Measurement of starch digestion of naturally ^{13}C-enriched weaning foods, before and after partial digestion with amylase-rich flour, using a ^{13}C breath test

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Malnutrition in infancy is a global problem which leads to retardation of childhood growth and development. There is a pressing need to improve weaning strategies for infants of the developing world. Traditional Gambian weaning foods are watery and of low energy density, but addition of energy in the form of fat and carbohydrate leads to thick, viscous gruels which are difficult to ingest. Partial digestion with amylase (*EC* 3.2.1.1)-rich flour reduces their viscosity while retaining their energy density. The aim of the present study was to measure the digestibility of a maize-based weaning food, before and after amylase digestion, in malnourished children using a ^{13}C breath test. Ten children (aged 7–16 months; mean weight-for-age Z score −0·8) received isovolumetric and isoenergetic quantities of a maize-based weaning food naturally abundant with ^{13}C. Breath samples were collected at intervals of 30 min for 5 h thereafter and $^{13}CO_2$ enrichment was measured by isotope-ratio mass spectrometry. Percentage dose of ^{13}C recovered increased from a mean 13·7 (SD 3·7)% before, to 18·3 (SD 5·6)% after ingestion of amylase-treated weaning foods ($P < 0·1$). There was a significant inverse relation between age and weight, and percentage dose of ^{13}C recovered in children receiving amylase-treated feeds. There were no differences in concentrations of amylase in saliva of infants or breast milk of their mothers. Partial digestion of supplementary foods may improve the nutrition of undernourished weaning children, not only by reducing their viscosity, thereby increasing ingestion, but also by improving their digestion and thereby their absorption.

Amylase: Cereal: Digestion: Weaning foods

More than half of the developing world's children are undernourished, with retardation of growth and development. Malnutrition begins in infancy during the transition from breast milk to solid diet (Weaver, 1994), and is often associated with depressed exocrine pancreatic function (Sauniere & Sarles, 1988).

Traditional infant weaning foods are gruels based on cereal flours (maize, rice, millet, sorghum and wheat), and it is the starch within them that provides most energy. When prepared by heating with water they become dilute, voluminous, and of low energy density (Hudson *et al.* 1980). Water constitutes over 90% of the total weight of such gruels which, while of acceptable viscosity for the infant to ingest, are of poor nutritional value. Addition of energy in the form of fat, oil or complex carbohydrates makes them thick, bulky, viscous and difficult to ingest (Hellstrom *et al.* 1981).

In several parts of the developing world, malting is used to reduce the viscosity of weaning foods by the enzymic action of naturally liberated amylase (*EC* 3.2.1.1). Cereal

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grains are germinated by soaking in the dark for 48–72 h, followed by drying, toasting, removal of potentially toxic vegetative parts and then milling to flour. This process releases amylase which partly digests the amylose and amylopectins of starch to dextrins and maltose which have less water-binding capacity (Anon., 1991). Using this method the energy density of cereal gruels may be doubled simply by the addition to the cereal of a small quantity of amylase-rich flour (ARF) (Nout, 1993). This results in a lowering of viscosity and hence the amount of water required to obtain a gruel easily ingested by infants and young children.

The aim of the present project was to measure the digestion of a cereal-based weaning food in Gambian infants and children using a non-invasive ^{13}C-breath test. A comparison was made between the digestion of an untreated maize-flour gruel, and a maize-flour gruel to which ARF was added.

SUBJECTS AND METHODS

Ten children (five boys, five girls) aged 7–16 months were studied. All were under the care of the MRC Dunn Nutrition Unit, at its overseas research station in The Gambia. They had already begun to take some home-made weaning foods, continued to breast-feed, and were free of gastrointestinal, respiratory and other disease. They were recruited at the regular clinics attended by all infants and children from birth to 3 years. Tests took place in a feeding centre with the cooperation of the infants' mothers. The sex, age, length and weight of the children are shown in Table 1.

Experimental design

Each child acted as his or her own control, undergoing two tests in random order using a starch-based feed rich in ^{13}C. The test meal, based on a locally prepared maize gruel, was prepared each morning by field staff. It consisted of maize flour of naturally high ^{13}C content, dried skimmed milk, beet sugar, groundnut oil and water, with an energy density of approximately 418 kJ (100 kcal)/100 g. The formula was palatable and well accepted by infants and children. Isovolumetric and isoenergetic quantities of the weaning food were made up either in the traditional way (maize-based feed with no added ARF; test feed 1), or modified by the addition of germinated maize flour: approximately 10 g ARF was added to 1 kg cooked weaning food to provide α- and β-amylase, but negligible extra ^{13}C (test feed 2).

The tests were performed on Mondays, Wednesdays and Fridays so that there was a rest day between each. To ensure that there was a relatively low and stable baseline ^{13}C abundance in breath, children and their mothers consumed a diet free of maize or other cereals enriched with ^{13}C for the day before and the day between each test. Rice-based foods (low in ^{13}C content) were provided free at the feeding centre (Dewit *et al.* 1990*b*).

Each child attended the feeding centre in the morning, following a breast feed, at about 06.00 hours. At 08.00 hours (after at least 2 h fast) a saliva sample was collected for analysis of amylase concentration, followed at 08.30 hours by a baseline breath sample. Each infant was encouraged to take 20 g/kg of one of the two test feeds (each of 418 kJ/100 g) representing approximately 20% of his or her daily energy requirements. Thereafter breath samples were collected at intervals of 30 min for 6 h. The mother breast-fed her infant again at 12.00 to 13.00 hours, when milk samples were obtained from each breast for analysis of amylase concentration. Supplements of water were provided for the infant throughout the test period.

Table 1. *Characteristics of children studied*

Subject	Sex	Age (months)	Length (mm)	Weight (kg)	Wt-for-age Z score*
1	F	9·39	692	8·67	0
2	F	10·48	676	6·76	−1·8
3	F	10·52	700	7·80	−1·0
4	F	10·71	715	8·48	−0·3
5	M	7·21	643	6·40	−2·0
6	M	10·46	743	8·80	−1·0
7	M	15·68	774	9·75	−1·0
8	F	15·87	712	8·05	−1·6
9	M	10·81	723	9·93	+0·5
10	M	16·14	797	11·45	+0·7
Mean		11·23	718	8·61	−0·8

* Weight for age calculated from National Centre for Health Statistics percentiles (Hammill *et al.* 1979).

Measurement of cereal-based feed intakes

The quantity of feed ingested by each child was measured by weighing the feeding cup and feeding bib before and after each test-feed, using an electronic balance accurate to 5 g (Digital Baby Scale, Seca 727, CMS Equipment, London).

Collection and analysis of samples

Saliva was collected with foam or cotton-wool sponges and expressed using a syringe into a container for storage and transport to Cambridge for analysis, using a method described elsewhere (Dewit *et al.* 1990*a*).

Samples of breast milk (2 ml), taken from each breast after the midday feed, were expressed directly into a container for storage and transport to Cambridge for analysis, using a method described elsewhere (Dewit *et al.* 1990*a*).

Breath samples were collected with a face-mask developed at the Dunn Nutrition Unit (Dewit *et al.* 1990*b*). It has a one-way inlet and outlet valve connected to a collecting bag. Approximately five respiratory cycles were sufficient to obtain breath samples for ^{13}C measurements, and each collection took about 30 s. Duplicate breath samples were stored in vacutainers (Becton-Dickinson, Rutherford, NJ, USA), and ^{13}C enrichment was measured by isotope-ratio mass spectrometry (ANCA, Europa Scientific Ltd., Crewe, Cheshire) at the Dunn Nutrition Unit in Keneba.

Principle and method of ^{13}C breath test

The method used to assess starch digestion involves the measurement of the ratio of C isotopes present in breath CO_2 after ingestion of a nutrient or meal containing ^{13}C. ^{13}C is a non-radioactive stable isotope which occurs naturally at a concentration of 1·1% of all C. Maize is naturally enriched in ^{13}C with a ^{13}C abundance higher than that of other cereals and most other foods. Some plants, particularly maize, incorporate more ^{13}C than others during photosynthesis (Harding *et al.* 1994). When a food that is relatively ^{13}C-rich is ingested, the ^{13}C contained in it is absorbed and enters oxidative metabolic pathways leading to enrichment of bicarbonate, protein, fat and carbohydrate within the body. The end-product of oxidative metabolism is $^{13}CO_2$ which, when expired in the breath, can be collected and its ^{13}C enrichment measured (Weaver *et al.* 1993).

^{13}C enrichment was calculated from the ^{13}C:^{12}C ratio compared with the international

limestone standard, PeeDee Belemnite, and the relative difference between the sample and standard was expressed as δ‰. Percentage dose recovery (PDR) was estimated for each time interval, from which cumulative PDR was calculated for the complete test duration (Weaver *et al.* 1993).

Statistics and ethical approval

The weights of children were expressed as standard deviation (Z) scores based on the National Centre for Health Statistics standards (Hammill *et al.* 1979). The data were normally distributed and Student's *t* tests were used to measure the significance of the differences between results. The study was undertaken with the informed consent of the infants' parents, and with approval of the local (Gambian–MRC) ethical committee. The methods were non-invasive and caused minimal disturbance or discomfort to the child. They had all been used previously to study children and proved acceptable and free of adverse effects (Dewit *et al.* 1992).

RESULTS

Test feed intakes

The test feed intakes of the ten subjects taking the two test feeds are shown in Table 2. On six occasions test 1 preceded test 2, and on four occasions *vice versa*. There were no significant differences between the mean feed intakes on each test day ($P > 0{\cdot}5$). There was no significant relation between age of infant and weight of feed ingested per kg (r 0·0004; $P > 0{\cdot}5$).

Breast and salivary amylase concentrations

The concentrations of amylase in the saliva and in the breast milk consumed by the children are shown in Table 2. There were no significant differences in the mean concentrations of amylase in saliva ($P > 0{\cdot}5$) or breast milk ($P > 0{\cdot}1$) consumed by the children on each test day. There was no significant relationship between age of infant and salivary amylase concentration (r 0·35; $P = 0{\cdot}3$), or breast milk amylase concentration (r 0·5; $P = 0{\cdot}5$).

Measurement of breath ^{13}C enrichment

Percentage recovery of breath ^{13}C. Mean ^{13}C recovery from the untreated cereal (test feed 1) was 13·7 (SEM 1·8)%, and that from the ARF-treated cereal (test feed 2) was 18·3 (SEM 1·7)%. There was 33% greater ^{13}C recovery from the ARF-treated cereal than from the untreated cereal ($P < 0{\cdot}1$). The PDR of ^{13}C in breath for tests 1 and 2 are shown in Table 3. Two children (9 and 10) with positive Z scores had ^{13}C recoveries which were less, rather than more, with the addition of ARF to untreated cereal. When the ^{13}C recovery data were analysed excluding these two children, there was a 49% increase in the mean recovery (from mean 13·6 (SEM 1·5) to 20·3 (SEM 1·4)) between test 1 and 2, which was significant ($P < 0{\cdot}005$).

Percentage ^{13}C recovery with age and weight. When the children were fed with untreated cereal (test 1) the correlation coefficient between percentage recovery of ^{13}C and age was $-0{\cdot}44$ ($P > 0{\cdot}5$), and that with weight was $-0{\cdot}33$ ($P = 0{\cdot}36$). When the children were given ARF-treated cereal (test 2) there was a significant reverse correlation between percentage recovery of ^{13}C and age (r $-0{\cdot}65$, $P < 0{\cdot}05$), and with weight (r $-0{\cdot}67$, $P = 0{\cdot}04$). When the differences in PDR between tests 1 and 2 were plotted against weight, age and weight-for-age, only the last approached significance ($P < 0{\cdot}08$).

Recovery of ^{13}C at peak and 6 h. Neither the mean time to peak recovery ($P > 0{\cdot}5$) nor the height of the peak ($P > 0{\cdot}5$) of ^{13}C was significantly different between the two groups (Table 4). On completion of each test (6 h) most children continued to excrete some ^{13}C in their breath. There was no significant difference in the mean δ recoveries at 6 h between the two test-feeds ($P > 0{\cdot}1$) (Table 4).

Table 2. *Intakes of test feeds by Gambian infants, and concentrations of amylase* (EC *3.2.1.1*) *in breast milk and saliva**†

(Mean values, standard deviations and standard errors for ten infants or mothers)

Test...	1			2		
	Mean	SD	SE	Mean	SD	SE
Feed intake (g/kg)	13·3	5·9	1·9	13·2	6·1	1·9
Breast milk amylase (IU/ml)	0·70	0·48	0·15	0·67	0·32	0·10
Salivary amylase (IU/g protein)	43·1	27·8	8·8	45·3	27·0	8·5

* For details of test feeds and procedures, see pp. 532–533.
† There were no significant differences between values for the two test feeds.

Table 3. *Percentage dose of* ^{13}C *recovered* (*PDR*) *after ingestion of* ^{13}C*-enriched flour untreated with an amylase* (EC *3.2.1.1*)*-rich flour* (*ARF*) (*PDR-1*) *or after treatment with ARF* (*PDR-2*)*

Subject	PDR-1	PDR-2	Change (%)
1	15·8	22·8	+44
2	18·2	20·6	+13
3	6·9	26·7	+84
4	14·6	17·8	+22
5	16·3	20·3	+25
6	15·3	22·6	+48
7	14·2	17·3	+22
8	7·3	14·2	+95
9	15·4	12·5	−19
10	13·2	7·7	−42
Mean	13·7	18·3	+29
SD	3·7	5·6	

* For details of feeds and procedures, see pp. 532–534.

Table 4. *Recovery of* ^{13}C *in the breath of Gambian infants after consumption of untreated maize flour* (*test 1*) *or maize flour treated with an amylase* (EC *3.2.1.1*)*-rich flour* (*test 2*)*

(Mean values, standard deviations and standard errors for ten infants)

Test...	1			2		
	Mean	SD	SE	Mean	SD	SE
^{13}C recovery (%)	13·7	3·7	1·8	18·3	5·6	1·7
Time to peak (min)	153	41	13	162	159	18
Recovery at peak (δ units)	3·1	0·7	0·2	3·8	1·5	0·5
Recovery at 6 h (δ units)	1·0	0·6	0·2	1·6	1·2	0·4

* For details of feeds and procedures, see pp. 532–534.

DISCUSSION

'Remarkably little progress has been made in our ability to advise mothers as to when they should supplement the diet of their offspring and there has been little attempt to improve traditional weaning foods in terms of consistency, shelf life and bioavailability of nutrients' (Rowland, 1980). The weanling infant of the developing world faces an impossible dilemma: introduction of weaning foods too late leads to malnutrition from insufficient breast milk alone to meet the requirements for growth, while introduction of weaning foods too early leads to undernutrition through the ingestion of watery, contaminated feeds with insufficient energy (Weaver, 1994).

The ideal weaning food should be of high energy and protein content, resistant to pathogenic micro-organisms, clean and hypoallergenic, easily digestible, culturally acceptable, available and cheap (Weaver, 1994). The most important factor determining the energy and nutrient content of traditionally prepared weaning foods in rural Gambia is their water content, which may approach 90% of total weight (Hudson *et al.* 1980). A high water content results in a low energy density, and consequently a low energy intake and undernutrition. In addition, weaning foods stored for up to 8 h after preparation are frequently infected with enteropathogenic bacteria (Rowland *et al.* 1978).

Small quantities of ARF when added to freshly prepared thick gruels reduce their viscosity while preserving their energy density. This may be doubled, from about 167–209 kJ/100 ml to 335–418 kJ/100 ml, permitting a doubling of energy and nutrient intake per unit volume ingested, or a halving of the amount of gruel consumed to achieve the same energy and nutrient intake. Such treatment may raise the energy intake to within the requirements for normal growth (Nout, 1993).

In the present study we kept the intake and energy density of the test meals constant, thereby measuring only their digestion, absorption and metabolism using the ^{13}C breath test. Each child at the time of each test was in the same metabolic state with regard to duration of fast, activity and general health. The children showed no significant differences in the time to peak recovery of ^{13}C nor in the height of the peak recovery between tests 1 and 2, so the increased recovery from the ARF-treated cereal was due not simply to more rapid absorption of starch. Many of the children studied continued to have some ^{13}C enrichment of the breath after 6 h. It was felt that this was as long as the child and mother should be asked to cooperate. The remaining ^{13}C enrichment of breath was compared with background (baseline) enrichment on each of the study days and there were no significant differences.

The increased ^{13}C recovery that followed ingestion of feeds treated with ARF did not appear to result from other sources of amylase. Concentrations of breast-milk and salivary amylase were not significantly different in the two tests, nor was the volume of cereal test feed ingested on the 2 test days. Breast milk from a mother consuming maize products may contain detectable amounts of ^{13}C. However, although the ^{13}C enrichment of breast milk was not analysed in the present study, previous work (Dewit *et al.* 1990*b*) has shown that a maize-free diet for 36 h lowers and stabilizes the ^{13}C content of breast milk, and as the volumes of milk taken were not significantly different between tests 1 and 2, we believe that this was not a significant source of difference.

Percentage recovery of ^{13}C following ingestion of ARF-treated feeds was significantly inversely correlated with age and with weight, suggesting that the younger children received the greater advantage from predigested feed. Pancreatic amylase secretion is low at birth, and increases gradually after 3 months, not reaching adult levels until at least 2 years (McClean & Weaver, 1993). In addition, malnutrition is associated with impaired exocrine pancreatic function. The acinar glands of the pancreas atrophy (Blackburn & Vinijchaikul, 1969) and the concentration of α-amylase is diminished in the duodenal contents of

children with kwashiorkor (Sauniere & Sarles, 1988), but returns to the normal range after nutritional rehabilitation (Sauniere *et al.* 1986). The median age of the children we studied was 11·5 months, and most were between 80 and 90% of weight-for-age. Only those with weight-for-age Z scores $\leqslant 0$ showed increased PDR values with ARF-treated feeds, suggesting that undernourished children enjoy the most nutritional benefit from predigestion of starch with ARF, which compensated for insufficiency of endogenous pancreatic amylase.

We have shown that, in a group of Gambian children taking isovolumetric and isoenergetic amounts of a maize-based weaning food, the addition of ARF benefited the youngest and lightest infants, those who are likely to have immature or compromised pancreatic exocrine function. Malting is a traditional practice in many developing countries and the present study shows that its value may lie not only in reducing the viscosity of weaning foods, thereby increasing their ingestion, but also in improving their digestion and absorption of energy. The wider use of ARF to enable partial digestion of weaning foods could have a significant effect in improving the nutritional status of malnourished children in The Gambia and elsewhere in the developing world.

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