Intestinal absorptive capacity, intestinal permeability and jejunal histology in HIV and their relation to diarrhoea

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Abstract

Intestinal function is poorly defined in patients with HIV infection. Absorptive capacity and intestinal permeability were assessed using 3-O-methyl-D-glucose, D-xylose, L-rhamnose, and lactulose in 88 HIV infected patients and the findings were correlated with the degree of immunosuppression (CD4 counts), diarrhoea, wasting, intestinal pathogen status, and histomorphometric analysis of jejunal biopsy samples. Malabsorption of 3-O-methyl-D-glucose and D-xylose was prevalent in all groups of patients with AIDS but not in asymptomatic, well patients with HIV. Malabsorption correlated significantly ($r=0{\cdot}34$–$0{\cdot}56$, $p<0{\cdot}005$) with the degree of immune suppression and with body mass index. Increased intestinal permeability was found in all subgroups of patients. The changes in absorption-permeability were of comparable severity to those found in patients with untreated coeliac disease. Jejunal histology, however, showed only mild changes in the villus height/crypt depth ratio as compared with subtotal villus atrophy in coeliac disease. Malabsorption and increased intestinal permeability are common in AIDS patients. Malabsorption, which has nutritional implications, relates more to immune suppression than jejunal morphological changes.

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Keywords: HIV, AIDS, intestinal permeability, intestinal function, intestinal infection, intestinal absorption.

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Patients infected with HIV may develop diarrhoea, weight loss, and wasting but the prevalence, severity, cause, and contribution of malabsorption to these symptoms is controversial.[1–6] Some workers suggest that malabsorption is infrequent, mild, and of little clinical importance[2] while others have reported severe malabsorption in patients with AIDS associated with cryptosporidial diarrhoea which is purported to correlate to the degree of villus atrophy.[1] It is nevertheless unclear when, in the natural history of HIV, disease malabsorption becomes evident and how it interrelates with symptoms of weight loss and diarrhoea.

We studied intestinal function with respect to absorption and permeability markers in a large group of patients with HIV infection at various stages and correlated the findings with symptoms of diarrhoea, weight loss, degree of immune suppression, and jejunal morphology. Patients with untreated coeliac disease have been used for comparison to enable functional-morphometric interpretation of results in HIV patients with malabsorption.

Subjects and methods

SUBJECTS

Fifty seven healthy volunteers (21 women) acted as controls for the intestinal absorption-permeability test (mean (SD) age 33 (7) years). Controls comprised 47 healthy, mainly medical and health service staff recruited for a number of previous studies conducted at Northwick Park Hospital and King's College Hospital and 10 men recruited from Chelsea Westminster Hospital during this study. None were at high risk for the HIV infection but they were not tested serologically before this study or subsequently.

Eighty eight HIV positive male homosexual patients were studied (mean (SD) age 39 (9) years). HIV was confirmed by both enzyme linked immunosorbent assay and western blot analysis. This group comprised 25 consecutive, well patients with HIV (out of 52 (48%) invited to participate) who had not had an AIDS defining illness and 22 (out of 43 (51%)) well patients with AIDS seeing a single physician in the Genitourinary Medicine outpatient department of Chelsea and Westminster Hospital. Patients who declined to participate in these studies did not differ significantly ($p>0{\cdot}4$) from those studied in respect of age, duration of AIDS, CD4 counts, body mass index, or serum albumin. The remainder comprised 34 consecutive inpatients with AIDS admitted to Chelsea and Westminster Hospital for gastrointestinal investigation who met the inclusion criteria (HIV positive, male homosexuals) and seven inpatients who were wasted but did not have gastrointestinal symptoms. Patients were specifically excluded if they had received a treatment course of antibiotics (including antimicrobial drugs) other than for *Pneumocystis carinii* prophylaxis in the preceding four weeks or if they required opioids for control of diarrhoea.

According to the revised classification system of the Centres for Disease Control,[7] and taking into account the European modification of this classification,[8] 25 patients were at stage 2 – asymptomatic and without an AIDS

TABLE I *Demographic details of patients infected by HIV (values median (range))*

		AIDS			
	HIV (well)	*Well*	*Wasted*	*Pathogen −ve diarrhoea*	*Pathogen +ve diarrhoea*
No of patients	25	22	7	7	27
Age (y)	37 (25–54)	39 (27–48)	36 (32–60)	39 (33–50)	35 (22–50)
Duration of AIDS (mth)*	–	11 (3–94)	6 (4–26)	15 (7–20)	23 (3–46)
CD4 counts†	295 (4–555)	67 (8–171)	40 (14–67)	18 (2–44)	29 (5–204)
Body mass index‡	24 (18–28)	21 (18–27)	16 (14–19)	19 (18–21)	18 (14–23)
Serum albumin§	39 (19–43)	37 (21–49)	26 (14–47)	32 (26–38)	33 (19–44)

*From the time of an AIDS defining illness. There was no significant difference ($p>0.34$) in the duration of AIDS between the subgroups.
†Lower limit of normal is 600. HIV well patients had significantly ($p<0.025$) greater CD4 counts than patients with AIDS apart from wasted AIDS patients ($p=0.18$). There was no significant difference between the subgroups of patients with AIDS ($p>0.09$).
‡Lower limit of normal is 20. The HIV well patients had significantly ($p<0.02$) higher BMI than the patients with AIDS apart from the AIDS well patients ($p=0.08$).
§Lower normal limit is 35 g/l. HIV well patients differed significantly ($p<0.05$) from wasted and pathogen positive patients with AIDS, but there was otherwise no significant difference between the various subgroups.

defining illness (infection with *P carinii*, *Candida albicans* oesophagitis, etc). All the remaining 63 patients were stage 4 and had AIDS (all but one had CD4 counts below 200×10^6/l). These patients were further subclassified clinically and on the basis of intestinal pathogen status as follows:
(i) AIDS well – 22 patients were asymptomatic with no history of weight loss or diarrhoea.
(ii) AIDS wasted – seven patients who had greater than 10% weight loss in the preceding month without any diarrhoea. Five had recently suffered from opportunistic infections (*P carinii* (n=4) and cytomegalovirus (n=1)). Two had Kaposi's sarcoma.
(iii) Thirty four patients had had diarrhoea (more than three liquid motions a day, for over a month). After investigation, detailed below, these were further separated into a group of seven in whom no intestinal pathogens were found (pathogen negative group) and the remaining 27 who had cryptosporidia (n=17), microsporidia (n=6), or both (n=4). Demographic details of the HIV infected patients are shown in Table I.

Most of the patients were on drug treatment. Those with CD4 counts below 200 received treatment with dapsone (100 mg/d), pentamidine aerosol (300 mg/every third week) or co-trimoxazole (960 mg twice a day, three times per week) for *P carinii* prophylaxis.

Five of 25 stage 2 HIV, well patients and 29 of the patients with AIDS had been on regular antiretroviral therapy (zidovudine) for over a month, but all of the AIDS patients had been on the drug at some stage. A further 15 patients with AIDS were receiving oral acyclovir (400–3000 mg/d) for perianal or genital Herpes and 20 were on keto-, flu- or itraconazole (50–200 mg/d) for antifungal prophylaxis. Drugs were withheld for 12 hours before the absorption-permeability tests. None of the drugs listed above are known to affect intestinal integrity. In particular, studies in six male homosexuals (HIV well without an AIDS defining illness) have shown that short term zidovudine (10 day treatment) does not interfere with intestinal permeability.

Ten patients with newly diagnosed and untreated coeliac disease acted as a comparative group for these studies. All had been diagnosed on the basis of significant diarrhoea and weight loss with a jejunal biopsy specimen showing subtotal villus atrophy, and all responded to a gluten free diet.

These studies were approved by the local ethical committees of Camberwell and Westminster Health Authorities and all patients gave informed consent.

INVESTIGATIONS

The AIDS patients underwent a standard diagnostic gastrointestinal protocol which has been detailed previously.[9 10] Briefly, each was investigated by six stool examinations with light microscopy and culture, gastroduodenoscopy with duodenal aspiration and low duodenal (part 3) biopsy specimens, and sigmoidoscopy with rectal biopsy specimens. Well, stage 2 HIV patients had the stool examinations but not the endoscopies.

Body mass index (BMI: normal greater than 20) was calculated as weight (kg)/height^2 (m).[11] CD4 counts were assessed by flow cytometry and expressed as number of cells$\times10^6$/l.

JEJUNAL BIOPSY SPECIMENS AND MORPHOMETRIC ANALYSES

Thirty six stage 4 patients with AIDS, and all the patients with coeliac disease, underwent jejunal biopsy (Crosby capsule) just distal to the ligament of Treitz. Fifteen were from the AIDS well group and 21 with pathogen positive (n=17) or pathogen negative (n=4) diarrhoea. Morphometric analyses of jejunal biopsy samples was carried out by interactive image analyses (Joyce-Loebl magiscan) as previously described.[12] The histological slides and morphometric analyses was shared equally between two histopathologist neither of whom had any knowledge of the diagnostic categories of the patients. An internal quality control showed an interobserver difference of less than 8% for the villus height and crypt depth measurements. Mucosal height was measured as the distance between muscularis mucosa and tips of well oriented, vertically sectioned villi. Crypt depth was measured as the distance between the basement membrane and the open 'mouth' of each crypt. At least eight measurements were made from each biopsy and the data were averaged.

Sixty patients in whom a jejunal biopsy had been carried out as a part of gastroenterological investigation and in whom a final diagnosis of irritable bowel syndrome had been made acted as controls for the morphometric studies. Biopsy specimens were not available from the HIV well patient group.

INTESTINAL ABSORPTION-PERMEABILITY TEST

Following an overnight fast, subjects drank a 100 ml test solution (240 mOsm/l) containing:
- 3-O-methyl-D-glucose (0·2G)
- D-xylose (0·5 g)
- L-rhamnose (1·0 g)

TABLE II *Five hour urine excretion of test substances (values median % of dose excreted in five hours (interquartile range))*

	3-O-m-glucose	*D-xylose*	*L-rhamnose*	*Lactulose*	*Lactulose/L-rhamnose*
Control (n=57)	42·5 (39·4–50·0)	29·7 (26·2–34·7)	9·9 (8·6–12·3)	0·23 (0·21–0·39)	0·03 (0·02–0·03)
HIV-well (n=25)	47·8 (42·8–57·5)	39·0 (29·0–43·8)	12·3 (7·4–13·7)	0·38 (0·25–0·60)	0·04 (0·03–0·07)
AIDS:					
Well (n=22)	41·2 (24·7–54·0)	23·3 (16·4–30·9)	5·1 (3·8–9·3)†	0·38 (0·19–0·52)*	0·06 (0·04–0·11)†
Wasting (n=7)	27·0 (16·0–42·5)†	15·0 (8·8–27·6)†	2·8 (2·2–8·4)†	0·61 (0·31–0·69)*	0·12 (0·07–0·16)†
Path −ve (n=7)	37·5 (23·6–40·4)†	14·2 (6·9–23·8)†	4·7 (1·1–7·0)†	0·32 (0·17–0·73)	0·15 (0·07–0·19)†
Path +ve (n=27)	27·7 (13·0–33·6)†	9·6 (6·5–17·2)†	2·2 (1·3–5·0)†	0·44 (0·21–1·10)*	0·26 (0·08–0·33)†
Coeliac disease (n=10)	29·2 (23·3–38·4)†	18·7 (13·1–22·4)†	4·8 (4·1–6·6)†	0·55 (0·22–1·01)*	0·12 (0·07–0·17)†

*Differs significantly from control $p<0·05$. †Differs significantly from control $p<0·01$.

• Lactulose (5·0 g) (7·5 ml Duphalac syrup; 67% lactulose w/v syrup)

The solution was designed to assess active and passive carrier mediated transport and intestinal permeability (lactulose/L-rhamnose), respectively. Food and fluid were allowed two hours later. Complete urine collections into a contained containing 1 ml (19% w/v) mercurithiosalicylate (Merthiolate) as preservative for the sugar markers were made for five hours. Analysis of the sugar probes was done by a thin layer technique, as previously described,[13] which is both accurate and sensitive. All subjects abstained from alcohol for at least a week before the test,[14] no patient was taking non-steroidal anti-inflammatory drugs[15] or had medical conditions which are known to be associated with increased intestinal permeability.[16 17]

STATISTICAL ANALYSIS

Statistical differences between groups was assessed by the Mann-Whitney U test. Spearman's correlation coefficient was employed for the assessment of correlation's between parameters.

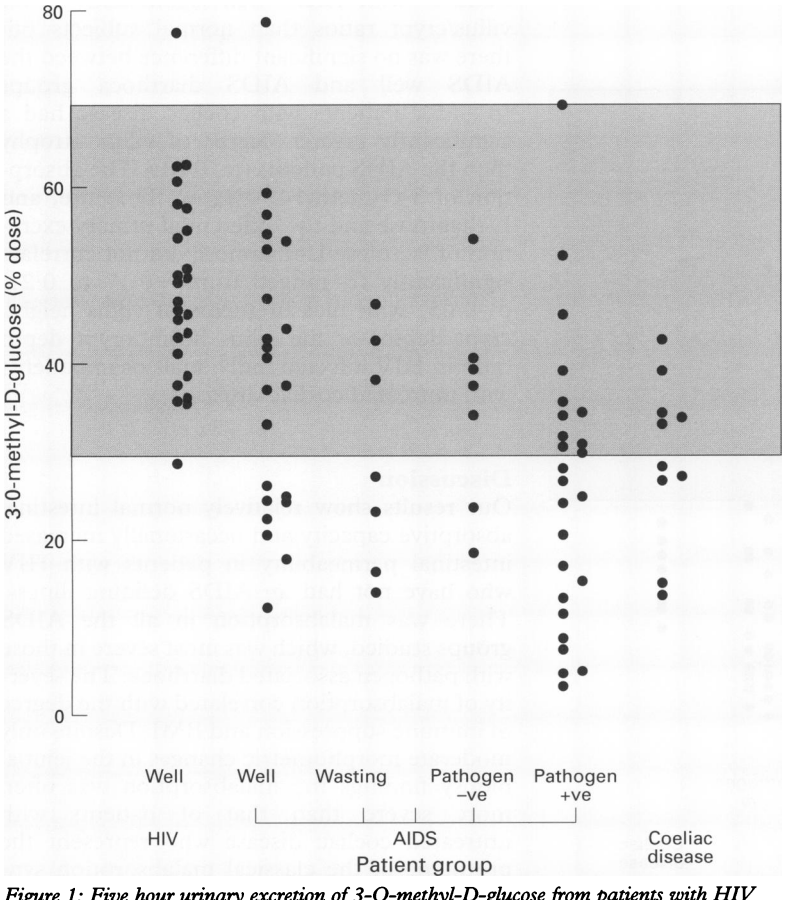

Figure 1: Five hour urinary excretion of 3-O-methyl-D-glucose from patients with HIV infection and coeliac disease. The shaded area represents the normal test range.

Results

INTESTINAL ABSORPTION AND PERMEABILITY

Table II shows the mean five hour urine excretion of the test substances from HIV infected patients and patients with coeliac disease. There was no significant difference between the HIV well patients and normal subjects. Patients with AIDS who were well had significant ($p<0·01$) malabsorption of L-rhamnose but not that of 3-O-methyl-D-glucose or D-xylose. Other patients with AIDS (wasted, pathogen negative and pathogen positive diarrhoea) had significant malabsorption of all three monosaccharides and to a similar or greater extent than patients with untreated coeliac disease.

Figures 1, 2, and 3 show the individual monosaccharide urine excretion data from the study groups. Malabsorption of monosaccharides was rare in patients with HIV stage 2. Patients with AIDS had variable prevalence of malabsorption of monosaccharides (Figs 1–3) ranging from 30% (seven of 23) AIDS well patients with malabsorption of D-xylose to 89% (24 of 27) AIDS patients with pathogen positive diarrhoea with malabsorption of L-rhamnose. AIDS patients with diarrhoea had significantly ($p<0·01$) greater malabsorption of the three monosaccharides than HIV well patients or AIDS patients without diarrhoea. Malabsorption in the AIDS patients was in many cases more severe than found in untreated coeliac disease.

The differential urinary lactulose/L-rhamnose excretion showed some striking changes (due to increased lactulose and decreased L-rhamnose urine excretion) and are shown in Figure 4. Five of 25 (20%) stage 2 patients had increased intestinal permeability. All the AIDS groups had significantly increased intestinal permeability with a frequency ranging from 50% (11 of 22) in well patients to nearly 100% in the other groups. AIDS patients with diarrhoea had significantly ($p<0·01$) greater changes in intestinal permeability than HIV well patients or AIDS patients without diarrhoea. The permeability changes in patients with AIDS were comparable to or more severe than those of the 10 untreated patients with coeliac disease.

There was a significant correlation between a rise in different urine excretion of lactulose/L-rhamnose and a depression in the absorption of

3-O-methyl-D-glucose ($r=-0.38$, $p<0.001$), and D-xylose ($r=-0.56$, $p<0.001$) in HIV infected patients. The permeability changes (lactulose/L-rhamnose) correlated significantly with BMI ($r=20.36$, $p<0.003$), and CD4 counts ($=-0.34$, $p<0.006$) but not with serum albumin ($r=-0.13$, $p>0.20$).

There was a significant correlation between BMI and the absorption of 3-O-methyl-D-glucose ($r=0.34$, $p<0.001$), D-xylose ($r=0.52$, $p<0.001$), and L-rhamnose ($=0.47$, $p<0.001$). There was also a significant correlation between CD counts and the absorption of 3-O-methyl-D-glucose ($r=0.43$, $p<0.001$). D-xylose ($r=0.54$, $p<0.001$), and L-rhamnose ($r=0.43$, $p<0.001$). Serum albumin concentrations correlated significantly with 3-O-methyl-D-glucose ($r=0.22$, $p<0.05$), D-xylose ($r=0.31$, $p<0.01$), and L-rhamnose ($r=0.27$, $p<0.03$).

The possible effect of zidovudine was assessed by comparing intestinal absorptive capacity and intestinal permeability between the AIDS patients who were taking ($n=29$) and not taking ($n=37$) the drug. There was, however, no significant difference ($p>0.17$) in these parameters between these groups.

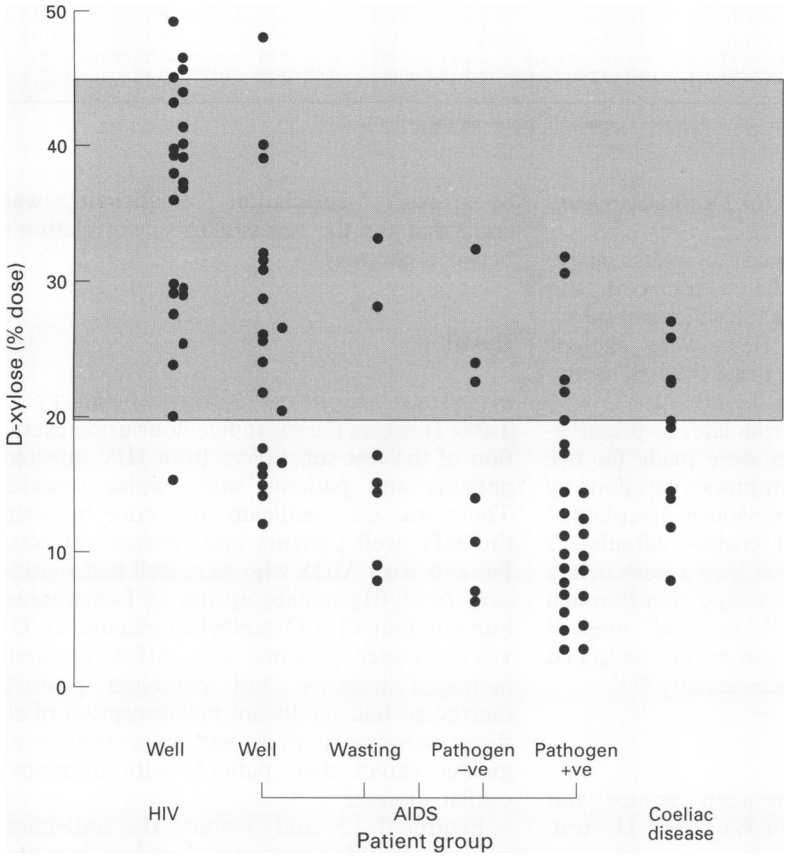

Figure 2: Five hour urinary excretion of D-xylose. The shaded area represents the normal test range.

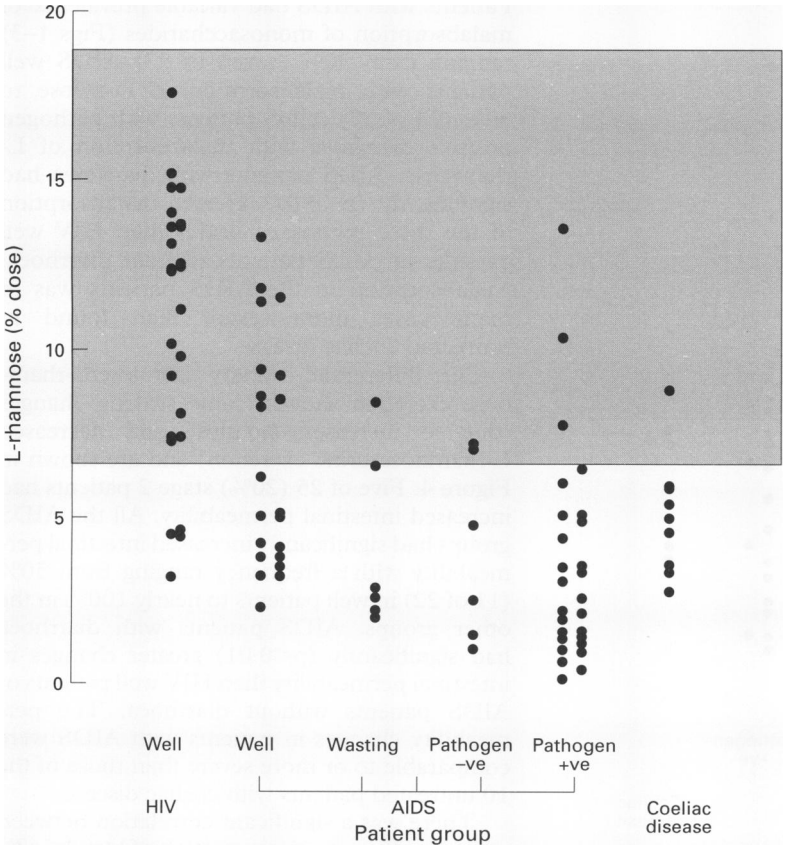

Figure 3: Five hour urinary excretion of L-rhamnose. The shaded area represents the normal test range.

HISTOMORPHOMETRIC ANALYSIS OF JEJUNAL BIOPSY SAMPLES

Table III shows the villus height and crypt depth from controls, patients with AIDS, and patients with coeliac disease. The villus height in patients with AIDS was within the control range but the crypt depth was significantly increased ($p<0.001$). Figure 5 shows the villus/crypt ratio, which is a proposed quantitative measure of the degree of villus atrophy. Patients with AIDS had significantly lower villus/crypt ratios than normal subjects but there was no significant difference between the AIDS well and AIDS diarrhoea groups ($p>0.5$). Patients with coeliac disease had a significantly greater degree of villus atrophy than the AIDS patients ($p<0.01$). The absorption of 3-O-methyl-D-glucose, D-xylose, and L-rhamnose and the differential urinary excretion of lactulose/L-rhamnose did not correlate significantly ($r=$ranged from -0.17 to 0.21, $p>0.05$) with measurements of villus height, crypt depth, or the villus height/crypt depth ratio in HIV infected individuals or in patients with untreated coeliac disease.

Discussion

Our results show relatively normal intestinal absorptive capacity and occasionally increased intestinal permeability in patients with HIV who have not had an AIDS defining illness. There was malabsorption in all the AIDS groups studied, which was most severe in those with pathogen associated diarrhoea. The severity of malabsorption correlated with the degree of immune suppression and BMI. Despite only moderate morphometric changes in the jejunal biopsy findings the malabsorption was often more severe than that of patients with untreated coeliac disease who represent the prototype of the classical malabsorption syndrome. Intestinal permeability was significantly increased in all the subgroups of AIDS

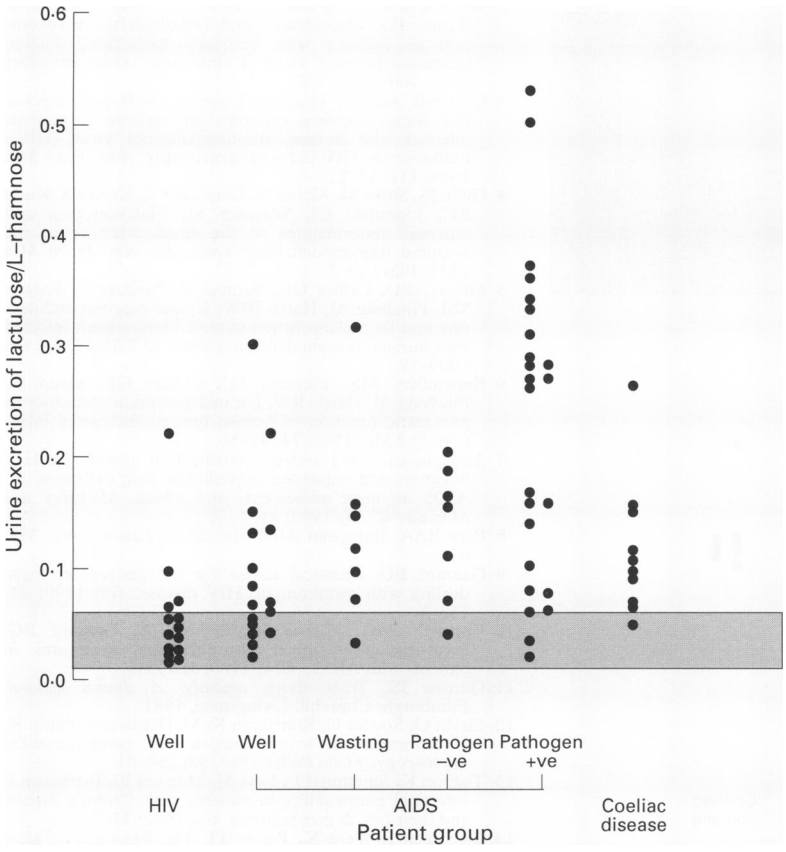

Figure 4: Differential urinary excretion of lactulose/L-rhamnose. The shaded area represents the normal test range.

and also correlated significantly with CD4 counts and BMI.

Malabsorption and increased intestinal permeability in patients with AIDS have different pathophysiological implications. The former may contribute to malnutrition and wasting while the latter may be important in predisposing to the development of a non-specific enteropathy.[18–20]

Previous studies assessing malabsorption in HIV infected patients have often employed a large test dose of D-xylose (25 g), which may in itself interfere with intestinal absorption.[21] Nevertheless, the impression is that patients with AIDS may have malabsorption, particularly those with cryptosporidial associated diarrhoea.[1–4] The prevalence of malabsorption has ranged from 28% to 100%, but the studies are confounded by the small numbers of patients studied and, at times, by inadequate description of the disease stage. We have not found significant malabsorption in asymptomatic HIV infected patients without AIDS. Many patients with AIDS, however, had evidence of malabsorption irrespective of symptoms, and in agreement with Kotler *et al*[1] and Miller *et al*[2] this was most severe in those with diarrhoea due to cryptosporidial or microsporidial infection. The jejunal morphometric findings frequently showed predominant crypt cell hyperplasia, in agreement with previous studies,[1 3–5 22] and only mild changes in the villus height/crypt depth ratio, which is a sensitive indicator of villus atrophy.[12] However, a previous study using different methods of histomorphometric analyses of jejunal biopsy samples, showed a significant correlation between the degree of mild partial villus atrophy and malabsorption parameters in HIV disease[5] suggesting a structure-function relationship. We and others[4] have not found this to be the case nor was there a significant correlation between the absorption or permeability parameters and the histological findings in patients with coeliac disease, in keeping with previous results.[23 24] If jejunal morphological changes (which may not be representative of the state of the more distal small intestine) were solely responsible for the malabsorption, it might be expected that patients with untreated coeliac disease would have more severe malabsorption than the AIDS patients. Indeed Ehrenpreis *et al*,[25] who assessed the kinetics of D-xylose absorption and disappearance following intravenous administration, concluded that the absorption constant of D-xylose was reduced out of proportion to the histological abnormalities of duodenal biopsy specimens in patients with AIDS.

The significant correlation in our study between the malabsorption parameters with the degree of immune suppression and BMI suggests that the reduced CD4 counts predispose to an intestinal infection or promote proliferation of the HIV within the intestine[26 27] (such as is the case in hepatitis A and rotavirus infections[28 29]) and the resulting malabsorption contributes to the wasting of these patients. This is clearly not the only explanation as some patients had wasting and normal absorptive capacity and vice versa, and many of those with severe diarrhoea had normal absorptive capacity while some without diarrhoea had malabsorption. This re-emphasises the multifactorial pathogenic mechanisms responsible for the clinical status of patients with AIDS.

Increased intestinal permeability was more common than malabsorption in patients with AIDS. Furthermore, the intestinal permeability changes were comparable or greater than those found in patients with untreated coeliac disease. There are only a few studies which have addressed intestinal permeability changes in HIV infection. Kapembwa *et al* reported increased intestinal permeability in AIDS patients with diarrhoea but findings in asymptomatic patients were normal.[30] However, there were methodological problems with analysis of sugars[31] and the question of 'topical enteropathy' in the Zambian subjects was not taken into account.[32 33] In agreement with our findings, Ott *et al* found an increased permeability in patients with AIDS regardless of intestinal symptoms, which was most noticeable in patients with intestinal cryptosporidial infection and quantitatively comparable with that of patients with Crohn's and coeliac

TABLE III *Morphometric analyses of jejunal biopsy samples (values median (interquartile range))*

Patient group (no)	*Villus height (μm)*	*Crypt depth (μm)*	*Villus/crypt ratio*
Normal (60)	510 (461–566)	133 (126–148)	3·65 (3·23–4·08)
AIDS well (15)	564 (483–629)	229 (192–294)*	2·40 (2·00–2·60)*
AIDS diarrhoea (22)	560 (500–638)	247 (174–290)*	2·25 (2·00–2·73)*
Coeliac disease (10)	422 (398–461)*	364 (334–392)*	1·19 (1·15–1·26)*

*Differed significantly from normals $p<0·001$.

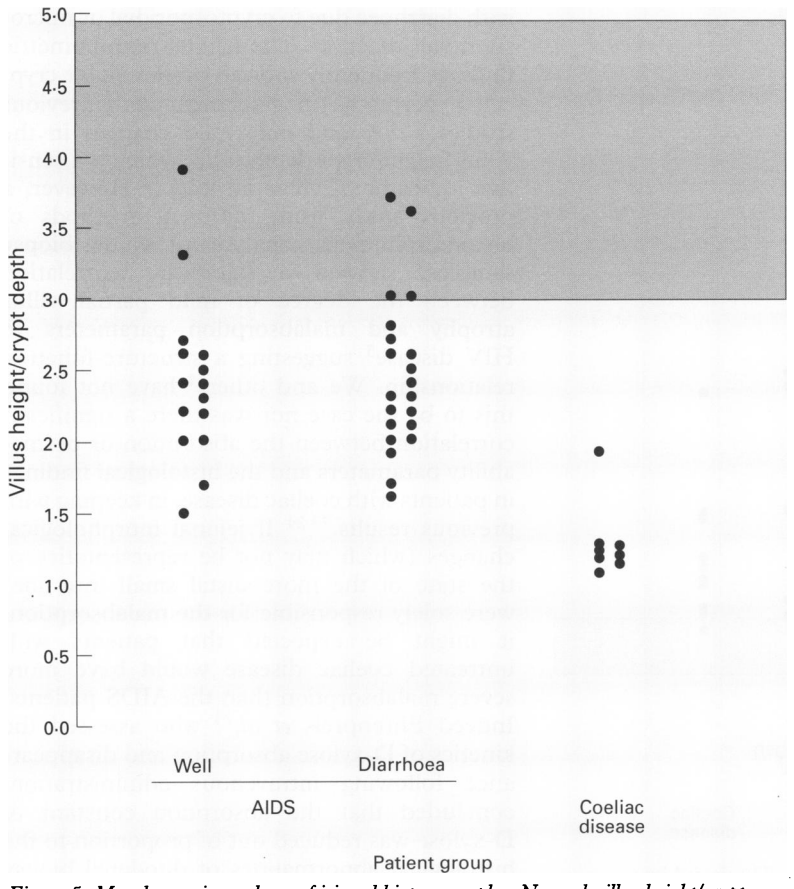

Figure 5: Morphometric analyses of jejunal biopsy samples. Normal villus height/crypt depth ratio is represented within the shaded box. Patients with AIDS have less severe changes than patients with coeliac disease.

diseases.[34] Similar findings were reported by Lim *et al.*[35]

While increased intestinal permeability is a non-specific finding, seen in a variety of situations,[13–15 28 36 37] it may be relevant in the present context that an increase in intestinal permeability is seen in some viral infections.[28 29] The consequences of increased intestinal permeability are the subject of speculation. In non-HIV related disease it may allow permeation of macromolecules, thereby promoting some of the systemic manifestations of disease,[38–41] but it is more likely to result in the development of a low grade enteropathy.[14 15 18]

In summary, intestinal absorptive capacity and integrity are usually maintained in asymptomatic homosexual patients before the development of AIDS. Increased intestinal permeability is almost invariably found in AIDS but the immediate clinical implications are uncertain. Intestinal malabsorption is common after the development of AIDS and has important implications for nutrition in these patients. Although our results can not be extrapolated to other groups of patients with AIDS (intravenous drug users, haemophiliacs, etc), it is suggested that the severity of malabsorption in homosexuals with AIDS is at times so great as to preclude attempts at supplementation by the enteral route.

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