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Age-related association of small intestinal mucosal enteropathy with nutritional status in rural Gambian children

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Small bowel enteropathy (assessed by the lactulose (L) : mannitol (M) permeability test) is a major factor in infant growth faltering and malnutrition in The Gambia. However, little is known about its persistence and nutritional effect beyond 2 years of age. This was addressed by two cross-sectional studies of intestinal permeability and nutritional status in 162 residents, aged 2–60 years, living in three villages in rural Gambia. L:M ratio was found to be highest in the youngest children and although there was a significant improvement with age ($P<0{\cdot}0001$), values were always greater than the range found in UK counterparts. M recovery (mean value 5·68 (SE 0·12) %) was at all times between one-third and one-half of expected UK values and showed no improvement with age. Gut barrier function, assessed by L uptake, improved with age ($P<0{\cdot}001$) and fell within the UK normal range beyond age 10 years. Both the L:M permeability ratio and L recovery were significantly associated with height-for-age z-scores (r − 0·31 and − 0·22 respectively, $P<0{\cdot}001$), a relationship that persisted throughout childhood and into adulthood. Change in height-for-age z-score beween the two visits was also related to the L:M ratio (r − 0·24, $P=0{\cdot}018$). The close within-subject correlation of permeability variabilities between the two visits suggests a long-term persistence of enteropathy within individuals. It appears that the small bowel enteropathy previously described in Gambian infants persists through to adulthood. Although the lesion improves with age, the relationship between attained height and L:M permeability raises the possibility that enteropathy may continue to limit growth throughout childhood and puberty.

Growth: Intestinal permeability: Enteropathy: Tropical: The Gambia

A persistent mucosal enteropathy of the small intestine has been demonstrated in rural Gambian infants by the lactulose (L) : mannitol (M) test and confirmed by biopsy studies (Behrens *et al.* 1987; Lunn *et al.* 1991*a*; Sullivan *et al.* 1991). The enteropathy has been shown to account for up to 43 % of growth faltering in both length and height of Gambian infants (Lunn *et al.* 1991*a*). Intestinal biopsies collected from adult inhabitants of other tropical climes also show histological evidence of the so-called tropical enteropathy (described by Lindenbaum *et al.* (1966) and Lindenbaum (1973)) associated with abnormal gastrointestinal function tests (Baker & Mathan, 1972), suggesting that the lesion seen in Gambian infants may persist into adulthood. However, there are no results to show how small intestinal mucosal status changes with age in a rural tropical environment or whether the enteropathy continues to be associated with poor growth beyond early childhood.

The L:M test is a non-invasive method of assessing aspects of small bowel function and integrity (Menzies *et al.* 1979; Travis & Menzies, 1992). L is a non-digestible disaccharide and is too large to be absorbed intact through a healthy small bowel mucosa. However, in disease situations that challenge mucosal integrity, the proportion of an oral dose absorbed and excreted into the urine increases (Ford *et al.* 1985). M, a non-digestible monosaccharide is sufficiently small to move passively across the small bowel mucosa in quantities directly proportional to the absorptive surface area (Menzies *et al.* 1979). Following an oral dose of the two sugars, the value of excretion of L:M in the urine (collected for 5 h) gives the intestinal permeability ratio (L:M). In addition, by measur-

Abbreviations: BMIZ, BMI z-score; HAZ, height-for-age z-score; L, lactulose; M, mannitol; WAZ, weight-for-age z-score.

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ing total urinary excretion of M and L it is possible obtain estimates of both small bowel absorptive capacity (M recovery) and barrier efficiency (L recovery). Studies in apparently healthy Gambian infants have shown intestinal permeability to be up to fivefold greater than in UK counterparts (Lunn *et al.* 1991*b*). The elevated values in these infants resulted from a combination of low M and high L uptake and excretion (Lunn, 2000).

The aims of this present study were twofold: (1) to use the L:M intestinal permeability test to assess small intestinal mucosal status of individuals from 2 to 60 years of age living in rural Gambian villages; (2) to define the association of small bowel function and integrity with growth and nutritional status from childhood through to adulthood.

Subjects and methods

Subject recruitment

Residents of Keneba and the surrounding villages of Kanton Kunda and Manduar, The Gambia, were stratified into the following age groups (years): 2–5, 5–10, 10–15, 15–20, 20–30, >30. Approximately thirty subjects were then selected at random (by computer generation) from each age group and invited to join the study (total recruited *n* 162). Informed consent was obtained from each subject through a trained field worker, as was permission from the village elders of each village. Ethical permission was obtained from the Medical Research Council and Gambian Government Joint Ethical Committee.

Of the recruits, 41 % were from Keneba, 30 % from Kanton Kunda and 29 % from Manduar. Table 1 gives details of the nutritional status of subjects at recruitment expressed as z-scores against the 1990 UK growth standards (Freeman *et al.* 1995). Anthropometric readings were taken at the beginning of the study (in June) and again 3·5 months later and growth was calculated as the change in weight over this period. Weight was measured using an electronic balance (Seca, Birmingham, UK) (accurate to 0·1 kg;) and height using a Harpenden measuring board (Holtain, Cymych, Dyfed, UK) for the 2–5-year-old group and a stadiometer (accurate to 0·001 m) for the other groups. All measurements were performed by one person (D.I.C.). Because of the pressures of gathering the harvest, it was not possible to repeat the study on more than 124 of the original subjects.

Methods

Subjects were given a brief medical examination and underwent an intestinal permeability test if they had been free from diarrhoea for the preceding week. The oral sugar dose contained 0·5 g M + 2 g L/10 ml water and the volume given varied according to subject age. Subjects aged 2–5 years old received 20 ml solution, those aged 5–10 years were given 40 ml, those aged 10–15 years were given 60 ml and those >15 years received 80 ml. All urine passed in the 5 h following the L + M dose was collected, the total volume was measured and, after mixing, a 2 ml sample was frozen at −40 °C. Samples were transported to Cambridge, UK, for measurement of L and M concentrations by automated enzymatic assays (Northrop *et al.* 1990; Lunn & Northrop-Clewes, 1992) and the L:M ratio was calculated. The actual dose of L and M administered was calculated following analysis of both sugars in the dosing solution. The recovery of each sugar excreted in the urine (%) was then calculated using the total urine volume and individual sugar concentrations.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) version 9·0 (SPSS Inc., Chicago, IL, USA). Most analyses were by ANOVA, but the paired *t* test was used to compare within-subject differences on the two study days. The L:M ratio had a skewed distribution which was normalised by calculation of $\log_e$ L:M (ln L:M) which was used in all statistical analyses.

Results

Age-related differences

Differences in weight-for-age z-scores (WAZ), height-for-age z-scores (HAZ) and BMI z-scores (BMIZ) with age are shown in Table 1. Both WAZ and HAZ were at their highest in the 20–30-year-old group, and poorest in the youngest children, i.e. the 2–5-year-old group. The 5–10-year-old group showed evidence of catch-up growth from earlier low weight and height, but this

Table 1. Age of mean subjects and their nutritional status at the onset of the study‡

Age group (years)	*n*	Age (years)		WAZ		HAZ		BMIZ	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
2–5	26	3·23	0·58	−1·67††	0·98	−1·76††	0·96	−0·59	1·13
5–10	24	8·00	0·95	−1·18	1·08	−0·88**	1·22	−0·88	0·66
10–15	29	13·35	1·61	−1·76††	0·99	−1·38	0·82	−1·47**††	1·06
15–20	27	18·19	7·03	−1·65††	1·50	−1·16	0·92	−1·15	1·40
20–30	31	25·79	5·05	−0·74**	0·98	−0·70**	1·11	−0·55	0·95
>30	25	48·27	6·44	−1·12	1·25	−1·08	1·11	−0·76	0·88

WAZ, weight-for-age z-score; HAZ, height-for-age z-score; BMIZ, BMI z-score.
Mean values were significantly different from those of the 2–5-year-old group (ANOVA): **$P<0·01$.
Mean values were significantly different from those of the 20–30-year-old group (ANOVA): ††$P<0·01$.
‡For details of procedures, see p. 500.

Table 2. Differences between the sexes in subject nutritional status at the onset of the study† (Mean values and standard deviations)

		WAZ		HAZ		BMIZ	
Sex	*n*	Mean	SD	Mean	SD	Mean	SD
Male	76	−1·79	1·03	−1·45	0·96	−1·19	1·14
Female	86	−0·96***	1·18	−0·89***	1·10	−0·65***	0·96

WAZ, weight-for-age z-score; HAZ, height-for-age z-score; BMIZ, BMI z-score.
Mean values were significantly different from those of the male group (ANOVA): ***$P<0·01$.
†For details of subjects and procedures, see Table 1 and p. 500.

improvement was lost in the 10–15- and 15–20-year-old groups, because of the late onset of the adolescent growth spurt. Overall however, both WAZ and HAZ improved with age ($P=0·004$ and $P=0·005$ respectively), but at all times, both z-scores were below 1990 UK standard values (Freeman *et al.* 1995). BMIZ fell with age until the 10–15-year-old group, but then recovered to give the highest value in the 20–30-year-old group. Overall however, both WAZ and HAZ improved with age ($P=0·004$ and 0·005 respectively).

There were also sex differences in WAZ, HAZ and BMIZ (Table 2), with nutritional status being better in females. Although no significant differences between the sexes were seen in subjects <15 years old, z-scores for women became substantially closer to the reference population (Freeman *et al.* 1990) than those of men.

The way in which the mean L:M ratio varied with age (after correction for sex and visit) is shown in Fig. 1. The highest value, 0·353 (SE 0·022) was found in the youngest, 2–5-year-old group, but this fell progressively with increasing age ($P<0·001$). However, mean L:M values never fell into the normal range seen in the UK (Travis & Menzies, 1992), with subjects >50 years old having a mean of 0·1 (SE 0·04). Most of the improvement in the value of the ratio was due to a reduction ($P<0·001$) in the absorption and excretion of L (Fig. 2). This also showed a progressive fall from high values in the youngest group, but in this case, mean levels were within the expected UK range from the 10–15-year-olds onwards. In contrast, although M uptake and excretion showed a small downward trend with increasing age, this did not reach statistical significance. In fact, M excretion levels (overall mean 5·68 (SE 0·12) % administered dose) were at all times between one-half and one-third of expected UK values (Travis & Menzies, 1992). This lack of improvement of M with age was responsible for the L:M ratio remaining elevated into adulthood. No further change in the L:M ratio was found with increasing age beyond 20 years (r 0·04, $P=0·86$). There was no overall difference in intestinal permeability between the sexes; however in the 5–10-year-old group, girls had greater values than boys (L:M ratios 0·178 (SE 0·011) and 0·269 (SE 0·029) respectively, $P<0·01$).

Intestinal permeability and nutritional status

Intestinal permeability expressed as ln L:M ratio was significantly and negatively associated with HAZ (r −0·31, $P<0·001$ after correction for age, sex and visit), but not with WAZ or BMIZ. The higher (i.e. the more abnormal) the ratio, the poorer the height attained. This association of the ratio with HAZ was mainly due to the higher L excretion in subjects with poorer HAZ scores (r −0·22, $P=0·001$). The relationship between ln L:M and HAZ held statistical significance across the age spectrum, even when age groups were divided at 15 years of age. There was no significant association between mean ln L:M ratio (mean of the two values) and change in WAZ or BMIZ between the study visits (r −0·24, $P=0·018$, omitting the 20–30-year-old and >30-year-old groups). However, neither mean M nor mean L urinary recovery values were related to changes in weight or BMI between study visits.

Changes between visits

During the 3·5 months between the two study visits, gains in height were recorded for subject groups <20 years old, but no change was seen in groups >20 years old. No significant change in weight was seen in the groups <20 years old, but in groups >20 years old, small weight losses were observed (Table 3). Overall, the nutritional status of the subjects showed some deterioration, with the reductions in WAZ and BMIZ but not HAZ being significant ($P<0·01$; Table 4). Table 4 also shows that there was a small improvement in intestinal permeability between the two study days: the L:M ratio decreased from 0·198 (SE 0·018) to 0·172 (SE 0·010), associated with an improvement in M recovery. However, no change was seen in L excretion.

Table 3. Changes in weight and height of age groups between study visits

		Weight change (kg)		Height change (m)	
Age group (years)	*n*	Mean	SD	Mean	SD
2–5	23	0·14	0·56	0·178***	0·098
5–10	22	−0·25	1·41	0·134***	0·113
10–15	25	0·61	2·43	0·147***	0·176
15–20	18	0·07	1·67	0·097***	0·077
20–30	19	−2·51**	3·05	−0·020	0·060
>30	18	−1·19*	1·90	−0·022	0·067

Mean values were significantly different between visits (paired *t* test): *$P<0·05$; **$P<0·01$; ***$P<0·001$.
†For details of subjects and procedures, see Table 1 and p. 500.

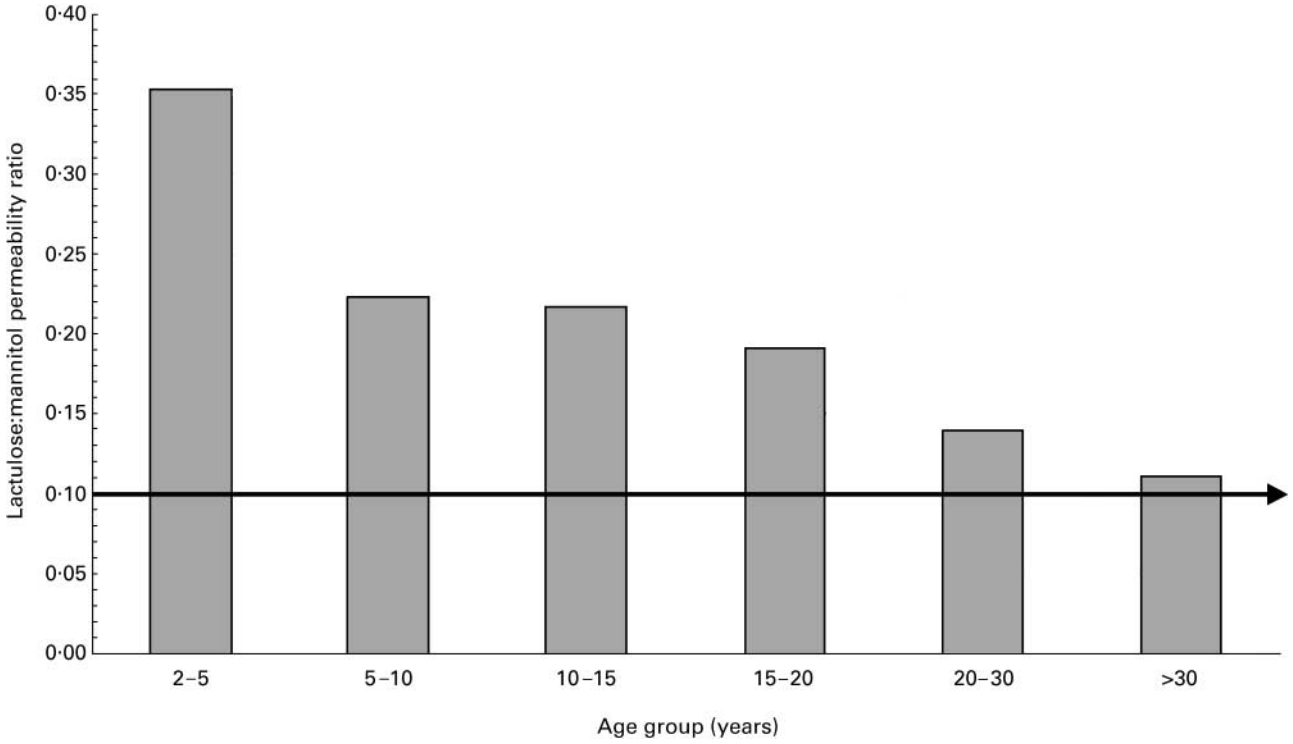

Fig. 1. Variation in lactulose: mannitol permeability ratio with age. For details of subjects and procedures, see p. 500. Values are means for about thirty subjects per age group with standard errors shown by vertical bars. →, UK upper limit of normal (Freeman *et al.* 1995).

Indices of intestinal permeability within subjects showed a high degree of correlation between the two visits. The correlation coefficients (r) for these associations in ln L:M ratio and recovery of M and L between the visits were 0·66 ($P<0·001$), 0·24 ($P<0·05$) and 0·55 ($P<0·001$) respectively.

Discussion

Previous work has demonstrated that the marked growth faltering of rural Gambian infants from 3–15 months of age is closely associated with the presence of a mucosal enteropathy in the small intestine. That work also showed that the severity of the enteropathy and its effect on growth could be assessed by the L:M intestinal permeability test (Lunn *et al.* 1991*a*). Similar abnormalities in intestinal morphometric structure and function in asymptomatic residents from tropical countries has been referred to as tropical enteropathy (Cook, 1980).

The poor growth of rural Gambian infants from 3–15 months of age resulted in their mean HAZ and WAZ falling to about −2 (Lunn, 2000). The present study, which was carried out in the same villages, showed that 2–5-year-old children still had HAZ and WAZ near to −2, but that some catch-up growth did occur during the 5–10-year-old period. However, HAZ and WAZ in adults (>20 years of age) remained below the 1990 UK standards (Freeman *et al.* 1995), especially in men, indicating that complete catch-up had not occurred even during the adolescent growth spurt.

There are several possible reasons for both the initial growth failure and subsequent incomplete catch-up in growth, including poor dietary intake, malabsorption,

Table 4. Differences in nutritional status and intestinal permeability between study visits*
(Mean values and standard deviations for 112 subjects)

	Visit 1		Visit 2		Statistical significance of difference (paired *t* test): *P*
	Mean	SD	Mean	SD	
WAZ	−1·33	1·12	−1·52	1·08	<0·001
HAZ	−1·10	1·01	−1·11	1·01	NS
BMIZ	−0·92	1·12	−1·16	1·13	<0·001
Ln L:M ratio	−1·62	0·66	−1·76	0·55	0·026
M recovery (%)	5·25	2·69	6·28	3·03	0·006
L recovery (%)	0·28	0·20	0·29	0·18	NS

WAZ, weight-for-age z-score; HAZ, height-for-age z-score; BMIZ, BMI z-score; L, lactose; M, mannitol.
*For details of subjects and procedures, see Table 1 and p. 500.

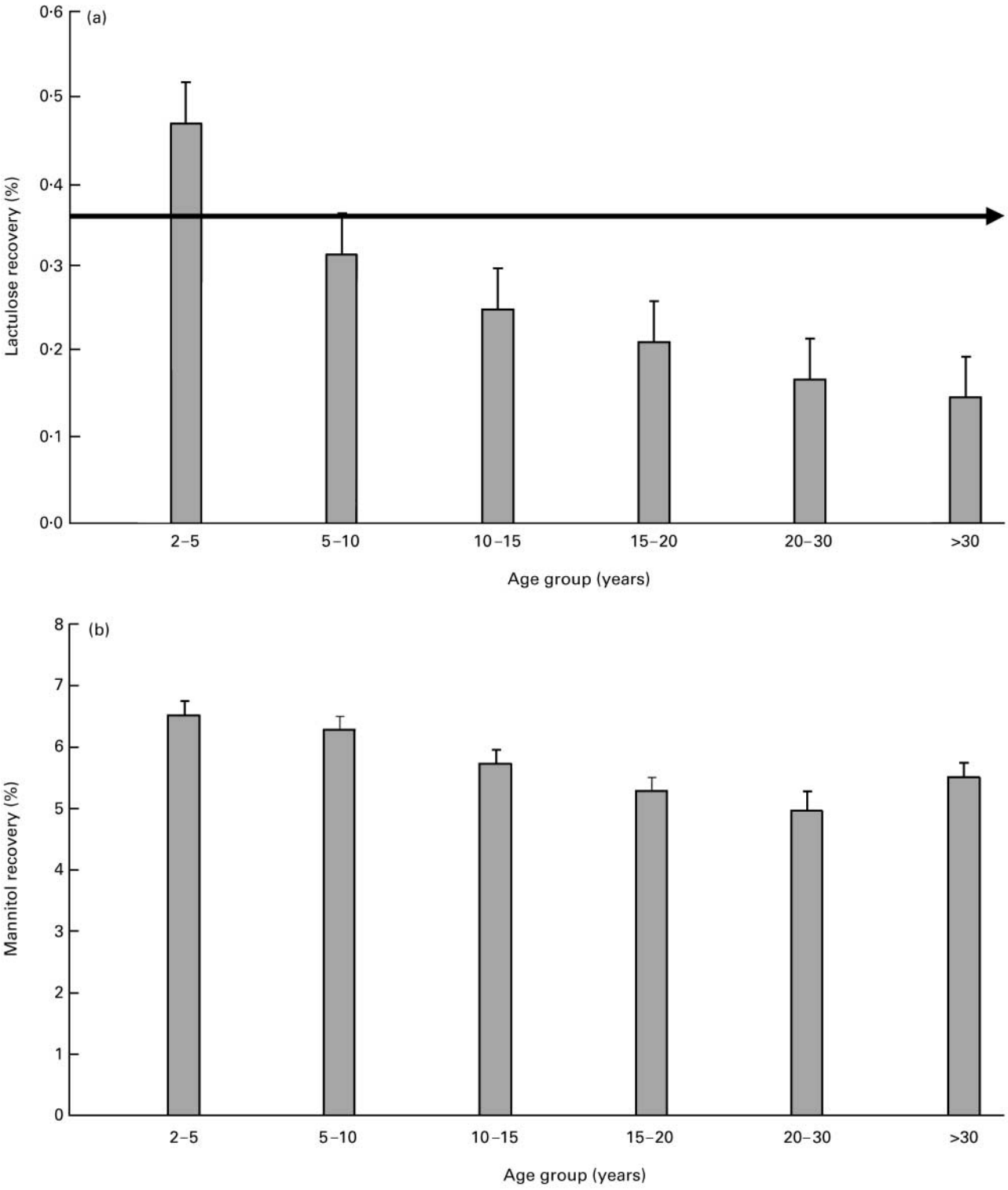

Fig. 2. Effect of age on the recovery of (a) lactulose and (b) mannitol with age. For details of subjects and procedures, see p. 500. Values are means for about thirty subjects per age group with standard errors shown by vertical bars. →, UK upper limit of normal (Freeman *et al.* 1995).

maldigestion and recurrent infections or other inflammatory illnesses. An alternative, though not mutually exclusive explanation concerns the enteropathy, which may not only cause malabsorption and/or maldigestion, but may also predispose to translocation of bacteria and toxins from the lumen of the gut into the systemic circulation (Lunn, 2000). The present study demonstrates for the first time that the enteropathy previously described in

infants (Lunn *et al.* 1991*a*) persists throughout childhood into adulthood. The L:M permeability ratio was particularly elevated in the 2–5-year-old group, who also had the poorest nutritional status. Although permeability then improved substantially with increasing age, mean values never fell into the range found in normal UK subjects (Travis & Menzies, 1992). This latter result is consistent with other studies that have shown that adults in tropical climes have significantly higher intestinal permeability (Menzies *et al.* 1999).

The recoveries of the individual permeability test sugars (%) indicate that L:M values in children aged 2–10 years were elevated because of both reduced M and elevated L uptake, suggesting that both barrier and absorptive functions of the small intestine were compromised. Beyond age 10 years however, L recovery fell into the normal UK range, which is consistent with the finding of Menzies *et al.* (1999) that L uptake in adults from tropical climes is not significantly different from European adults. M uptake by the small intestine did not improve with age and resulted in the persistently elevated L:M ratios in the 10–15-year-old and older age groups (Travis & Menzies, 1992).

Across the age range, intestinal permeability was associated with height attained; the greater the L:M ratio, the less the subjects height-for-age, assessed as HAZ. In addition, the correlation of L recovery with HAZ suggests that most of the relationship with height growth was associated with compromised barrier function, a finding also in agreement with studies on infants (Lunn, 2000). Despite the remarkably low uptake of M, which suggests reduced small intestinal absorptive capacity, there was no relationship between this variable and either HAZ or WAZ between the two study visits. One explanation for this result could be that despite a substantially reduced absorptive surface area (Sullivan *et al.* 1991), the digestive and absorptive capacity of the small intestine of these older children and adults may remain within its functional reserve until the abnormality becomes severe.

The change in HAZ (although not in WAZ or BMIZ), which occurred between the two study days, was also related to the mean of the two L:M ratio estimated, the higher the ratio, the poorer the growth. These results show that the mucosal enteropathy, although less severe than that seen in infants, continues to be associated with poor height growth of these older children. Although the time between visits was only 3·5 months, significant increases in height were recorded in all age groups <20 years old. The increments were small, but assuming a precision of 0·0057 m for a change in height (0·0049 m for a single measurement of height), it can be calculated that measurement error accounts for only 34, 25, 10 and 55 % of the associated variance in the four youngest age groups shown in Table 4 respectively. A study involving a mean change in height as small as 0·010 (SD 0·011) m (n 20) has the power to detect a significant result ($P<0·05$) with a power of 97 %, although there is obviously less confidence in the results of individual subjects.

Previous studies in Gambian children have reported that intestinal permeability is more closely related to impaired growth in height (stunting), than weight or BMI (wasting) (Lunn *et al.* 1991*a*; Lunn, 2000). This may be due to substantial short-term fluctuations in weight that results from diarrhoea and infective illnesses. Thus, intestinal permeability was not related to the deterioration in WAZ and BMIZ that occurred between the study visits. This reduction in weight status was expected since the first visit was in the dry season and the second within the rainy part of the year (Prentice & Cole, 1994)

It is surprising that the relationship between this intestinal permeability and HAZ persists even in young adults, when growth has ceased. One interpretation of this finding is that after restraining growth during the growth period, the mucosal damage may persist, so that the L:M ratio remains elevated in individuals of short stature. The present study also showed that the indices of intestinal status persisted in the same subjects over the 3·5-month period between the studies (r 0·66, 0·55 and 0·24 for the L:M ratio, L and M recoveries respectively) even though virtually all values were abnormal. Since small intestinal mucosal enteropathy is at its most severe during the first year of life (Lunn *et al.* 1991*b*), it is important to know whether the intestinal changes that occur at this very early stage are persistent in the same individuals and restrain growth throughout the growth period.

In the population studied, there was a difference in anthropometric status between the sexes from adolescence onwards. However, intestinal permeability values did not differ between the sexes at this age; thus, differences in small intestinal status could not explain why women had a better nutritional status than men in the study villages.

In conclusion, the results confirm the presence of small bowel mucosal enteropathy (as assessed by the L:M permeability test) in rural Gambians, and demonstrate for the first time that it persists at all ages from 2 to 60 years. They also demonstrate that the relationship between intestinal permeability and attained height is present at all ages from childhood to adulthood. Until now, such relationships had only been reported during the first 18 months of life. The present study, therefore, raises the possibility that a persistent intestinal enteropathy may be the cause of poor growth throughout the growth period and may be responsible for short stature in adults. However, a direct causal relationship can only be made on the basis of an intervention study. Reduced absorptive capacity and impaired barrier function have both been demonstrated in the small intestine and both abnormalities may contribute to impaired growth as previously described (Lunn, 2000).

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