

RESPONSES OF SMALL INTESTINAL ARCHITECTURE AND FUNCTION OVER TIME TO ENVIRONMENTAL FACTORS IN A TROPICAL POPULATION

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Abstract. To determine the response of the small intestinal mucosa to environmental conditions, we studied changes in mucosal architecture and function in a longitudinal cohort study in African adults. Over three consecutive years, 238 adults submitted monthly stool samples for parasitologic and bacteriologic analysis and underwent an annual endoscopic jejunal biopsy for mucosal morphometry. Absorption and permeability assays were performed on the same day as the enteroscopy. Variation in mucosal architecture and function was correlated with environmental factors and stool microbiology. The whole cohort had structural and functional evidence of tropical enteropathy, but structure and function were only weakly correlated. There were marked changes over time, and seasonal variation was observed in villous height (16%), xylose recovery (16%), and permeability (28%). Asymptomatic intestinal infections were common. Enteropathy was more severe in participants with *Citrobacter rodentium* or hookworm ova in the stool sample taken one month before the investigations were performed.

INTRODUCTION

The development of the crypt-villus unit depends critically on the microflora, which is the environment of the enterocyte at its luminal surface. *In utero*, finger-like villi develop first, followed by crypts.^{1,2} However, as the neonate is exposed to the rapidly established intestinal flora, villous blunting occurs, together with a change to broader villous morphologic forms such as leaves and ridges. During childhood, in temperate but not tropical populations, these villi subsequently change back to the adult finger-like phenotype.³ These changes occur contemporaneously with maturation of the mucosal immune system and exposure to the intestinal flora is required for this maturation to occur.⁴ Physical and immunologic components of the mucosal barrier thus depend for their development on the interaction between the host and its luminal environment. In tropical populations, small intestinal mucosal architecture and function appear to be enteropathic, i.e., resemble an apparently damaged phenotype, referred to as tropical enteropathy.^{5,6} Studies in migrants indicate that these changes are partially reversible,^{7,8} suggesting that environmental factors are important in influencing mucosal structure and function. These environmental factors are probably infective, but undernutrition may contribute. In children recovering from persistent diarrhea and malnutrition, the gross morphology of the proximal small intestinal mucosa (i.e., the pattern of leaves and ridges) does not change during recovery,⁹ but morphometric analysis indicates that both mucosal thickness and crypt cell proliferation increase.¹⁰ In adults with primary undernutrition, structure and function can recover on a solely nutritional therapeutic program.¹¹ In general terms, the plasticity of mucosal architecture in humans has not been the focus of much research because of the need for repeated biopsies, and it cannot be precisely predicted from intestinal permeability measurements.¹²

We postulated that tropical enteropathy is a small intestinal mucosal response to environmental influences in adult life. To test this hypothesis, we performed a longitudinal cohort study in healthy adults in a tropical population and related small intestinal structure and function to the frequency and

type of asymptomatic intestinal infection. In this population, the seroprevalence of human immunodeficiency virus (HIV) is stable at approximately 20–30%,¹³ so the influence of HIV was also analyzed.

METHODS

Study design and participants. A cohort study of adults (greater than 18 years of age) was set up in one sector of Misisi township in Lusaka, the capital of Zambia. The study was reviewed and approved by the Research Ethics Committees of both the University of Zambia and the London School of Hygiene and Tropical Medicine. The cohort was reasonably representative of the population from which it was drawn except that young men were somewhat under-represented.¹⁴ Participants were enrolled in a program of investigation and treatment of each episode of ill-health for three years. They were then asked to attend once a year for investigation, when blood tests, enteroscopy with jejunal biopsy, and sugar absorption/permeability tests were carried out over the course of a whole day in the endoscopy unit. Testing for HIV was conducted and CD4 counts were determined each year of the study using a FACSCount analyzer (Becton Dickinson, Franklin Lakes, NJ) according to the manufacturer's instructions.¹⁴ Endoscopy was deferred in participants who were pregnant or lactating, and also in those who had experienced diarrhea and had taken antibiotics or non-steroidal anti-inflammatory drugs until one month had elapsed.

During the setting up of the study, monosaccharide/disaccharide absorption and permeability testing was conducted by members of the study team on themselves. These subjects, drawn from the same ethnic group but of higher socioeconomic status, were subject to the same exclusion criteria listed earlier and were used as a control group solely for comparison of sugar test results.

On the day of investigation for cohort participants, enteroscopy was performed with an Olympus SIF-10 enteroscope (KeyMed, Ltd., Southend on Sea, Essex, United Kingdom) under sedation. No significant adverse events were encountered. Three biopsies were collected under direct vision from

proximal jejunum and placed in normal saline. One hundred milliliters of a solution of four test sugars was then instilled immediately into the jejunum down the biopsy channel and flushed through with another 100 mL of water. At the end of the procedure, biopsies were mounted on to cellulose acetate filter paper under a dissecting microscope and fixed in 10% formalin in saline ready for processing.

Morphometry. Biopsies were all processed by a single operator (CN) and orientated into wax prior to cutting 3- μ m sections for staining with hematoxylin and eosin. Sections were cut at three levels at 25- μ m intervals. Slides were evaluated by a senior pathologist (RF) before morphometry was performed by a single observer (PK). Morphometry was performed using a Zeiss (Thornwood, NY) KS400 image analysis system and a macro (written by SG) available from <http://www.smd.qmul.ac.uk/morbidanatomy/code/villi4.html>. This macro was designed to allow morphometry of mucosa with varying degrees of enteropathy (Figure 1). From each individual, one level of sectioning with the best orientation was chosen, and all mucosa were measured where the muscularis mucosae and epithelium were intact and where crypts could be seen to have been sectioned longitudinally. Where two villi were partly or fully fused, they were treated as one. In each field, a length of muscularis mucosae was identified underlying all mucosa where these criteria were satisfied and measured. The first two measurements were expressed in relation to a 100- μ m length of muscularis mucosae¹⁵ at a magnification of 63 $\times$. The following variables were then measured: 1) cross-sectional area of the villous compartment, which is representative of villous compartment volume (VCV); 2) total

length of epithelium, which is used as a measure of epithelial surface area (ESA); 3) villous height (VH), which is measured from villus tip to crypt-villus junction as judged by eye (care was taken to include every recognizable villous unit of well-orientated mucosa); 4) crypt depth (CD), which was measured in the same way, one for one with each villus; and 5) villous width (VW), which was measured automatically as the maximum width of the villus along a line perpendicular to VH in each unit of the villous compartment.

To define the reproducibility of the morphometric technique, measurements were repeated on eight biopsies to obtain confidence limits around these estimates (Table 1). To assess the variation between biopsies taken from the same individual, morphometric measurements were made on each biopsy individually in biopsies taken in triplicate from the same area of the jejunum. The variance ratio between biopsies from the same individual was 0.4 compared with 3.1 between individuals, indicating that only a small proportion (13%) of the variation seen between individuals could be accounted for by sampling variation between biopsies. Expressed another way, only 4 of 23 biopsies showed significant differences in VH between biopsies from the same individual (Figure 2). In biopsies taken in the second and third years, only VH and CD were measured.

Sugar testing of absorption and permeability. The four sugar test solution instilled into the small intestine contained 0.5 g of xylose, 1 g of rhamnose, 5 g of lactulose, and 0.2 g of 3-O-methyl D-glucose (Sigma Chemicals, Poole, Dorset, United Kingdom) in 100 mL of distilled water. Urine samples were collected prior to endoscopy and for exactly three hours afterwards and preserved with merthiolate (final concentration of approximately 0.04%). Preliminary work established that the ratios of recoveries of the four sugars over a three-hour period was not different from the results obtained over a five-hour period (Menzies I and Crane R, unpublished data). The three-hour test period was therefore used to allow several participants to complete all investigations over the course of one day. Urine samples were frozen at -80°C for transport and analyzed by thin-layer chromatography as previously described.¹⁶ In the presentation of results, xylose recovery, rhamnose: 3-O-methyl D-glucose (R:G) ratio, and the lactulose:rhamnose (L:R) ratio are presented and compared in Misisi participants and staff members against historical data.¹⁶

Environment: measures of exposure. To assess the influence of the environment on mucosal structure and function, several indicator variables were used: seasonality, a household hygiene score, and direct measures of exposure to both pathogenic and non-pathogenic intestinal bacteria, protozoa, and helminths.

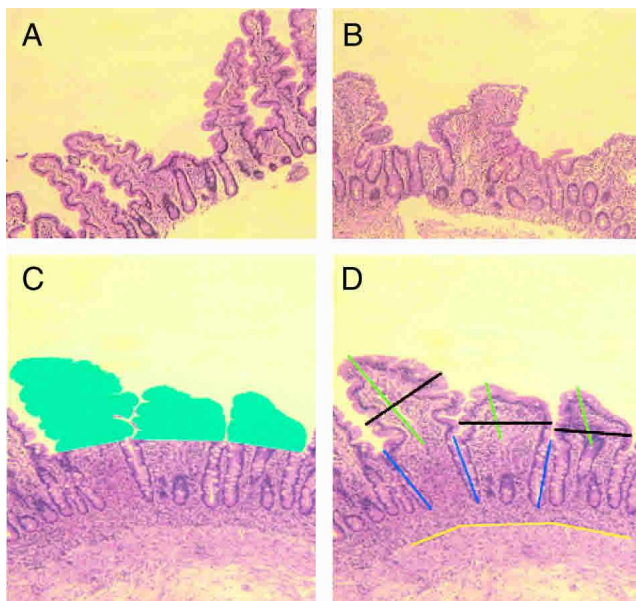


FIGURE 1. Morphometry of the jejunal mucosa in sections stained with hematoxylin and eosin and viewed on a video monitor at a magnification of 63 $\times$. Representative sections of mild (A) and more advanced enteropathy (B) are shown. A minority of sections showed more severe abnormalities. To quantify the architectural changes, automatic threshold definition was used (C) to measure villous compartment volume (VCV) and epithelial surface area (ESA), which were related to length of muscularis mucosae. Villous height, crypt depth, villous width, and muscularis mucosal length (used as a denominator for VCV and ESA measurements) were measured by eye using the cursor on the video screen (D).

TABLE 1
Reproducibility of morphometric measurements*

Measurement	Unit	95% CI	CV
VH	μ m	+25	5%
CD	μ m	+9	3%
VCV	μ m ²	+4,475	11%
VW	μ m	+41	13%
ESA	μ m	+83	10%

* Data were obtained from repeated measurements on eight biopsies. CI = confidence interval; CV = coefficient of variation; VH = villous height; CD = crypt depth; VCV = villous compartment volume (measured as area but representative of volume); VW = villous width; ESA = epithelial surface area (measured as length but representative of area). CI, confidence interval; CV, coefficient of variation.

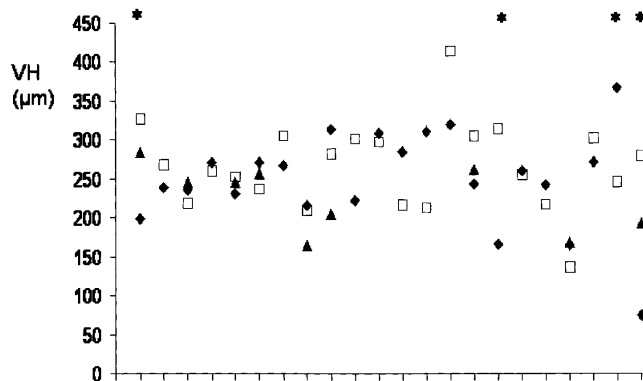


FIGURE 2. Scatter plot of villous height (VH) in individual biopsies taken from the same part of the proximal jejunum under direct vision from different study participants. Each tick mark on the x axis represents one individual, and 2–3 biopsies (shown by squares, triangles, and diamonds) were individually measured from each individual. Where individual biopsies differ significantly in VH, an asterisk is shown over the column for that individual (total of 4).

The household hygiene score used was constructed for the purpose of this study to allow comparison of the living conditions of each participant, and scores were given by the two nurses who visited the households on a daily basis. A scale of 1–10 was used, with up to two points being given for each of the following: overall cleanliness of the house, water storage facilities, food storage facilities, handwashing facilities and their use, and sanitation facilities.

Diarrhea incidence was measured by recall using a simple questionnaire administered every two weeks, and by active case-finding while offering investigation and treatment of each episode. Every month, participants submitted a stool sample that was analyzed for the presence of protozoa, bacteria, and helminths. Samples were plated onto MacConkey, xylose-lactose-sucrose, sorbitol-MacConkey, *Yersinia* selective, and thiosulfate-citrate-bile salt agar plates for culture of non-typhoid *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Citrobacter rodentium*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, and *Vibrio cholerae*. No isolates of *Yersinia* or *Plesiomonas* were obtained and they are not considered further. Colonies of non-lactose fermenting bacteria were subjected to biochemical characterization using triple-sugar-iron, lysine-iron, sulphide-indole-motility, urea, and Simmons citrate media, and interpreted using Cowan & Steel's manual.¹⁷ Isolates picked at random were confirmed using the API 20E system (BioMerieux, Inc., Hazelwood, MO). Micro-aerophilic conditions for isolation of *Campylobacter jejuni* were difficult to maintain and results for this organism are omitted. Protozoa and helminths (*Cryptosporidium parvum*, *Isospora belli*, microsporidia, *Giardia intestinalis*, *Entamoeba histolytica/dispar*, *Blastocystis hominis*, *Endolimax nana*, *Chilomastix mesnili*, *Iodamoeba butschlii*, *Entamoeba hartmanni*, *Hymenolepis nana*, *Strongyloides stercoralis*, *Ascaris lumbricoides*, hookworm, and *Taenia saginata*) were identified using wet preparation smears and smears stained with modified Ziehl-Neelsen and modified trichrome stains (for microsporidia).

Data analysis. Analysis was carried out using STATA version 7.0 (Stata Corp., College Station, TX). Morphometric variables and three of the sugar recoveries were normally distributed, but lactulose recovery was markedly skewed.

Therefore, for most variables, parametric tests (*t*-test or analysis of variance) and linear correlations were used, but for comparisons involving lactulose recovery, a non-parametric test (the Kruskal-Wallis test) and Spearman's rank correlation coefficient were used. Correlations are only presented when statistically significant ($P < 0.05$).

Longitudinal changes were analyzed as continuous variables and by identification of individuals in whom changes exceeded pre-specified confidence limits. These were 25 μm or 9 μm for VH and CD, respectively, being the 95% confidence limits of reproducibility (Table 1), and 0.018 and 0.040 for L:R ratio and R:G ratio, respectively, being the 95% confidence limits around measurements in healthy residents of the United Kingdom.¹⁶ To detect seasonal variation, measurements were made at different times of the year and analyzed in two-month periods that were related to rainfall and temperature (National Meteorological Office, Lusaka, Zambia). Matching between seasonal patterns was assessed by using the putative independent variable (e.g., incidence of diarrhea) to rank the two-month periods in ascending order and using a trend test to assess the effect of the ranked seasons on the dependent variable (e.g., VH).

Logistic regression models were constructed to evaluate the independent contributions of demographic, nutritional, and environmental variables. Morphometric or functional variables (VH, CD, xylose recovery, L:R ratio, and R:G ratio) were dichotomized around the median for use as outcome variables.

RESULTS

Two hundred two participants underwent investigation in the first year, 173 in the second year, and 101 in the third year. Thus, 238 adults (81 men, mean age = 40 years and 157 women, mean age = 32 years) underwent investigation on one or more occasions during the study. Biopsies were adequate for morphometric analysis in 173, 158, and 90, respectively. Sugar tests were completed in 180, 131, and 80 respectively. One hundred eighty study participants gave consent to HIV testing at some point and 57 (32%) were seropositive. The CD4 cell counts in HIV-seropositive participants (median = 319, interquartile range [IQR] = 167–467 cells/ μL) were lower than in HIV-seronegative participants (median = 774, IQR = 660–960 cells/ μL ; $P < 0.0001$).¹⁴ For further analysis, the cohort was divided into three groups: HIV seronegative ($n = 143$), HIV seropositive with CD4 cell counts ≥ 200 cells/ μL ($n = 50$), and HIV seropositive with CD4 cell counts < 200 cells/ μL ($n = 25$).

Morphometry: cross-sectional assessment at entry. Stereo-microscopy was performed after 164 enteroscopies to orientate the biopsies. None of these jejunal biopsies showed finger-like villi as the predominant form, but the severity of enteropathy varied considerably (Figure 1). Morphometric measures did not differ between men and women, nor were they related to age. The CD and VW were significantly greater in HIV-infected individuals (Table 2). In HIV-seropositive adults, VH was positively correlated with CD4 cell count ($r = 0.33$, $P = 0.03$).

Absorption and permeability: cross-sectional assessment at baseline. Xylose recovery (mean $\pm$ SD = $16.9 \pm 6.3\%$) and the R:G ratio (mean $\pm$ SD = 0.123 ± 0.052) were lower in 182

TABLE 2

Architectural and functional parameters in relation to human immunodeficiency virus (HIV) infection: cross-sectional data at baseline*

	HIV infection status				P
	HIV seronegative	HIV seropositive CD4 count ≥ 200	HIV seropositive CD4 count < 200	HIV unknown	
n	110	38	14	11	
VH (μm)	265 (49)	255 (47)	247 (39)	261 (48)	0.26
CD (μm)	149 (29)	173 (26)	174 (33)	158 (29)	< 0.0001
VW (μm)	193 (39)	209 (56)	217 (47)	158 (29)	0.05
VCV (μm^2)	16,659 (4,082)	16,733 (4,074)	18,195 (7,055)	15,717 (3,293)	0.47
ESA (μm)	458 (112)	425 (101)	415 (74)	438 (55)	0.13
n	114	41	16	12	
Xylose recovery (%)	17.5 (6.8)	17.1 (4.5)	13.3 (6.1)	14.6 (6.2)	0.047
Rhamnose recovery (%)	3.5 (1.5)	3.8 (1.8)	3.0 (2.8)	3.2 (1.7)	0.32
R:G ratio	0.118 (0.038)	0.129 (0.066)	0.131 (0.095)	0.128 (0.044)	0.04
L:R ratio	0.058 (0.038–0.096)	0.069 (0.054–0.100)	0.091 (0.064–0.232)	0.073 (0.045–0.097)	< 0.001

* Values are the mean (SD), except for the L:R ratio, which being markedly negatively skewed, is given as the median (interquartile range). Statistical testing was performed using analysis of variance, except for the L:R ratio, for which the test used was the Kruskal-Wallis test. R:G = rhamnose:glucose; L:R = lactulose:rhamnose. For definitions of other abbreviations, see Table 1.

Misisi study participants than in control tests undertaken by 13 team members (mean $\pm$ SD xylose recovery = $25.5 \pm 4.4\%$, mean $\pm$ SD R:G ratio = 0.164 ± 0.038 ; $P < 0.0001$ and $P = 0.006$, respectively). The L:R ratio was higher (median = 0.064, IQR = 0.043–0.105) in Misisi than in controls (median = 0.029, IQR = 0.025–0.033; $P = 0.004$). In a previous study of 68 healthy participants in a study in London,¹⁶ the mean (SD) xylose recovery was 33.1% (0.7%), the mean (SD) R:G ratio was 0.216 (0.5), and the mean (SD) L:R ratio was 0.026 (0.001). Both xylose recovery and the L:R ratio were significantly different in our staff members from London residents ($P < 0.001$ in both cases), but the R:G ratio did not differ. Functional studies therefore showed enteropathy in the Misisi cohort as a whole compared with local controls, and they in turn when compared with London residents.

Within the study population, recoveries of rhamnose, D-xylose, and 3O-methyl-D-glucose, and the R:G ratio were normally distributed and there was no difference between men and women. Lactulose recovery and the L:R ratio were higher in women than men ($P = 0.0001$). The L:R ratio, but not the R:G ratio, was higher in HIV-seropositive adults, more so in those with low CD4 cell counts (Table 2). The higher permeability in young women was still statistically significant after stratification by HIV status. In HIV-seropositive individuals only, xylose recovery was correlated with CD4 cell counts ($r = 0.53$, $P < 0.001$).

Structure: function correlation at baseline. There were few significant correlations between structure and function, and these correlations were modest: xylose recovery and VH ($r = 0.22$, $P < 0.01$), rhamnose recovery and ESA ($r = 0.19$, $P < 0.05$), L:R ratio and VH ($\rho = -0.26$, $P = 0.01$), and L:R ratio and ESA ($\rho = -0.30$, $P = 0.004$). Other possible pairwise correlations were not significant.

Longitudinal changes in architectural and functional parameters. Structure and function varied substantially within individuals over time (Figure 3). Over the two-year period between initial and final tests, 14 (24%) of 59 individuals with satisfactory biopsies at both time points showed an increase in VH over 25 μm , and 28 (47%) showed a decrease of greater than 25 μm . The CD varied in 69% of the participants over this time. Over two years, permeability (L:R ratio) increased in 21 (38%) of 55 adults in whom measurements were available throughout and decreased in 16 (29%). The R:G ratio showed less change, with 58% remaining within the limits

described earlier. The HIV-seropositive participants were more likely to demonstrate a decrease in VH over one (risk ratio [RR] = 2.5, 95% confidence interval [CI] = 1.03–6.2, $P = 0.03$) or two (RR = 3.0, 95% CI = 1.3–6.9, $P = 0.006$) years. Permeability changes did not differ significantly in HIV-seropositive and HIV-seronegative participants. Changes over one year in VH and CD were positively correlated ($r = 0.26$, $P = 0.008$) and changes in VH and permeability were negatively correlated ($\rho = -0.32$; $P = 0.01$).

Seasonality and diarrhea incidence. Seasonal changes were observed in VH (range of variation = 16%), L:R ratio (28%), and xylose recovery (16%), but not in CD or R:G ratio (Table 3). Diarrhea incidence was 0.52 episodes per year in HIV-negative participants and 1.33 episodes per year in HIV-seropositive participants. Diarrhea incidence showed a seasonal pattern with a peak in December–January (peak rainfall months) and a nadir in June–July (cold, dry season), which did not match the seasonality of VH or permeability. Only in HIV-seropositive adults was there an association between diarrhea incidence and mucosal parameters: the L:R ratio and R:G ratio were correlated with the rate of diarrhea two months prior to the date of investigation ($\rho = 0.54$ and $r = -0.48$, respectively, $P < 0.001$ for both).

Asymptomatic infections were detected in approximately half the stool samples examined (Table 4). Isolation rates of several organisms were also seasonal, i.e., *C. parvum*, microsporidia, *S. stercoralis*, *E. nana*, *H. nana*, *I. butschlii*, *E. hartmannii*, *Shigella* spp., *A. hydrophila*, and *C. rodentium*. The seasonal variation in VH matched that seen in *C. rodentium* ($P < 0.0001$).

Impact of environmental factors and asymptomatic infection. In logistic regression models, a hygiene score below the median was independently associated with CD above the median (odds ratio [OR] = 1.6; $P = 0.04$) and L:R ratio (permeability) above the median (OR = 2.5, $P < 0.001$). Infection with HIV was associated with VH below the median (OR = 2.1, $P = 0.005$) and CD above the median (OR = 4.2, $P < 0.001$). Thus, multivariate analysis showed an effect of HIV on VH not seen in univariate analysis (Table 2).

We analyzed the association between asymptomatic infection detected in stool samples (Table 4) and enteropathy as assessed on the day of investigation one month later. Organisms isolated during diarrheal episodes were not included because only participants who had been asymptomatic for at

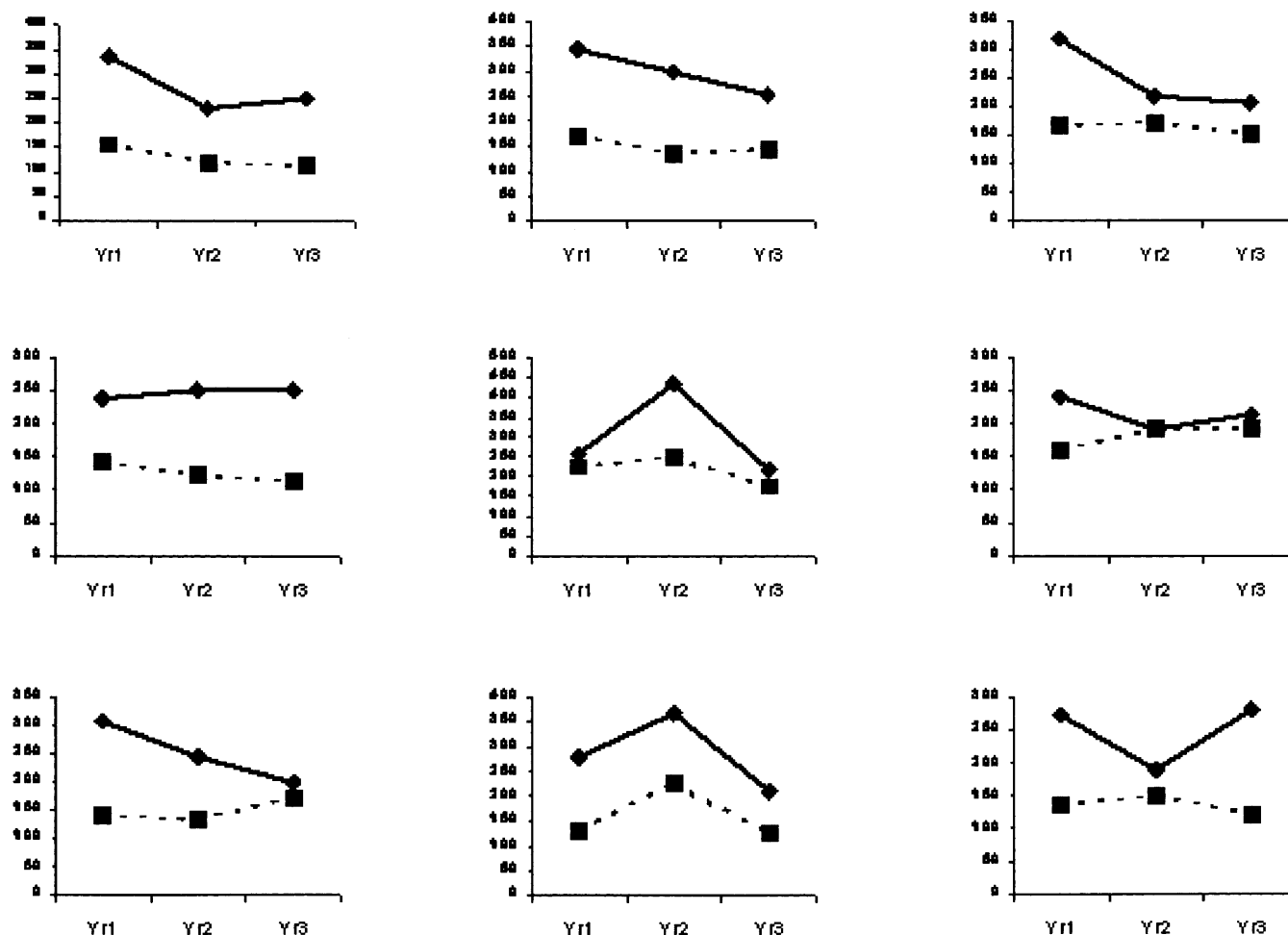


FIGURE 3. Changes in villous height (VH) (in μm) over time in nine selected participants to show the range of changes observed. $\blacklozenge$ = VH; $\blacksquare$ = crypt depth; Yr = year.

least one month (usually much longer) underwent small intestinal investigations. The VH was reduced in participants from whom *C. rodentium* had been isolated the month prior to biopsy (mean $\pm$ SD = $239 \pm 5 \mu\text{m}$) compared with those free of this infection ($257 \pm 3.5 \mu\text{m}$; $P = 0.03$), and permeability was higher in those who had had *C. rodentium* (mean = 0.101, IQR = 0.06–0.13) compared with those who had not (0.06, 0.05–0.09; $P = 0.008$). Crypt depth was increased in people with hookworm infection (mean $\pm$ SD = $193 \pm 13 \mu\text{m}$) compared with those without this infection ($155 \pm 2 \mu\text{m}$; $P < 0.001$). The negative effect of *C. rodentium* infection on VH,

the positive effect of *C. rodentium* on the L:R ratio, and of hookworm on CD were confirmed in logistic regression models.

DISCUSSION

The etiology and significance of tropical enteropathy have been uncertain since the description of this phenomenon more than three decades ago. To our knowledge, these are the first data on changes in both mucosal architecture and function over time in an unselected adult population, and

TABLE 3
Seasonal variation in jejunal architecture and absorption/permeability*

Months	n	VH (μm)	CD (μm)	Xylose (% recovery)	L:R	R:G
Jan, Feb	31	234 (44)	146 (23)	16.4 (6.5)	0.077 (0.051–0.138)	0.115 (0.086)
Mar, Apr	100	277 (54)	162 (35)	19.5 (7.4)	0.080 (0.055–0.110)	0.104 (0.043)
May, Jun	146	262 (45)	156 (30)	16.7 (6.3)	0.060 (0.042–0.093)	0.115 (0.049)
Sep, Oct	122	254 (47)	154 (27)	18.4 (7.2)	0.058 (0.041–0.086)	0.126 (0.037)
Nov, Dec	22	244 (48)	154 (21)	18.4 (7.4)	0.076 (0.059–0.112)	0.120 (0.025)
P		<0.0001	0.12	0.04	0.006	0.07

* Values are the mean (SD) except for the L:R ratio, which is the median (interquartile range). P values refer to results of analysis of variance across all groups, except for the L:R ratio, which was derived using the Kruskal-Wallis test across all groups. No sampling was performed in July or August. Note the parallel seasonal variation in VH, CD, and xylose recovery, but not the other variables. For definitions of abbreviations, see Tables 1 and 2.

TABLE 4

Intestinal infections detected in 3,260 monthly samples from asymptomatic participants*

Organism	Frequency of isolation	Frequency of isolation one month before investigation
<i>Cryptosporidium parvum</i>	31	1
<i>Isospora belli</i>	11	0
Microsporidia	5	0
<i>Giardia intestinalis</i>	40	5
<i>Blastocystis hominis</i>	236	19
<i>Ascaris lumbricoides</i>	489	33
Hookworm	92	13
<i>Strongyloides stercoralis</i>	11	0
<i>Schistosoma mansoni</i>	12	0
<i>Trichuris trichiura</i>	6	0
<i>Taenia saginata</i>	7	0
<i>Iodamoeba butschlii</i>	120	5
<i>Entamoeba histolytica/dispar</i>	12	0
<i>Entamoeba hartmanni</i>	47	4
<i>Chilomastix mesnili</i>	208	25
<i>Endolimax nana</i>	259	12
<i>Hymenolepis nana</i>	19	2
<i>Salmonella</i> spp.	44	4
<i>Shigella</i> spp.	2	3
<i>Aeromonas hydrophila</i>	13	1
<i>Citrobacter rodentium</i>	608	42
<i>Vibrio cholerae</i>	3	0

* The table shows which organisms were isolated from asymptomatic participants and which organisms were isolated from participants in the month prior to investigations carried out (jejunal biopsy and sugar testing). It is apparent that for many organisms it is not possible to determine any effect on the mucosa since there were too few isolates in the month prior to investigation.

indicate that the human small intestine responds to environmental conditions in the tropics in a seasonal manner. The variation observed in this population was not explained by variation related to sampling. In this and many other tropical populations in the third millennium, HIV-related enteropathy is superimposed on tropical enteropathy, but this variability is common to both.

By European and North American norms,¹⁶ mucosal absorptive capacity and permeability were abnormal, and these abnormalities were more severe in adults in the cohort than in controls of higher socioeconomic status, probably reflecting their greater exposure to environmental noxa. This is consistent with the effect of socioeconomic conditions reported at a population level by Menzies and others.¹⁶ In that study, a tropical population of high economic status (Qatar) had minimal or no increase in permeability, and this is supported by more recent data from Singapore (Lim S and Menzies I, unpublished data). The enteropathy therefore seems to be a consequence of contaminated environmental conditions rather than climate *per se*. The relationship between the severity of enteropathy and asymptomatic infection during the month prior to investigation indicates that exposure to organisms of modest pathogenicity may be an important environmental factor contributing to tropical enteropathy. Our study was not designed to detect the influence of diarrhea-causing organisms and we deferred participants who had experienced diarrhea until one month had elapsed.

There were two organisms notably associated with more severe enteropathy in biopsies taken one month later: *Citrobacter rodentium* and hookworm. The frequency of isolation of *C. rodentium* was surprisingly high and we took care to confirm the identity of the isolates using the API system read manually. However, this finding needs further confirmation in

other geographic locations. These infections were not associated with diarrhea, nor with HIV, so it is unlikely that they merely behave as markers of a more generalized susceptibility to intestinal pathogens. Our analysis suggests that the effects of these organisms were specific, but an alternative possible explanation is that other organisms were isolated too infrequently to analyze their role with the same power. Indeed, the frequency of isolation of helminths is lower than in many tropical populations and our findings may be specific to this population. However, the seasonality of VH and *Citrobacter* incidence matched closely, giving further evidence to the idea that the association is specific. Enteropathy is well-recognized in patients with hookworm anemia,¹⁸ but *C. rodentium* is not known to be an intestinal pathogen in humans. It does cause intestinal damage in mice, a feature attributed to the bacterial expression of β -intimin^{19,20} and host expression of Th1 cytokines.²¹ Whether isolates of *C. rodentium* from our population alter small intestinal structure and function through β -intimin expression is a question for further work. There are other intestinal infections which would be worth examining in such a study, including enterovirulent *Escherichia coli* and viruses, but time and resources did not permit their inclusion.

There was a lack of correlation between architectural and functional measures, and a lack of parallelism in their associations with other variables. Permeability was higher in women, but no sex difference was evident in architectural parameters. It was clear that CD was higher in HIV-infected adults irrespective of CD4 cell counts, but permeability was increased only in the group with low CD4 cell counts. This dissociation has previously been documented in HIV-infected adults living in London.²² Correlations between the temporal changes in structural and functional parameters were also weak. Although there is some evidence that permeability predicts architecture in jejunal biopsies,²³ more recent evidence suggests that reduction in permeability precedes restoration of villous architecture in patients with celiac disease after gluten elimination.¹² One explanation for the weak correlation between structure and function may be that functional studies provide information about the whole jejunum and ileum, beyond the biopsy site in the proximal jejunum. Interestingly, the correlation between VH and xylose recovery was closer than that with rhamnose or 3-O-methyl-D-glucose recovery, which might reflect the fact that xylose is largely absorbed in the proximal jejunum. Another explanation for the lack of correlation could be that the dynamics of villous and crypt architecture are determined by different cellular and molecular processes than those that control absorptive capacity and permeability. Permeability reflects tight junction function, which is under the control of at least one newly described molecule, zonulin.²⁴

In this population, VH and CD were positively correlated at baseline, over time, and seasonally, but the process which leads to these parallel changes is unclear. T cell activation *in vitro* induces either an enteropathy characterized by villous atrophy and crypt hyperplasia or a mucosal destruction,²⁵ and the same pattern is seen in celiac disease.¹⁵ T cell activation was associated with reduced VH and increased CD observed in Zambia compared with a South African black population without tropical enteropathy.²⁶ However, in our cohort, the positive correlation between VH and CD suggests that T cell activation is not the principal determinant of the variation in enteropathy within this population. In animal models, starva-

tion alters function,²⁷ and undernutrition leads to true mucosal atrophy (i.e., reduced VH and CD in parallel). In humans in the short term, starvation can induce reduced absorptive capacity and increased permeability.^{28,29} Malnutrition in children is seasonal.³⁰ We have previously reported that CD and body mass index were positively correlated in adults with advanced acquired immunodeficiency syndrome (AIDS)-related diarrhea and malnutrition,³¹ but further work is required to confirm and explain this finding.

Infection with HIV was associated with reduced VH and increased CD, but not with permeability or absorption. This is consistent with previous morphometric analysis in HIV-infected adults with or without AIDS.^{32,33} However, xylose recovery and permeability were altered when CD4 cell counts were reduced, again consistent with previous studies.³⁴ It remains to be established whether the mucosal changes seen in HIV infection are due to HIV itself, to immunologic changes,³⁵ or to secondary opportunistic infection, but it is well-established that anti-retroviral therapy leads to improvement in many of the pathologic changes observed.³⁶ Our data suggest that in this population the earliest significant change in the mucosa of HIV-infected adults is increased CD.

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