously.[14] The ^{13}C content of each stool from the three day stool collection was determined following each ^{13}C labelled TAG. For a single day from the three day collection, those stool specimens with the highest level of ^{13}C were used for the extraction of the TAG and FA fractions. The lipid was extracted from stool by a modification of the method of Folch and colleagues[15] with prior acidification. The TAG and FA were then separated by thin layer chromatography. The enrichment of ^{13}C in stool and TAG and FA fractions at baseline and after label administration were analysed by continuous flow isotope ratio mass spectrometry (CF-IRMS; ANCA-NT GSL; Europa Scientific Ltd, Crewe, UK). For the ^{13}C labelled glycocholate studies, abundance levels and change in ^{13}C enrichment was measured by CF-IRMS in baseline specimens and in stool passed each day.

Enrichment was expressed as atom % and then as atom % excess by subtracting the average background ^{13}C abundance (+2 SD) at baseline from the ^{13}C enrichment of the stool specimens containing the label.

The proportion of the orally administered ^{13}C label that was recovered in total stool (over three days) and as TAG and FA in stool was expressed as a percentage of label administered. For the ^{13}C labelled TAG studies, the label recovered in stool was compared to a reference value of 6% administered dose from a previous study in normal healthy children given orally administered ^{13}C labelled tripalmitin.[16] The upper limit of normal ^{13}C excretion has not been established following ^{13}C labelled glycocholate in children. We have applied a recovery of less than 7% of label in stool following an oral dose of ^{13}C labelled glycocholate as the upper limit from previous observations in adults.[11]

Presentation of results and statistical analysis

The results are reported as median and range. Comparisons were made between phases for each ^{13}C labelled TAG by the repeated measures Friedman test so that the subjects would act as their own control. The Wilcoxon rank signed test was used to compare label recovered in FA and TAG within a given phase for each labelled TAG. Associations between the variables were tested by the Spearman rank correlation coefficient.

RESULTS

Total excretion of ^{13}C in stool

Taking the labelled TAG together, total excretion of ^{13}C in stool was 9% (median), range 1–29% at phase 1 (table 1). The amount of label excreted in stool was significantly reduced during phase 2 and 3 for each labelled TAG ($p < 0.05$). When all the phases were considered together, there was a significant association between total lipid and the amount of ^{13}C label in stool for trilaurin ($r = 0.59$, $p < 0.01$) and triolein ($r = 0.53$, $p < 0.01$), but not for trilinolein ($r = 0.24$, NS).

Excretion of ^{13}C in stool as FA or TAG

The most enriched stool specimens selected from all the phases for ^{13}C FA and TAG analysis contained 54.1–100% of the total ^{13}C excreted in stool over the three day stool collection.

Figure 1 shows the recovery of label in stool as TAG and FA as a percentage of the administered dose for each labelled TAG at phase 1. Taking the labelled TAG together, label recovered as

Table 2 Recovery of ^{13}C label in stool in the form of TAG and FA (% administered dose) at each of the three phases

	Phase 1		Phase 2		Phase 3	
	FA	TAG	FA	TAG	FA	TAG
Trilaurin	2.2 (0–11.7)	1.7, 2.8, 6.0	0.4 (0–2.8)	ND	0.4 (0–3.2)	ND
Triolein	2.5 (0–8.1)	0.2 (0–3.5)	0.4, 0.8	ND	1.1, 3.8	ND
Trilinolein	2.2 (0–6.4)	0.1 (0–0.3)	0.8 (0–9.6)	ND	0.9, 2.0, 2.3	ND

Values are given either as median (ranges in parentheses) or individual values if label was detected in three or less children in the group.
ND, no label detected in all children.

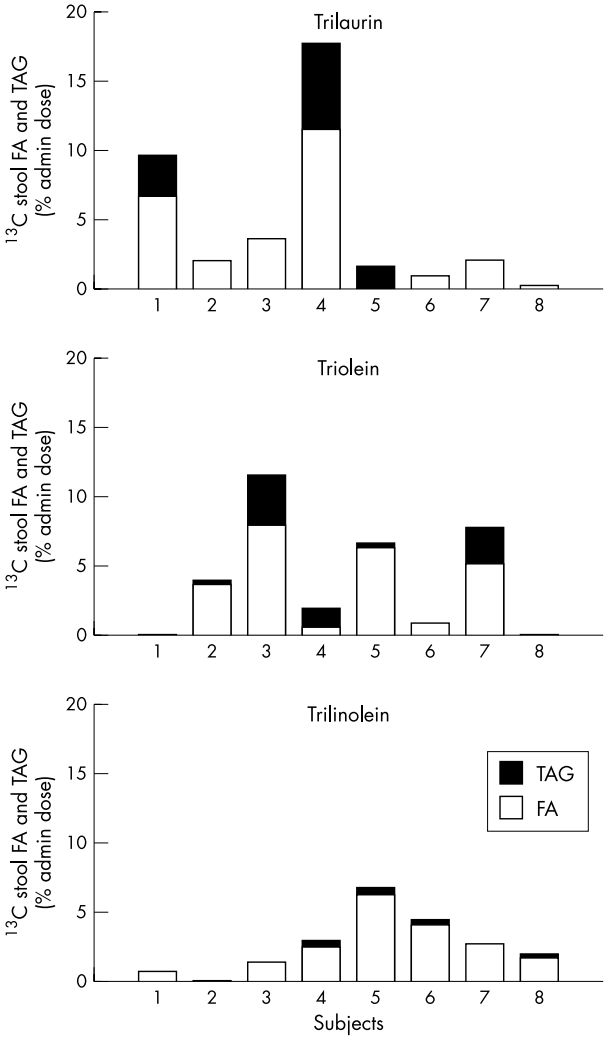

Figure 1 Recovery of ^{13}C label in the form of TAG and FA isolated from stool, as a percentage of an oral dose of ^{13}C labelled trilaurin, triolein, and trilinolein in children at phase 1. Each bar represents the results for a single child for each ^{13}C labelled TAG.

FA was about 2%, range 0–12% (table 2). Up to 6% of the label was recovered as TAG in some of the children. Relatively more of the label in stool was recovered as FA than TAG for trilaurin (FA, 55.3%, 0–100%; TAG, 0%, 0–43.8%; $p < 0.05$), triolein (FA, 26.8%, 0–79.5%; TAG, 1.5%, 0–33.8%; $p < 0.05$), and trilinolein (FA, 26.8%, 0–79.5%; TAG, 1.5%, 0–33.8%; $p < 0.05$). During phases 2 and 3, less label was recovered as FA than at phase 1. No ^{13}C label was detected as TAG in stool for all the children during phases 2 and 3.

^{13}C labelled glycocholate

At phase 1, label was detected in 17 children, and this exceeded the upper limit of normal in 13 (fig 2). There was no increase in stool enrichment in seven children. At phase 2, five children still exhibited raised stool ^{13}C. One child excreted as much as 27% of the administered dose at phase 2 and still had increased losses even by phase 3. There were only two other children who had slightly raised losses of label in stool at phase 3.

DISCUSSION

The aim of the present study was to determine whether the predominant FA in the oils (coconut and corn oil) used to fortify the diet during the treatment of severely malnourished

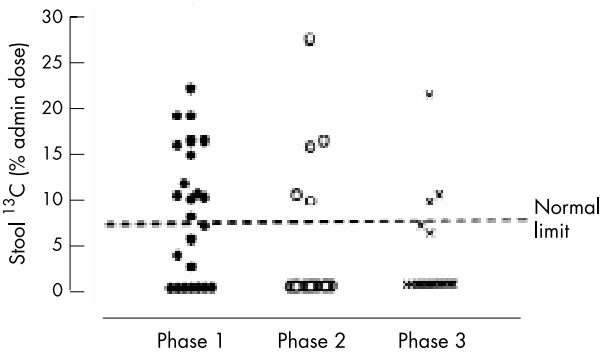

Figure 2 Recovery of ^{13}C label in stool as a percentage of an oral dose of ^{13}C labelled glycocholate in children during rehabilitation for severe malnutrition at each of the three phases. Each point represents the results for a single child.

children, can be effectively processed by digestion and absorption in the bowel. Wide differences were shown among children for label excreted in stool at admission. For individual children the response was far greater than for healthy children,[16] with substantial losses in half of the group reflecting impaired digestion and absorption. Notably in four children, more than 20% of the label was recovered in stool. Medium chain TAG have been reported to undergo rapid, and near complete absorption with a reduction in stool lipid excretion.[17] The present study shows that the medium chain TAG, trilaurin was not handled differently to the longer chain unsaturated TAGs, triolein and trilinolein in the bowel, similar to findings in animal studies.[18] While we did not set out to directly compare the different TAG, it would appear that under these circumstances the administration of this medium chain TAG did not confer any benefit.

As treatment progressed there was notable improvement in the gastrointestinal handling for each labelled TAG. However, we have previously reported impaired absorption of orally administered ^{13}C labelled palmitic acid (given as tripalmitin) in children during recovery from malnutrition.[19] Compared with tripalmitin, there was much less variability between children for the digestion and absorption of triolein, trilinolein, and trilaurin in the present study. Differences in the physicochemical properties of each TAG will influence the way in which they are handled in the bowel, for example, through different affinities for FA binding proteins or differences in the propensity for further handling by re-esterification into TAG for assembly into chylomicrons.

It was possible to determine the extent to which lipid in stool reflected impaired digestion, or was a limitation in some aspect of absorption by determining whether the label recovered was present in the form of TAG, or as a FA following hydrolysis. Most of the label was recovered in the FA fraction (median 30%), suggesting that impaired absorption was the main problem. Label as TAG was detected when the children were severely malnourished and could reflect difficulties with solubilisation or hydrolysis. Label as TAG was observed in 12 children and was equivalent to up to 6% of the administered dose and accounted for more than a third of the label in stool. There was a reduction in label excreted as FA and TAG as the clinical condition of each child improved, suggesting that the underlying lesion was reversible.

The possibility of further hydrolysis of TAG by bacterial lipases in the colon and stool should not be overlooked. Although bacterial lipases have been described,[20 21] the relative contribution that lipases may have to hydrolysis in vivo within the colon has not been directly determined. While other studies have confirmed continuing TAG hydrolysis in collected and stored stool specimens,[22] in the present study particular care was taken to limit further hydrolysis by immediately freezing

samples and maintaining storage conditions at −20°C. FA loss in excess of TAG suggests that the primary problem is either one of impaired absorption, or possibly impaired TAG digestion with continued hydrolysis in the colon.

Bacterial overgrowth in the small bowel has been documented in children with severe malnutrition, and might contribute directly to ineffective solubilisation, digestion, and absorption of lipid.[7 8 23] By determining the recovery of label in stool following orally administered ^{13}C labelled glycocholate, we previously failed to show significant bile salt deconjugation as a consequence of small bowel overgrowth in a group of eight children with severe malnutrition.[19] However, in the present study we extended these observations to a larger group and showed excess label in stool following ^{13}C glycocholate in about one third of cases. It was possible to identify the nature of the infectious insult (from stool culture) in most of the children with increased losses of lipid, and about half of these children showed substantial recovery of label in stool. This would indicate that although significant bile salt malabsorption in some children contributes to impaired digestion and absorption of lipid, the relation was not close, suggesting the likelihood of other important factors which have not been defined in the present study. Bile salt handling improved as treatment progressed, except for one child who exhibited notable bile acid malabsorption at the time of an intercurrent infection, pneumonia with fever, and increased stool frequency during phase 3.

The present work shows that more than half of the children with severe malnutrition had abnormal lipid handling in the gastrointestinal tract. Lipid in stool was largely attributed to malabsorption although impaired digestion could have been a contributory factor. There were abnormalities in bile salt metabolism, but this alone could not account for all the problems observed. Intercurrent infection may further impair lipid handling in the bowel.

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